

ACETYLATOR PHENOTYPING OF TUBERCULOSIS PATIENTS USING MATRIX ISONIAZID OR SULPHADIMIDINE AND ITS PROGNOSTIC SIGNIFICANCE FOR TREATMENT WITH SEVERAL INTERMITTENT ISONIAZID-CONTAINING REGIMENS

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1 The acetylator phenotype of over 600 pulmonary tuberculosis patients treated with intermittent isoniazid-containing regimens in two controlled clinical trials was determined using either sulphadimidine or a slow-release isoniazid formulation.

2 Both methods unequivocally classified over 99% of the patients as being either slow or rapid acetylators.

3 Rapid and slow acetylators did not differ in their ability to hydrolyse acetylisoniazid to isonicotinic acid plus monoacetylhydrazine, or to conjugate isonicotinic acid with glycine.

4 Rapid acetylators acetylated the monoacetylhydrazine liberated *in vivo* more rapidly than slow acetylators, demonstrating that this compound is also polymorphically acetylated in man.

5 The acetylator phenotype of the patients was without prognostic significance when they were treated on a twice-weekly basis with isoniazid plus streptomycin plus pyrazinamide, or with isoniazid plus rifampicin. However, when patients were treated once every week for 12 months with isoniazid plus rifampicin, 5% of the rapid acetylators had an unsatisfactory response as contrasted to the complete success of the treatment in the slow acetylators.

Introduction

Isoniazid (isonicotinyl hydrazine) continues to be the most widely used chemotherapeutic agent for the treatment of tuberculosis (Fox, 1972; Fox & Mitchison, 1975). Its pharmacology has been extensively investigated in man (Evans, 1968; Peters, 1968; La Du, 1972; Ellard & Gammon, 1976). The metabolism of isoniazid appears to be non-inducible and the most important metabolic pathway is acetylation to acetylisoniazid. Large differences have been demonstrated between individuals in the rates at which isoniazid is acetylated and the great majority of subjects can be clearly characterized as being either 'slow' or 'rapid' acetylators of isoniazid. Rapid acetylators also acetylate monoacetylhydrazine, certain other hydrazines, and some sulphonamides more rapidly than slow acetylators. The polymorphic acetylation of isoniazid is genetically determined in a simple Mendelian fashion and the frequencies of the genes controlling the slow or rapid acetylation of isoniazid vary among different racial populations. Other important metabolites of isoniazid include the acid-labile hydrazones formed from

isoniazid by conjugation with pyruvic and α -keto-glutaric acid, monoacetylhydrazine and isonicotinic acid formed by hydrolysis of acetylisoniazid, and their further metabolic products diacetylhydrazine and isonicotinyglycine. All the metabolites of isoniazid are devoid of anti-tuberculosis activity and most are much less toxic than the parent drug. Evidence has however been adduced to suggest that monoacetylhydrazine might play a crucial role in isoniazid-induced hepatitis (Mitchell, Thorgeirsson, Black, Timbrell, Snodgrass, Potter, Jollow & Keiser, 1975).

The results of a series of controlled clinical trials have shown that the isoniazid-acetylator status of tuberculosis patients treated with isoniazid-containing regimens is of no prognostic significance when treatment is given daily. It may however be of significance when twice-weekly regimens are employed and is of considerable importance in once-weekly treatment, rapid acetylators always having fared considerably worse than slow acetylators on all the regimens evaluated so far (Ellard, 1976a). Recent studies have shown

that slow and rapid acetylators can be efficiently and conveniently characterized by determining the relative proportions of acetylated to free drug in the plasma or urine after giving oral doses of either sulphadimidine (Evans, 1969; Rao, Mitchison, Nair, Prema & Tripathy, 1970; Víznerová, Slavíková & Ellard, 1973), or a slow release formulation of isoniazid (Smith & Nephew, HS 82) developed for use in the intermittent treatment of pulmonary tuberculosis and referred to hereafter as 'matrix isoniazid' (Ellard, Gammon, Polansky, Víznerová, Havlík & Fox, 1973; Ellard, Gammon & Tiitinen, 1975; Kailasam, Immanuel, Nair, Radhakrishna & Tripathy, 1975; Ellard, 1976b; Tripathy, 1976).

This paper describes the results obtained when sulphadimidine and matrix isoniazid were used to phenotype over 600 pulmonary tuberculosis patients who were being treated in Hong Kong and Singapore in controlled clinical trials with intermittent regimens containing either isoniazid plus streptomycin plus pyrazinamide or isoniazid plus rifampicin. Studies undertaken to assess the relative abilities of slow and rapid acetylators to hydrolyse acetylisoniazid to isonicotinic acid plus monoacetylhydrazine, to conjugate isonicotinic acid with glycine, and to acetylate monoacetylhydrazine are also reported.

