RODENT MODELS OF THE HUMAN ISONIAZID-ACETYLATOR POLYMORPHISM

ROBERT H. TANNEN[1] AND WENDELL W. WEBER

Department of Pharmacology, University of Michigan Medical School

(Received April 2, 1979)

ABSTRACT:

Inbred strains and subpopulations of rats, laboratory mice, and deer mice were examined for individual variation in the ability to metabolize several arylamines (p-aminobenzoic acid, sulfamethazine, aniline, α-naphthylamine, and aminofluorene) by N-acetylation. Individual differences within species were found to be dependent upon the tissue source of N-acetyltransferase activity and the acetyl acceptor employed. Long-Evans rats possessed about 2-fold more p-aminobenzoic acid N-acetyltransferase activity in blood and liver than Sprague-Dawley rats; no strain differences could be found with sulfamethazine. Nine strains of laboratory mice (Mus musculus) were found to have considerable liver p-aminobenzoic acid N-ace-

tyltransferase activity but only slight activity towards sulfamethazine. No strain differences were apparent in regard to liver N-acetyltransferase activity. Blood p-aminobenzoic acid N-acetyltransferase activity was distinctly polymorphic in laboratory mice; of the nine strains tested, only A/J mice did not have this activity. Partially inbred deer mice (Peromyscus maniculatus) showed a narrower phenotypic range than random-bred stock from which they were obtained, which suggests the existence of distinct subpopulations with respect to N-acetylation capacity. Presumptive evidence for multiple forms of N-acetyltransferase in liver and blood was obtained through a study of substrate specificity.

The acetylating capacity of humans for isoniazid (INH),[2] SMZ, and several other arylamine drugs is a bimodally distributed, inherited trait (1, 2). Rapid and slow acetylator phenotypes are both subject to differential toxicity from drugs and foreign compounds that are metabolized in this way (3). We are particularly interested in the slow acetylator phenotype as a predisposing factor in spontaneous and drug-induced systemic lupus erythematosus (3, 4) and in carcinogenesis induced by arylamines (5). The only animal model presently available for the human INH-acetylator polymorphism is the rabbit (6, 7). A rodent model of this human trait would facilitate the study of inherited susceptibility to drugs and toxicants that are metabolized by N-acetylation.

Measurements of individual variation in ability to N-acetylate arylamine drugs and toxic chemicals were carried out in populations of rats, mice, and deer mice. Our criteria for selection of species included availability, ease of genetic manipulation, and potential for variation. All three species are readily available and suitable for genetic manipulation on a large scale. The inbred mouse strains were chosen to maximize genetic unrelatedness on the basis of diverse originating stock (8). Rats were selected primarily on the basis of availability. Deer mice were selected because they possessed the greatest potential for individual vari-

ation, inasmuch as available colonies have not been extensively inbred; two genetically related populations were available to us.

PABA and SMZ were the principal substrates employed; they show, respectively, unimodal and bimodal distributions of acetylation capacity in populations of rabbits and humans. Rats and deer mice were also tested for their ability to acetylate α-naphthylamine, a paradigm of carcinogenic arylamines, because its metabolism has recently been found to parallel that of SMZ in rabbits and humans (9).

Materials and Methods

Substrates and Reagents. Sulfamethazine (free base) was obtained from Lederle Labs., Pearl River, N. Y.; p-aminobenzoic acid (potassium salt) and dithiothreitol from Sigma Chemical Co., St. Louis, Mo.; aniline from Fisher Scientific Co., Fairlawn, N. J., α-naphthylamine hydrochloride from Eastman Organic Chemicals, Rochester, N. Y.; acetylsulfamethazine from ICN Pharmaceuticals, Cleveland, Ohio; acetyl-coenzyme A from P-L Biochemicals, Milwaukee, Wis.; tritiated acetyl coenzyme A, Aquafluor, and Omnifluor from New England Nuclear, Boston, Mass. N-1-Naphthylethylenediamine dihydrochloride for the diazotization procedure was obtained from Matheson Coleman and Bell, Norwood, Ohio.

Animals. Mice (Mus musculus) were obtained from Jackson Labs., Bar Harbor, Me. (A/J, C57BL/6J, DBA/1J, and Balb/c), from Spartan Farms Jackson, Mich. (Swiss-Webster) and from the colonies of Dr. Sara Walker, University of Michigan, Ann Arbor (NZB, NZB/W, NZW, and Palmerston-North). Deer mice (Peromyscus maniculatus) were obtained from Jackson Labs. and from the colonies of Dr. Morris Foster, The University of Michigan, Ann Arbor. Rats (Rattus norvegicus) were obtained from Rockland Farms, Gilbertsville, Pa. (Sprague-Dawley, Sherman, and Wistar) and from Blue Spruce Farms, Altamount, N. Y. (Long-Evans). All animals were housed in a fluorescent lighted room on a 12-hr light-dark cycle for at least 7 days prior to experimental use. Rations consisted of sulfonamide-free Purina rat or mouse chow and tap water ad lib.

