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Genetic Variation in N-Acetylation of Carcinogenic Arylamines by Human and Rabbit Liver

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SUMMARY

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The arylamine carcinogens aminofluorene, α-naphthylamine, β-naphthylamine, benzidine and methylene bis-2-chloroaniline are acetylated by the same polymorphic N-acetyltransferase (EC 2.3.1.5) as isoniazid and sulfamethazine in human and rabbit populations. Apparent K_m values for these carcinogens determined with human and rabbit N-acetyltransferases obtained from rapid and slow isoniazid acetylator individuals are approximately one order of magnitude smaller than for the polymorphic drug substrate, sulfamethazine. The apparent K_m values for all the carcinogens and sulfamethazine were also strongly correlated with their octanol-water partition coefficients indicating that their kinetic properties are highly dependent upon their hydrophobic nature. Since differences in susceptibility to toxicity from isoniazid, hydralazine and procainamide are associated with different acetylator phenotypes, the possibility is raised that rapid and slow isoniazid acetylator populations may differ in susceptibility to chemical carcinogenicity from exposure to arylamines.

INTRODUCTION

Biotransformation of arylamines to carcinogens is a complex process (Fig. 1) involving at least two enzymatically mediated steps including N-hydroxylation and esterification (1, 2). Certain N-hydroxy derivatives of arylamines are more carcinogenic than the parent compounds at usual sites of tumor formation in experimental animals and are also carcinogenic at sites where the parent compounds are not (3). The guinea pig, relatively deficient in the ability to N-hydroxylate arylacetamides, is also refractory to carcinogenesis induced via this pathway (3). In other experimental animals (4, 5) and man (6), N-hydroxylation has been regarded as the rate-determining step for activation. Recently, Thorgeirsson *et al.* (7) have shown that the induction of aryl hydrocarbon hydroxylase activity, the formation of new cytochrome P-450 and the nitrogen oxidation of 2-AAF[2] by aromatic

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[2] Abbreviations used: PABA, p-aminobenzoic acid; INH, isoniazid; NAT, N-acetyltransferase; SMZ, sulfamethazine; Ac, acetyl; PAS, p-aminosalicyclic acid; MOCA, methylene bis-2-chloroaniline; AF, aminofluorene; 2-AAF, 2-acetyl aminofluorene; N-OH-AF, N-hydroxyaminofluorene; N-OH-2-AAF, N-hydroxy-2-acetyl aminofluorene.

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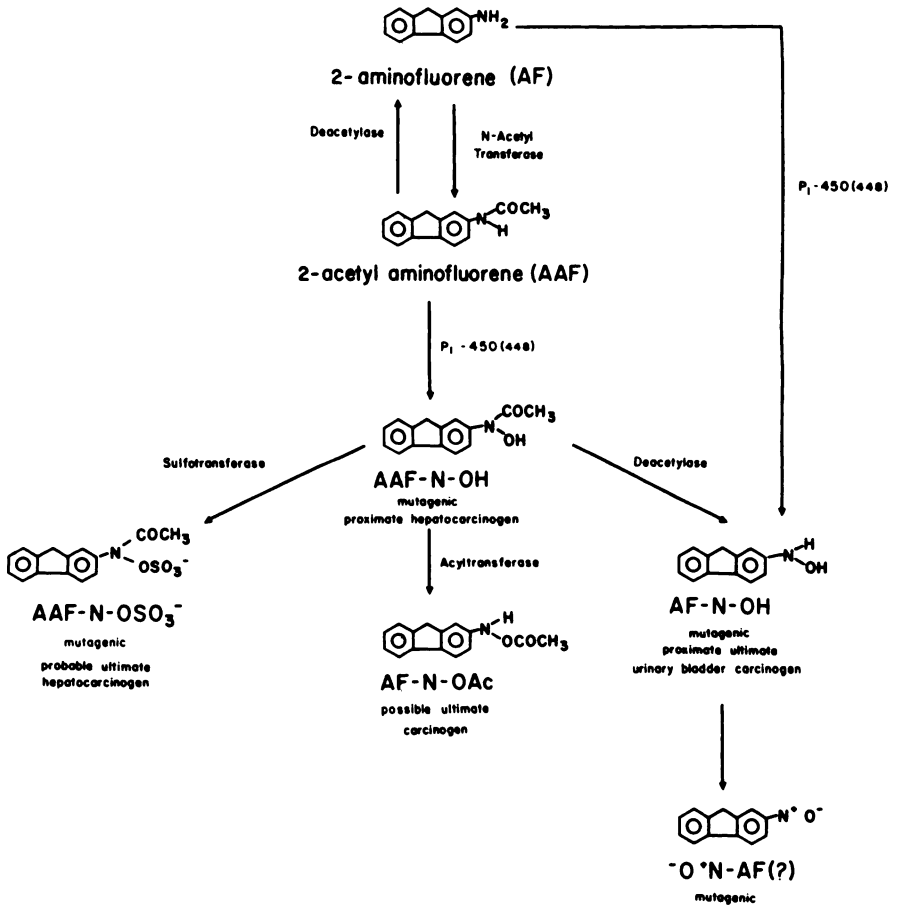

