Biochemical Genetics, Vol. 18, Nos. 9/10, 1980

Characterization of Human Lymphocyte *N*-Acetyltransferase and Its Relationship to the Isoniazid Acetylator Polymorphism

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Received 6 Sept. 1979—Final 28 Nov. 1979

Characterization of human lymphocyte N-*acetyltransferase (NAT) for specific activity, substrate specificity, inhibition, pH optimum, apparent K_m, kinetic mechanism, trypsin stability, freezing stability, and heat stability was carried out in rapid and slow isoniazid (INH) acetylators. There is a statistically significant difference in the heat stability of lymphocyte NAT from rapid and slow INH phenotypes. The lymphocyte enzyme from rapid INH acetylators is less heat stable than the lymphocyte enzyme from slow INH acetylators. This is an indication of a structural, possibly polymorphic, difference in lymphocyte NAT from the two acetylator phenotypes.*

KEY WORDS: *N*-acetyltransferase; isoniazid acetylator polymorphism; heat stability.

INTRODUCTION

Acetylation is an important metabolic route for inactivation of drugs and carcinogens that are arylamines or hydrazine derivatives. The reaction, catalyzed by *N*-acetyltransferases (NAT), involves the transfer of the acetyl group from acetyl coenzyme A (AcCoA) to the arylamine substrate (Lipmann, 1945). Individuals are classified as rapid or slow acetylators according to the rate at which drugs such as isoniazid (INH) or sulfamethazine (SMZ)

This work was supported by U.S. Public Health Service Grant GM 27028 and GM 21723. A preliminary report of this work was presented to the American Society of Human Genetics, San Diego, California, October 1977.

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are acetylated by liver NAT (Knight *et al.,* 1959; Evans *et al.,* 1960). The slow acetylator phenotype is homozygous recessive while the rapid acetylator phenotype is heterozygous or homozygous dominant. Both man and the rabbit possess this INH acetylator polymorphism (Knight *et al.,* 1959; Evans *et al.,* 1960; Frymoyer and Jacox, 1963; Gordon *et al.,* 1973).

An extrahepatic polymorphism has also been described in the rabbit that involves the acetylation of p-aminobenzoic acid (PABA) and is related to the INH phenotype (Weber *et al.,* 1975, 1976; Szabadi *et al.,* 1978). Rapid INH acetylator animals have low blood PABA NAT while the slow phenotype has high blood PABA NAT. This relationship is also seen in lymphocytes as well as red blood cells (Weber *et al.,* 1976).

Although previous studies have failed to detect any relationship between extrahepatic NAT and the INH phenotype in man, activity has been the only criterion used (Motulsky and Steinmann, 1962; Drayer *et al.,* 1974; Hearse *et al.,* 1970). Alterations in the enzyme could affect any of its biochemical properties (Paigen, 1971). This study was undertaken to determine whether there are differences in the genetic and biochemical characteristics of human lymphocyte NAT that are associated with different INH acetylator phenotypes.

MATERIALS AND METHODS

Determination of Acetylator Phenotype

Individuals were phenotyped as rapid or slow INH acetylators by a slight modification of the method of Evans (1969). Blood and urine samples were obtained from healthy adult volunteers who had not eaten for 2 hr prior to the test. SMZ (10 mg/kg) was given orally, and blood and urine samples were collected 4½ hr later. The percent of acetylated drug was then determined (Weber and Brenner, 1974).

Separation of Peripheral Lymphocytes

The method developed by Böyum (1967) and modified by Lopes *et al.* (1973) was used to separate lymphocytes from peripheral blood samples. The gradient mixture was prepared by combining 2.4 parts of 9% Ficoll 400 (9 g/100 ml 1×10^{-2} Tris-HCl, *p*H 7.4) with 1 part Hypaque mixture (45.3 ml Hypaque-M75, 1 ml of 1 M Tris-HCl, *p*H 7.4, and 53.7 ml H_2O). The blood sample was diluted 1:1 with phosphate-buffered saline (PBS), *p*H 7.4, layered on the gradient mixture (ratio of sample to gradient mixture is 2:1) and centrifuged for 30 min in a GLC II (Sorvall, Norwalk, Connecticut) at 400*g*. After the mononuclear layer had been washed, the cells were counted,

divided into aliquots, and centrifuged for 10 min at 1300*g* in a GLC II. The supernatant was removed and the cell pellet stored at −70C in an ultrafreezer.