Blood Hemolysate. Blood was collected from the orbital sinus or upon decapitation into heparinized beakers. Whole blood (50 μl) was pipetted into 500 μl of distilled water and allowed to hemolyze for 5 min. Buffer (1 ml; 10^{-4} M EDTA and 10^{-4} M dithiothreitol in 0.1 M phosphate, pH 6.8) was added and the hemolysate kept on ice until used.

[1] Present address: Department 6104, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, N. Y. 10021. This work was performed in partial fulfillment of the Ph.D. degree in Pharmacology at The University of Michigan.

[2] Abbreviations: PABA, p-aminobenzoic acid; SMZ, sulfamethazine; ANI, aniline; α-N, α-naphthylamine; INH, isoniazid; NED, N,N-dimethyl-1-naphthylamine; NAT, N-acetyltransferase; AcSMZ, N-acetylsulfamethazine; AF, aminofluorene; TCA, trichloroacetic acid.

Send reprint requests to: Dr. Wendell W. Weber, Department of Pharmacology, The University of Michigan Medical School, Ann Arbor, Mich. 48109.

Liver Homogenate. Livers were removed from decapitated animals, minced, and homogenized with four volumes of buffer (see above) in a ground-glass homogenizer (20 strokes). The homogenate was centrifuged at 30,000 g for 30 min and the supernatant fraction was used in the assay procedure.

Colorimetric Assay for PABA-NAT. Blood assays contained 20 μl of acetyl-coenzyme A (10 mM in water), 20 μl of PABA (0.2 mM in water), and 75 μl of suitably diluted hemolysate; reference samples contained 20 μl of water instead of acetyl-coenzyme A. Reactions were started by the addition of enzyme and conducted at approximately 22°C. The reactions were terminated by the addition of 50 μl of 10% TCA (in water) and free PABA determined by the Bratton-Marshall diazotization procedure (10) as modified by Weber et al. (11).

Radioassay for SMZ, Aniline, and α-Naphthylamine NAT.[3] Standard reaction mixtures contained 250 μl of each of the following: buffer (see above), substrate (1 mM in 0.1 M phosphate buffer, pH 6.8), enzyme and ^{3}H-acetyl-coenzyme A (10 mM in water with a specific activity of 0.5 mCi/mmol). Blank values were obtained by substituting buffer for the substrate solution. Reactions were started by the addition of ^{3}H-acetyl-coenzyme A and conducted at 37°C. At 30-sec intervals 200-μl aliquots were removed and vigorously mixed with 1 ml of ethylene dichloride to terminate the reaction and extract the acetylated product. An aliquot (500 μl) of the ethylene dichloride layer was placed in a 4-ml glass vial and evaporated to dryness. Omnifluor (3 ml) was added and the samples were counted in a Packard Tricarb scintillation counter (model 3356). Stock solution of ^{3}H-acetyl-coenzyme A (50 μl containing 500 nmol) was added to 3 ml of Aquaflor and counted with each experiment as a standard; 1 cpm corresponds to 6.25 pmol of acetylated product. Activities were obtained from the linear portion of a plot of radioactivity extracted vs. time and then corrected for counting efficiency (28%) and efficiency of extraction (SMZ, 62%; ANI, 87%; α-N, 92%; AF, 98%; see reference 9).

Enzyme Activity. Enzyme activity is expressed as nmol of substrate acetylated per min per mg of protein unless otherwise stated.

Urinary Excretion of AcSMZ. Animals were injected with 20 mg of SMZ per kg ip in a total volume of 1 ml of physiologic saline solution and confined to 15-cm diameter discs of Whatman no. 3 filter paper. Urine samples produced over the next 3 hr were extracted from the paper, first by adding one volume of 10% TCA in water followed by gentle shaking for 5 min, by three volumes of distilled water and by shaking again for 15 min. The percent of acetylated SMZ was then determined by the method of Weber and Brenner (12).

The presence of SMZ and AcSMZ was confirmed by thin-layer chromatography. Undiluted urine (50 μl) was placed on a 2-cm line at the origin in five 10-μl portions. The TLC plate was glass coated with 0.25-mm thick silica gel containing fluorescent indicator (Merck & Co.; other brands and materials were not equivalent in this separation) and the solvent system consisted of chloroform/acetone (1:1,v/v). Two major spots at R_F 0.65 and 0.77 cochromatographed with AcSMZ and SMZ, respectively. Spots were visualized under UV light, scraped off individually, extracted in a manner identical to the filter paper technique, and diazotized. The spot corresponding to SMZ produced color whereas the material in the spot at R_F 0.67 had to be boiled for 30 min in the presence of HCl before diazotization produced a colored product.

