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INTER-INDIVIDUAL VARIATION OF HUMAN BLOOD *N*-ACETYLTRANSFERASE ACTIVITY *IN VITRO*

R. M. LINDSAY and J. D. BATY*
Department of Biochemical Medicine (University of Dundee), Ninewells Hospital and Medical School, Dundee, Scotland DD1 9SY, U.K.

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Abstract—Inter-individual variation in the *in vitro* acetylation of the antibacterial drug sulphamethazine by human whole blood was studied using reverse phase HPLC. The mean (range) values of blood *N*-acetyltransferase activity *in vitro* were 0.50 (0.29–0.83) nmol per 10^9 red blood cells (rbc) (N = 23), 3.33 (2.22–5.27) nmol per 10^9 rbc (N = 27) and 9.36 (6.72–15.76) nmol per 10^9 rbc (N = 23) at initial sulphamethazine concentrations of 0.018 mM, 0.18 mM and 1.44 mM respectively. The mean (range) values of the initial rate of sulphamethazine acetylation at these substrate concentrations were 28.1 (20.9–35.0) pmol/hr per 10^9 rbc (N = 11), 0.26 (0.18–0.42) nmol/hr per 10^9 rbc (N = 19) and 0.91 (0.61–1.50) nmol/hr per 10^9 rbc (N = 14) respectively. The mean (range) half life of thermal inactivation of blood acetylation capacity at 50° was 0.91 (0.59–1.27) min (N = 12) at an initial substrate concentration of 0.18 mM. In each of these cases, there was no significant differences between the values obtained using blood samples from rapid and slow acetylators.

Intra-individual variation of blood *N*-acetyltransferase activity was studied in a single subject on 24 separate occasions during a two year period and was less than 10% at each of the three sulphamethazine concentrations studied. The correlation between the *in vitro* blood *N*-acetyltransferase activity of eight volunteers measured on two separate occasions at least 6 weeks apart was 0.84, 0.98 and 0.98 at initial sulphamethazine concentrations of 0.018 mM, 0.18 mM and 1.44 mM respectively.

Increasing the acetyl-CoA concentration of blood samples from 4 subjects by 0.34, 0.85 and 1.67 mM significantly increased both the initial acetylation rate of sulphamethazine and the amount of acetylsulphamethazine produced after an incubation time of 24 hr (initial sulphamethazine concentration = 0.18 mM).

N-acetylation of foreign compounds containing arylamine or hydrazino groups is catalysed by the group of enzymes known as *N*-acetyltransferases (EC 2.3.1.5) and requires acetyl-CoA as the acetyl group donor. Drugs such as sulphamethazine, isoniazid and hydralazine display a genetic polymorphism in man, a metabolic feature which has important therapeutic and toxic implications [1]. Since detoxification of foreign compounds is principally associated with the liver, most studies have been directed towards characterisation of liver *N*-acetyltransferase. Correlation between the activity of this enzyme *in vitro* and the *in vivo* acetylator phenotype has been demonstrated in man using all the above substrates [2].

Extrahepatic sites of *N*-acetyltransferase activity such as the intestine, kidney and peripheral blood have also been identified [3]. Human blood has been shown to acetylate dapsone [4], *p*-aminobenzoic acid [5] and *p*-aminosalicylic acid [6] although there is no significant correlation between the *in vitro* acetylation of these substrates and the *in vivo* acetylator phenotype of the blood donors.

An unusual case of polymorphic acetylation has been reported using rabbit blood. The *in vitro* blood *N*-acetyltransferase activity towards *p*-aminobenzoic acid is significantly higher in rabbits which are phenotypically slow acetylators *in vivo*. Discrimination between the two groups is clear, especially if familially related animals are studied when no substantial overlap occurs [7]. Preliminary studies using human reticulocytes also appear to exhibit similar phenotypic differences in *p*-aminobenzoic acid acetylation *in vitro*. These differences are not apparent in either erythrocyte preparations which have not been fractionated according to age or in polymorphonuclear leucocytes, lymphocytes and whole blood [3]. Phenotypic differences in the apparent rate of thermal inactivation at 44° of human lymphocyte *N*-acetyltransferase have been identified using *p*-aminobenzoic acid as substrate [8] although discrimination of the two phenotypes is not complete and there is considerable overlap between the two groups.