Methods

Patients

In Singapore 481 adult Chinese, Malays and Indians with newly diagnosed smear-positive pulmonary tuberculosis were allocated at random to four regimens of intermittent rifampicin plus isoniazid. All patients received an initial 2 weeks of daily streptomycin plus isoniazid plus rifampicin. This was followed either by twice-weekly isoniazid (15 mg/kg) plus rifampicin 900 mg (HR2 regimen), or 600 mg (LR2 regimen), or by once-weekly isoniazid (15 mg/kg) plus rifampicin 900 mg (HR1 regimen) or 600 mg (LR1 regimen). The conduct of the trial and the results obtained at 12 months are described in detail elsewhere (Singapore Tuberculosis Service/British Medical Research Council, 1975). Of these patients 479 were phenotyped with sulphadimidine and 188 with matrix isoniazid.

In Hong Kong 193 adult Chinese tuberculosis patients were randomly allocated to 6 or 9 months twice-weekly treatment with streptomycin (0.75 or 1 g) plus isoniazid (15 mg/kg) plus pyrazinamide (3 or 3.5 g) according to their age or weight ($S_2H_2Z_2$ regimen). Of these patients 184 were phenotyped with matrix isoniazid. Similar

numbers of patients were also randomly allocated to 6 or 9 months treatment with the same drugs given either three times a week ($S_3H_3Z_3$ regimen) or daily (SHZ regimen). Full details of the trial are reported elsewhere (Hong Kong Tuberculosis Treatment Services/British Medical Research Council, 1975).

Collection of samples

Urine samples were collected over a period of 48 h for a preliminary evaluation of the feasibility of phenotyping subjects using matrix isoniazid from a slow (G.A.E.) and a rapid (P.T.G.) acetylator after oral dosage with matrix isoniazid (30 mg/kg). The metabolism of isoniazid in both subjects had been extensively investigated previously (Ellard & Gammon, 1976). Urine samples were also collected from 49 British subjects (30 slow and 19 rapid acetylators) 23-24 h after dosage with 600 mg matrix isoniazid (Ellard *et al.*, 1975), and from 20 Czech subjects (12 slow and 8 rapid acetylators) after giving 30 mg/kg matrix isoniazid (Ellard *et al.*, 1973).

In the main study patients were phenotyped with sulphadimidine by collecting urine (5-6 h) and blood samples (6 h) after dosage with 40 mg/kg^{0.7} sulphadimidine (sulphadimethylpyridine, sulphamethazine) given according to the dosage schedule of Evans (1969). For phenotyping with matrix isoniazid, urine collections (23-24 h) were obtained after giving 30 mg/kg matrix isoniazid (Smith & Nephew, HS 82). All doses of sulphadimidine and matrix isoniazid were taken on an empty stomach. Samples of plasma and urine were preserved by the addition of a crystal of thymol, stored frozen and then flown to London for analysis.

Analytical methods

Sulphadimidine and acid-hydrolysable (free plus acetylated) sulphadimidine were determined colorimetrically by the Bratton & Marshall procedure (Varley, 1962). Acid-labile isoniazid and acetylisoniazid were determined fluorimetrically using salicylaldehyde (Ellard & Gammon, 1976) following extraction into butan-1-ol and thence into 0.1 N HCl (Ellard, Gammon & Wallace, 1972). Acid-labile isoniazid was also determined colorimetrically by two alternative methods, either using trinitrobenzene sulphonic acid by a modification (Ellard *et al.*, 1973) of the Dymond & Russell (1970) procedure (method 1) or with vanillin (Ellard *et al.*, 1972) (method 2). Acetylisoniazid was also determined colorimetrically using a modification (Ellard *et al.*, 1975) of the method of Venkataraman, Eidus & Tripathy

(1968) in which the interference of acid-labile hydrazones (Sarma, Immanuel, Kailasam, Kannapiran, Nair & Radhakrishna, 1974) was largely eliminated by prior acid treatment.

Isonicotinic acid and its glycine conjugate, and diacetylhydrazine were extracted and determined colorimetrically by reaction with cyanogen chloride and barbituric acid, and with *p*-dimethylaminobenzaldehyde, respectively (Ellard *et al.* 1972). Creatinine was determined colorimetrically with alkaline picrate.

Results

In order to facilitate comparison with the results of other studies, the ratios of the absolute concentrations of acetylisoniazid to acid-labile isoniazid are given; all other ratios are however expressed on a molar basis.