Results

Variation in the capacity to N-acetylate various aromatic amine substrates within and between species is summarized in table 1.

Rattus norvegicus. Rat liver homogenates contained both PABA- and SMZ-NAT activity, whereas blood hemolysates contained only PABA-NAT activity. Blood and liver PABA-NAT activity of all strains correlated very well ($N = 22$; $r = 0.70$; $p < 0.001$). Long-Evans rats were at the upper end of the range and Sprague-Dawley, the lower (fig. 1); there was little overlap between these groups. Liver SMZ-NAT activity did not correlate with either liver PABA-NAT or blood PABA-NAT activity.

Liver homogenates from Sprague-Dawley rats were tested for

the ability to acetylate ANI, α-N, and AF (table 2). All of these substrates were acetylated at a much higher rate than either SMZ or PABA when all were assayed at equimolar concentration of acetyl-acceptor substrate.

Mus musculus. N-Acetylating capacity of nine inbred strains of the common laboratory mouse were studied. Swiss-Webster, C57BL/6J, Balb/c, and DBA/1J were selected for maximum genetic unrelatedness (8) and NZB, NZW, NZB/W F1 hybrid, and Palmerston-North were selected because they are potential models of systemic lupus erythematosus or other autoimmune phenomena. A/J mice were chosen for a combination of these two reasons. The autoimmune-susceptible strains other than A/J were available in limited quantities and are included in table 1 for the purposes of qualitative comparison.

Liver PABA-NAT activity was very high in all strains but interstrain variation was slight. Blood hemolysates had undetectable[4] SMZ-NAT activity but possessed considerable PABA-NAT activity. The A/J strain was unique in having no detectable blood PABA-NAT activity.

Presumptive evidence was obtained in four strains of mice for the presence in urine of AcSMZ by using the method of Weber and Brenner (12) and confirmed in the urine of A/J and C57BL/6J mice by thin-layer chromatography. Urinary excretion of AcSMZ was > 20% in all strains except A/J, which excreted about 10% AcSMZ. The presence of urinary AcSMZ strongly indicates the presence of SMZ-NAT activity, even though no such activity was found under our standard assay conditions. By increasing the specific activity of the ^{3}H-acetyl-coenzyme A (less unlabeled coenzyme), some NAT activity could be detected vs. sulfamethazine (about 0.06 unit in each of three A/J mice). When the apparently more avid substrate, ANI, was used, a value of 0.9 unit could be obtained. Thus mice do have NAT activity in liver towards substrates other than PABA, but it is much lower than in other species.

Peromyscus maniculatus. Deer mice were drawn from two populations. One was maintained outbred at The University of Michigan by Dr. Morris Foster and designated as Foster mice by us. The other was derived originally from Foster stock and partially inbred for 5 years at Jackson Laboratories; the latter strain we designate Jackson mice.

Individual variation in NAT activity in liver and blood is depicted in fig. 2 and urinary AcSMZ in fig. 3. It is clear that the Foster mice occupy the upper end of the distributions and that they overlap the Jackson mice. Nevertheless, the difference between these populations is statistically significant.

A subgroup of the Jackson mice was also tested for NAT activity towards ANI and α-N (table 3). The results show that these compounds are acetylated at a much higher rate than either PABA or SMZ as was the case in rats. Correlation plots comparing NAT activity in pairs of tissue sources from each individual mouse were constructed for each of the substrates in table 3. The substrates may be arranged into three categories based upon the results of these correlations (fig. 4). Two noncorrelating extremes were represented by PABA-NAT activity of blood and liver on the one hand and ANI-NAT and α-N-NAT activity on the other. A third group is a universally correlating group represented by liver SMZ-NAT activity.

---

[3] Procedure modified from Glowinski et al. (9).

TABLE I

Comparison of NAT activity within species

Data represent means ± SD. *ND*, not detected (below sensitivity of assay).