Assay for PABA NAT Activity

Samples were assayed for enzyme activity using a spectrophotometric microassay system (Hearse and Weber, 1973). A cell pellet was resuspended in 0.1 M phosphate buffer, *p*H 7.0, with 10^{-3} M dithiothreitol (DTT) and 10^{-3} M ethylenediamine tetraacetic acid (EDTA) to give a concentration of approximately 10×10^6 cells/ml. This suspension was subjected to eight to ten rounds of freezing in a dry ice and methanol bath and thawing in a 37C water bath in order to lyse the cells. Fifty microliters of the lysate was used to determine enzyme activity.

Culture of Human Peripheral Lymphocytes

Lymphocytes were maintained in culture according to the method of Bloom and Iida (1967). The cell culture medium was prepared by adding fetal calf serum (10% v/v), penicillin (50 units/ml), and streptomycin (50 μg/ml) to RPMI 1640, minus PABA (Flow Laboratories, Rockville, Maryland). Isolated lymphocytes were added to the medium to give a concentration of approximately 5×10^5 cells/ml. Ten milliliters of the cell and medium suspension was pipetted into a T25 flask with 0.1 ml of phytohemagglutinin (Burroughs Wellcome, Research Triangle Park, North Carolina) and incubated at 37C in a 5% CO_2 atmosphere.

Protein Determinations

The amount of protein in a cell lysate was determined by a microbiuret reaction (Gornall *et al.*, 1949).

Experimental Conditions for Comparative Determinations of Lymphocytes NAT from Rapid and Slow INH Acetylators

Substrate Specificity

All compounds were assayed for substrate potential for lymphocyte NAT by the procedure described for determination of PABA NAT activity. The final concentration of substrate was $4.4. \times 10^{-5}$M in all cases.

Inhibition

Cell pellets were resuspended with 0.1 M potassium phosphate buffer, *p*H 7.0, to a concentration of approximately 20×10^6 cells/ml and lysed by freezing

and thawing as previously described. Twenty-five microliters of the cell lysate was used in the enzyme assay. The test compound (25 μl) at the appropriate concentration was added to the incubation mixture and the procedure followed as previously described.

pH Optimum

*p*H optimum curves were determined in 0.1 M potassium pyrophosphate buffer adjusted to the desired *p*H with HCl. Cell samples, isolated from a single individual, were each resuspended to a final concentration of 10×10^6 cells/ml in buffer at several specific *p*H's, and lysed by repeated freezing and thawing. Enzyme activity was determined as previously described.

Stability to Freezing

The cell lysate was divided into aliquots and stored at −20C. Enzyme activity was determined on a freshly thawed sample at specified time intervals.

Trypsin Inactivation

The cell pellet was resuspended in 0.1 M phosphate buffer, *p*H 7.0, with 10^{-3} M DTT and 10^{-3} EDTA to a concentration of approximately 17×10^6 cells/ml. Thirty microliters of the cell lysate was added to a 400-μl tube, and a 10-μl aliquot of 0.01% trypsin solution (Worthington Biochemical Corp., Freehold, New Jersey) was then added. The mixture was incubated in a 33C water bath for specified times, and the action of the trypsin was stopped by the addition of 10 μl of 0.01% soybean trypsin inhibitor (Worthington Biochemical Corp., Freehold, New Jersey). The samples were then assayed for residual PABA NAT activity by the procedure previously described.

Stability to Heat

Fifty-microliter aliquots of the cell lysate (10×10^6 cells/ml) were placed in 400-μl tubes. The samples were then placed in an Aquatherm water bath (New Brunswick Scientific Co., New Brunswick, New Jersey) at 44C, heated for a specified time, removed, and placed on ice. Blanks and zero-time samples were kept on ice and then assayed with the experimental samples for PABA NAT activity by the assay previously described.

RESULTS

Healthy adult volunteers were phenotyped as rapid or slow acetylators of SMZ by determining the percent of acetylated drug in blood and urine

(Evans, 1969; Weber and Brenner, 1974). Slow acetylators had less than 75% acetylated drug in urine and 40% in blood, while rapid acetylators had greater than 80% in urine and 45% in blood.