Two of the most commonly used compounds for acetylator phenotyping *in vivo* are isoniazid and sulphamethazine. However, *in vitro* studies on blood *N*-acetyltransferase using these substrates have shown little, if any, activity. Isoniazid is not acetylated by either rabbit blood *N*-acetyltransferase purified over 300-fold [9] or human red cell resuspensions supplied with the acetyl-CoA precursors acetate, CoA and ATP [6]. Previous studies on the acetylation of sulphamethazine by human blood have either been unable to detect any activity [10, 11] or have failed to detect activity in all the samples analysed [12]. The inability to reliably detect and quantify blood *N*-acetyltransferase activity using sulphamethazine has prevented investigations on inter-individual variation and therefore possible polymorphism of this enzyme has not been studied with

this substrate. In this study, we have used a reverse phase HPLC assay developed in our laboratory [13] to study variation of this enzyme activity *in vitro* amongst healthy volunteers. The principal aim was to determine if the use of this more sensitive and specific analytical method would permit detection of any significant differences in the *in vitro* blood acetylation capacity of samples from donors of known acetylator phenotype.

MATERIALS AND METHODS

Materials. Sulphamethazine and acetic anhydride were obtained from the Aldrich Chemical Co. Ltd (Gillingham, U.K.). Sulphapyridine and acetyl-CoA were purchased from the Sigma Chemical Co. Ltd (Poole, U.K.). Potassium dihydrogen phosphate and disodium hydrogen phosphate were obtained from the BDH Chemical Co. Ltd (Poole, U.K.). HPLC-grade acetonitrile and ethyl acetate were supplied by Rathburn Chemicals (Peebleshire, U.K.) and May & Baker (Dagenham, U.K.) respectively. All reagents were of the highest grade commercially available. Acetylsulphamethazine was synthesised from sulphamethazine by heating with acetic anhydride and was recrystallised from aqueous ethanol (m.p. 254–255°). Its structure was confirmed by mass spectrometry and its purity was 100% by HPLC analysis. Sulphamethazine tablets (500 mg) were obtained from the Department of Pharmacy, Ninewells Hospital and Medical School, Dundee.

Subjects. Blood samples were donated by healthy volunteers (15 males and 12 females) from Ninewells Hospital and Medical School. These subjects were not currently receiving any medication and their mean age and range was 28 years and 19–58 years respectively.

Acetylator phenotyping. Ethical approval for the acetylator phenotyping procedure was granted by the Ethics Committee of the Faculty of Medicine, University of Dundee and written informed consent was obtained from all subjects. To determine the acetylator phenotype of the volunteers, the subjects fasted overnight and then orally ingested 750 mg (compressed tablet) of sulphamethazine. Blood samples were collected 6 hr later by venepuncture and dispensed into lithium–heparin anticoagulant tubes, extracted immediately and analysed by HPLC [13]. The acetylating capacity of each subject was expressed quantitatively as the percentage of sulphamethazine acetylated. This value was calculated by dividing the amount of acetylsulphamethazine present by the total amount of sulphonamide present (i.e. sulphamethazine and acetylsulphamethazine), expressed as a percentage. The percentage acetylation for rapid acetylators ranged from 70 to 89% (mean = 79%) and that for slow acetylators ranged from 23 to 43% (mean = 31%).

In vitro *blood* N-*acetyltransferase assay*. The incubation protocol was as documented previously [13] and involved mixing 0.2 ml of heparinised whole blood and 0.2 ml of sulphamethazine in 33.3 mmol/l phosphate buffer (pH 7.4) to produce final substrate concentrations of either 0.018, 0.18 or 1.44 mM. The samples were incubated at 37° for periods up to 24 hr then extracted with ethyl acetate and analysed by HPLC as previously described [13]. The limit of detection of this assay (at a signal-to-noise ratio = 3) is approximately 3 pmol (1 ng) of acetylsulphamethazine. The coefficient of variation for analysis of replicate samples ($N > 4$) in this study did not exceed 5%. Since variations in the number of blood cells may result in differences in the observed *N*-acetyltransferase activity, the results obtained for each individual were corrected for the number of red blood cells present.