| Species	Strain	N	NAT Activity			Urinary AcSMZ	
			Liver		Blood	N	Percent
			SMZ	PABA	PABA		
			nmol/min/mg protein				
Rattus	Sprague-Dawley	8	0.98 ± 0.67	0.80 ± 0.40	0.035 ± 0.019		
norvegicus	Sherman	4	0.40 ± 0.16	1.05 ± 0.22	0.048 ± 0.016		
	Wistar	4	2.01 ± 1.38	1.32 ± 0.10	0.056 ± 0.023		
	Long-Evans	6	0.56 ± 0.16	1.56 ± 0.18	0.064 ± 0.014		
Mus musculus	Swiss-Webster	6	ND	16.4 ± 4.76	1.45 ± 0.32	10	21.4 ± 2.5
	DBA/1J	4	ND	8.15 ± 0.32	1.39 ± 0.30	6	25.2 ± 3.7
	Balb/c	4	ND	7.29 ± 1.28	2.11 ± 0.86		
	C57BL/6J	4	ND	13.0 ± 3.29	1.11 ± 0.13	17	28.8 ± 7.0
	A/J	7	ND	10.6 ± 1.71	0.0	15	9.9 ± 5.4
	NZB	2	ND	12.3	1.17		
	NZW	2	ND	11.0	0.94		
	NZB/W F1	2	ND	7.61	0.88		
	Palmerston-North	2	ND	8.89	0.96		
Peromyscus	Jackson	80	1.51 ± 0.71	3.93 ± 1.91	0.14 ± 0.08	56	23.7 ± 6.7
maniculatus	Foster	20	5.36 ± 2.64	5.79 ± 1.98	0.27 ± 0.12	51	28.1 ± 7.2
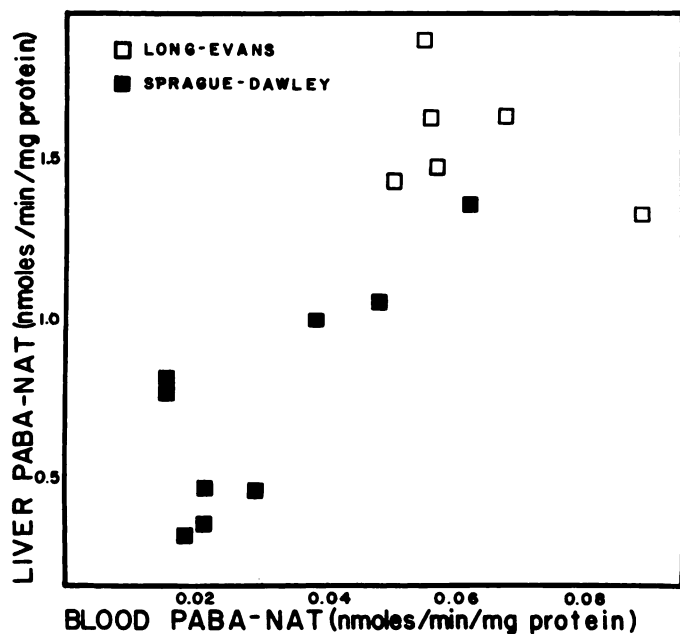
FIG. 1. *Correlation of PABA-NAT activity in liver and blood of rats.*

Liver and blood PABA-NAT activity were found to correlate ($N = 15$, $r = 0.61, p < 0.02$).

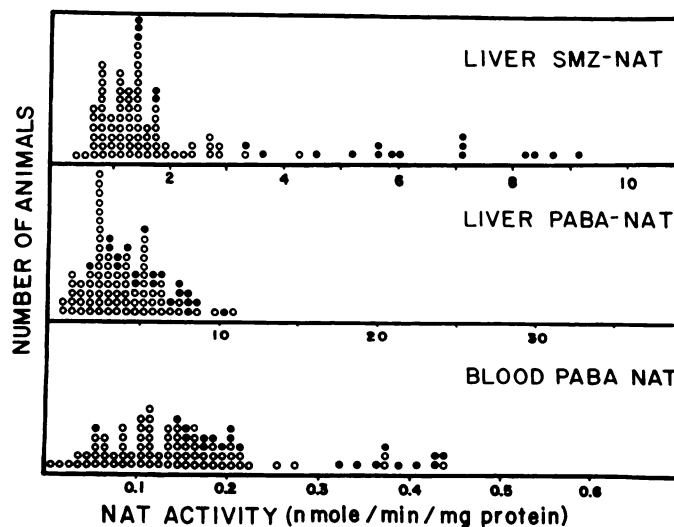## TABLE 2

NAT activity in Sprague-Dawley rat liver

Substrate	Rat 1	Rat 2	Rat 3	Rat 4
	nmol/min/mg protein			
Sulfamethazine	1.00	1.10	1.69	2.35
Aniline	42.5	37.7	25.8	60.7
α-Naphthylamine	75.0	61.5	111.	79.8
Aminofluorene	40.0	50.2	71.8	52.5
p-Aminobenzoic acid	0.46	0.45	0.76	0.80
p-Aminobenzoic acid (blood)	0.02	0.03	0.02	0.02

FIG. 2. *NAT activity in deer mice from two genetically related populations.*

The population of Jackson mice (O) was derived from Foster mice (●) 5 years previously and inbred since then. Foster mice were found to possess greater activity in all measures of NAT: liver SMZ-NAT, $\chi^2 = 53$, $p < 0.005$; liver PABA-NAT, $\chi^2 = 10.7, p < 0.005$; blood PABA-NAT, $\chi^2 = 21.4, p < 0.005$. Males possessed greater activity than females in liver SMZ-NAT measurements ($\chi^2 = 11.0, p < 0.005$), but no sex difference could be seen in the other measures of NAT activity. Each circle represents one animal; each animal is represented in the three distributions.