Differential cell counts were done on the mononuclear layer obtained in the Ficoll Hypaque gradient, and almost equal numbers of lymphocytes and monocytes were present. After this fraction had been washed, almost 90% of the monocytes were removed, so the final preparation contained appproximately 6% monocytes. NAT activity was sought in a purified preparation of monocytes and none was detected. Therefore, the monocyte contribution to PABA NAT activity in the mononuclear cell fraction was negligible.

The biochemical characteristics of lymphocyte NAT from rapid and slow INH acetylators were determined. Comparisons of specific activity, substrate specificity, kinetic properties, inhibitors, *p*H optimum, and stability were made.

Table I. Specific Activity of Lymphocyte PABA NAT from Rapid and Slow INH Acetylators

Rapid INH acetylators		Slow INH acetylators	
Donor	nmol AcPABA/hr/mg	Donor	nmol AcPABA/hr/mg
ML	105	BW	106
AA	114	EP	108
PS	116	GG	113
SL	119	CAS	113
LB	120	RT	121
IG	131	LH	124
VZ	134	CS	127
SB	136	FC	130
WB	141	WV	136
PN	142	CM	144
DK	146	KL	145
HE	150	JS	148
MV	169	DG	149
JB	171	SH	152
DR	178	CH	158
		HR	158
		KM	170
		BB	172
		PDS	172
		OL	173
		DH	174
		MAL	207
		WW	215
		RV	225
MEAN	138		151
SD	22		32
N	15		24

Specific Activity

The activity of lymphocyte NAT (nmol acetyl p-aminobenzoic acid (AcPABA) formed/hr/mg protein) was determined for both INH acetylator phenotypes. The range of NAT activity in the slow phenotype was 106–225 and was 105–178 in the rapid phenotype (Table I). No relationship was observed between the specific activity of NAT in the lymphocyte and the acetylator phenotype.

Substrate Specificity

The ability of lymphocyte NAT to acetylate SMZ, benzocaine, and procainamide as well as PABA was tested. PABA was the only acetyl acceptor found. NAT activity for SMZ, benzocaine, or procainamide was not detected in either acetylator phenotype.

Kinetic Properties

Initial velocities were determined for PABA and AcCoA using lymphocytes from six individuals of both acetylator phenotypes and graphed as double reciprocal (Lineweaver-Burk) plots. No differences were detected in the K_m or V_{max} values obtained from each acetylator phenotype. Average K_m values of 1.71×10^{-4} M and 1.82×10^{-4} M were obtained for PABA and AcCoA, respectively, while average V_{max} values of 574 and 199 nmol AcPABA/hr/mg protein were obtained for PABA and AcCoA, respectively. The kinetic constants for PABA were determined at an AcCoA concentration of 2.43×10^{-3} M while the kinetic constants for AcCoA were determined at a PABA concentration of 4.4×10^{-5} M. A series of initial velocity curves was run varying both substrates and yielded a series of parallel lines.

Innhibitors

Various metal ions were tested for their effect on lymphocyte NAT activity. The metal concentrations ranged from 10^{-3} M to 10^{-7} M. Fe^{3+}, Mn^{2+}, Ca^{2+}, and Fe^{2+} had no effect on enzyme activity, but 50% inhibition was seen at 10^{-5} M Cu^{2+}, 1.4×10^{-4} M Zn^{2+}, and 5.5×10^{-6} M Hg^{2+}. *N*-Ethylmaleimide, a sulfhydryl group inhibitor, was a potent inhibitor of the enzyme with a concentration of 1×10^{-6} M resulting in a 50% loss of activity. The products of the enzyme reaction, coenzyme A and AcPABA, were poor inhibitors. The addition of 10^{-3} M AcPABA resulted in a 60% loss of enzyme activity and of 10^{-3} M CoA an 18% loss. No activation of the enzyme was seen. The effects of all the compounds tested were the same for both acetylator phenotypes.

