ANTIPYRINE CLEARANCE IN INDIAN VILLAGERS

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- 1 Antipyrine clearance has been measured using saliva samples in 50 Maharashtrians from a village to the north of Bombay.
- 2 All subjects were very lean and were also anaemic probably as a result of hookworm infestation.
- 3 Antipyrine clearance was 36% faster than in White Londoners studied previously and more than twice as fast as in Asian immigrants living in London.
- 4 Clearance was 25% faster in men than in women but volume of distribution was also greater in men and mean half-lives did not differ significantly between the sexes.
- 5 There was a negative correlation between antipyrine clearance and haemoglobin concentration.
- 6 The study has identified geographic differences in antipyrine metabolism which could be clinically important. The role of anaemia merits further study.

Introduction

There are large interindividual differences in the rate at which drugs are oxidized in man. Both genetic and environmental factors have been shown to be important in determining the rate of drug oxidation. Among identical twins, not exposed to other drugs, there is a very high degree of concordance in rates of drug metabolism (Alexanderson, Price-Evans & Sjöqvist, 1969). Comparisons of identical and fraternal twins have led to estimates of the genetic contribution to the variance in rates of oxidation as high as 99% (Vesell, 1972). However, identical twins tend to share a similar environment as well as an identical genome and there is now considerable evidence that environmental factors such as other drugs, cigarette smoking, alcohol intake and exposure to halogenated hydrocarbon insecticides make a substantial contribution to the variance (Fraser & Dollery, 1978).

The vast majority of drugs have been developed in Western countries and pharmacokinetic studies and

drug trials have usually been carried out in well-nourished European or North American volunteers. In spite of the apparent importance of genetic factors, it has generally been assumed that there are no important ethnic differences in rates of drug oxidation and little attention has been paid to the variation in environmental factors in different populations. If genetic and environmental differences bring about major changes in drug kinetics, the universal use of dose ranges determined in subjects living in Western countries may be inappropriate.

We have shown in a previous study (Fraser, Bulpitt, Kahn, Mould, Mucklow & Dollery, 1976) that nutritional factors and social drug use account for a significant proportion of the variance in antipyrine elimination in West African villagers, a sample of whom were studied in Gambia. In a subsequent study of Asian and White subjects living in London (Fraser, Mucklow, Bulpitt, Kahn, Mould & Dollery, 1979) we identified a larger number of factors contributing to variation in clearance including tobacco smoking, the contraceptive pill and race. The mean clearance of the Asians, most of whom were vegetarians, was 40% slower than that of the White Londoners. We thought it would be useful to investigate another population of Asians living in

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the Indian subcontinent and we were able to carry out a study in a Maharashtra village.

Methods

Patients and procedure

Dapchari is a small village in Maharashtra, about 150 kilometres north-east of Bombay. The inhabitants, who speak Marathi, live in a relatively poor agricultural community but there have been no recent major droughts or food shortages. Hookworm infestation affects the majority of the population.

Some villagers were known to scientific workers as a result of previous genetic studies and they and their families agreed to participate. Sixty-one subjects (33 men) gave informed consent to take part. All were healthy, on no medication and gave no past history of jaundice, liver disease or thyroid disease.

Weight and height were measured and all volunteers were questioned about use of alcohol and tobacco. The local alcohol is a crude product of fermented palm tree juice, known as toddy, with an alcohol content of between 6 and 9 g/100 ml. Toddy consumption was estimated in litres per week. Tobacco use was estimated as the number of native cigars (biddi) smoked per day or the weight (in g) of tobacco chewed.

Diet histories were taken from each subject by a qualified dietician who was asked to assess both the total calorie intake and the meat intake. The diet was uniform and comprised three staple items, rice, Indian bread (bhakari) and ground lentils (wadi dal), with a modest amount of meat, mainly poultry.

After an overnight fast each subject received 600 mg antipyrine orally with 150 ml of drinking water. No food or drink was allowed until after the first saliva sample had been obtained. Samples were collected at 3, 5, 8, 24 and 32 h after the dose, using a piece of Parafilm (Gallenkamp) to stimulate salivary flow. The particulate matter in the samples was allowed to settle and the supernatant decanted, packed and stored frozen for subsequent despatch to London for analysis.

