THE RELATIONSHIP BETWEEN INDIVIDUAL DIETARY CONSTITUENTS AND ANTIPYRINE METABOLISM IN INDO-PAKISTANI IMMIGRANTS TO BRITAIN

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- 1 Antipyrine clearance has been measured from serial saliva samples in 36 healthy adult Indo-Pakistani immigrants to Britain, to assess the effect of dietary differences within this population.
- 2 Clearance (mean $\pm$ s.e. mean) was significantly slower in 16 lactovegetarians (0.54 ± 0.06 ml min⁻¹ kg⁻¹) than in the subjects who ate meat regularly (0.91 ± 0.07 ml min⁻¹ kg⁻¹).
- 3 The absence of meat from the diet was associated with a significantly smaller daily intake of dietary protein, which was abnormally low by Western standards.
- 4 It is likely that the contrast in daily protein intake between the dietary subgroups was largely responsible for the differences observed in antipyrine clearance.

Introduction

Among the numerous environmental factors which are known to influence microsomal mono-oxygenase activity in man (Alvares, 1978; Conney, 1967; Fraser & Dollery, 1978; Vesell, 1972), the importance of diet and the means by which food is prepared have recently been recognised. An increased intake of dietary protein accelerates elimination of antipyrine and theophylline while increased dietary carbohydrate produces the opposite effect (Kappas *et al.*, 1976). Deliberate inclusion in the diet of cruciferous vegetables, such as Brussels sprouts and cabbage, stimulates the metabolism of antipyrine and the presystemic metabolism of phenacetin (Pantuck *et al.*, 1979). Phenacetin, antipyrine and theophylline are all metabolised more rapidly if the diet contains beef which has been charcoal-broiled (Conney *et al.*, 1976; Kappas *et al.*, 1978).

A recent study of antipyrine metabolism in a large urban population (Fraser *et al.*, 1979) showed that antipyrine clearance was 40% slower in Indo-Pakistani immigrants to Britain than in native White subjects. A large majority of the immigrants ate a vegetarian diet and this could have contributed to the ethnic variation. However, multiple regression

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analysis could not dissociate diet from race as an independent correlate with antipyrine clearance.

The present study was designed to disclose the relationship between dietary meat and antipyrine metabolism, in a more homogeneous population, and to examine the effects of individual dietary constituents.

Method

Subjects and procedure

Enquiries made through the local Community Relations Council resulted in the recruitment of 43 Indo-Pakistani immigrants (27 men) living in or around Newcastle upon Tyne. An attempt was made to select equivalent numbers of vegetarians and non-vegetarians. A past history of jaundice or liver disease were contraindications to inclusion in the study, and all volunteers were in good health and receiving no regular medication.

The nature of the study was explained to each subject, using an interpreter where necessary, and each volunteer gave written informed consent to take part. Subjects were weighed and provided informa-

tion about their use of tobacco, alcohol, coffee and tea. Use of tobacco was recorded as cigarettes per day, and weekly consumption of alcohol in units equivalent to one half-pint of beer, one glass of wine, or one measure of sherry or spirits (each containing approximately 12 g of alcohol; Laurence, 1973). A coffee/tea index (cups of coffee per day + 0.6 × cups of tea per day) was also estimated (Nagy, 1974).

A diet history was obtained from each volunteer and the daily intake of total kilocalories, carbohydrate, fat and protein were subsequently estimated (McCance & Widdowson, 1978). The weekly intake of meat was also recorded.

After an overnight fast, each subject swallowed a single capsule (600 mg) of antipyrine, followed by a glass of drinking water. No food or drink were permitted for a further 2 h. Subjects were shown how to produce a saliva sample, using a small piece of Parafilm (Gallenkamp) as a sialogogue, and were asked to produce samples (5 ml) at 3, 5, 8, 13, 24 and 32 h after the dose, recording the precise time on the instruction sheet provided. Venous blood was drawn for the measurement of haemoglobin, albumin, bilirubin, alkaline phosphatase and aspartate transaminase concentrations. An aliquot was used for determination of 12 blood group loci (ABO, Rh, MNS, P, Kell, Duffy, Kidd, Lutheran and Lewis) and 10 red cell enzymes (adenosine deaminase, phosphoglucomutase 1 and 2, acid phosphatase, adenylate kinase, 6-phosphogluconate dehydrogenase, glucose 6-phosphate dehydrogenase, lactate dehydrogenase, malate dehydrogenase and esterase D). Alleles within these categories together occupy loci on a total of 12 chromosome pairs.

Subjects were requested not to take proprietary

medicines for 24 h before, or during the study and to return their saliva samples after completion of the series. The samples were stored frozen prior to analysis.