Inter-individual variation of blood *N*-acetyltransferase was investigated by three methods. An end point study using initial sulphamethazine concentrations of 0.018 mM, 0.18 mM and 1.44 mM was conducted by incubating the blood samples for 24 hr. Kinetic acetylation studies at these three sulphamethazine levels were also performed in which the initial rates of acetylsulphamethazine production were measured. Finally the thermal inactivation of blood sulphamethazine *N*-acetyltransferase at 50° was investigated using an initial sulphamethazine concentration of 0.18 mM as described below.

The effect of added acetyl-CoA on blood N-*acetyltransferase activity* in vitro. In a further set of experiments using blood samples from four volunteers only, the substrate solution was augmented with acetyl-CoA to increase the incubation concentration of this cofactor by either 0.34, 0.85 or 1.67 mM.

Thermal inactivation of blood acetylation capacity at 50°. The apparent thermal inactivation of blood *N*-acetyltransferase was monitored by pre-incubating blood samples from 12 individuals at 50° for periods of 0, 1, 3, 5, 7 and 9 min prior to incubation with sulphamethazine at 37°. A semi-log regression program was used to calculate the apparent half life of *N*-acetyltransferase inactivation.

Haematological analysis. Determination of packed cell volume and counting of red and white blood cells were performed by the Department of Haematology at Ninewells Hospital and Medical School using an Ortho Diagnostics Systems coulter counter (Model ELT-800/WS).

RESULTS

Intra-individual variation in human blood N-*acetyltransferase activity* in vitro

The coefficient of variation for the amounts of acetylsulphamethazine produced by 24 blood samples collected during 2 years from a single healthy volunteer was less than 10% at each of the three substrate concentrations studied. The reproducibility of *in vitro* blood *N*-acetyltransferase activity was also studied using samples from eight individuals collected on two separate occasions at least 6 weeks apart. The correlation between the values obtained in the initial and repeat experiments at initial sulphamethazine concentrations of 0.018 mM, 0.18 mM and 1.44 mM were 0.84, 0.98 and 0.98 respectively. Intra-individual variation in the rate of acetylsulphamethazine production at each of the three substrate levels using blood samples obtained from a single individual on seven occasions over a period of approximately 1 year was slight (coefficient of variation < 7%).