FIG. 1. *Metabolic activation of AF and AAF*

hydrocarbon treatment are under genetic control in the mouse and display Mendelian inheritance apparently involving the same or closely linked genes. The rate-limiting step proposed for the activation of 2-AAF is hydroxylation by cytochrome P-450 (8). Observations of McCann *et al.* (9) obtained with the *Salmonella* mutagenicity test provide further evidence that both N-OH-AF and N-OH-2-AAF are mutagenic even without further metabolism. Conjugation of the N-hydroxyl function with acetate (10), sulfate (11), or glucuronic acid (2) can then lead to the formation of reactive N-esters which are capable of interacting covalently with DNA, RNA and protein. Bartsch *et al.* (12) present evidence that the activation of N-OH-2-AAF results from N-O transfer of the acetyl group of arylhydroxamic acids yielding reactive N-acetoxy arenes that can react with tissue nucleophiles. Studies in rat by King support this hypothesis and also show that an acyltransferase may be responsible for this intramolecular reaction (13). It is suggested that diminution in hepatocarcinogenicity of N-OH-2-AAF when administered concurrently with acetanilide or p-hydroxyacetanilide results from the inhibitory action of these substances on the acyltransferase *in vivo* (14). In another esterification reaction involving sulfate conjugation, the activity of N-OH-2-AAF sulfotransferase *in vivo* correlates with the susceptibility in the rat to hepatocarcinogenesis (15). Therefore metabolic activation of arylamines appears to involve N-hydroxylation followed by esterification.

N-acetylation, another step in the biotransformation of arylamines, may also be capable of modulating susceptibility to car-

cinogenesis from these substances (5, 16). Dogs develop tumors of both liver and bladder upon administration of acetylated arylamines but only urinary bladder tumors when the unacetylated parent compound is administered. This is particularly interesting since the dog is incapable of acetylating arylamines (5, 16). Thus the capacity for N-acetylation may regulate both the occurrence of tumors in specific organs and the susceptibility of different tissues to carcinogenesis. It is also of interest that 2-AAF is capable of binding to ribosomal RNA isolated from livers of rats fed 2-AAF suggesting that the ultimate carcinogen in this instance may involve N-acetylated forms of the arylamine (17).

The rate of N-acetylation of INH and several other arylamine drugs including SMZ, sulfapyridine, hydralazine, and procainamide in man and rabbit are subject to a genetic polymorphism which is determined by differences in the amount of NAT activity in liver (18). This may be pertinent to a consideration of enzymatic factors affecting carcinogenesis from arylamines. As a consequence of this genetic polymorphism, individuals in human and rabbit populations are distributed bimodally according to their capacity for N-acetylation as either rapid or slow acetylators of these drugs. Genetic differences in the rate of N-acetylation are associated with individual susceptibility to toxicity from chronic administration of drugs such as INH. Slow INH-acetylators are more likely to develop peripheral neuropathies (19) and a lupus erythematosus-like syndrome with chronic administration of these drugs than are rapid acetylators (20); INH-induced skin reactions also occur preferentially in slow acetylators (21). The acetylator phenotype may also be a determinant of liver damage although further studies are needed to clarify the nature of that relationship more precisely. Mitchell *et al.* (22) have obtained strong evidence that rapid acetylators are more susceptible to chemical hepatitis from INH while other clinical studies show that slow acetylators who have received INH in combination with rifampicin tend to develop hepatocellular damage more readily (23–25). It should be noted, however, that some drugs which contain the arylamine group are nevertheless not polymorphically acetylated in man and rabbit. PABA (26–29) and PAS (30) which are monomorphically acetylated in these species are examples of such drugs.