Preliminary investigations

After giving an oral dose of matrix isoniazid (30 mg/kg) to the slow acetylator, the ratios of the urinary excretion of acetylisoniazid to acid-labile isoniazid increased from about 0.5 at 1 h to 1.1 at 12 h, 1.3 at 18 h and 1.8 at 24 h. In the rapid acetylator the corresponding values were 1.1, 2.5, 4.7 and 7.0, respectively, indicating the suitability of urine samples collected from 23-24 h for phenotyping subjects using matrix isoniazid. The ratios of the urinary excretion of isonicotinyglycine to isonicotinic acid excreted by the two subjects were virtually identical and changed only very slowly with time, averaging about 0.65 1 h after dosage and rising to about 0.75 after 24 h. The ratios of the excretion of isonicotinic acid plus isonicotinyglycine to acetylisoniazid in the slow and rapid acetylator were also very similar averaging about 0.95 from 0-12 h and rising to about 1.2 by 24 h. By contrast the ratio of diacetylhydrazine to acetylisoniazid (or of diacetylhydrazine to isonicotinic acid plus isonicotinyglycine) excreted at 24 h by the rapid acetylator was approximately 2.5 times that of the slow acetylator and was increasing by about 20% per h as compared to an increase of only about 10% per h in the slow acetylator.

Comparison of the acetylation of sulphadimidine and isoniazid and of the further metabolism of isoniazid by slow and rapid acetylators from Singapore

The results of a series of analyses undertaken on the urine and plasma samples collected from the first 100 Singapore patients allocated to the LR1

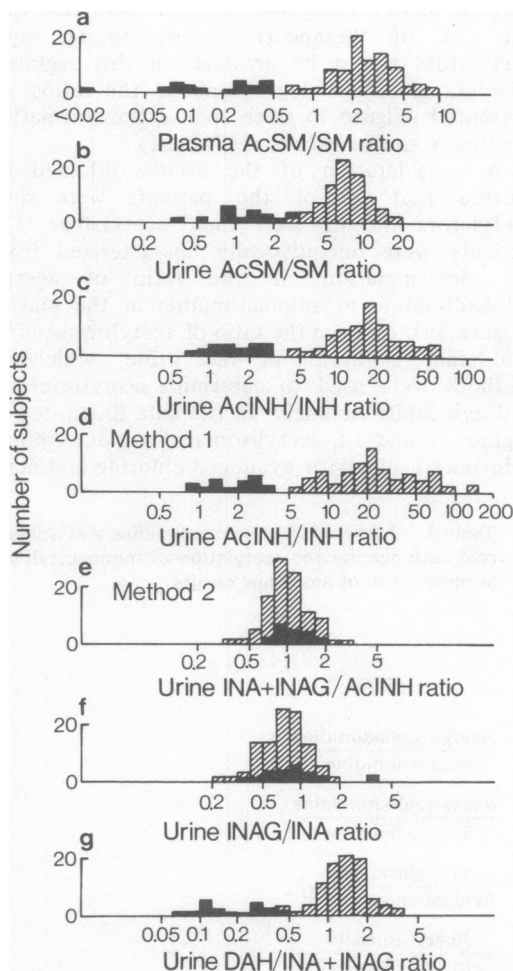


Figure 1 Distributions of 100 Singapore patients according to the ratios of drugs and metabolites determined in the plasma or urine after dosage with sulphadimidine or matrix isoniazid. SM, sulphadimidine; AcSM, acetylsulphadimidine; INH, acid-labile isoniazid; AcINH, acetylisoniazid; DAH, diacetylhydrazine; INA, isonicotinic acid; INAG, isonicotinyglycine; ■ slow acetylators; ▨ rapid acetylators.

regimen are illustrated in Figure 1 and summarized in Table 1. These analyses enabled the efficiency of various potential methods of phenotyping subjects with sulphadimidine or matrix isoniazid to be compared and provided evidence of the relative ability of slow and rapid acetylators to hydrolyse acetylisoniazid, conjugate isonicotinic acid with glycine and acetylate monoacetylhydrazine. Samples from the Singapore patients allocated to the LR1 regimen were selected for

this intensive investigation since it was likely that the risk of therapeutic failure among rapid acetylators would be greatest on this regimen. Histograms of the logarithms of the ratios are presented (Figure 1) since such a transformation gave more similar variances (Table 1).

A consideration of the results obtained indicated that 19 of the patients were slow acetylators and 81 were rapid acetylators. The patients were unequivocally characterized from the determination of the ratio of acetyl-sulphadimidine to sulphadimidine in the plasma (Figure 1a), or from the ratio of acetylisoniazid to acid-labile isoniazid in the urine, whichever methods were used to determine acetylisoniazid and acid-labile isoniazid. In the data illustrated in Figures 1c and 1d, acetylisoniazid was determined colorimetrically with cyanogen chloride and acid-

labile isoniazid was estimated colorimetrically with trinitrobenzene sulphonic acid ('Method 1') or fluorimetrically with salicylaldehyde ('Method 2'). Results based on the fluorimetric determination of acetylisoniazid with salicylaldehyde and the colorimetric determination of acid-labile isoniazid with vanillin have not been presented since they gave virtually identical results to those obtained with the simpler colorimetric methods. If the patients had been phenotyped solely according to the ratio of acetylsulphadimidine to sulphadimidine in the urine, one subject (a slow acetylator) would probably have been misclassified (Figure 1b).