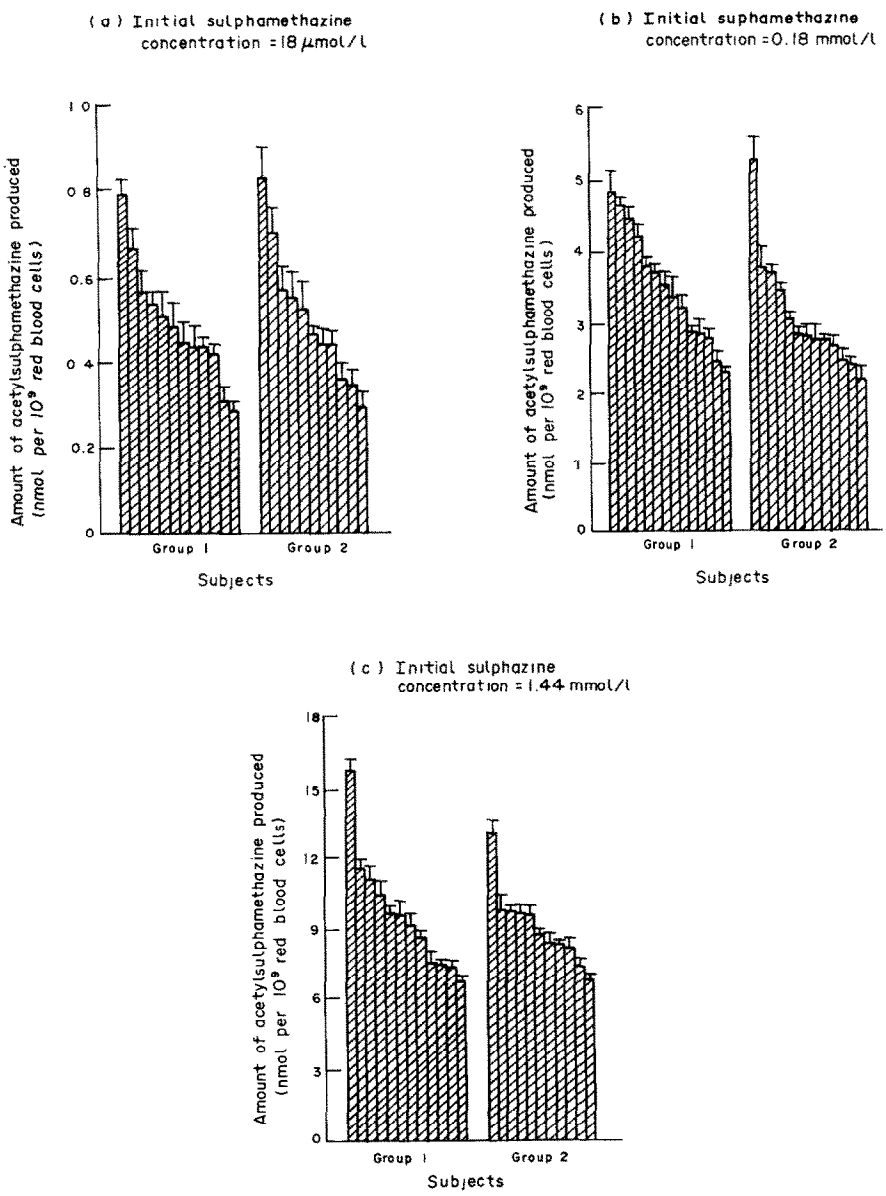

Fig. 1. The inter-individual variation of the *in vitro* acetylation of sulphamethazine by human whole blood from rapid and slow acetylator subjects. Values shown represent the mean of at least four replicates (error bars represent the upper 95% confidence limits). Subjects in Group 1 are rapid acetylators of sulphamethazine *in vivo*. Subjects in Group 2 are slow acetylators of sulphamethazine *in vivo*.

Inter-individual variation of human blood N-*acetyltransferase activity* in vitro

End point study. The mean (range) values of blood *N*-acetyltransferase activity *in vitro* were 0.50 (0.29–0.83) nmol per 10^9 red blood cells (rbc) (N = 23), 3.33 (2.22–5.27) nmol per 10^9 rbc (N = 27) and 9.36 (6.72–15.76) nmol per 10^9 rbc (N = 23) at initial sulphamethazine concentrations of 0.018 mM, 0.18 mM and 1.44 mM respectively. The difference in the amount of product obtained using blood from rapid acetylators and slow acetylators was insignificant ($P > 0.1$, Student's *t*-test; Fig. 1a–c). There was also no significant difference in the *in vitro* blood *N*-acetyltransferase activities of samples obtained from male and female volunteers ($P > 0.1$, Student's *t*-test). No significant correlation existed between the *in vitro* blood *N*-acetyltransferase activity and the age of the donor ($r = 0.20$, $P > 0.1$).

Kinetic acetylation study. The mean (range) values of the initial acetylation rate at sulphamethazine concentrations of 0.018 mM, 0.18 mM and 1.44 mM were 28.1 (20.9–35.0) pmol/hr per 10^9 rbc (N = 11), 0.26 (0.18–0.42) nmol/hr per 10^9 rbc (N = 19) and 0.91 (0.61–1.50) nmol/hr per 10^9 rbc (N = 14)