Since inherited differences in N-acetylation rates are important determinants of chronic drug toxicity it seems reasonable to examine their role in relation to susceptibility to arylamine carcinogenesis. We have investigated this possibility by determining whether AF and certain other proven mutagenic and/or carcinogenic arylamines are acetylated by NAT and to see if the rates of N-acetylation of these substances are subject to the same genetic polymorphism as INH and SMZ.

MATERIALS AND METHODS

Chemicals and reagents. Reagent grade sulfamethazine (free acid) was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio, p-Aminobenzoic acid from Sigma, St. Louis, Missouri, N-(1-naphthyl) ethylene diamine dihydrochloride from Fisher Scientific Co., Springfield, N.J., [Acetyl-^{3}H]-Acetyl Coenzyme A (3 Ci/mmol) and Omnifluor from New England Nuclear, Boston, Massachusetts, [1-^{14}C]-acetyl-CoA (58.5 mCi/mmole) from Amersham Searle, Arlington Heights, Illinois, 2-Aminofluorene and β-Naphthylamine from K and K Labs, Plainview, N. Y., Benzidine from Matheson, Coleman-Bell, Cincinnati, Ohio, α-Naphthylamine HCl and silica gel thin layer chromatography plates from Eastman Kodak, Rochester, N. Y. Methylene bis-2-chloroaniline (MOCA) was a gift of The Anderson Development Co., Adrian, Michigan. All organic solvents used were reagent grade.

Rabbit liver samples. Selected lines of phenotypically rapid and slow INH acetylator New Zealand white rabbits (greater than 2.5 kg) of both sexes maintained in our laboratory were used as the source of rabbit liver (27). Rabbits were caged individually at room temperature (18–23°) on a light-dark schedule with lights on from 6 a.m. to 9 p.m. and fed sulfonamide-free rabbit chow with water *ad lib.*

Human liver samples. Fresh liver biop-

sies were obtained from individuals undergoing exploratory laparotomy and were used for determination of initial N-acetylation rates. Optimum conditions for assay of each arylamine substrate tested were established previously with autopsy liver samples (Table 3).

Autopsy liver specimens were obtained from nine individuals from approximately 2 to 18 hours after death. Examination of liver samples obtained beyond 3 hours usually revealed that PABA acetylation activities were undetectable using the colorimetric assay described below, while those for SMZ acetylation ranged from appreciable levels 9 hours after death to barely detectable levels at 18 hours. Those specimens obtained within 2.5 hours after death were used for determinations of Michaelis-Menten constants because the levels of PABA and SMZ acetylating capacity were still within 30% of that observed in fresh biopsy samples. Autopsy liver was kept on ice for about 20 minutes prior to enzyme isolation.

Fresh liver biopsy samples of up to 0.8 g wet weight were obtained from 9 individuals (5 females, 4 males). Gross visual appearance of all biopsies was normal. Histological examination of all biopsies was also normal except for that of #6 (Table 2) which revealed 2 or 3 non-caseating granulomas per low power field; the amount of liver parenchyma replaced by the granulomas was insignificant. Each piece of liver was kept on ice for about 20 minutes prior to homogenization as described below.

Enzyme Preparation

Rabbit liver NAT purification. Animals were killed by injection of approximately 20 ml air into a marginal ear vein and the liver was removed and immediately homogenized (20%, w/v) in a 0.1 M potassium phosphate buffer, pH 7.4 at 4°C. The enzyme was then partially purified according to a method previously published (31) and optimum assay conditions for each arylamine substrate were established (Table 3).

Human liver NAT isolation. Autopsy liver specimens (70–85 g) were homogenized (20%, w/v) in 0.1 M potassium phosphate buffer, pH 7.4 at 4°C, and centrifuged at 10,000 × *g* for 20 min and at 100,000 × *g* for 1 hr.

Liver biopsies (0.5–1 g) were homogenized (11.1%, w/v) in 0.1 M potassium phosphate buffer, pH 7.4 at 4°C by 20 strokes of a motor driven teflon pestle in a plexiglass vessel followed by centrifugation at 10,000 × *g* for 20 min and 100,000 × *g* for 1 hr.