Significant differences were not demonstrated between slow and rapid acetylators in the ratios of the excretion of isonicotinic acid plus isonicotinylglycine to acetylisoniazid or of isonicotinylglycine to isonicotinic acid (Table 1), and the distribution

Table 1 Acetylation of sulphadimidine and isoniazid, hydrolysis of acetylisoniazid, conjugation of isonicotinic acid with glycine and acetylation of monoacetylhydrazine by 100 Singapore patients. The results are expressed as mean \pm s.d. of individual results.

Ratio	Fluid	Acetylator phenotype	
		Slow (n = 19) Mean log ₁₀ ratios	Rapid (n = 81) Mean log ₁₀ ratios
$\frac{\text{Acetylsulphadimidine}}{\text{Sulphadimidine}}$	Plasma	-0.74 \pm 0.37	0.41 \pm 0.21
$\frac{\text{Acetylsulphadimidine}}{\text{Sulphadimidine}}$	Urine	0.06 \pm 0.21	0.86 \pm 0.18
$\frac{\text{Acetylisoniazid}}{\text{Acid-labile isoniazid}^1}$	Urine	0.20 \pm 0.18	1.24 \pm 0.25
$\frac{\text{Acetylisoniazid}}{\text{Acid-labile isoniazid}^2}$	Urine	0.26 \pm 0.20	1.37 \pm 0.32
$\frac{\text{Isonicotinic acid} + \text{Isonicotinylglycine}}{\text{Acetylisoniazid}}$	Urine	0.04 \pm 0.12	-0.02 \pm 0.15
$\frac{\text{Isonicotinylglycine}}{\text{Isonicotinic acid}}$	Urine	-0.07 \pm 0.22	-0.15 \pm 0.17
$\frac{\text{Diacylhydrazine}}{\text{Isonicotinic acid} + \text{Isonicotinylglycine}}$	Urine	-0.73 \pm 0.24	0.13 \pm 0.16

¹ Determined colorimetrically with trinitrobenzene sulphonic acid

² Determined fluorimetrically with salicylaldehyde.

Table 2 Logarithms₁₀ of the ratios of the urinary excretion of diacylhydrazine to acetylisoniazid 24 h after dosage with matrix isoniazid (mean \pm s.d. of individual results).

Subjects		Acetylator phenotype	
		Slow	Rapid
British	30	-0.40 \pm 0.16	19 0.10 \pm 0.12
Czech	12	-0.43 \pm 0.23	8 0.24 \pm 0.17
Singaporean	19	-0.71 \pm 0.27	81 0.13 \pm 0.17

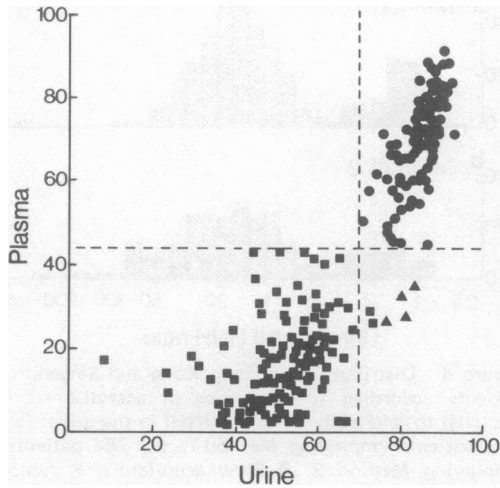


Figure 2 Distribution of 479 Singapore patients according to the percentage of sulphadimidine acetylated in the plasma and urine. For clarity, the results from only a quarter of the rapid acetylators are included. ■ Slow acetylators; ● rapid acetylators; ▲ unclassified.

of both these ratios was unimodal (Figures 1e, 1f). These findings indicated that there is no correlation between the rates at which individuals acetylate isoniazid and the rates at which they hydrolyse acetylisoniazid to isonicotinic acid plus monoacetylhydrazine or conjugate isonicotinic acid with glycine.