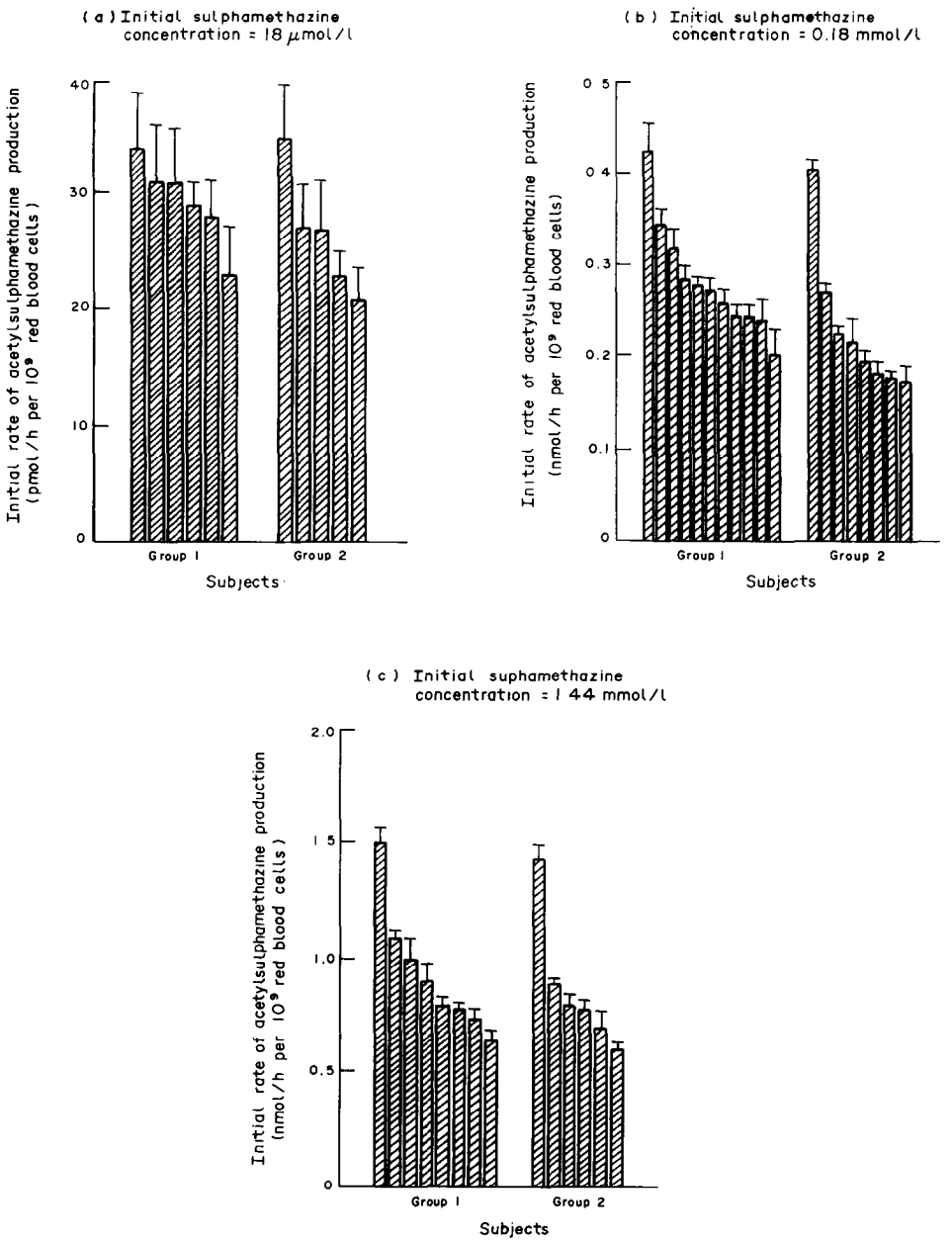

Fig. 2. The inter-individual variation of the initial acetylation rate of sulphamethazine by human whole blood from rapid and slow acetylator subjects. Values shown represent the mean of at least four replicates (error bars represent the upper 95% confidence limits). Subjects in Group 1 are rapid acetylators of sulphamethazine *in vivo*. Subjects in Group 2 are slow acetylators of sulphamethazine *in vivo*.

respectively. There was no significant difference ($P > 0.1$, Student's *t*-test) in the rates of acetylsulphamethazine production by blood samples from rapid or slow acetylators (Fig. 2a–c). A study using blood from a single volunteer established that in the absence of added acetyl-CoA, the reaction is linear up to 17, 10 and 7 hr at sulphamethazine concentrations of 0.018 mM, 0.18 mM and 1.44 mM respectively (Fig. 3). The limit of reaction linearity was not studied using blood samples from other volunteers. The maximum incubation times used to calculate the initial acetylation rates shown in Fig. 2(a–c) ranged from 4.5 to 7.5 hr and therefore the reaction was linear up to these times. Linearity may have extended beyond these times but this was not investigated since the only other incubation time studied was 24 hr. Linearity did not extend to 24 hr with any of the blood samples received.

Thermal inactivation of blood acetylation capacity at 50°. There was no significant difference ($P > 0.1$, paired *t*-test) in *N*-acetyltransferase activity between untreated samples and those heated at 50° for 1 min.