Determination of Acetylating Activity

Radioactive assay. The reaction mixture (0.20 ml) consisted of 0.10 ml of suitably diluted enzyme, 0.05 ml aqueous radioactive AcCoA (2×10^{-3} M) and 0.05 ml amine substrate solution in 1.5 ml capped polypropylene micro sample tubes (Kew Scientific Inc., Columbus, Ohio). [^{3}H]AcCoA was used for the majority of the experiments; specific activity of a typical stock solution was 4.5×10^{-3} μCi/nmole. [^{14}C]AcCoA was used for the remainder of the experiments (specific activity 8×10^{-4} μCi/nmole). The composition of amine substrate solution for each amine tested is given in Table 1. The amine substrate was omitted from controls. Reaction mixtures were preincubated at 37°C for 3 min. The reaction was initiated by the addition of radioactive AcCoA incubated for 15 sec, 30 sec, 1 min, 2 min, 3 min and 5 min, and terminated by vortexing for at least 30 sec with 1.0 ml N-ethyl maleimide (2×10^{-3} M) in ethylene dichloride for all substrates used except β-naphthylamine. For β-naphthylamine 1.0 ml N-ethyl maleimide (2×10^{-3} M) in chloroform: ethylacetate (2:1) was used. Mixtures were shaken for 20 min on a platform rotator to extract acetylated product into the organic phase and then centrifuged in a Brinkmann Eppendorf Centrifuge 3200 for 1 min to effect a separation into two layers. The upper layer (aqueous phase) was removed by aspiration and 500 μl of the organic layer pipetted into 5 ml glass scintillation vials and evaporated to dryness under vacuum. Omnifluor scintillation fluid (3 ml) was added and the radioactivity was counted in a liquid scintillation counter (Hewlett Packard Tricarb Model 3375).

Under the conditions of this assay 5000 dpm corresponded to the acetylation of 1 nmole of substrate using [^{3}H]AcCoA as donor; 1,875 dpm corresponded to the acetylation of 1 nmole of substrate using [^{14}C]-AcCoA, corrected for extraction efficiency of acetylated product. Protein was deter-

TABLE 1

Conditions for measurement of enzymatic N-acetylation of arylamines by radioassay

SMZ was dissolved in a slightly basic aqueous solution. α-Naphthylamine HCl was dissolved in 0.1 M potassium phosphate buffer, pH 7.4. All other substrates listed were dissolved in dimethyl sulfoxide.

The radioactive assay (see METHODS), modified by employing a higher specific activity AcCoA (3 μCi/nmole) and run to completion (approximately 30 minutes), was used to determine the % extraction efficiency of each acetylated product formed. After centrifugation, aliquots of the organic and aqueous phases were spotted on acetate-backed silica gel TLC sheets and chromatographed using ethylene dichloride: methanol (90:10) for 10 cm. Acetylated products appeared to move as single spots with R_f values of 75, 68, 70, 70, 61 and 42 corresponding to Ac-MOCA, Ac-Benzidine, Ac-β-Naphthylamine, Ac-α-Naphthylamine, Ac-AF and Ac-SMZ, respectively. Percent efficiency was calculated from:

$$\frac{\text{N-acetylated product (cpm organic phase)}}{\text{N-acetylated product (cpm organic \& aqueous phase)}} \times 100.$$

Arylamine substrate	Extraction of acetylated product	
	Solvent	% Efficiency
Sulfamethazine	ethylene dichloride	62
α-Naphthylamine HCl	ethylene dichloride	92
β-Naphthylamine	chloroform:ethylacetate (2:1)	95
Benzidine	ethylene dichloride	76
2-Aminofluorene	ethylene dichloride	98
MOCA	ethylene dichloride	95

mined by the method of Warburg and Christian (32).

Colorimetric Assay for Acetylation of PABA. The amount of PABA NAT-activity was determined by the procedure of Hearse and Weber (28). All incubations were carried out at 37°C in 0.4 ml capped polyethylene microtest tubes (Kew Scientific Inc., Columbus, Ohio). Absorbance was measured at 540 nm in 400 μl cuvettes of 1 cm light path against a water blank in a Beckman DU Spectrophotometer. The extent of acetylation was obtained by subtracting the experimental from the control absorbance reading. Under the conditions of this assay a decrease of 164.5 absorbance units at 540 nm corresponds to the acetylation of 1 μmole of PABA. Specific activity is expressed as nmoles acetyl-PABA formed/min/mg protein.