All subjects had capillary blood samples taken for haemoglobin estimation. Thirty subjects provided a venous blood sample, serum from which was also sent to London for estimation of serum albumin, globulin, bilirubin, alkaline phosphatase and aspartate transaminase.

Methods

Antipyrine estimations were performed by a gas chromatographic method described previously (Fraser, Mucklow, Murray & Davies, 1976).

Elimination rate constants (K_{el}) and half-lives ($T_{1/2}$) were obtained by least squares regression analysis. After back-extrapolation of the terminal β phase of the log saliva concentration-time curve (to give the apparent concentration at zero time C_0) the apparent volume of distribution (V_d) was calculated by dividing the dose by C_0 . Clearance (Cl) was then given by:

$$Cl = \frac{0.693}{T_{1/2}} \times V_d$$

Haemoglobin was estimated by Sahli's method (Wintrobe, 1974) and protein and liver function tests using a Technicon SMA plus autoanalyser. Differences between groups were tested for significance using Student's *t*-test. Multiple regression analyses were carried out using a stepwise regression programme (Biomedical Computer Programme BMD 02R).

Results

Saliva sampling or antipyrine estimation were technically unsatisfactory for eleven subjects, leaving 50 (30 men and 20 women) for analysis and presentation. The majority of both men and women were aged between 20 and 30 years, with a range of 20–50 and a mean of 26.5 years. Anthropometric, haemoglobin and protein values are shown in Table 1 according to sex. Although men were taller and heavier than women, ponderal indices were significantly greater in women than in men ($P < 0.001$), where ponderal index (P.I.) was given by $P.I. = \text{Weight (g)} \div \text{Height (cm)}^2$.

Haemoglobin values were low throughout the sample, no individual value exceeding $10 \text{ g } 100 \text{ ml}^{-1}$. Values were significantly lower in women ($7.5 \text{ g } 100 \text{ ml}^{-1} \pm 0.7 \text{ s.d.}$) than in men (8.4 ± 1.0) ($P < 0.001$). Mean serum albumin and globulin values were within the normal range in the 27 subjects sampled although four subjects had a low albumin ($< 30 \text{ g l}^{-1}$) and 11 a high globulin ($> 30 \text{ g l}^{-1}$). Men and women had similar mean albumin and globulin values. With the exception of one elevated alkaline phosphatase value (175 i.u. l^{-1}) none of the 27 subjects examined had abnormalities of plasma bilirubin or liver enzymes.

The diet was fairly uniform throughout the population sampled. Calorie intake from the staple food sources and meat intake are shown in Table 2. The three staples contributed an estimated average total of 1232 k calories to the daily calorie intake. All subjects ate meat on several occasions during the week, but the average total weekly intake of 252 g was low by European standards and no individual ate more than 300 g per week. Men ate significantly more meat, rice and bread than did women.

Table 1 Nutritional indices (mean) of fifty subjects according to sex

	Men (n=30)	Women (n=20)	All (n=50)
Weight (kg)	45.0	41.6*	43.6
Height (cm)	162	151**	157
Ponderal index (Weight. Height ⁻² : g cm ⁻²)	1.71	1.84**	1.76
Haemoglobin (g 100 ml ⁻¹)	8.4	7.5**	8.0
	(n=17)	(n=10)	(n=27)
Albumin (g l ⁻¹)	38.1	40.0	38.8
Globulin (g l ⁻¹)	29.3	29.5	29.4

Significance of difference between men and women **P* < 0.01, ***P* < 0.001.

Table 2 Calorie and meat intake (mean ± s.d.) according to sex, assessed by diet histories

	Men (n=30)	Women (n=20)	All (n=50)
<i>K cal per day</i>			
as rice	785	596**	709
	± 98	± 153	± 153
as bread	428	291**	373
	± 44	± 111	± 102
as lentils	150	150	150
	± 0	± 0	± 0
Total as rice, bread and lentils	1363	1037**	1232
	± 134	± 163	± 214
Meat intake (g per week)	266	233*	253
	± 43	± 41	± 45

Significance of difference between men and women **P* < 0.01, ***P* < 0.001.

Toddy was consumed regularly by 40 subjects although by a much larger proportion of men (97%) than of women (55%). The weekly consumption by both sexes ranged from 0.25 to 7 l (mean 1.5 l). Eleven subjects (ten men) smoked biddi and four chewed tobacco. One man used tobacco by both routes. The average number of biddi smoked each day ranged from 2.5 to 25 in the smokers. Three men chewed 3.5 g and one chewed 3 g of tobacco each day.