Polymorphic acetylation of monoacetylhydrazine

The ratios of diacetylhydrazine to acetylisoniazid excreted in the urine by healthy British subjects and by tuberculosis patients from Czechoslovakia and Singapore after dosage with 600 mg (about 8 mg/kg) or 30 mg/kg matrix isoniazid are summarized in Table 2. Among each group of subjects significant (3-to-7-fold) higher ratios of diacetylhydrazine/acetylisoniazid were excreted by the rapid acetylators ($P < 0.001$). Logarithmically transformed data are shown since this resulted in similar variances among both acetylator phenotypes. Significant positive correlations were also demonstrated within each phenotype between the ratios of diacetylhydrazine to acetylisoniazid excreted in the urine and the concomitant ratios of acetylisoniazid to acid-labile isoniazid ($P = 0.003$ for the 30 British slow acetylators and $P < 0.001$ for the 81 Singapore rapid acetylators).

In order to make allowances for differences between individuals in the rates of hydrolysis of acetylisoniazid which could have played a part in

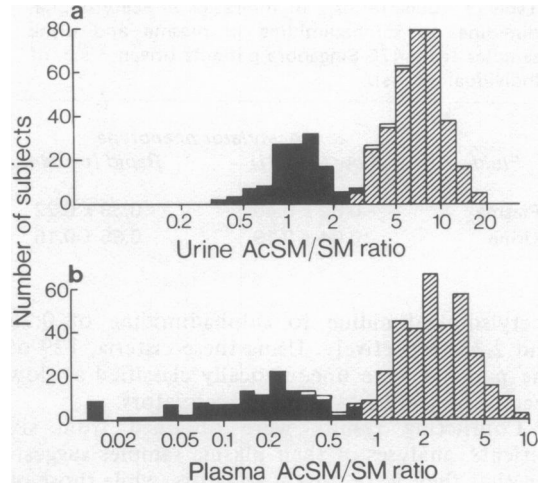


Figure 3 Distribution of 479 Singapore patients according to the ratio of acetylsulphadimidine (AcSM) to sulphadimidine (SM): (a) in the urine, (b) in the plasma. ■ Slow acetylators; ■ rapid acetylators; □ unclassified.

influencing the ratios of diacetylhydrazine to acetylisoniazid, further estimates of the relative abilities of the Singapore patients to acetylate monoacetylhydrazine were obtained by determining the ratios of the excretion of diacetylhydrazine to isonicotinic acid plus isonicotinylglycine 24 h after dosage with matrix isoniazid. In these circumstances, when the rate of formation of isonicotinic acid by direct hydrolysis of isoniazid is minimal (Ellard & Gammon, 1976), the rate of excretion of isonicotinic acid plus isonicotinylglycine would be expected to closely parallel the rate of formation of monoacetylhydrazine *in vivo*. The bimodal distribution of these ratios is illustrated in Figure 1g, those of rapid acetylators of isoniazid and sulphadimidine averaging about seven times those of slow acetylators (Table 1).

Acetylator phenotyping with sulphadimidine

The results obtained when 479 Singapore patients were phenotyped with sulphadimidine are illustrated in Figures 2 and 3 and summarized in Table 3. In Figure 2 the percentages of sulphadimidine acetylated in the plasma at 6 h have been plotted against the percentages acetylated in the urine at 5-6 h. From the scatter of the points it was concluded that the most efficient criteria for distinguishing between rapid and slow acetylators were 43% sulphadimidine acetylated in the plasma and 70% acetylated in the urine, or ratios of

Table 3 Logarithms₁₀ of the ratios of acetylsulphadimidine to sulphadimidine in plasma and urine samples from 475 Singapore patients (mean \pm s.d. of individual results).

Fluid	Acetylator phenotype	
	Slow (n=131)	Rapid (n=344)
Plasma	-0.82 \pm 0.40	0.39 \pm 0.22
Urine	0.04 \pm 0.19	0.85 \pm 0.16

acetylsulphadimidine to sulphadimidine of 0.75 and 2.33 respectively. Using these criteria, 129 of the patients were unequivocally classified as slow inactivators and 344 as rapid acetylators.

Conflicting results were obtained from six patients, analyses of their plasma samples suggesting that they were slow acetylators, while those of their urine samples indicated that they were rapid acetylators. Two of these patients were provisionally classified as slow acetylators since the proportions of sulphadimidine acetylated in the plasma were considerably less than the chosen discrimination value while their urine values were only marginally above the selected cut. The other four patients could not be classified. These six patients were therefore also phenotyped with matrix isoniazid. The results obtained confirmed the provisional classification of the two slow acetylators and indicated that the other four subjects probably consisted of two slow and two rapid acetylators. Approximately one-third of the patients unequivocally classified with sulphadimidine were also phenotyped using matrix isoniazid. In every case the sulphadimidine classification was confirmed.