Determination of octanol-water partition coefficients. Substrates were dissolved in octanol (except SMZ and PABA which were dissolved in 0.01 M potassium phosphate buffer pH 7.4) and standard curves were determined. Nine milliliters of aqueous solution (pH 7.4) was then inverted 50 times with 1 ml octanol solution at room temperature and centrifuged at 2000 rpm for 10 min. An aliquot of the organic layer was suitably diluted (except for SMZ and PABA for which an aliquot of the aqueous layer was used) and the concentration in the solution obtained at the UV maximum wavelength for each compound. The concentration of substrate in the unmeasured layer was obtained by difference and the octanol water partition coefficients calculated as the ratio of the concentrations in each layer.

Data treatment. Initial velocities were determined by extrapolating the time-activity curves (Fig. 2, Table 2) to the time of addition of AcCoA and measuring the slope of the tangent to the curve at that point. Apparent K_m values (Table 3) were calculated with the aid of a modified Hyperprogram (33) which uses a least squares linear regression method.

RESULTS AND DISCUSSION

Arylamines selected for investigation include a number of well established human and animal carcinogens (11). Urinary bladder tumors develop in animals after oral administration of AAF (34). Benzidine and α- and β-naphthylamines, components in dye synthesis, also produce urinary bladder tumors in man following chronic industrial exposure (35). MOCA, which is a chlorinated arylamine structurally similar to benzidine that exhibits the general toxicity characteristic of aromatic amines, is a urinary bladder carcinogen in humans and a hepatocarcinogen in experimental animals (36). Steinhoff and Grundman (37) have shown that rats develop liver tumors when raised on a diet containing MOCA, and Mastromatteo (38) has implicated MOCA as the causative agent in urinary bladder neoplasia for two Canadian workers. It has

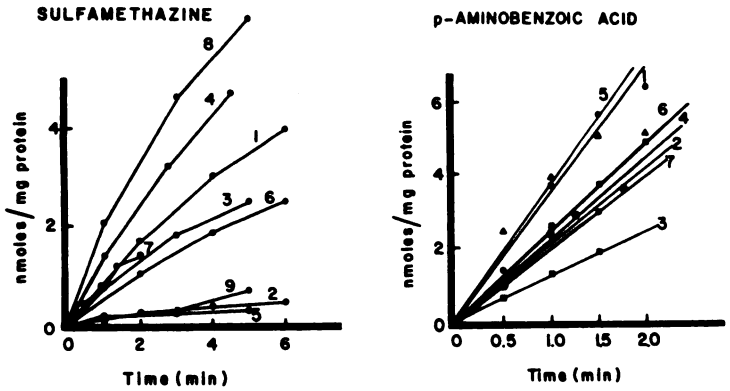

FIG. 2. *Time course of SMZ and PABA acetylation by human liver biopsies*

Partially purified (100,000 × *g*) preparations of human liver from 9 individuals were incubated with SMZ (2 × 10^{-4} M) and ^{3}H-AcCoA (5 × 10^{-4} M) as described in Methods. The first seven liver biopsies were also assayed with PABA (2.22 × 10^{-5} M) and AcCoA (2.22 × 10^{-3} M) using the modified Bratton-Marshall procedure (28).

TABLE 2

Initial rates of formation of N-Ac-SMZ and N-Ac-PABA by human liver biopsies

Samples were homogenized in 8 ml 0.1 M potassium phosphate buffer, pH 7.4, per g liver biopsy. The 100,000 × *g* supernatant fraction was used for assay of NAT activity. The initial AcCoA concentration in the incubation mixture for the SMZ determinations was 5 × 10^{-4} M and for the PABA determinations 2.2 × 10^{-3} M. The initial concentration of SMZ in the assay medium was 2 × 10^{-4} M. All assays were performed at 37°C.

Sample no.	*SMZ* Initial rate (nmoles Ac-SMZ formed/min/mg protein)	*PABA* Initial rate (nmoles Ac-PABA formed/min/mg protein)
2	0.11	2.76
5	0.13	4.80
9	0.13	—
6	0.51	2.50
3	0.70	1.23
7	0.74	2.10
1	0.84	3.69
4	1.37	2.50
8	2.07	—

recently been used extensively in industry as a curing agent in the manufacture of various polymers and resins.