Methods

Antipyrine concentrations were measured using a gas chromatographic method (Prescott *et al.*, 1972). Elimination half-life ($T_{1/2}$) was estimated using the method of least squares and assuming a one-compartment model. The saliva antipyrine concentration at zero time (C_0) was obtained by back extrapolation of the terminal regression line. Bioavailability of antipyrine was assumed to be complete (Andreassen & Vesell, 1974; Mucklow, 1980). Total body clearance was calculated using the formula:

$$\text{Clearance} = \frac{0.693 \cdot \text{Dose}}{T_{1/2} \cdot C_0 \cdot \text{body weight}} \text{ ml min}^{-1} \text{ kg}^{-1}$$

Red cell enzymes were typed using horizontal starch gel electrophoresis and enzyme staining (Papiha *et al.*, 1972).

Multiple regression analysis, incorporating a stepwise regression procedure, was carried out using the Statistical Package for the Social Sciences (SPSS) regression subprogram. Differences between groups were tested for significance using either Student's *t* or chi-square tests.

Results

Six subjects failed to return their samples despite repeated reminders, and one labelled her samples

Table 1 Distribution of subjects according to sex and religion, with mean ($\pm$ s.d.) values for the daily intake of kilocalories and major dietary constituents and for nutritional indices, Results shown for vegetarians, non-vegetarians and all subjects together.

Variable	No meat (n = 16)	Meat (n = 20)	All subjects (n = 36)
Sex			
Men	7	15	22
Religion			
Hindu	16	7	23
Muslim	0	12	12
Sikh	0	1	1
Diet			
Kcals	2155 $\pm$ 852	2507 $\pm$ 906	2350 $\pm$ 888
Protein (g)	52 $\pm$ 21	78* $\pm$ 17	66 $\pm$ 21
Carbohydrate (g)	292 $\pm$ 114	281 $\pm$ 120	286 $\pm$ 116
Fat (g)	87 $\pm$ 46	118 $\pm$ 50	104 $\pm$ 50
Nutritional indices			
Weight (kg)	62.7 $\pm$ 15.3	63.6 $\pm$ 11.0	63.2 $\pm$ 12.9
Haemoglobin (g 100 ml ⁻¹)	13.8 $\pm$ 1.7	14.5 $\pm$ 1.8	14.2 $\pm$ 0.8
Albumin (g l ⁻¹)	47.9 $\pm$ 3.1	48.3 $\pm$ 2.8	48.1 $\pm$ 2.9

**P* < 0.001.

incorrectly. Complete data were available for only 36 subjects (22 men). There were 23 Hindus, whose families originated in Gujarat (20), Uttar Pradesh (2) or East Punjab (1), 12 Muslims from Pakistan (4), Bangla Desh (4), Bihar (2), East Punjab (1) or Uttar Pradesh (1) and one Sikh from East Punjab. The Hindus, 19 of whom had been born in Africa, had arrived in Britain between 1971 and 1975, while the remaining subjects had lived in Britain for at least 10 years. With the exception of three married couples, one with their son, and a mother with her two children, the volunteers were unrelated to each other. Their ages ranged from 18–64 (mean 31.3) years.

Twenty subjects ate meat regularly, the estimated weekly intake varying from 200–1400 g (mean 684 g), while the remaining 16 were lactovegetarians. Table 1 shows the distribution of subjects according to diet, sex and religion (the latter being also largely indicative of the state of origin and the length of time spent in Britain). The mean ages of the vegetarians (33.5 years) and non-vegetarians (29.8 years) did not differ significantly.

The estimated intake of kilocalories and major dietary constituents is also shown in Table 1 for vegetarians, non-vegetarians and all subjects together. Mean values for kilocalories and carbohydrate intake did not differ significantly. Mean daily protein was significantly greater ($P < 0.001$) in subjects eating meat. The difference in daily fat intake between the two groups did not quite achieve statistical significance ($0.05 < P < 0.1$). The mean coffee/tea index was significantly greater ($P < 0.05$) in those eating meat (3.3 ± 0.5 s.e. mean) than in vegetarians (2.1 ± 0.2). Six subjects (3 men) drank alcohol (weekly consumption 12–144 g) and six men smoked cigarettes (consumption 12–40 per day). None of the women used an oral contraceptive.

Nutritional indices are shown in Table 1 for vegetarians, non-vegetarians and all subjects together. There were no significant differences between the dietary subgroups and apart from two isolated abnormalities of alkaline phosphatase (121, 146 IU l⁻¹), one of bilirubin (25 μ mol l⁻¹), and one of

aspartate transaminase (121 IU l⁻¹), all values were within normal limits for the local laboratory.