Discussion

Previous studies of the N-acetylation of arylamines have dealt with the capacity of a species to perform this reaction, but comparatively little attention has been paid to individual or genetic variability of this metabolic pathway within a species (13) other than man or rabbit (1, 2, 6, 7). One of our primary goals in surveying several species and strains of rodents was to uncover intraspecies variation in the capacity to N-acetylate arylamines which could be developed into new genetic models of the human
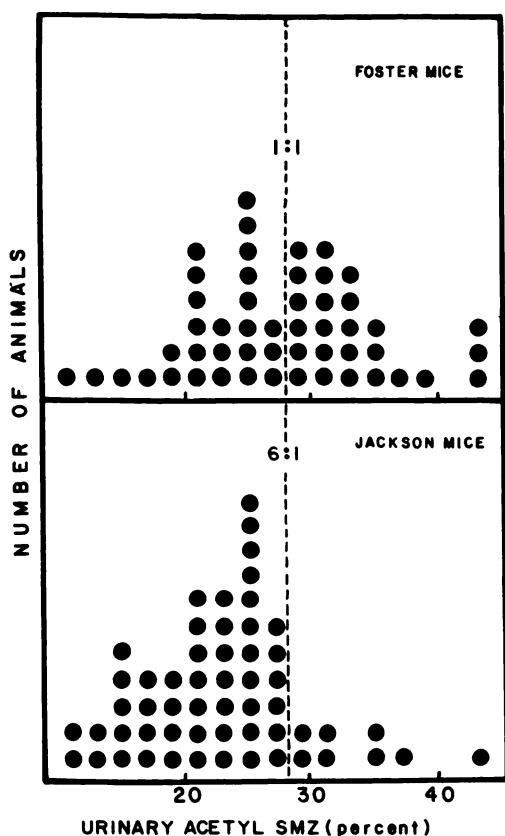
FIG. 3. *Urinary excretion of AcSMZ in two populations of deer mice.*

Jackson mice, derived from Foster mice by inbreeding, excreted less AcSMZ in urine after ip administration of SMZ (20 mg/kg) than did Foster mice ($p < 0.002$ by Student's t-test). Each circle represents one animal. Ratios emphasize the shift in population mean (Foster, 28.1 ± 7%; Jackson, 23.7 ± 7%).

TABLE 3

NAT activity in Jackson Colony deer mouse liver

Data represent means ± SD.

Substrate	N	NAT Activity
		nmol/min/mg protein
Sulfamethazine	16	1.37 ± 0.71
Aniline	16	80.8 ± 33.6
α-Naphthylamine	15	368 ± 140
p-Aminobenzoic acid	16	3.8 ± 1.5
p-Aminobenzoic acid (blood)	16	0.13 ± 0.10

INH-acetylator polymorphism. These models could provide new opportunities to investigate the hereditary predisposition of individuals to toxic effects of arylamine drugs and carcinogens of this chemical class which cannot be conveniently carried out in man or rabbit. The extent to which genetically variant NAT's may account for differences in the disposition of these substances was another relevant consideration for making these observations.

The characteristics of a population distribution of N-acetylating capacity in mammals depends upon the substrate employed and the choice of *in vitro* or *in vivo* measurements (see table 4).

The occurrence of bimodality in the urinary AcSMZ distribution reflects the existence of distinct differences in the N-acetylating capacity of individuals within each of the species studied. Liver SMZ-NAT activity is widely variable and distinctly bimodal
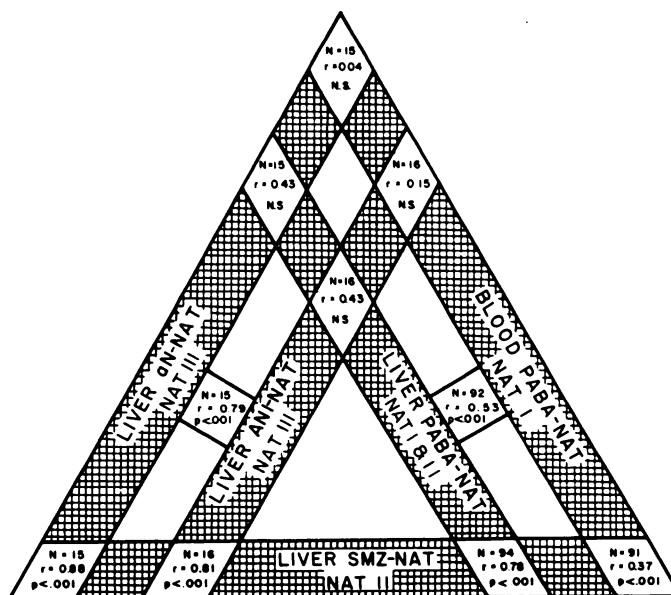