It was necessary to establish the acetylator phenotype of the human liver preparations in order to determine whether these carcinogenic agents were substrates for polymorphic liver NAT. Initial rates of formation of N-Ac-SMZ and N-Ac-PABA by fresh human liver biopsies are shown in Table 2. The time course of formation of N-Ac-SMZ (Fig. 2) becomes nonlinear within the first two minutes after the reaction is initiated. As a consequence, initial rates for SMZ acetylation are determined by drawing tangents to the velocity curves at zero time. There is approximately 20-fold variation in these values and subjects fall into two groups corresponding to slow (2, 5, and 9) and rapid (1, 3, 4, 6, 7 and 8) acetylators. Our observations with SMZ agree well with observations by Evans who used INH as an alternate polymorphic substrate to study the acetylating capacity in fresh liver biopsies from eight individuals (26). Evans's values showed a similar range of variation in SMZ-NAT activity (16-fold) and the values were bimodally distributed. Rapid acetylators (Table 2) average more than eight times greater liver SMZ-NAT activity than the slow acetylators. The activities obtained with PABA in the same set of biopsies differ from those of SMZ with respect to kinetic properties as well as in the range of values and their distribution. Initial velocities for PABA acetylation are linear with time for at least two minutes after the reaction is initiated (Fig. 2). Values for different individuals show less than 4-fold variation and it is apparent that there is no correlation between these rates of acetylation and those for SMZ. In the studies of Evans previously mentioned, PABA acetylation by fresh biopsies of jejunal mucosa was also measured in the same individuals.

TABLE 3

Michaelis-Menten constants and octanol-water partition coefficients for arylamine substrates

Apparent K_m values for rabbit enzyme were determined using a partially purified liver NAT (DEAE cellulose) fraction obtained from a rapid acetylator (see MATERIALS AND METHODS). The apparent K_m values for human enzyme were determined with a partially purified liver NAT preparation (100,000 × *g* supernatant fraction) from a rapid acetylator, after homogenization in 4 volumes 0.1 M potassium phosphate buffer, pH 7.4. The apparent K_m value for benzidine was also determined using a similar preparation of human liver NAT from a slow acetylator. The apparent K_m value obtained was $7.2 \pm 2.1 \times 10^{-5}$. Log P is the logarithm of the octanol water partition coefficient which was determined as described under MATERIALS AND METHODS.

Substrate	Rabbit liver NAT	Human liver NAT	Log P
Sulfamethazine	$4.3 \pm 0.91 \times 10^{-4}$	$1.5 \pm 0.77 \times 10^{-3}$	−0.43
p-Aminobenzoic Acid	$6.9 \pm 0.59 \times 10^{-5}$	$1.8 \pm 0.40 \times 10^{-4}$	−1.62
α-Naphthylamine	$8.1 \pm 0.08 \times 10^{-5}$	$4.7 \pm 1.20 \times 10^{-5}$	–
MOCA	$2.2 \pm 1.80 \times 10^{-5}$	$6.5 \pm 1.03 \times 10^{-5}$	1.38
β-Naphthylamine	$4.0 \pm 0.36 \times 10^{-5}$	$2.4 \pm 0.18 \times 10^{-5}$	1.37
2-Aminofluorene	$7.5 \pm 0.75 \times 10^{-6}$	$5.0 \pm 1.10 \times 10^{-5}$	1.49
Benzidine	$5.9 \pm 0.87 \times 10^{-6}$	$1.2 \pm 0.36 \times 10^{-4}$	0.65

The values varied approximately 3-fold and there was a similar lack of correlation with liver-SMZ acetylating activity (26). The latter observation on jejunal mucosa is of interest in connection with our findings using liver since a high correlation has been demonstrated between the NAT activities in liver and jejunum with the polymorphic substrate INH (30). Thus, while SMZ-acetylating capacity of liver (and jejunum) is bimodally distributed in human populations, the distribution of PABA acetylating capacity in both these tissues is unimodal.