The phenotype frequencies in respect of the 12 blood group loci and 10 red cell enzymes were examined in vegetarians and non-vegetarians. There were no significant differences between the two dietary subgroups.

Mean values for antipyrine clearance, half-life and volume of distribution are shown in Table 2 according to meat intake and for all subjects together. Clearance was significantly faster ($P < 0.001$) in subjects eating meat regularly than in lactovegetarians. Volume of distribution was also significantly greater ($P < 0.01$) in the meat eaters, however, so that mean half-lives did not differ between the two groups. Mean ($\pm$ s.e. mean) clearance was significantly faster ($P < 0.001$) in men (0.88 ± 0.05 ml min⁻¹ kg⁻¹) than in women (0.56 ± 0.05) and also significantly faster ($P < 0.005$) in smokers (1.17 ± 0.34) than in non-smokers (0.66 ± 0.25). However, all the smokers ate meat and Figure 1 shows antipyrine clearance in relation to both diet and smoking status. Clearance was faster in smokers irrespective of diet and faster amongst non-vegetarians irrespective of smoking status.

Antipyrine clearance was regressed upon 14 observed variables to identify linear correlation (Table 3). Variables without an interval scale were classified by number: 1 for non-smokers, 2 for smokers; 1 for Hindus, 2 for Muslims and Sikhs; 1 for men, 2 for women. Significant correlations were observed between clearance and age, sex, religion, smoking, haemoglobin and the daily intake of meat, kilocalories, protein, carbohydrate and fat. No significant correlation was observed with weight, coffee/tea index, alcohol intake or plasma albumin.

When non-vegetarians alone were considered, there was no significant correlation between antipyrine clearance and daily meat intake, raising the possibility that one or more other dietary factors might be of greater importance. Moreover, many of the variables correlated significantly with each other as well as with clearance. Stepwise multiple regression analysis was therefore performed and identified

Table 2 Mean ($\pm$ s.d.) antipyrine clearance, half-life and volume of distribution for vegetarians, non-vegetarians and all subjects together.

	No meat (n = 16)	Meat (n = 20)	All subjects (n = 36)
Clearance (ml min ⁻¹ kg ⁻¹)	0.54 $\pm$ 0.25	0.91** $\pm$ 0.3	0.74 $\pm$ 0.33
Half-life (h)	14.5 $\pm$ 6.5	11.9 $\pm$ 4.3	13.0 $\pm$ 5.4
Volume of distribution (l kg ⁻¹)	0.65 $\pm$ 0.21	0.85* $\pm$ 0.25	0.76 $\pm$ 0.25

* $P < 0.02$, ** $P < 0.001$.

Figure 1 Antipyrine clearance (mean $\pm$ s.e. mean) in vegetarians and non-vegetarians, smokers $\blacksquare$ and non-smokers $\square$. Amongst non-smokers, vegetarians: non-vegetarians: $P < 0.05$. Amongst non-vegetarians, smokers: non-smokers; $P < 0.01$.

smoking status, age and fat intake as independent correlates with clearance (Table 4). Clearance was faster in smokers than in non-smokers, fell with age and increased with total daily fat intake. These three variables accounted for 71% of the total variance in antipyrine clearance ($r = 0.84$, $r^2 = 0.71$).

Discussion

The subjects who took part in this study were deliberately selected by virtue of their ethnic origin

Table 4 Independent correlates with antipyrine clearance, showing partial regression coefficients (r) with standard error (s.e.) and significance (F) values, for 36 subjects. The regression equation constant was 0.27.

Correlates	r	s.e.	F
Smoking status	0.45	0.09	26.6**
Age	-0.01	0.002	14.2**
Dietary fat	0.002	0.0007	12.1*

* $P < 0.01$, ** $P < 0.001$.

and dietary habits. The sample was therefore more homogenous than a true population sample and the results obtained do not necessarily apply to the Indo-Pakistani immigrant population at large. Although all subjects were resident in Britain, their origins within the Indian subcontinent varied, as did their religions. The determination of blood group and red cell enzyme phenotypes revealed no unexpected variation within the sample, but the small numbers involved made it impossible to infer genetic homogeneity with great confidence.

The small number of smokers in the sample and the in excessive consumption of alcohol and caffeine-containing beverages resembled the pattern of social habits found in the Indo-Pakistani immigrants studied previously in London (Fraser *et al.*, 1979). It therefore proved possible to examine the effect of dietary variables independent of many other environmental factors.