Highly significant correlations were found for both the 131 slow and 344 rapid acetylators between the percentage of sulphadimidine acetylated in the urine at 5-6 h and the percentage acetylated in the plasma at 6 h (correlation coefficients 0.495 and 0.646, respectively), indicating that within each acetylator group substantial differences did not exist between

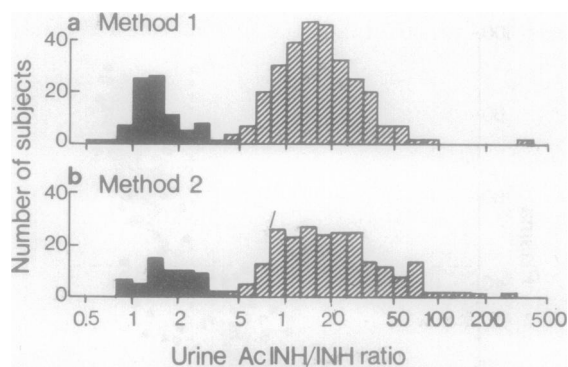


Figure 4 Distribution of Hong Kong and Singapore patients according to the ratios of acetylisoniazid (AcINH) to acid-labile isoniazid (INH) in the urine: (a) 372 patients employing Method 1, (b) 284 patients employing Method 2. ■ Slow acetylators; ▨ rapid acetylators.

individuals in the relative plasma renal clearances of sulphadimidine and acetylsulphadimidine. The results are illustrated separately in Figure 3 in the form of histograms of the ratios of acetylsulphadimidine/sulphadimidine displayed on a logarithmic scale since such a transformation gave more similar variances (Table 3). Significant amounts of acetylsulphadimidine were not detected in the 6 h plasma samples of nine of the slow acetylators. From the accuracy of the Bratton & Marshall procedure it was concluded that probably less than 2% of the drug was acetylated in these samples, and they were assigned a value of 1.9% acetylated for calculating the means shown in Tables 2 and 3.

Acetylator phenotyping with matrix isoniazid

The results obtained by the two methods used to determine the ratios of acetylisoniazid to acid-labile isoniazid are summarized in Table 4 and illustrated in Figure 4. In the first method isoniazid was determined colorimetrically with

Table 4 Logarithms₁₀ of the ratios of acetylisoniazid to acid-labile isoniazid in urine samples from Hong Kong and Singapore patients (mean \pm s.d. of individual results).

Method ¹	Slow acetylators		Rapid acetylators	
	Number of samples	Mean \pm s.d.	Number of samples	Mean \pm s.d.
1	86	0.16 \pm 0.16	286	1.20 \pm 0.26
2	59	0.23 \pm 0.17	225	1.30 \pm 0.33

¹ For details see text.

trinitrobenzene sulphonic acid, while in the second the more sensitive fluorimetric salicylaldehyde method was employed. In both methods acetylisoniazid was determined colorimetrically with cyanogen chloride. Both methods were applied to the samples obtained after giving matrix isoniazid to the first 100 Singapore patients allocated to the LR1 regimen and to those from the 184 Hong Kong patients, while a further 88 samples from Singapore patients allocated to other regimens were also analysed by the first method. Although similar results were given by the two isoniazid methods for the samples from the slow acetylators and for most of those from the rapid acetylators, the concentrations of acid-labile isoniazid determined colorimetrically in Method 1 were substantially higher than those determined using the more sensitive and specific fluorimetric method (Method 2) in about a fifth of the samples from the rapid acetylators. The discrepancy between the results obtained by the two methods was greatest in those samples in which the ratios of fluorimetrically-determined acid-labile isoniazid to creatinine were lowest. Thus in those samples containing less than 0.1 μg acid-labile isoniazid/mg creatinine, the results obtained with the colorimetric method were on average about 65% higher than those given by the fluorimetric method, whereas for all the other samples the discrepancy was only about 10%. As a consequence there was a considerably greater spread of the ratios of acetylisoniazid to acid-labile isoniazid among rapid acetylators determined using Method 2 as compared to Method 1 (Figure 4).

Proportion of slow and rapid acetylators and frequency of very rapid acetylators

Identical proportions (22%) of the Chinese patients from Singapore and Hong Kong were classified as being slow acetylators (83/386 and 40/184, respectively) whether the sulphadimidine (Singapore) or matrix isoniazid (Hong Kong) methods were employed. Smaller numbers of Malay (75) and Indian (57) patients were also phenotyped from Singapore, the proportions of slow acetylators among these groups being 42% and 73%, respectively. Seventy-nine of the 272 Chinese patients (29%) phenotyped from Singapore and Hong Kong using matrix isoniazid (Method 2) were very rapid acetylators with acetylisoniazid/acid-labile isoniazid ratios 25 or more, a proportion almost identical to that calculated using the Hardy-Weinberg Law for homozygous rapid acetylators in this population (Kailasam *et al*, 1975).