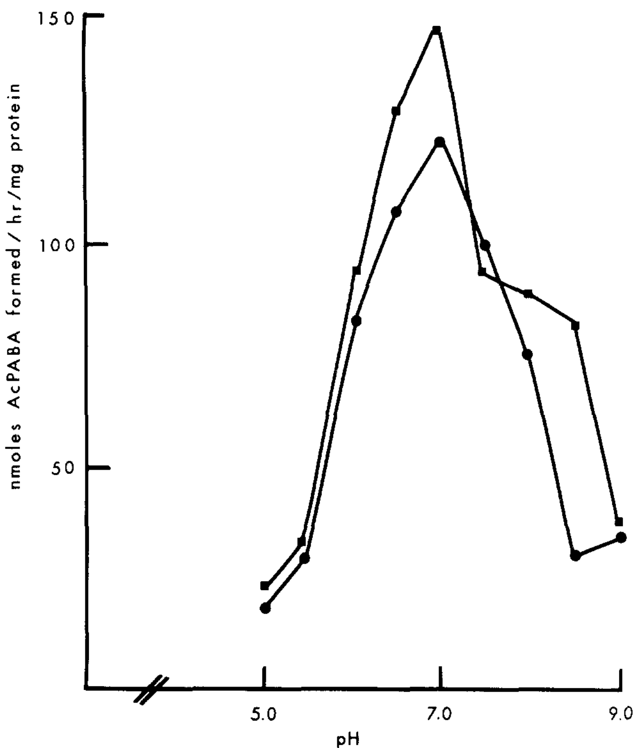

Fig. 1. Activity at *p*H 5.0–9.0 of lymphocyte NAT from rapid (●) and slow (■) acetylators. The cells were lysed in 0.1 M pyrophosphate buffer of varying *p*H's and assayed for enzyme activity according to the procedure in Materials and Methods.

*p*H optimum

*p*H optimum curves were determined for human lymphocyte PABA NAT from rapid and slow INH acetylators. A *p*H range of 5.0–9.0 (0.5 *p*H unit intervals) was used initially and both phenotypes had a peak of activity at *p*H 7.0 (Fig. 1). When a *p*H range of 6.2–7.8 (0.2 *p*H unit intervals) was used, the peak of activity was found to be broader, approximately 6.6–7.4. No significant differences were found between the *p*H curves from the two acetylator phenotypes.

Stability

The stability of lymphocyte PABA NAT to a variety of agents and conditions was tested. Lymphocyte lysates from both acetylator phenotypes were stored at −20C, then assayed for NAT activity. No differences were observed in the

stability of activity during the first 18 days, and an average of 60% of the activity was still detectable between 21 and 72 days. Prior addition of AcCoA 2.85×10^{-3} M) to the lysate did not exert a protective effect against storage at −20C.

Treatment of lymphocyte NAT from six individuals, three rapid and three slow acetylators, with 0.01% trypsin for varying time periods resulted in a 50% loss of activity in 13–22 min. The average $t_{1/2}$ for the slow acetylators was 17 min, the same average obtained for the rapid phenotype.

The effect of heating on lymphocyte NAT was initially determined at temperatures ranging from 30 to 53C for 5 min. At temperatures less than 40C, at least 90% of the enzyme activity was still present after 5 min of