Initial acetylation rates were determined for each substrate with fresh liver biopsies from rapid and slow acetylator rabbits and humans. Apparent K_m values for PABA, SMZ and each of the carcinogenic substrates tested had been determined to establish optimal assay conditions (Table 3). Rabbit liver NAT has a smaller apparent K_m value than the human liver enzyme for both SMZ and the carcinogenic arylamine substrates (Table 3). Despite these species differences in apparent K_m values between rabbit and human liver NATs, the apparent K_m values for rapid and slow acetylators within each species do not differ (39). Thus comparisons between the rapid and slow enzymes were made at the same concentrations of each substrate. Activity ratios (Table 4, column 4) were calculated from this information. Since the range of ratios for carcinogens (190–580) overlaps the range of SMZ ratios (60–464) one may ask whether a carcinogen with an activity ratio of less than any SMZ activity ratio can be considered a polymorphic substrate. However, if comparisons are made between a carcinogen and SMZ within the same pair of rapid and slow acetylator rabbits, the activity ratio for the carcinogen is always greater than the ratio for SMZ (cf. the benzidine activity ratio in rabbits 18 and 49 with their SMZ activity ratio vs. the benzidine activity ratio in rabbits 58 and 49 with their SMZ activity ratio). Human liver NAT shows a trend similar to that observed in rabbits with respect to the drug and carcinogenic substrates, although the magnitude of the ratio is not as great (5–13 fold). We conclude from this information that the carcinogenic compounds show differences in acetylation rates in rapid and slow acetylators that are greater than those for SMZ and thus are polymorphically acetylated in both rabbit and man. While the activity ratio for PABA acetylation in man closely approximates one, the corresponding ratio in rabbit is somewhat more variable ranging from 1.5 to 5. It should be noted that previous observations in rabbit show that such ratios are not inconsistent with the conclusion that PABA behaves as a monomorphic substrate (28).

The apparent K_m values for the carcinogenic compounds are much smaller for both rabbit and human liver NATs than for SMZ (Table 3). The apparent K_m value for enzyme-substrate interactions can be influ-

enced by the nature and degree of binding of the substrate to the enzyme and other factors including the structure of the substrate. Hydrophobic bonding is important in protein-small organic molecule interactions (40). Hansch found that the binding of biologically active compounds to enzymes parallels their octanol-water partition coefficients rather closely (41). For example, Helmer *et al.* showed that the binding of a wide variety of organic compounds (including β-naphthylamine) to bovine serum albumin supported this concept (42). There is also information suggesting that differences in hydrophobicity may play a role in determining differences in kinetic properties of enzymes. Hansch *et al.* concluded that hydrophobic bonding accounts for almost 40% of the variance in the rate of acetylation of a series of aromatic amines by pigeon liver NAT (41). The chemical structures of the carcinogenic compounds in this study indicate they are more hydrophobic than SMZ. Substrate structure may also affect the apparent K_m value. In a study of the inhibition of the enzyme α-chymotrypsin by a series of aromatic compounds, Wallace *et al.* showed that the K_i's for α- and β-naphthylamines were two orders of magnitude smaller than for the simple aromatic compound benzoate (43). They suggested that all other factors being equal, a structure with a larger planar area of interaction will result in a greater binding energy and therefore a lower value of K_i. It is apparent that the carcinogenic substrates we studied (Table 4) have a larger planar area than SMZ and therefore would be expected to have a smaller apparent K_m value. Octanol-water partition coefficients were measured for each substrate (Table 3) and the logarithms of these values for the polymorphic compounds correlate with the apparent K_m values for both human (r =

TABLE 4

N-Acetyltransferase activity for arylamine substrates in liver from rapid and slow INH-acetylator rabbits and humans

Initial N-acetylation rates for SMZ and the carcinogenic substrates listed were determined using the radioactive enzyme assay, as described under MATERIALS AND METHODS, with an initial concentration of AcCoA of 5×10^{-4} M. Initial N-acetylation rates for PABA were determined using the modified Bratton-Marshall colorimetric procedure (28) with an initial AcCoA concentration of 2.2×10^{-3} M. Substrate concentrations used were at or near the apparent K_m value for each substrate (Table 3). DEAE-cellulose fractions were used for rabbit and 100,000 × g supernatant fractions of autopsy liver were used for human.