Mean (± s.d.) values for antipyrine clearance, half-life and volume of distribution are shown according to sex in Table 3. The overall mean ± s.e. mean clearance was 0.94 ± 0.04 ml min⁻¹ kg⁻¹ and the overall mean half-life 11.9 ± 0.4 h. Clearance was significantly faster in men than in women (*P* < 0.001) but volume of distribution was also greater in men (*P* < 0.001) and half-lives did not differ significantly.

Antipyrine clearance and half-life were regressed upon 11 observed variables and the first-order correlation coefficients are shown in the correlation matrix (Table 4). Significant correlation (*P* < 0.01)

Table 3 Antipyrine clearance, half-life and volume of distribution (mean ± s.d.) for fifty subjects according to sex

	Men (n=30)	Women (n=20)	All (n=50)
Clearance (ml min ⁻¹ kg ⁻¹)	1.06	0.77**	0.94
	± 0.24	± 0.28	± 0.29
Half-life (h)	11.5	12.5	11.9
	± 3.0	± 2.7	± 2.9
Volume of distribution (l kg ⁻¹)	1.02	0.78**	0.92
	± 0.23	± 0.13	± 0.23

Significance of difference between men and women ***P* < 0.001.

was found between clearance and sex (*r* = 0.5), serum albumin (*r* = -0.4) and smoking status (*r* = 0.3), while smoking status alone correlated significantly (*P* < 0.05) with half-life (*r* = 0.29). In these and subsequent analyses, sex is classified as 1 for men and 2 for women and smoking status as 1 for non-smokers and 2 for smokers. Mean clearance did not differ significantly between smokers and chewers of tobacco and all tobacco users were therefore classified as smokers.

In this study, as in others of its type, simple correlation coefficients do not necessarily reflect the independent effect of the observed variables upon clearance and half-life. Multiple regression analysis was therefore performed in an attempt to determine independent correlates with clearance and half-life. In view of the apparent effect of sex upon clearance, separate analyses were carried out for men, women and all subjects together. Tables 5 and 6 show the independent correlates identified in all these analyses with their partial regression coefficients and, for each analysis, the multiple correlation coefficient and its square. The constants for the multiple regression equations are also shown.

Haemoglobin concentration featured prominently in virtually all analyses, while age, ponderal index and smoking status appeared at least twice. In women alone, weight, height and meat intake emerged as independent correlates with clearance.

Discussion

The population of Dapchhari differed from Asians living in London (Fraser *et al.*, 1979) in many ways. The Maharashtrians were both shorter and lighter and had ponderal indices markedly lower (*P* < 0.001) than their London counterparts. Both men and women were extremely lean, consistent with their low calorie intake.

Table 4 Correlation matrix

Variable	Sex	Age	Weight	Height	Ponderal index	Hb	Alb	Glob	Smoke	Alcohol	Meat	V _d	T _{1/2}	Cl
Sex	1.00	0.33*	-0.43*	-0.73*	0.44*	-0.42*	0.17	0.06	-0.42*	-0.24	-0.37*	0.51*	0.18	-0.50*
Age		1.00	-0.08	-0.22	0.22	-0.08	0.14	-0.001	0.17	-0.15	-0.30*	-0.16	0.17	-0.26
Weight			1.00	0.66*	0.29*	0.41*	0.02	0.07	0.08	-0.03	0.07	0.28*	0.10	0.16
Height				1.00	-0.53*	0.32*	0.11	0.20	0.24	0.11	0.15	0.34*	0.02	0.23
Ponderal index					1.00	0.05	-0.11	-0.16	-0.20	-0.18	-0.11	-0.12	0.06	-0.09
Haemoglobin						1.00	0.12	-0.21	0.14	-0.14	0.12	0.15	0.26	-0.14
Albumin							1.00	0.50	0.24	-0.40	-0.36*	-0.13	0.22	-0.40*
Globulin								1.00	-0.32*	-0.13	-0.08	0.10	-0.04	0.08
Smoking									1.00	0.16	0.22	0.06	-0.29*	0.30*
Alcohol										1.00	0.08	0.16	-0.07	0.17
Meat											1.00	-0.001	-0.25	0.21
V _d												1.00	0.20	0.55*
T _{1/2}													1.00	-0.67*
Clearance														1.00