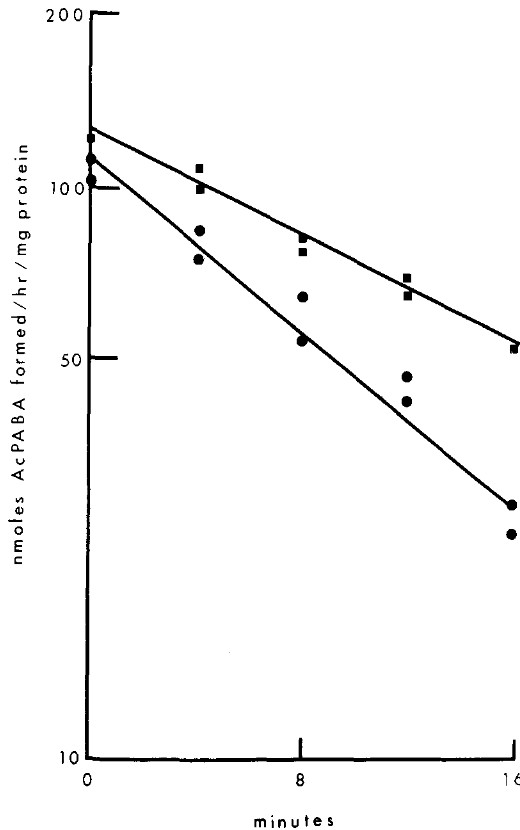

Fig. 2. Inactivation of lymphocyte NAT at 44C. The cell lysate was heated at 44C for 0, 4, 8, 12, and 16 min, then placed on ice. Enzyme activity was determined according to the procedure in Materials and Methods. The log of the product was plotted against the time at 44C. The $t_{1/2}$ and k were determined by regression analysis. Rapid INH acetylator (●) $t_{1/2}$ = 15 min, k = 0.048 min^{-1}, r = 0.99; slow INH acetylator (■) $t_{1/2}$ = 8.3 min, k = 0.083 min^{-1}, r = 0.97.

Table II. Inactivation at 44 C of Lymphocyte NAT from Rapid and Slow INH Acetylators

Slow INH acetylators			Rapid INH acetylators		
Donor	k(min^{-1})	$t_{1/2}$(min)	Donor	k(min^{-1})	$t_{1/2}$(min)
GG	0.034	21	VZ	0.048	15
BB	0.037	19	SL	0.057	12
CH	0.043	16	AA	0.058	12
WW	0.044	16	LB	0.061	11
RT	0.046	15	DK	0.066	11
CS	0.048	15	SB	0.072	10
RV	0.048	15	DR	0.073	10
OL	0.049	15	PN	0.078	8.8
BW	0.050	14	IG	0.081	8.7
HR	0.051	14	PS	0.081	8.6
DG	0.053	13	SH	0.089	8.1
FC	0.056	12	HE	0.091	8.0
PDS	0.056	12	ML	0.106	6.6
DW	0.057	12	WB	0.110	6.6
LH	0.058	12	JB	0.183	3.8
KM	0.058	12			
WV	0.061	12			
EP	0.061	12			
DH	0.065	11			
KL	0.066	10			
JS	0.070	10			
CM	0.071	10			
MAL	0.076	9.2			
CAS	0.107	6.6			
Mean	0.056	13		0.084	9
SD	0.015	3.2		0.033	2.7
N	24	24		15	15

heating, while at 46C, only 20% of the enzyme activity remained. Subsequently, the stability of the enzyme was determined at 44C. Lymphocytes from 39 healthy adult volunteers, 17 females and 22 males, were used. Semilog plots of NAT activity vs. time of heating yielded straight lines (Fig. 2). The rate of inactivation (k min^{-1}) and the half-life ($t_{1/2}$ min) were calculated using a semilog regression program. Lymphocyte PABA NAT from rapid acetylators was more sensitive to heating than enzyme from slow acetylators. The mean $t_{1/2}$ value from rapid acetylators was 9 min ($k = 0.084$ min^{-1}) and for slow acetylators was 13 min ($k = 0.056$ min^{-1}) (Table II and Fig. 3). This difference was statistically significant ($p < 0.001$) in a Student's t test.

The reproducibility of the $t_{1/2}$ was tested in the same cell sample and in different cell samples from the same individual. A ratio of the lowest $t_{1/2}$ to the highest $t_{1/2}$ was calculated for each individual. The average ratio for