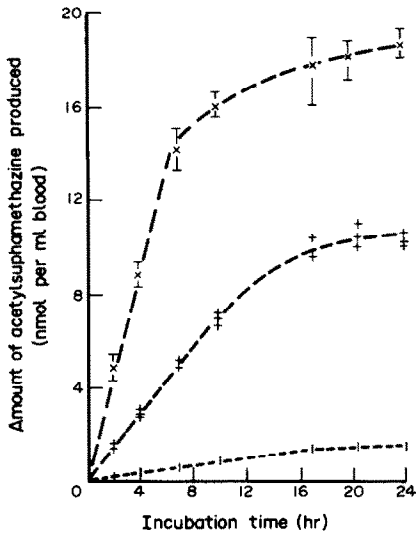

Fig. 3. The *in vitro* acetylation of sulphamethazine by human whole blood from a single healthy volunteer over a 24 hr period: initial substrate concentration = 0.018 mmol/l (---), 0.18 mmol/l (+——+), 1.44 mmol/l (×——×). Mean and 95% confidence limits are shown for points with more than three samples.

The amount of acetylsulphamethazine produced by untreated samples was significantly higher than those incubated at 50° for 3 min ($P < 0.01$, paired *t*-test). Heating at 50° for 9 min resulted in no detectable *N*-acetyltransferase activity. Semi-log plots of *N*-acetyltransferase activity against pre-incubation time at 50° were linear ($r > 0.87$, $N = 16$, $P < 0.001$). In constructing these graphs the point at zero time was omitted due to the insignificant change in activity after heating for 1 min. The mean (range) values for the apparent half life of *N*-acetyltransferase inactivation *in vitro* using samples from rapid ($N = 7$) and slow ($N = 5$) acetylators were 0.85 (0.59–0.99) min and 1.00 (0.70–1.27) min respectively. The values for the two groups were not significantly different ($P > 0.1$, Student's *t*-test).

The effect of added acetyl-CoA on blood N-acetyltransferase activity in vitro. The addition of acetyl-CoA significantly raised both the initial acetylation rate of sulphamethazine ($P < 0.05$, paired *t*-test; Table 1) and the amount of acetylsulphamethazine produced after an incubation time of 24 hr ($P < 0.05$, paired *t*-test; Table 2).

DISCUSSION

Intra-individual variation of blood acetylation capacity was slight and the relative constancy of the values suggest that blood *N*-acetyltransferase activity or the blood acetyl-CoA level or both is a characteristic of the individual. A similar result has been obtained using rabbit blood with *p*-aminobenzoic acid as substrate [7].

The absence of any significant relationship between blood *N*-acetyltransferase activity *in vitro* and the *in vivo* acetylator phenotype of the blood donor suggests that blood *N*-acetyltransferase and liver *N*-acetyltransferase may be under independent genetic control. However, studies in the rabbit, a species which also exhibits the acetylation polymorphism, have shown that an inverse relationship exists between these parameters. It has been proposed that the two enzymes are probably products of the same gene but undergo post-translational modification which is tissue-specific to produce the different isoenzymes [14]. As described earlier, the available evidence using human blood suggests that

Table 1. The effect of added acetyl-CoA on the initial rate of sulphamethazine acetylation *in vitro* by human whole blood

Subject	Acetylator phenotype	Increase in incubation acetyl-CoA concentration (mmol/l)	Rate of acetylsulphamethazine production (nmol/hr per 10^9 rbc)	Incubation time (hr)
1	Slow	0	0.18 (0.16–0.21)	0, 4.5, 8, 12
		0.34	0.26 (0.25–0.27)*	
		0.85	0.31 (0.27–0.35)*	
		1.67	0.33 (0.32–0.35)*	
2	Slow	0	0.19 (0.16–0.21)	0, 4, 7.5, 12
		0.34	0.28 (0.27–0.30)*	
		0.85	0.30 (0.28–0.32)*	
		1.67	0.34 (0.32–0.36)*	
3	Rapid	0	0.19 (0.17–0.20)	0, 4.5, 8, 12
		0.34	0.26 (0.25–0.27)*	
		0.85	0.32 (0.29–0.35)*	
		1.67	0.36 (0.33–0.38)*	
4	Rapid	0	0.26 (0.24–0.28)	0, 3, 6, 9
		0.34	0.28 (0.27–0.29)†	
		0.85	0.32 (0.29–0.34)†	
		1.67	0.34 (0.32–0.36)*	

Initial sulphamethazine concentration = 0.18 mmol/l (72 nmol per sample).
Rate values are the mean and 95% confidence limits of at least four replicates performed at the indicated times.
The values were significantly different from the control samples (no acetyl-CoA added) by paired *t*-test.
* $P < 0.01$.
† $P < 0.05$.