*Significance of r values $r > 0.279$ $P < 0.05$, $r > 0.361$ $P < 0.01$, $r > 0.452$ $P < 0.001$
D.F. = 48

Table 5 Independent correlates with antipyrine clearance

Subjects	Correlates	Partial regression coefficients	Constant	Multiple R R ²	
All (50)	Sex	-0.50***	1.78	0.68	0.46
	Haemoglobin (g 100 ml ⁻¹)	-0.15***			
	Ponderal index (g cm ⁻²)	0.60*			
Men (30)	Haemoglobin	-0.15***	2.28	0.58	0.34
Women (20)	Age (years)	-0.02**	8.25	0.76	0.58
	Weight (kg)	0.05**			
	Height (cm)	-0.06***			
	Meat intake (g week ⁻¹)	-0.003*			

Sex = 1 for men, 2 for women **P* < 0.05 ***P* < 0.01 ****P* < 0.001

Table 6 Independent correlates with antipyrine half-life

Subjects	Correlates	Partial regression coefficients	Constant	Multiple R R ²	
All (50)	Smoking	-2.44**	4.31	0.50	0.25
	Haemoglobin (g 100 ml ⁻¹)	1.01*			
	Age (years)	0.95*			
Men (30)	Haemoglobin (g 100 ml ⁻¹)	1.25*	4.10	0.57	0.32
	Smoking	-2.14*			
Women (20)	Ponderal index (g cm ⁻²)	-13.0**	20.25	0.69	0.48
	Age (years)	0.15*			
	Haemoglobin (g 100 ml ⁻¹)	1.53*			

Smoking status = 1 for non-smokers, 2 for smokers

P* < 0.05 *P* < 0.01

Haemoglobin values were low in all subjects. The villagers are known to suffer from a high level of infestation with hookworm (*Necator americanus*) although stool examinations were not carried out during this study. Hookworm is a common cause of iron deficiency anaemia in the tropics. Its severity is related to the burden of worms and is most marked in women and in those having deficient or borderline diets.

In spite of the low calorie diet and severe anaemia, subjects did not appear to be deficient in protein. They obtained protein from both meat and lentils, in excess of the minimum daily requirements recommended in Britain, and only four of the 27

subjects whose serum was examined had a low serum albumin.

The pattern of social drug use was very different from that of the Asians in London. Alcohol was taken liberally in Dapchari and one-third of the men used tobacco, whereas in London, 84% of the Asians took neither alcohol nor tobacco.

Comparison of antipyrine clearance between these subjects and Asians and Whites living in London reveals markedly faster rates in the Maharashtrians, mean clearance being more than twice that of London Asians and 36% greater than London Whites (Figure 1). As almost half the London Asians originated from either Maharashtra or neighbouring

Figure 1 Frequency distributions of antipyrine clearance in (a) White British subjects, (b) Maharashtrians and (c) Asian immigrants to Britain. Mean values (indicated by arrows) are all significantly different ($P < 0.001$) from each other.

Gujarat, the difference between the two Asian populations is unlikely to be simply ethnic.

The apparent volume of distribution (corrected for body weight, V_d) in the Maharashtrians was both greater than expected and greater than that found in previous studies. The most likely explanation for this is the extreme leanness of the subjects (mean ponderal index 1.76) by comparison with Whites (2.38) and Asians (2.40) living in London (Fraser *et al.*, 1979). A paucity of adipose tissue would result in a high apparent volume of distribution (when corrected for body weight). However, in calculating V_d and clearance, the bio-availability of antipyrine is assumed to be complete (Andreassen & Vesell, 1974) and absorption to be rapid compared with elimination rate. This assumption may be invalid for subjects with hookworm infestation. *Necator americanus* invades the jejunal mucosa and produces

a marked inflammatory response, partial villous atrophy, and in severe cases, complete loss of the normal villous pattern (Sheehy, Meroney, Cox & Soler, 1962). This is associated with impaired intestinal absorption of fat, vitamin A and folate. Absorption of lipid soluble drugs may also be impaired but as no evidence exists to support this possibility, absorption was assumed to be complete in this study.