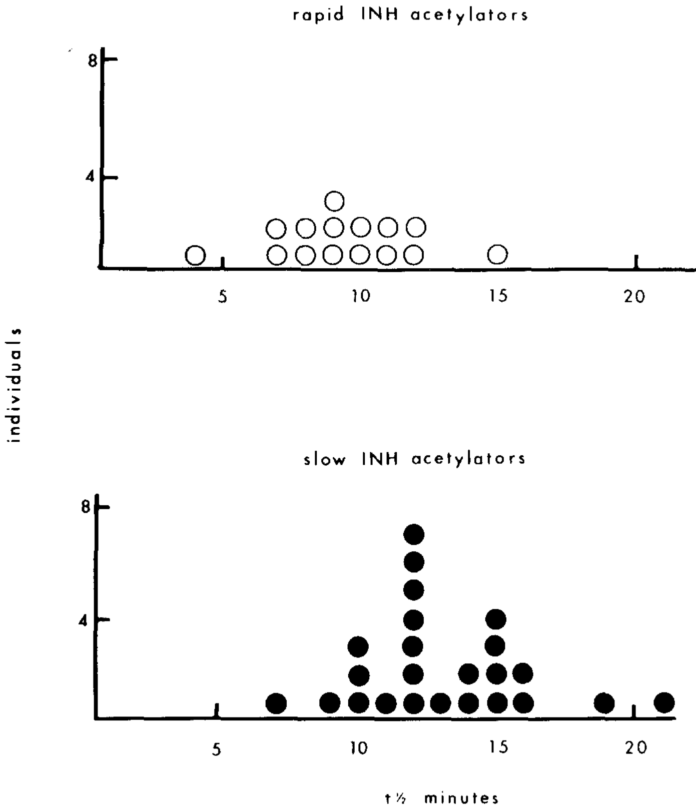

Fig. 3. Distribution of the half-life ($t_{1/2}$) at 44C of lymphocyte NAT in rapid and slow INH acetylators.

multiple determinations using the same cell sample was 0.83 and for different cell samples obtained from the same individual it was 0.76. This indicates that the variation seen in repeated $t_{1/2}$ determinations on an individual were not due to changes in the cell population of the blood sample.

The $t_{1/2}$ in either phenotype was not effected by dialysis of the cell lysate against 0.1 M potassium phosphate, *p*H 7.0, plus 10^{-3} M DTT plus 10^{-3} M EDTA before the $t_{1/2}$ determination. Lysing the cells in buffer with or without 10^{-3} M DTT and/or 10^{-3} M EDTA did not alter the heat inactivation curves.

The lymphocyte lysate from a rapid acetylator was mixed with lysate from a slow acetylator, and the $t_{1/2}$ and k at 44C were determined. The k value of 0.059 min^{-1} obtained was approximately the average of the values for the two phenotypes, 0.045 min^{-1} for the slow acetylator and 0.069 min^{-1} for the rapid acetylator. The $t_{1/2}$ of the mixture was 12 min, with values of 15 min for the slow acetylator and 11 min for the rapid acetylator.

Table III. Effect of Compounds on the Stability of Lymphocyte NAT at 44 C

		Rapid INH acetylator $t_{1/2}$(min)			Slow INH acetylator $t_{1/2}$(min)		
Compound	M concentration	A	B	$\frac{B}{A}$	A	B	$\frac{B}{A}$
AcCoA	2.86×10^{-3}	14	—	—	12	153	13
AcCoA	2.86×10^{-4}	14	206	15	12	123	10
AcCoA	2.86×10^{-5}	14	134	10	12	127	11
AcCoA	2.86×10^{-6}	14	221	16	12	91	8
AcCoA	2.86×10^{-7}	14	38	3	12	18	2
AcINH	1.30×10^{-3}	7	7	1	11	13	1
Acthiocholine	2.86×10^{-4}	11	12	1	15	20	1
Actryptamine	2.86×10^{-4}	8	9	1	11	15	1
INH	1.30×10^{-3}	7	15	2	11	29	3
Melatonin	2.86×10^{-4}	8	10	1	12	10	1
Nicotinamide	2.86×10^{-4}	11	8	1	15	12	1
PABA	5.70×10^{-4}	7	31	5	11	23	2
Serotonin	2.86×10^{-4}	8	13	2	15	21	1
Tryptamine	2.86×10^{-4}	8	8	1	11	11	1

A variety of compounds were screened for their ability to stabilize lymphocyte NAT against heat denaturation. The test compound was added to the cell lysate and heated at 44C, and the residual enzyme activity was determined. A ratio of the $t_{1/2}$ with the compound to the $t_{1/2}$ of the enzyme alone was calculated. A value of 1 was expected if no stabilization occurred, with the greatest stabilization giving the highest value. AcCoA was the most effective compound (Table III). A concentration of approximately 3×10^{-5} M resulted in a tenfold increase in the $t_{1/2}$; even a concentration of 3×10^{-7} M had an appreciable effect. PABA and INH were less effective while acetylthiocholine, serotonin, nicotinamide, acetylisoniazid (AcINH), melatonin, tryptamine, and acetyltrptamine had no effect.

