

# Microsomal enzymes in malnutrition as determined by plasma half life of antipyrine

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## Summary

Nutritional-pharmacological interactions were studied in a group of malnourished subjects. Antipyrine was used to evaluate mixed-function oxidase in man. The results indicated that the rate of disappearance of antipyrine from plasma was strongly influenced by the nutritional status of the individual. The half life of antipyrine was modified in undernourished subjects and those with nutritional oedema.

This finding indicates that drug regimens may have to be adjusted in patients who have antipyrine half lives that are shorter or longer than normal. Otherwise drug treatment may be inadequate or, in patients with impaired microsomal enzyme activity, potentially dangerous.

## Introduction

The nutritional status of an individual may affect his drug metabolism. The activity of the drug metabolising enzymes located in the microsomal fraction of the liver is important in determining blood and tissue concentrations of drugs. Many reports suggest that malnutrition, which is prevalent in the developing countries, may lead to changes in the activity of mixed-function oxidases.<sup>1,2</sup> Most of these studies have been done in animals, however, and studies evaluating the capacity of malnourished human subjects to metabolise drugs are scanty. Poskitt<sup>3</sup> recently reviewed studies on the clinical problems related to the use of drugs in malnutrition. Abnormalities in the structure and function of the endoplasmic reticulum, consequent on malnutrition, may be expected to change drug metabolism in man. We have already reported that the plasma half life of tetracycline is significantly changed in undernourished people.<sup>4</sup> But tetracycline is an antibiotic that does not undergo biotransformation. We therefore considered it possible that drugs that are metabolised by the microsomal enzymes may behave differently in malnourished subjects.

We evaluated the microsomal enzyme systems in man using antipyrine half life as an index of drug metabolism. Antipyrine is widely used to predict the metabolism of drugs in man, since it is rapidly and almost completely absorbed from the gastrointestinal tract, is slowly metabolised (6% per hour), and only the major metabolite—4-hydroxy antipyrine—is excreted in urine.<sup>5</sup> Also, less than 10% of the parent drug is bound to plasma proteins, and hence the kinetics are not likely to be seriously affected by changes in plasma protein concentrations.

## Patients and methods

Four groups of subjects were chosen for the study. Seven healthy male laboratory workers volunteered to serve as controls (group 1). They did not smoke or drink. Eight other healthy men who smoked were also studied (group 2). Thirteen undernourished men with weight deficits of varying degrees formed group 3. Fifteen men suffering from nutritional oedema—a severe manifestation of protein calorie deficiency—formed group 4. The oedema in these subjects was due to inadequate food intake and consequent hypoalbuminaemia. All subjects with nutritional oedema received frusemide, a diuretic, before the investigation to remove as much extracellular fluid as possible. Frusemide was given for only three days.

After an overnight fast antipyrine, 18 mg/kg body weight, was given by mouth with 300 ml of water. No food was permitted for three hours thereafter to ensure complete absorption of the drug. Blood was collected four, six, nine, and 12 hours after drug administration and the samples were assayed for antipyrine.<sup>6</sup> Log concentrations versus time were plotted and elimination rate constants and half times were calculated by the method of least squares. The pharmacokinetic data were calculated by regression analysis of the log of the plasma concentrations with respect to time in each group. The statistical analysis was carried out by analysis of covariance technique.

## Results

The anthropometric data and serum albumin concentrations are indicated in table I. The mean weight in the controls was 58.0 kg, whereas mean weights in the malnourished subjects in groups 3 and 4 were 42 kg and 39 kg respectively. The heights of the subjects were similar, thus modifying the weight:height ratio. In normal subjects the ratio was over 0.20. In the undernourished subjects and those with nutritional oedema it was only 0.15, indicating the considerable weight deficit. Similarly, serum albumin concentrations were significantly different in the control and experimental groups, the patients with nutritional oedema having the lowest concentrations.