Table 2. The effect of added acetyl-CoA on the *in vitro* acetylation of sulphamethazine by human whole blood

Subjects (number)	Increase in incubation acetyl-CoA concentration (mmol/l)	Mean (range) amount of acetyl-sulphamethazine produced (nmol/per 10^9 rbc)
Healthy volunteers (4)	0	2.76 (2.57–3.06)
	0.34	3.29 (3.14–3.67)*
	0.85	4.14 (3.89–4.41)*
	1.67	4.81 (4.40–5.35)*

Initial sulphamethazine concentration = 0.18 mmol/l (72 nmol per sample).
Incubation time = 24 hr.
The values were significantly different from the control samples (no acetyl-CoA added) by paired *t*-test.
* $P < 0.05$.

the *in vitro* *N*-acetyltransferase activity obtained with *p*-aminobenzoic acid as substrate is also inversely related to the acetylator phenotype of the blood donor although this relationship is only apparent when reticulocyte preparations are compared [3].

Further evidence for the non-identity of blood and liver *N*-acetyltransferase in the rabbit has been provided by thermal inactivation [15], isoelectric focusing [7] and immunochemical [16] studies. None of these studies have been conducted using human blood or liver preparations.

Previous investigations on inter-individual variation of human blood acetylation capacity have been performed using a single substrate concentration [4–6]. These studies appear to have involved end-point methods although the limits of reaction linearity are not always reported. The use of a range of substrate concentrations to enable calculation of V_{max} and K_m has only been reported by one group [8]. An *in vitro* phenotyping assay which involves only a single substrate concentration would be simpler and quicker to both perform and interpret although more detailed information about the catalytic capacity and substrate affinity of blood *N*-acetyltransferase would be obtained by using a range of substrate concentrations. In the current study, a compromise between these approaches was used. The three initial sulphamethazine concentrations studied were chosen since preliminary experiments demonstrated that they represent concentrations at which the acetylation reaction is approximately first order, a mixture of first and zero order and zero order respectively.

The inter-individual variation of blood *N*-acetyltransferase activity *in vitro* in any of the studies reported here was approximately 2–3-fold. This variation is slightly less than the approximately 4–5-fold variation observed *in vivo* in this study and in others [17, 18] but is much lower than the 56-fold ranges reported for *N*-acetyltransferase preparations from both human liver and intestinal mucosa [19]. Since the variation in blood *N*-acetyltransferase activity may be due to either differences in the enzyme activity or the amount of acetyl-CoA present, the effect of added acetyl-CoA was investigated in a small number of subjects. These limited studies suggested that blood *N*-acetyltransferase was not saturated by the endogenous level of this cofactor although a substantial increase in acetyl CoA level (relative to the initial sulphamethazine concentration) was needed to significantly affect the acetylation capacity. However, in the absence of published data on the influence of blood levels of this cofactor on *in vitro* acetylation capacity, this explanation should not be overlooked.

Compared to previous studies on blood *N*-acetyltransferase activity *in vitro*, the period of reaction linearity is long (Fig. 3). The time course of *p*-aminobenzoic acid acetylation using human whole blood lysates in the presence of added acetyl-CoA is linear up to 30 min using a substrate concentration of 0.2 mM [12]. This shorter period of reaction linearity may be due to the higher blood *N*-acetyltransferase activity towards *p*-aminobenzoic acid than sulphamethazine. The acetylation of dapsone (initial concentration = 0.013 mM) [4] and procainamide (initial concentration = 0.084 mM) [20] by human whole blood in the absence of added acetyl CoA is linear up to at least 5 hr and 8 hr respectively. The limit of reaction linearity therefore depends on the substrate studied.

The rate at which *N*-acetyltransferase is thermally inactivated was studied since previous studies on human lymphocyte *N*-acetyltransferase demonstrated that the enzyme preparation from rapid acetylators was more heat labile at 44° than that from slow acetylators [8]. However, the two phenotypes were poorly resolved by this method. The current study did not find any significant difference in the thermal inactivation rate of blood sulphamethazine *N*-acetyltransferase at 50° from rapid acetylators and slow acetylators.