FIG. 4. *Presumptive evidence for multiple forms of NAT in deer mice.*

Each leg of the triangle represents an NAT activity measured in a single tissue with the indicated substrate. Intersections of legs indicate the presence or absence of a correlation between the two activities.

in man, rabbit, and deer mouse. The capacity for urinary AcSMZ excretion is correlated with the level of liver SMZ-NAT activity in both man and rabbit (6, 14). Genetically distinct subpopulations termed rapid and slow SMZ or INH acetylators have been identified by these criteria in both species (19). It seems reasonable to suppose from the information collected in the deer mice with use of SMZ that similar subpopulations may also exist in this species as well. Overlapping strain differences in the rat and barely detectable levels of activity in the mouse preclude a definite statement about the existence of different subgroups with respect to liver SMZ-NAT activity within these two species at the present time. Individual variation in SMZ-NAT activity is not reflected in blood NAT activity; indeed, the blood NAT from these mammalian species does not seem to be able to metabolize SMZ to any appreciable extent.[4]

The level of NAT activity as measured by PABA is quite different from that obtained using SMZ. Liver PABA-NAT activity is unimodally distributed in all five species. Distributions of blood PABA-NAT activity suggest the presence of two acetylator phenotypes in each species except for man. The distribution of blood PABA-NAT activity in several strains of the laboratory mouse is especially noteworthy, because the A/J strain stands alone in having no blood PABA-NAT activity (table 4). In the rat and deer mouse, distributions are continuously variable with tendencies toward bimodality (figs. 1 and 2). The occurrence of genetically controlled differences in blood PABA-NAT activity has been demonstrated in rapid and slow INH-acetylator rabbits (18). Man is the only species tabulated which shows a unimodal distribution of blood PABA-NAT activity, although the distributions in several nonhuman primates, including the baboon, chimpanzee, and monkey, also appear to be unimodal.[5]

It has been previously shown in rabbits that not only does relative NAT activity vary from individual to individual but it also varies from tissue to tissue within the same individual (20). This relationship can be expressed by constructing scatter dia-

[5] G. S. Drummond and W. W. Weber, unpublished observation.

grams in which two different measures of NAT activity in the same animal provide the x and y values. For example, one might compare two substrates in the same tissue, the same substrate in two different tissues, or the metabolite pattern of some tissue with that of urine. Correlation coefficients computed from these plots then give an indication of the degree of relatedness of the two measures of NAT activity. When no significant correlation is found it suggests that the NAT activities may belong to two or more functionally distinct entities. Correlation of two measures of NAT activity suggests that either identical or closely related enzymes account for both or that two different enzymatic activities are genetically linked.

Results of scatter plots treated in this way are summarized in fig. 5 and provide several pieces of evidence for multiple forms of NAT in each of the rodent species studied, as well as for man and rabbit: (1) The most striking evidence for the existence of multiple forms of NAT is the observation that liver homogenates can generally N-acetylate a multiplicity of arylamine substrates,

whereas blood hemolysates are active primarily, if not solely, towards PABA; this is true for all species studied so far. (2) In rat populations blood PABA-NAT activity significantly correlates with liver PABA-NAT activity but not with liver SMZ-NAT activity. This suggests that rat liver contains at least two forms of NAT, one of which is closely allied to the enzyme found in blood. (3) In mouse populations we find that NAT activity is largely directed towards PABA as substrate in both blood and liver. The fact that A/J mice do not show NAT activity in blood suggests that this enzyme, if not totally distinct from liver NAT, is at least subject to differential tissue expression. (4) In deer mice we find a more complex set of interrelationships among the several measures of NAT activity. As in rat populations, liver and blood NAT activities correlate when measured by PABA acetylation; unlike the rat, however, deer mice also show a correlation when the blood enzyme is measured with PABA and liver enzyme is measured with SMZ. In a small sample of these animals we found correlations among NAT activities with SMZ, ANI, and α-N in liver (fig.

TABLE 4

Comparative population distribution of N-acetylating capacity in mammalian species: substrate and tissue dependency

Species	Urinary AcSMZ	Tissue NAT Activity			
		Liver		Blood	
		SMZ	PABA	SMZ	PABA
Man	Bimodal (14)	Bimodal (15, 16)	Unimodal (15, 16)	No activity[a]	Unimodal (17)
Rabbit	Bimodal (6)	Bimodal(7)	Unimodal (9)	No activity[a]	Bimodal (18)
Rat	Not determined	Unknown; completely overlapping strain differences[b]	Unimodal; partly overlapping strain differences[b]	No activity[b]	Bimodal; slightly overlapping strain differences[b]
Mouse	Bimodal[b]	Unknown; activity too low to assess[b]	Unimodal[b]	No activity[b]	Bimodal[b]
Deer mouse	Bimodal, much overlap[b]	Bimodal[b]	Unimodal with skewness, possibly bimodal[b]	No activity[b]	Unimodal with a tendency towards bimodality[b]