TABLE I—Mean ( $\pm$  SE) body weight, height, weight:height ratio, and serum albumin concentration

Group	No of subjects	Weight (kg)	Height (cm)	$\frac{\text{Weight}}{\text{height}^2} \times 100$	Serum albumin (g/l)
1	7	58.0 $\pm$ 3.63	164.8 $\pm$ 1.26	0.22 $\pm$ 0.013	36.7 $\pm$ 0.91
2	8	58.1 $\pm$ 2.08	167.6 $\pm$ 2.25	0.21 $\pm$ 0.010	37.5 $\pm$ 0.87
3	13	41.9 $\pm$ 1.80	162.4 $\pm$ 2.65	0.16 $\pm$ 0.005	24.5 $\pm$ 0.11
4	15	38.6 $\pm$ 1.76	164.0 $\pm$ 3.88	0.15 $\pm$ 0.006	20.4 $\pm$ 0.123

For weight, weight:height, and serum albumin, group 1 v 2: NS; group 1 v 3:  $P < 0.001$ ; group 1 v 4:  $P < 0.001$ ; group 2 v 3:  $P < 0.001$ ; group 2 v 4:  $P < 0.001$ . For serum albumin, group 3 v 4:  $P < 0.05$ . Differences in height were not significant.

Pharmacokinetic data are shown in table II and fig 1. The extrapolated plasma concentrations of antipyrine at 0 hour were similar in all groups (table II). The rate of decline of antipyrine concentrations in plasma, as indicated by the regression coefficient, was significantly different between the groups. The concentration declined faster in control smokers than in control non-smokers. Similarly, the regression coefficient was significantly lower in undernourished subjects than in controls who did not smoke, but the decline was similar to that seen in smokers. The half life calculated from these regression equations indicated that the half life was 11.22 hours in normal non-smokers, and 8.60 hours in undernourished people. In patients with nutritional oedema, the regression coefficient and half life were similar to those of normal non-smokers but significantly different from those of smokers and undernourished subjects. The adjusted mean con-

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TABLE II—Pharmacokinetic data on antipyrine: concentration at 0 hour, regression coefficient, and plasma half life

Group	Plasma antipyrine at 0 hour ( $\mu\text{mol/l}$ )	Regression coefficient	Standard error of regression coefficient	Half life (hours)	Adjusted mean concentration* ( $\mu\text{mol/l}$ )	Correlation coefficient (serum level $\times$ time)
1	6.44	-0.0268 P < 0.05	0.001752	11.22 8.94 P < 0.05	3.99 3.59 P < 0.05	0.8033
2	6.39	-0.0337 P < 0.01	0.001383	8.60 P < 0.001	3.48 4.17 P < 0.001	0.7293
3	6.48	-0.0350 P < 0.001	0.002942	12.82 P < 0.001	3.48 4.17 P < 0.001	0.7223
4	6.18	-0.0235	0.002230			0.8195

\*At mean time 7.7641 hours. \*P < 0.001 for each correlation coefficient.  
Conversion: SI to traditional units—Antipyrine: 1  $\mu\text{mol/l}$   $\approx$  0.188  $\mu\text{g/ml}$ .

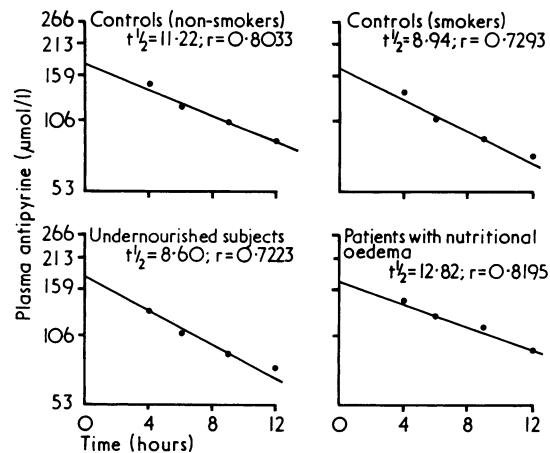


FIG 1—Rate of decline of plasma antipyrine concentrations.  
Conversion: SI to traditional units—Antipyrine: 1  $\mu\text{mol/l}$   $\approx$  0.188  $\mu\text{g/ml}$ .

centrations at the mean time of 7.76 hours were significantly different in the four groups.