The inability of this study to distinguish rapid and slow acetylators *in vitro* may be due to the relatively crude *N*-acetyltransferase preparation used. Further purification of the enzyme may permit segregation of the two phenotypes but would also lengthen and complicate the assay. The use of a readily accessible tissue such as peripheral blood as a means of phenotyping individuals *in vitro* is attractive since it would be simpler and more economical to perform on a large scale than the current *in vivo* methods.

REFERENCES

1. Weber WW and Hein DW, *N*-acetylation pharmacogenetics. *Pharmacol Rev* **37**: 25–79, 1985.

2. Evans DAP and White TA, Human acetylation polymorphism. *J Lab Clin Med* **63**: 394–403, 1964.
3. Weber WW, Radtke HE and Tannen RH, Extrahepatic *N*-acetyltransferases and *N*-deacetylases. In: *Extrahepatic Metabolism of Drugs and Other Foreign Compounds* (Ed. Gram TE), pp. 493–531. MTP Press, Lancaster, 1980.
4. Drayer DE, Strong JM, Jones B, Sandler A and Reidenberg MM, In vitro acetylation of drugs by human blood cells. *Drug Metab Dispos* **2**: 499–505, 1974.
5. Hearse DJ, Weber WW and Pretorius HJ, Multiple N-acetyltransferases and their relation to drug toxicity. *Am J Hum Genet* **22**: 15a, 1970.
6. Motulsky AG and Steinmann L. Arylamine acetylation in human red blood cells. *J Clin Invest* **41**: 1387, 1962.
7. Weber WW, Drummond G. Miceli JN and Szabadi R, Genetic control and isozymic composition of drug acetylating enzymes. In: *Isozymes* (Ed. Market CL), Vol. 4, pp. 813–828, Academic Press, New York, 1975.
8. McQueen CA and Weber WW, Characterisation of human lymphocyte N-acetyltransferase and its relationship to the isoniazid acetylator polymorphism. *Biochem Genet* **18**: 889–904, 1980.
9. Drummond GS, Kelker HC and Weber WW, N-acetylation of drugs: Observations on the properties of partially purified N-acetyltransferase from peripheral blood of rabbit. *Biochem J* **187**: 157–162, 1980.
10. Tannen RH and Weber WW, Rodent models of the human isoniazid acetylator polymorphism. *Drug Metab Dispos* **7**: 274–279, 1979.
11. Gollamudi R, Rackley RJ and Autian J, A new substrate for the measurement of N-acetyltransferase activity. *Enzyme* **30**: 155–161, 1983.
12. Mandelbaum-Shavit F and Blondheim SH, Acetylation of *p*-aminobenzoic acid by human blood. *Biochem Pharmacol* **30**: 65–69, 1981.
13. Baty JD, Lindsay RM and Sharp S, Use of high-performance liquid chromatography in the measurement of *in vitro* acetylation in man. *J Chromatogr* **353**: 329–337, 1986.
14. Szabadi RS, McQueen CA, Drummond GS and Weber WW, *N*-acetylation of drugs. A genetically controlled reciprocal relationship between drug *N*-acetylating enzymes of rabbit liver and peripheral blood cells. *Drug Metab Dispos* **6**: 16–20, 1978.
15. Hearse DJ and Weber WW, Multiple N-acetyltransferases and drug metabolism: Tissue distribution, characterisation and significance of mammalian *N*-acetyltransferase. *Biochem J* **132**: 519–526, 1973.
16. Patterson E, Radtke HE and Weber WW, Immunochemical studies of rabbit N-acetyltransferases. *Mol Pharmacol* **17**: 367–373, 1980.
17. Olson W, Miceli J and Weber WW, Dose-dependent changes in sulfamethazine kinetics in rapid and slow isoniazid inactivators. *Clin Pharmacol Ther* **23**: 204–211, 1978.
18. Whelpton R, Watkins G and Curry S, Bratton-Marshall and liquid chromatographic methods compared for determination of sulphamethazine acetylator status. *Clin Chem.* **27**: 1911–1914, 1981.
19. Jenne JW, Partial purification and properties of the isoniazid transacetylase in human liver: Its relationship to the acetylation of p-aminosalicylic acid. *J Clin Invest* **44**: 1992–2002, 1965.
20. Chen M-L, Lee MG and Chion WL, Pharmacokinetics of drugs in blood. iii: Metabolism of procainamide and storage effect of blood samples. *J Pharm Sci* **72**: 572–574, 1983.