Cell Culture

Cultures of freshly isolated peripheral lymphocytes, stimulated with PHA, had PABA NAT activity which peaked at 96–120 hr. The amount of activity was comparable to that in uncultured cells.

The stability at 44C of cells cultured for 96 hr was tested and found to be the same as for uncultured cells. For a rapid acetylator, a $t_{1/2}$ of 9 min $k = 0.080$ min^{-1}) was obtained after cell culture while the uncultured cells had a $t_{1/2}$ of 8 min ($k = 0.082$ min^{-1}). The values seen for slow acetylators were 9 min ($k = 0.080$ min^{-1}) for the cultured cells as compared to 11 min ($k = 0.065$ min^{-1}) for the uncultured cells.

DISCUSSION

Rabbits exhibit a polymorphism in hepatic (Frymoyer and Jacox, 1963; Gordon *et al.*, 1973) as well as extrahepatic NATs (Weber *et al.*, 1975, 1976; Szabadi *et al.*, 1978). The hepatic enzyme determines the rate of acetylation of drugs such as INH, and animals are designated as rapid or slow acetylators (Frymoyer and Jacox, 1963; Gordon *et al.*, 1973). Blood NAT metabolizes PABA, and animals are designated as having high or low levels of activity (Weber *et al.*, 1975, 1976). A relationship exists between these two polymorphisms, with rapid INH acetylators having low blood PABA NAT activity and slow INH acetylators having high blood PABA NAT. Fractionation of rabbit blood cell samples shows that the difference in PABA NAT activity is present in the red cell and lymphocyte (Szabadi *et al.*, 1978).

Since rabbit and man both express the liver NAT polymorphism, it was reasonable to see if the extrahepatic polymorphism is present in man as well. Because polymorphic differences can be expressed in an alteration of activity, thermal stability, *p*H optimum, or any of the biochemical properties of an enzyme (Paigen, 1971), a variety of parameters of lymphocyte NAT were tested. The purposes of this study were to characterize human lymphocyte NAT of rapid and slow INH acetylators and to seek correlations between genetically determined INH acetylator phenotypes and one or more biochemical properties of lymphocyte NAT. A comparison of the properties of human lymphocyte NAT from the two acetylator phenotypes revealed no differences in the specific activity, substrate specificity, kinetic properties, inhibition, *p*H optimum, stability to trypsin, or stability to freezing. Heat stability studies did show that lymphocyte PABA NAT from human rapid acetylators was more sensitive to inactivation at 44C than enzyme from slow acetylators (Table II).

The differential heat stabilities observed may be accounted for in several ways:

1. By the presence of an inhibitor of PABA NAT.
2. By the presence of a stabilizing agent of PABA NAT.
3. By the posttranslational modification of the enzyme.
4. By a structural gene difference.

Results of mixing experiments and the similarities in specific activities of the enzyme from the two acetylator phenotypes argue against the first two possibilities. When lymphocyte NAT from a rapid INH acetylator was mixed with that from a slow acetylator, the k at 44C of the mixture was the average of the two phenotypes. If an inhibitor were present, the expected $t_{1/2}$ or k would be like that of the less stable type, while if a stabilizing agent were

present, the value would be more like that of the more stable phenotype. The presence of a stabilizer or an inhibitor should also be reflected in the specific activity of lymphocyte NAT. The phenotype with the inhibitor would be expected to have a low specific activity while the phenotype with the stabilizer would have a high specific activity. No such differences were seen in the specific activity of human lymphocyte NAT in the rapid or slow INH phenotypes (Table I), although rabbit blood NAT did show differences in specific activity which, it has been postulated, may be due to a stabilizing agent inactivated by the polymorphic NAT. Greater amounts of this substance would be present in slow acetylators since they have a lower liver NAT activity than rapid acetylators (Szabadi *et al.,* 1978). The only substance that had a substantial effect on the heat stability of human lymphocyte NAT was the acetyl donor substrate of the reaction, AcCoA (Table III). A stable acetyl enzyme intermediate could be formed by the lymphocyte enzyme which seems to have the same kinetic mechanisms as the rabbit liver enzyme. Initial velocity studies of both rabbit liver NAT (Weber and Cohen, 1963; Weber *et al.,* 1968) and human lymphocyte NAT showed a series of parallel lines. Differences in the concentration of AcCoA in rapid and slow acetylators, then, could effect the stability of the lymphocyte enzyme. Dialysis of the enzyme, however, did not change the stability of lymphocyte NAT, ruling out the presence of a dialyzable stabilizer, like AcCoA, or an inhibitor.