The rate of decline of antipyrine in plasma was about 6.2% per hour in normal non-smokers, 7.8%/hour in smokers, about 9%/hour in undernourished subjects, and 5.7%/hour in patients with nutritional oedema. While analysing the data pooled variations between groups were taken into consideration and the differences between individuals (probably genetic) were not excluded from the total variation.

Fig 2 shows antipyrine half lives in each subject. The variation in non-smokers was higher than that in either smokers or undernourished subjects. The half life in those with nutritional oedema varied from 7.55 hours to 22.62 hours. Most of the patients with nutritional oedema had prolonged half lives compared with the mean

half life in normal subjects (11.22 hours) or undernourished subjects (8.6 hours). Four of the 15 subjects had a half life of over 16 hours, which was the highest value we encountered in the controls. The mean half life in patients with nutritional oedema was, however, comparable to that of normal subjects.

## Discussion

There is no general biochemical test to detect slow drug metabolisers. The normal laboratory tests used to assess liver function are of little value in predicting the capacity of an individual to metabolise drugs. The current trend in drug treatment is towards fitting treatment to the individual, and it is therefore essential to study the kinetics of drug elimination. An effective regimen may then be designed.<sup>7</sup> We therefore attempted to determine the capacity of the liver in malnourished subjects to handle drugs.

The liver is the major organ responsible for drug metabolism and consequently for drug disposition and elimination. Hence hepatic disease obviously affects drug kinetics.<sup>8</sup> The clinical implications of such changes are obvious. Vessel *et al*<sup>9</sup> have shown that there is interindividual variation in drug metabolism, as indicated by antipyrine and phenylbutazone metabolism. Our results confirm their finding. Despite individual variations, however, there were significant differences between the groups we studied.

Smokers clear antipyrine faster than non-smokers. Both nicotine and alcohol stimulate the microsomal enzymes.<sup>10</sup> Recently Vestel *et al*<sup>11</sup> showed that caffeine and cigarette use are positively correlated with the rate of antipyrine metabolism in human subjects. Our data confirm this finding. Drug metabolism seems to be about 15-20% faster in smokers, probably because of enzyme induction by nicotine. The half life of antipyrine was also short in our undernourished subjects, who were smokers and drank alcohol. Some of the subjects in this group were agricultural labourers, and careful questioning disclosed that some were exposed to pesticides as well. Kolmodin *et al*<sup>12</sup> showed that the plasma half life of antipyrine in workers exposed to chlorinated hydrocarbons is significantly low. Thus the microsomal enzymes in our undernourished subjects seemed to be in an induced state. Undernutrition itself might also have acted as a stress and induced the enzymes. Basu *et al*<sup>13</sup> have shown that restricted feeding of animals for 14-20 days results in an induction of microsomal enzymes, and Mgbodile<sup>14</sup> has shown increased deoxycorticosterone concentrations in semi-starved rats.

On the other hand, over half the subjects with severe protein calorie malnutrition had half lives prolonged by 12-13%, and in four the half lives were prolonged by 44-88%. In patients with liver disorders drugs like antipyrine, acetaminophen, chloramphenicol, and phenylbutazone have a prolonged half life.<sup>8</sup> But although fatty infiltration and hepatic dysfunction are characteristic features in children with kwashiorkor, hepatic involvement is minimal in adults with nutritional oedema.<sup>15</sup> Involvement of the endoplasmic reticulum and its consequences cannot, however, be ruled out.