Therapeutic efficacy and acetylator phenotype

All of the 218 Singapore patients who could be assessed after 12 months treatment with the twice-weekly regimens of isoniazid plus rifampicin (HR2 and LR2 regimens) had a favourable bacteriological response to chemotherapy. However, 11 of the 214 patients treated with the once-weekly rifampicin plus isoniazid regimens had an unfavourable response at 12 months (3 on the HR1 regimen, and 8 on the LR1 regimen). All of the 11 patients with an unfavourable status were rapid acetylators. Thus, 11 of 310 rapid acetylators had an unfavourable response compared with none of 117 slow acetylators, a significant difference ($P = 0.05$). The influence of the rate of acetylation of isoniazid on the response of the Hong Kong patients to short course treatment with twice-weekly streptomycin plus isoniazid plus pyrazinamide ($S_2H_2Z_2$ regimen) was assessed by amalgamating the results for patients treated for both the 6- and 9-month periods. Of the patients with fully sensitive tubercle bacilli pretreatment, 3 of the 30 slow acetylators (15%) and 11 of the 70 rapid acetylators (16%) had an unsatisfactory response in that they failed to achieve culture negativity during chemotherapy or relapsed after stopping treatment. For patients with pretreatment strains resistant to isoniazid, streptomycin, or both drugs four of five slow acetylators had an unfavourable response compared with 14 of 26 rapid acetylators. In both the Singapore and Hong Kong studies the response of treatment of very rapid acetylators with acetylisoniazid/acid-labile isoniazid ratios (Method 2) of 25 or more did not differ significantly from that of the other rapid acetylators with ratios of less than 25.

Discussion

The excellence of the results obtained when the 479 Singapore patients were phenotyped with sulphadimidine is in accord with those obtained by Víznerová *et al* (1973) from among over 400 patients in Czechoslovakia. In both studies approximately 99% of the subjects were classified with confidence and it was estimated that if the interpretation of the test had been based solely on the urine results, only about 1% of the subjects would have been misclassified. The results obtained using matrix isoniazid to phenotype the subjects were as excellent as those achieved with sulphadimidine. It was not possible to make the formal comparison of the relative abilities of the two methods to discriminate between slow and

rapid acetylators since the distribution of acetylisoniazid/acid-labile isoniazid ratios among rapid acetylators was not normal (Figure 4b). However, a comparison of the proportions of patients with ratios within $\pm 30\%$ of the antimodes of the distributions illustrated in Figures 3 and 4 (about 2.3% for sulphadimidine and 1.4% for matrix isoniazid) suggested that the discriminant ability of the matrix isoniazid method might be slightly superior to that of the sulphadimidine method.

Both methods are clearly very satisfactory and their relative suitability will depend primarily on the situation in which they are employed. The timing of the urine collection in the matrix isoniazid method (23-24 h after dosage) makes it particularly suitable for phenotyping tuberculosis out-patients. In other circumstances, for example in obtaining data on the frequencies of slow and rapid acetylators in different populations, the sulphadimidine method would be preferred since subjects need to be seen on only a single occasion.

The results obtained with the matrix isoniazid method demonstrate that the ratios of acetylisoniazid/acid-labile isoniazid were underestimated in very rapid acetylators when acid-labile isoniazid was determined colorimetrically with trinitrobenzene-sulphonic acid, probably because of significant urine blanks. Thus very rapid acetylators could only be properly identified when acid-labile isoniazid was determined fluorimetrically with salicylaldehyde as in Method 2. This method is fundamentally similar to that employed by Kailasam *et al* (1975) for phenotyping South Indian patients with matrix isoniazid. The similarity between the results they obtained and those described in this report from Chinese populations are noteworthy. Thus the geometric means of the ratios of acetylisoniazid/acid-labile isoniazid excreted 24 h after dosage with 30 mg/kg matrix isoniazid were 1.70 for slow acetylators (both studies), 13.2 and 12.7 for rapid acetylators (ratios of less than 25) and 37 and 45 for very rapid acetylators (ratios of 25 or more), respectively. Furthermore, although the proportions of very rapid acetylators in the two studies were very different (6% among the South Indians and 29% among the Chinese), in each case the proportion was similar to that predicted by the Hardy-Weinberg law for homozygous rapid acetylators. It is nevertheless clear from the distribution of the ratios of acetylisoniazid/acid-labile isoniazid among rapid acetylators illustrated in Figure 4b that there was probably a considerable overlap in the results obtained from homozygous and heterozygous rapid acetylators. This finding is in accord with evidence obtained by Scott, Wright & Weaver (1969).