Substrate	Rabbit[a]			Human[b]		
	Activity		Ratio	Activity		Ratio
	Rapid	Slow		Rapid	Slow	
	nmoles/min/mg			*nmoles/min/mg*		
Sulfamethazine	8.62 (72)	0.030 (66)	290	0.837	0.226	3.7
	8.26 (72)	0.030 (49)	275			
	3.02 (18)	0.029 (49)	104			
	7.43 (58)	0.016 (49)	464			
	4.71 (58)	0.078 (63)	60			
p-Aminobenzoic Acid	0.71 (18)	0.34 (49)	2	0.150	0.157	0.96
	1.61 (58)	0.32 (49)	5			
	1.10 (58)	0.83 (63)	1.5			
Carcinogenic Arylamines						
α-Naphthylamine	12.40 (72)	0.023 (66)	540	1.17	0.225	5.2
Benzidine	8.88 (58)	0.016 (49)	560	0.173	0.019	9.0
	4.14 (18)	0.022 (49)	190			
β-Naphthylamine	8.73 (72)	0.28 (66)	310	0.231	0.026	8.9
2-Aminofluorene	7.53 (72)	0.013 (49)	580	0.285	0.021	13.0
MOCA	—	—	—	0.144	0.015	9.6

[a] Numbers in parentheses refer to individual rabbits.

[b] One rapid acetylator human (#4) and one slow acetylator human (#9) were used for initial rate determinations.

−0.93) and rabbit ($r = -0.90$) liver NAT. All other things being equal, these findings support the concept that the differences in apparent K_m values we observed may be related, at least in large part, to differences in hydrophobicity.

The induction of cancer in individuals by chemical carcinogens obviously depends upon the nature of the chemical agent involved and host susceptibility. In view of the evidence regarding enzymatic factors affecting the activation of arylamines to carcinogens, it is reasonable to ask whether a relationship exists between the hydrophobicity of these compounds and their carcinogenicity. Franke stated that hydrophobic interactions of polycyclic aromatic hydrocarbons with proteins do influence the process of chemical carcinogenesis, but they appear secondary compared to chemical reactivity. He found that any alteration of a particular hydrocarbon which leads to a decrease in binding strength within that series, results in a decrease in carcinogenic activity provided there is no change in chemical reactivity (44). Considering the fact that the carcinogenic compounds are substrates for polymorphic NAT, the possibility exists that hydrophobicity and acetylator phenotype are both important in determining individual susceptibility to arylamine carcinogenesis. It is interesting to note that studies by Lower *et al.* suggest a higher incidence of slow INH-acetylators among bladder cancer patients (45). Acetylator phenotype may also influence the site of tumor formation. Rabbits develop liver tumors rather than bladder tumors from compounds like AF[3]. Rapid acetylator rabbits might be expected to develop this type of tumor more readily than slow acetylators due to the production of greater amounts of the acetylated amine. The metabolic activation proposed in Fig. 1 does involve N-acetylation at an early stage of the process and subsequent N-hydroxylation to N-OH-AAF which can then be conjugated to liver carcinogens. On the other hand, studies in dogs indicate that the capacity for N-acetylation may be required for hepatoma formation but not for cancer of the urinary bladder (see INTRODUCTION). Other studies in this species indicate how factors affecting the balance between N-acetylation and deacetylation may play an important role in determining tissue and species susceptibility to arylamine carcinogenesis. Thus, after oral administration of 4-aminobiphenyl one sees primarily urinary bladder tumors (46–48) while administration of 4-acetylaminobiphenyl leads to tumors of both liver and bladder (49). In contrast, oral administration of 2-acetyl naphthylamine fails to induce urinary bladder tumors (50), even though 2-naphthylamine (β-naphthylamine) is a well-established canine urinary bladder carcinogen (51). *In vitro* studies indicate that 4-acetyl-aminobiphenyl has an apparent K_m value almost an order of magnitude smaller with canine liver deacetylase than does 2-acetyl-naphthylamine (46). Thus the lack of carcinogenicity of 2-acetyl-naphthylamine in the urinary bladder appears to be related to the inability of this species to deacetylate this substance. These phenomena suggest that tissue concentrations of arylamines and arylacetamides and their interconversion can be influential determinants of carcinogenesis from these substances and their derivatives.

Occurrence of genetic variability in at least two steps in carcinogenic arylamine metabolism suggests the existence of subpopulations which may differ significantly with respect to susceptibility to tumor formation. Genetic differences in the levels and inducibility of hydroxylating enzymes involved in carcinogenic arylamine metabolism has been documented (7). We now report genetic differences in the capacity of human and rabbit liver to acetylate carcinogenic arylamines. Such differences in metabolic capacity which may lead to differences in susceptibility to the development of cancer from arylamines deserve critical evaluation.

[3] Lower, G. M. Personal communication.

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