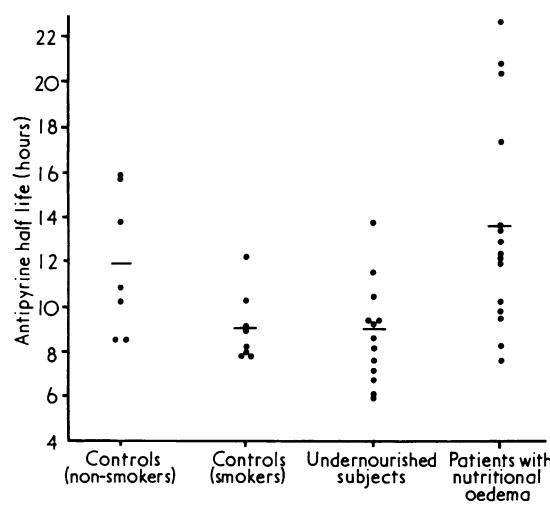


FIG 2—Antipyrine half life in each subject studied.

The half life of antipyrine reflects the metabolic capacity of the hepatocytes and is not influenced by plasma protein binding or hepatic drug delivery rate. Therefore the antipyrine clearance rates in our subjects indicated each individual's capacity to metabolise drugs.

Although a diuretic had been given to the patients with nutritional oedema before the study, some degree of expansion of the extracellular fluid volume remained. Nevertheless, Reidenberg *et al*<sup>16</sup> have shown that the metabolism of antipyrine and tolbutamide is not changed in obese fasting subjects despite significant changes in body water. The prolonged half life seen in patients with nutritional oedema was therefore unlikely to have been due to changes in body water. This view was also supported by the observations that relative volume distribution was similar in all the groups investigated.

These changes in the metabolism of antipyrine in malnourished subjects indicate that in certain conditions drug regimens may have to be changed. A reduction in half life may call for more frequent administration of drugs. On the other hand, if microsomal enzyme activity is impaired, and the half life prolonged, patients may be at a greater risk of accumulating potent drugs.

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## Controlled trial of active immunotherapy in management of stage IIB malignant melanoma

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### Summary

The prognosis for patients who undergo surgery for stage IIB malignant melanoma is poor. Animal studies have suggested that BCG and tumour cell vaccines given together may provide effective immunotherapy. To assess the effectiveness of this treatment 15 patients with stage IIB malignant melanoma who had had their tumour excised were studied. Seven were treated conservatively, and eight were vaccinated with BCG and autologous irradiated cells. Three vaccinated patients suffered widespread recurrence within three months. All four vaccinated patients who suffered a recurrence within the first year died, while none of the three controls with recurrent disease died. In view of this alarming trend the trial was stopped after a year. BCG and the tumour cells may have enhanced the tumour growth, although

there was no apparent reason for this. The results of uncontrolled or unrandomised trials that have suggested that this treatment is beneficial should be treated with scepticism.

### Introduction

Within two years of operation about three-quarters of all patients with malignant melanoma who have affected regional nodes (stage IIB) will have obvious recurrences and over half will have died.<sup>1</sup> Results are therefore likely to improve only if some other treatment can be added to excision.

The demonstration of tumour-associated antigens on melanoma cells and of circulating antibodies and cytotoxic lymphocytes directed against them in patients with the disease has suggested that immunotherapy may be useful,<sup>2</sup> but there have been few good controlled clinical trials of such treatment.<sup>3</sup> Bacillus Calmette-Guérin (BCG) has been included in the treatment of malignant melanoma as a non-specific immunostimulant. Intralesional BCG will often produce tumour regression,<sup>4</sup> and intradermal BCG, given repeatedly, may delay recurrence after surgery<sup>5</sup> and augment the response to chemotherapy.<sup>6</sup> In contrast, specific immunotherapy with irradiated tumour cells, while increasing cytotoxic antibody production and the proportion of circulating cytotoxic lymphocytes, has no apparent effect on the outcome in patients with widespread disease.<sup>7</sup> Nevertheless, animal studies indicate that host resistance to transplantable tumours is enhanced when BCG and tumour cell vaccines are given together as contact immunotherapy provided that the tumour load is small. This suggests

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