The biochemical characteristics of an enzyme such as heat stability can be altered by posttranslational modifications of the enzyme. Studies of murine kidney catalase isolated from inbred strains C3H and C57B1/6 indicated that they were electrophoretically different (Hoffman and Grieshaber, 1976). Breeding studies done with these strains showed the electrophoretic pattern from the F_1 generation to be identical to that of C57B1/6 parent. If enzyme from the parental strains was mixed and then subjected to electrophoresis, the pattern of the C57B1/6 parent was seen. This suggests that some posttranslational modification had converted C3H type to the C57B1/6 type.

A similar pattern of events was seen with human adenosine deaminase, where different tissues give different electrophoretic patterns. Mixing of homogenates from one tissue (e.g, RBCs) with another (e.g., kidney) was followed by electrophoresis which resulted in a single pattern (kidney) (Hirshhorn, 1975).

Although it is possible that the differential heat stability seen in lymphocyte NAT could be due to a posttranslational modification, the results of mixing experiments previously described were not consistent with this. Electrophoresis, the technique used in the studies described above, was not

done with lymphocyte NAT because a staining technique for this enzyme is not available.

The final explanation for the differential heat stability seen in human lymphocyte NAT is a structural gene difference. While evidence from this study is consistent with that hypothesis, further work is necessary to demonstrate the nature of the differences. The ultimate way to determine an alteration in a structural gene is amino acid sequencing, which requires a highly purified preparation of the enzyme. Even techniques such as peptide mapping that could detect amino acid differences require a more highly purified enzyme than is presently available.

Studies with bovine carboxypeptidase A in which the amino acid sequences were determined do support the possibility of a structural gene difference accounting for differential heat stability. Two forms of the enzyme having the same specific activities were isolated from pancreatic tissue. One form of the enzyme had a $t_{1/2}$ at 50C of about 25 min and the other a value of about 50 min. Sequencing of the terminal peptide showed they differed only in the *C*-terminal amino acid. The more stable form of the enzyme contained valine, and the less stable form contained leucine (Walsh *et al.*, 1966).

A limited study was done to determine if the differential heat stability had a genetic component. Data obtained on three human families were consistent with the $t_{1/2}$ and k of lymphocyte NAT being determined by a two-allele system, but more extensive family studies would be necessary to confirm this hypothesis.

Among the several species of mammals which have been studied, a relationship is always observed between liver INH phenotype and extrahepatic NAT, but the nature of this relationship varies from one species to another. Deer mice show a direct correlation between liver NAT activity and blood PABA NAT (Tanner and Weber, 1977). Rabbits, on the other hand, show an inverse relationship, which rapid INH acetylators having low levels of blood PABA NAT and slow INH acetylators having high blood PABA NAT activity (Weber *et al.*, 1976; Szabadi *et al.*, 1978). Biochemical studies of rabbit liver NAT and blood NAT indicate common structural features for the enzyme from these tissues (Weber *et al.*, 1975). Recent studies of the heat stability and immunological characteristics of hepatic NAT from rapid and slow INH acetylator rabbits provide strong evidence for a structural difference accounting for the INH acetylator polymorphism (Weber *et al.*, 1978). In this report, we described a relationship between the human liver INH acetylator phenotype and the heat stability of lymphocyte NAT: rapid acetylators have a lymphocyte NAT that is less heat stable than the lymphocyte enzyme from a slow acetylator (Table II). In view of the correlation between human lymphocyte NAT and acetylator phenotype and

the evidence from rabbit studies, it may be anticipated that there is also a structural difference in human hepatic NAT which accounts for the INH acetylator polymorphism. Further studies are necessary to clarify the relationship between lymphocyte NAT and the hepatic enzyme.

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