The proportion of slow acetylators among the

Chinese patients phenotyped from both Hong Kong and Singapore was 22% which is identical to that found among Chinese in Taiwan by Sunahara, Urano, Lin, Cheg & Jarumilinda (1963). Forty-two per cent of the 75 Malay patients and 73% of the 57 Indian patients phenotyped from Singapore were slow acetylators. Studies of the prevalence of the acetylator phenotype among Malay populations do not appear to have been conducted before. The proportion of slow acetylators among the relatively small number of Indians studied was not significantly different from that of 59% determined among over 1400 South Indian tuberculosis patients in the Madras area (Tuberculosis Chemotherapy Centre, Madras, 1970, 1973a, 1973b, Tripathy, 1974).

Determination of the ratios of the excretion of isonicotinyglycine to isonicotinic acid 24 h after dosage with matrix isoniazid (Figure 1f, Table 1) confirms previous evidence that slow and rapid acetylators do not differ in their ability to conjugate isonicotinic acid with glycine (Peters, Miller & Brown, 1965; Ellard & Gammon, 1976). From the ratios of the excretion of isonicotinic acid plus isonicotinyglycine to acetylisoniazid (Figure 1e), it was also apparent that the two phenotypes do not differ in their ability to hydrolyse acetylisoniazid to isonicotinic acid plus monoacetylhydrazine.

In the absence of an analytical method capable of determining monoacetylhydrazine in the presence of isoniazid, the acetylation of monoacetylhydrazine was studied by measuring the ratios of diacetylhydrazine to acetylisoniazid (or to isonicotinic acid plus isonicotinyglycine) excreted after giving matrix isoniazid (Tables 1 and 2, Figure 1g). The results obtained from the 61 slow and 108 rapid acetylators studied, confirm previous evidence from a small group of subjects for the polymorphic acetylation of monoacetylhydrazine (Ellard & Gammon, 1976). These findings are pertinent to the current discussion concerning the potential role of monoacetylhydrazine in isoniazid-induced hepatitis. Two main lines of evidence implicate the possible involvement of monoacetylhydrazine in isoniazid-associated liver damage. Firstly it has been shown to be a powerful inducer of hepatic necrosis in the rat (Mitchell, Zimmerman, Ishak, Thorgeirsson, Timbrell, Snodgrass & Nelson, 1976), especially in phenobarbital-treated animals with raised levels of cytochrome P-450 metabolizing enzymes. Secondly, in a retrospective study Mitchell *et al* (1975) found an unexpectedly high proportion of rapid acetylators among North American patients who had recovered from hepatitis that had apparently been caused by the self-administration of isoniazid for prophylaxis. Mitchell and his

colleagues argued that since rapid acetylators formed acetylisoniazid in the liver more rapidly than slow acetylators, they would therefore initially produce larger amounts of monoacetylhydrazine and from this generate higher concentrations of the active chemical species postulated as being ultimately responsible for causing hepatic necrosis. The demonstration that monoacetylhydrazine is polymorphically acetylated and as a consequence is more speedily eliminated from the body by rapid acetylators (Ellard & Gammon, 1976) suggests however that the maximal levels of monoacetylhydrazine eventually achieved after giving isoniazid to slow and rapid acetylators might be similar. This possibility has been discussed elsewhere in connection with the possible role of monoacetylhydrazine in causing the delayed giddiness encountered when high doses (40 mg/kg or more) of matrix isoniazid are given (Ellard, 1976b; Tripathy, 1976). It is also of interest that in a retrospective European study of the hepatitis encountered among subjects who had received isoniazid chemoprophylactically, it was concluded that the acetylator phenotype did not influence the incidence of isoniazid-induced hepatitis (Riska, 1976).

Evidence from previous controlled clinical trials for the prognostic significance of the acetylator

phenotype for the treatment of pulmonary tuberculosis with isoniazid-containing regimens has been reviewed elsewhere (Ellard, 1976a). The results obtained in the studies reported here are fully in accord with the pattern demonstrated by previous investigations. Thus in the Hong Kong study, when streptomycin and pyrazinamide were given together with isoniazid twice-weekly for either 6 or 9 months, the acetylator phenotype of the patients was without prognostic significance. This parallels the results obtained in previous studies when isoniazid and streptomycin were given twice-weekly for periods of a year or more with or without an initial period of daily chemotherapy. Previous studies have shown that when isoniazid is given once-weekly combined with either ethambutol, streptomycin, streptomycin plus PAS, or streptomycin plus pyrazinamide, rapid acetylators always fared worse than slow acetylators. Similarly the Singapore study, in which isoniazid was combined with rifampicin the most potent companion drug available, demonstrated the same phenomenon even though the overall level of therapeutic success was very high (95% for the HR1 and LR1 regimens combined).

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