Antipyrine clearance was significantly associated with three independent variables, sex, haemoglobin and ponderal index. Men cleared antipyrine 25% faster than women. There is no consistent evidence in the literature for a sex difference in drug metabolism in man and it may be that this finding reflects greater cigarette use, alcohol consumption and meat intake in males. As meat, alcohol and tobacco intake were all larger in men, a strong correlation between male sex and antipyrine clearance would be anticipated on the basis of earlier findings with these variables. When male sex is taken into the regression equation before meat, alcohol and tobacco intake, these factors no longer appear as significant independent variables. This problem needs to be borne in mind when interpreting the results of the regression analyses.

The multivariate regression analyses consistently identified haemoglobin as an independent correlate with both clearance (–ve) and half-life (+ve). A similar relationship was seen in black women studied in Gambia. In that sample only the women were significantly anaemic. A negative correlation between clearance and haemoglobin might be anticipated by analogy with animal data as iron deficiency in rats produces marked stimulation of metabolism of Type I (aminopyrine) and Type II (aniline) substrates, both in vivo and in vitro (Becking, 1976). It is not yet clear how iron deficiency controls microsomal oxidation but the observation of this effect in two very different populations with anaemia suggests that it could be clinically important. The apparent effect of anaemia upon antipyrine metabolism may be the main reason for the differences in clearance and half-life between the Maharashtrians and the Asian immigrants in London, whose haemoglobin values were within the normal range.

Smoking status, age and ponderal index emerged as independent correlates with one or both kinetic variables, each appearing in at least two of the six multivariate analyses. Antipyrine is a drug which has a low intrinsic hepatic clearance (Branch, Nies & Shand, 1973) and factors affecting its metabolism should therefore produce changes in both total body clearance and half-life. Similarly, there is no reason to expect that any of the above factors will affect antipyrine metabolism differently in men and women. The apparent lack of consistency between the correlates identified by the different multivariate

analyses therefore reflects, most probably, the small numbers of subjects in the samples analysed.

Smokers had shorter half-lives than non-smokers. This supports our earlier finding among White subjects in London that clearance was faster in smokers, regardless of sex, and is consistent with earlier work (Vestal, Norris, Tobin, Cohen, Shock & Andres, 1975).

Half-life increased with increasing age in all subjects and clearance fell with age in women. This also supports the findings of earlier workers in relation to antipyrine half-life (O'Malley, Crooks, Duke & Stevenson, 1971; Vestal *et al.*, 1975).

The effect of ponderal index on clearance and half-life cannot easily be explained. In a European population an increase in ponderal index reflects increased adiposity. Since antipyrine is distributed in body water, the apparent volume of distribution falls with increasing obesity and may reduce half-life, but a positive correlation between clearance and ponderal index must reflect factors other than the volume of distribution.

Studies of variation in rates of drug metabolism in population samples are useful to identify factors which may affect inter-individual variation. Proof of a cause-and-effect relationship is more difficult and it is usually necessary to proceed to controlled studies in which single factors are examined in isolation, others being held constant. Alvares, Anderson, Conney & Kappas (1976) showed, using this technique, that high protein diets accelerated both antipyrine and

theophylline elimination, and a similar approach will be necessary as other environmental factors, with a potential for altering drug clearance, are identified.

If those environmental factors affecting antipyrine metabolism are shown to affect other drugs, this would have important clinical consequences. Two-fold changes in clearance rate would significantly alter steady state plasma concentrations and, by implication, the average dose requirement of a drug metabolized by oxidation would differ by this amount: comparison of Asians in London and Maharashtrians in Dapchari reveals a two-fold difference in mean clearance, which has not been recognized previously and which may relate to the difference in haemoglobin concentration. Furthermore, major variation in the incidence of adverse drug reactions could result following exposure of differing populations to drugs and environmental chemicals which cause toxicity, mutagenicity and carcinogenicity by means of activated intermediates of metabolism (Dollery, Fraser, Mucklow & Bulpitt, 1979; McLean, Chenery, Greaves & Fisher, 1978).

The study formed part of a cooperative investigation of rates of drug metabolism sponsored by the Clinical Pharmacology Section of the International Union of Pharmacology.

We would like to thank Miss Pamela Clifton of the London School of Hygiene and Tropical Medicine, for help in data processing, and Mr Geoffrey Mould, for performing the biochemical analyses. J.C.M. and H.S.F. were supported by Wellcome Trust Fellowships in Clinical Pharmacology.

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(Received June 8, 1979)