[a] D. J. Hearse and W. W. Weber, unpublished observation.
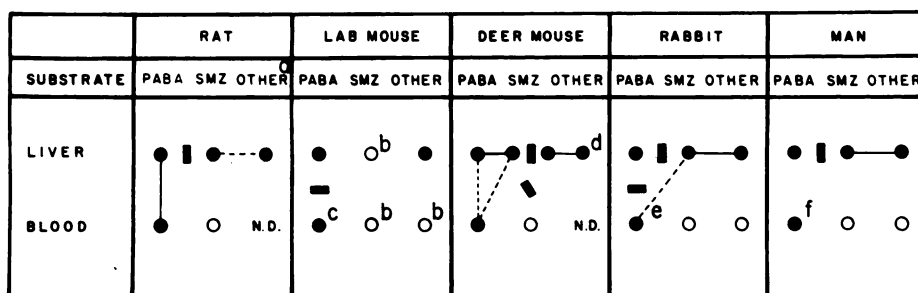[b] Data presented in this paper.

FIG. 5. NAT multiplicity as indicated by substrate specificity.

Solid circles indicate the presence of measurable NAT activity. Significant correlations are indicated by a solid line between circles and tentative correlations by a broken line. A bar between circles indicates a lack of correlation between activities. Open circles indicate undetectable NAT activity under normal assay conditions (see Materials and Methods section for exceptions). ND indicates activity not determined.

[a] ANI, α-N, and/or AF.
[b] NAT activity detectable under special assay conditions (see Results section).
[c] A/J mice have no detectable activity under normal assay conditions.
[d] NAT activity with ANI and α-N only.
[e] Activities of blood PABA-NAT and liver SMZ-NAT are reciprocally related (18).
[f] Data are insufficient to determine whether correlations between blood PABA-NAT and liver NAT activities exist with any of the substrates studied.

4). We also found, however, that PABA-NAT activity did not correlate with ANI or α-N-NAT activity in liver. The simplest hypothesis which explains all of these observations in deer mice entails three NAT's. One of these NAT's is specific for PABA and is located in blood and liver. Blood hemolysates acetylate only PABA and none of the other substrates to any appreciable extent. An NAT with similar specificity to that in blood was also found in liver homogenates of Jackson mice and corresponds to the horizontal line in fig 6. We have called this PABA-specific NAT, NAT I. It accounts for the positive correlation ($r = 0.53$) between blood and liver NAT activity with PABA (fig. 4). In fig. 6 there is another component of PABA-acetylating activity seen primarily in Foster mice which shows a correlation with SMZ acetylation in liver. This activity is attributed to another enzyme which we call NAT II. NAT II is responsible for the correlation ($r = 0.78$) observed between PABA and SMZ acetylation by liver homogenates (fig. 4). Liver homogenates can also acetylate α-N and ANI. These measures of NAT activity correlate with each other ($r = 0.79$) and with SMZ ($r = 0.88$ and 0.81, respectively) but not with PABA acetylation in liver ($r = -0.43$) or blood ($r = 0.04$ and 0.15, respectively) (fig. 4). These findings indicate that the liver must contain another enzyme with specificity for α-N and ANI, as well as for SMZ, but not for PABA. We have called this enzyme NAT III. There is also a relatively weak correlation ($r = 0.37$) between PABA acetylation in blood and SMZ acetylation in liver which may be a result of genetic linkage between NAT I and NAT II. The presence or absence of correlations between these various NAT measures may reflect differences in the presence of endogenous inhibitors, differences in the lability of proteins, or differences in the extrahepatic handling of the test substrates and metabolites; definitive proof for multiple forms of NAT awaits the isolation and purification of these enzymes.

It is quite clear from studies in rabbit that extrahepatic NAT can play an important role in the disposition of drugs that are N-acetylated (20). The present study confirms this possibility in another species, the laboratory mouse. Thus, we found liver SMZ-NAT activity to be very low in laboratory mice, yet significant amounts of AcSMZ appeared in the urine after parenteral admin-

istration. Additionally, the amount of AcSMZ in the urine of deer mice was approximately the same as that found in laboratory mice, in spite of the considerably higher SMZ-NAT activity in the livers of deer mice. To resolve this question it would be necessary to conduct a complete tissue distribution of NAT activity in both species. Attention might also be paid to the presence or absence of an N-deacetylase as the cause of the species difference.