Retrospective dietary assessments made at a single interview, even when this is conducted by an experienced dietitian, are open to criticism since they rely heavily upon the memory of the subject interviewed, the estimates of quantity made by the dietitian and the subsequent expression of those quantities in mass units. The opportunity for error is clearly increased when the interview is conducted through an interpreter. Nevertheless, standardisation was attempted in this study by ensuring that all interviews were conducted by the same dietitian and, in most instances, through the same interpreter.

Table 3 Significant correlates with antipyrine clearance with simple correlation coefficients and significance levels.

Variable	Correlation coefficient	Significance
Fat intake	0.62	$P < 0.001$
Smoking	0.59	
Religion	0.56	$P < 0.01$
Protein intake	0.55	
Kilocalories	0.54	
Meat intake	0.49	
Age	0.47	$P < 0.05$
Sex	0.46	
Haemoglobin	0.39	
Carbohydrate intake	0.33	

In previous population studies of antipyrine clearance, sex has not emerged as a consistent correlate and only one study (O'Malley *et al.*, 1971) has suggested that clearance is slightly faster in men. It is likely that the difference between men and women in this present study was due to the disproportionate number of non-vegetarian men. The apparent effect of tobacco smoking on antipyrine clearance is consistent with the findings of previous studies (Vestal *et al.*, 1975; Hart *et al.*, 1976; Fraser *et al.*, 1979). The correlation between antipyrine clearance and age is also in accord with earlier findings. O'Malley and his colleagues (O'Malley *et al.*, 1971; O'Malley, 1972) demonstrated prolongation of antipyrine half-life and reduced clearance in elderly subjects, and this has been confirmed by Vestal and co-workers (Vestal *et al.*, 1975).

Forty-eight of the 56 Indo-Pakistani immigrants studied in London (Fraser *et al.*, 1979) were lacto-vegetarian and antipyrine clearance values lay in the range 0.2–0.7 ml min⁻¹ kg⁻¹. Only three of the 16 lactovegetarians studied in Newcastle had values which lay outside this range although the mean ($\pm$ s.e. mean) clearance in the Newcastle subjects (0.54 ± 0.06 ml min⁻¹ kg⁻¹) was faster than the London subjects (0.42 ± 0.02 ; $P < 0.05$). Clearance values in the 20 non-vegetarians resembled earlier findings amongst White non-vegetarians (Fraser *et al.*, 1979).

The difference in antipyrine clearance between vegetarians and non-vegetarians, independent of smoking habit, suggests that the presence (or absence) of meat in the diet on a regular basis may modify microsomal enzyme activity. It also lends support to the argument that the difference in antipyrine clearance found in the earlier study in London (Fraser *et al.*, 1979) between White British subjects and Indo-Pakistani immigrants may have been at least partly due to their contrasting diets. The apparent effect of meat could relate to the difference of protein intake, fat intake or both. Daily protein intake was significantly lower amongst vegetarians, while daily fat intake was similar in the two subgroups and it was surprising that regression analysis identified fat intake as the only independent dietary correlate with antipyrine clearance. It is unwise to

attach too great importance to the results of multiple regression analysis in a small population sample in which several of the independent variables are so closely associated. Moreover, recent studies provide support for the hypothesis that protein is the dietary constituent most likely to be responsible.

Brodie and co-workers (Brodie *et al.*, 1980) found that in White subjects native to Britain, a vegetarian diet is not characterised by a low protein intake, and mean antipyrine clearance values in vegetarians and non-vegetarians do not differ significantly. A study among Hindus living in East Punjab (Chaudhury, personal communication) has likewise shown that a vegetarian diet does not result in reduction of antipyrine clearance: the vegetarian diet of the Punjab is characterised by its rich protein content, and differs markedly in that respect from the diet enjoyed by the Gujarati. Furthermore deliberate manipulation of dietary protein in man has been shown to modify the clearance of both antipyrine and theophylline (Kappas *et al.*, 1976) whereas manipulation of dietary fat has no such effect (Anderson *et al.*, 1979; Mucklow *et al.*, 1980). It therefore seems likely that the difference in antipyrine clearance between vegetarian and non-vegetarian immigrants studied here resulted from the contrasting amounts of protein in the two diets. Mean daily protein intake amongst the vegetarian immigrants was low by Western standards (Documenta Geigy, 1970) and if this is the explanation for their low antipyrine clearance values, there are potential therapeutic implications. Although individual rates of antipyrine clearance may not relate directly to the rate at which other drugs are oxidised, it is likely that lower average daily doses of drugs metabolised by hepatic oxidation would be required by Indo-Pakistani lactovegetarians.

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