Two potentially interesting animal models of the human INH acetylator polymorphism have been identified by this survey of N-acetylation capacity in three rodent species. Deer mice demonstrate considerable variation in the capacity to acetylate arylamines, including the carcinogenic arylamines. Consequently, this species may, with further development, serve as a useful tool for identification of hereditary factors affecting the capacity of humans to acetylate these substances and to explore the possible association between acetylator phenotype and bladder cancer in workers exposed to carcinogenic arylamines (5). The acetylation polymorphism found in inbred strains of the laboratory mouse has been particularly relevant to the study of the association of spontaneous and drug-induced forms of lupus erythematosus with phenotypically slow INH-acetylator humans (21), inasmuch as the A/J mouse is more susceptible to development of spontaneous and drug-induced antinuclear antibodies (22) in addition to being a slow-acetylator phenotype.

References

1. J. P. Biehl, *Trans. Conf. Chemother. Tuberc.* **16**, 108 (1957).
2. R. A. Knight, J. M. Selin, and H. W. Harris, *Trans. Conf. Chemother. Tuberc.* **18**, 52 (1959).
3. D. E. Drayer and M. M. Reidenberg, *Clin. Pharmacol. Ther.* **22**, 251 (1977).
4. D. Alarçon-Segovia, *Mayo Clin. Proc.* **44**, 644 (1969).
5. G. M. Lower, T. Nilsson, and G. T. Bryan, *Proc. Am. Assoc. Cancer Res.* **17**, 203 (1973).
6. J. W. Frymoyer and R. F. Jacox, *J. Lab. Clin. Med.* **62**, 891 (1963).
7. J. W. Frymoyer and R. F. Jacox, *J. Lab. Clin. Med.* **62**, 905 (1963).
8. J. Staats, in "Biology of the Laboratory Mouse," 2nd ed. (E. L. Green, ed.), p. 2. McGraw-Hill, New York, 1966.
9. I. B. Glowinski, H. E. Radtke, and W. W. Weber, *Mol. Pharmacol.* **14**, 940 (1978).
10. A. C. Bratton and E. K. Marshall, Jr., *J. Biol. Chem.* **128**, 537 (1939).
11. W. W. Weber, J. M. Miceli, D. J. Hearse, and G. S. Drummond. *Drug Metab. Dispos.* **4**, 94 (1976).
12. W. W. Weber and W. Brenner, *Am. J. Human Genet.* **26**, 467 (1974).
13. R. T. Williams, *Fed. Proc.* **26**, 1029 (1967).
14. D. A. P. Evans, *J. Med. Genet.* **6**, 405 (1969).
15. D. A. P. Evans, *Ann. N. Y. Acad. Sci.* **123**, 178 (1965).
16. J. Jenne, *J. Clin Invest.* **44**, 1992 (1965).
17. W. W. Weber, H. E. Radtke, and R. H. Tannen, in "Extrahepatic Metabolism of Drugs and Other Foreign Compounds." (T. E. Gram, ed.). Spectrum Publications, Jamaica, N. Y., in press.
18. R. R. Szabadi, C. McQueen, G. S. Drummond, and W. W. Weber, *Drug Metab. Dispos.* **6**, 16 (1978).
19. W. W. Weber, in "Metabolic Conjugation and Metabolic Hydrolysis," vol. 3 (W. H. Fishman, ed.), pp. 249–296. Academic Press, New York, 1973.
20. D. J. Hearse and W. W. Weber, *Biochem. J.* **132**, 519 (1973).
21. R. H. Tannen, Doctoral Dissertation, University of Michigan, Ann Arbor, 1978.
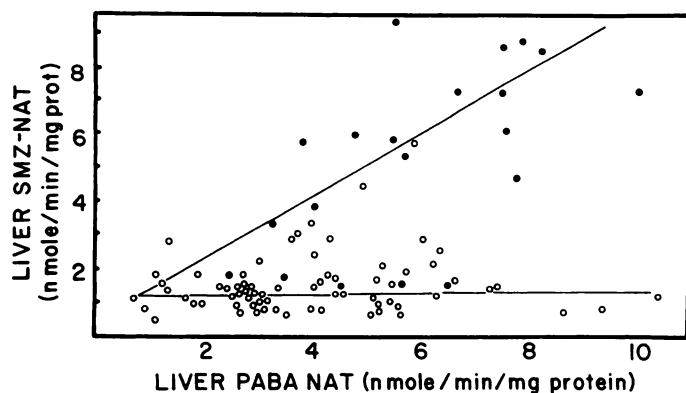22. J. H. TenVeen and T. E. W. Feltkamp, *Clin. Exp. Immunol.* **11**, 265 (1972).

FIG. 6. *Multiple forms of NAT in deer mice.*

O, Jackson mice; ●, Foster mice. Plotting liver PABA-NAT activity for each mouse against liver SMZ-NAT activity reveals the presence of two NAT activities: one enzyme which can metabolize both PABA and SMZ fairly well (NAT II) and one which metabolizes primarily PABA (NAT I). Lines have been drawn to emphasize these correlations.