0031-6997/85/3701-0025$02.00/0
PHARMACOLOGICAL REVIEWS
Vol. 37, No. 1
Printed in U.S.A.

N-Acetylation Pharmacogenetics

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I. Introduction

NEARLY 30 years have elapsed since the occurrence of isoniazid (INH)[1]-induced nerve damage in "slow" inactivators (acetylators) of INH led to the discovery of the human acetylation polymorphism. A review of acetylation pharmacogenetics published in 1973 by Weber (430) dealt with the acetylation of drugs in intact animals and man, inherited variation in acetylation, the properties of drug acetylating enzymes, and the mechanism of enzymic drug acetylation. The scope of the field has expanded considerably since then and much that is new has been learned about these and additional topics. Reviews that have appeared since the mid-1970s include one by Drayer and Reidenberg (103) and another by Lunde, Frislid, and Hansteen (254) on clinical disorders associated with the acetylator status. Others by Alarcon-Segovia (4), Uetrecht and Woosley (417), and a symposium (184) have considered the problem of drug-induced lupus. Reviews by Reece (333) and by Ludden et al. (253), examined the chemistry and the clinical pharmacokinetics of hydralazine. Reviews of the clinical pharmacokinetics of INH in 1979 were published by Weber and Hein (433), and of drug acetylation in 1980 by Weber and Glowinski (434). The problem of metabolic activation of aromatic amines, in which N-acetylation plays a role, has been the subject of many papers. It has been reviewed by several authors (e.g., refs. 68, 69, 304), but it has not usually been considered from the pharmacogenetic point of view. King and Weber (228) presented a short paper in which acetylator status was cited as a hereditary metabolic factor of potential importance in determining susceptibility to arylamine-induced bladder cancer. None of these

[1] Abbreviations used are: A, A/J inbred mice; AAF, 2-acetylaminofluorene; AAMU, 5-acetylamino-6-amino-3-methyl uracil; AcCoA, acetylcoenzyme A; AF, 2-aminofluorene; AFMU, 5-acetyl-6-formylamino-3-methyluracil; AHAT, N,O-arylhydroxamic acid acyltransferase; ANA, antinuclear antibodies; B6, C57BL/6J inbred mice; CoA, coenzyme A; CoASAc, acetylcoenzyme A; DDS, dapsone; HP, hydralazine; HPPAH, hydralazine pyruvic acid hydrazone; HPLC, high performance liquid chromatography; INH, isoniazid; MADDS, monoacetyldapsone; MAH, monoacetylhydrazine; MTP, 3-methyl-5-triazolo-(3,4-a)phthalazine; NAc-HPZ, 4-(2-acetylhydrazino)pthalazine-1-one; NAPA, N-acetylprocainamide; NAPADE, desethyl-N-acetylprocainamide; NAT, N-acetyltransferase; 3-OH-MTP, 3-hydroxymethyl-5-triazolo-(3,4-a)phthalazine; PA, procainamide; PABA, *p*-aminobenzoic acid; PADE, desethyl-procainamide; PAS, P-aminosalicylic acid; PZ, phthalzine-1-one; SGOT, serum aminotransferase; SMZ, sulfamethazine; TP, s-triazolo-(3,4-a)phthalazine; 1U, 1-methyl uric acid; 3U, 3-methyl uric acid; 7U, 7-methyl uric acid; 13U, 1,3-dimethyl uric acid; 17U, 1,7-dimethyl uric acid; 37U, 3,7 dimethyl uric acid; 137U, caffeine; 1X, 1-methylxanthine; 3X, 3-methylxanthine; 7X, 7-methylxanthine; 13X, 1,3-methylxanthine; 17X, 1,7-methylxanthine; 37X, 3,7-dimethylxanthine; XO; xanthine oxidase.

papers brings the story of acetylation pharmacogenetics up-to-date in a comprehensive manner. For this reason, and for several additional reasons, we believe that the time for a critical review of certain advances in this area is appropriate.

Progress in drug metabolism and disposition in man and experimental animal models has revealed remarkable relationships between genetically determined individual differences in acetylation capacity and drug toxicity. The diverse character of clinical toxicities induced by aromatic amine and hydrazine drugs, which are metabolized mainly by N-acetylation (fig. 1), has caused us to examine the metabolic capacity and specificity of the manifold processes of the cells and tissues affected in relation to acetylation differences they express. Evidence is accumulating that individual susceptibility to cancer induced by aromatic amine carcinogens may be modulated to an important extent by hereditary differences in acetylator status. A variety of reports have appeared that potentially implicate acetylator status as a determinant of susceptibility to other human illnesses. Observations of a more basic or experimental nature have recently kept pace with those in clinical areas. From new animal model studies, hereditary acetylation polymorphisms are known to occur in several mammalian species (rabbit, mouse, hamster, rat) other than man. Biochemical studies have revealed an assortment of qualitative variants of the acetylating enzymes that account for these hereditary polymorphisms. Studies in intact animals of known acetylator phenotype and genotype, and in cells isolated from these animals and grown in isolated primary culture have enabled investigators to assess more completely the pharmacological and toxicological significance of acetylator status.

We have attempted to describe and evaluate the current place of N-acetylation processes in the metabolism of foreign compounds as they relate to the objectives and scope of pharmacogenetics. Section II reviews the effects of acetylator status on the disposition, metabolism, and elimination of drugs and other foreign compounds in intact individuals, isolated cells, and in cell-free systems. Primary aromatic amine and hydrazine drugs (INH, hydralazine, procainamide, dapsone, phenelzine, aminoglutethimide), drugs which are converted in the body to primary amines and are acetylated (sulfasalazine, nitrazepam, clonazepam, acebutolol, caffeine) (fig. 2), and arylamine mutagens and carcinogens (aminofluorene, benzidine, methylene bis-2-chloroaniline, methylene dianiline) are considered. Section III considers the human acetylator status as a determinant of drug toxicity and human illness. Associations between acetylator status and selected adverse drug reactions that involve polymorphically acetylated drugs and other drugs, arylamine-induced cancer, and spontaneous illness are dealt with. In the latter category putative associations between acetylator status and spontaneous systemic lupus, breast

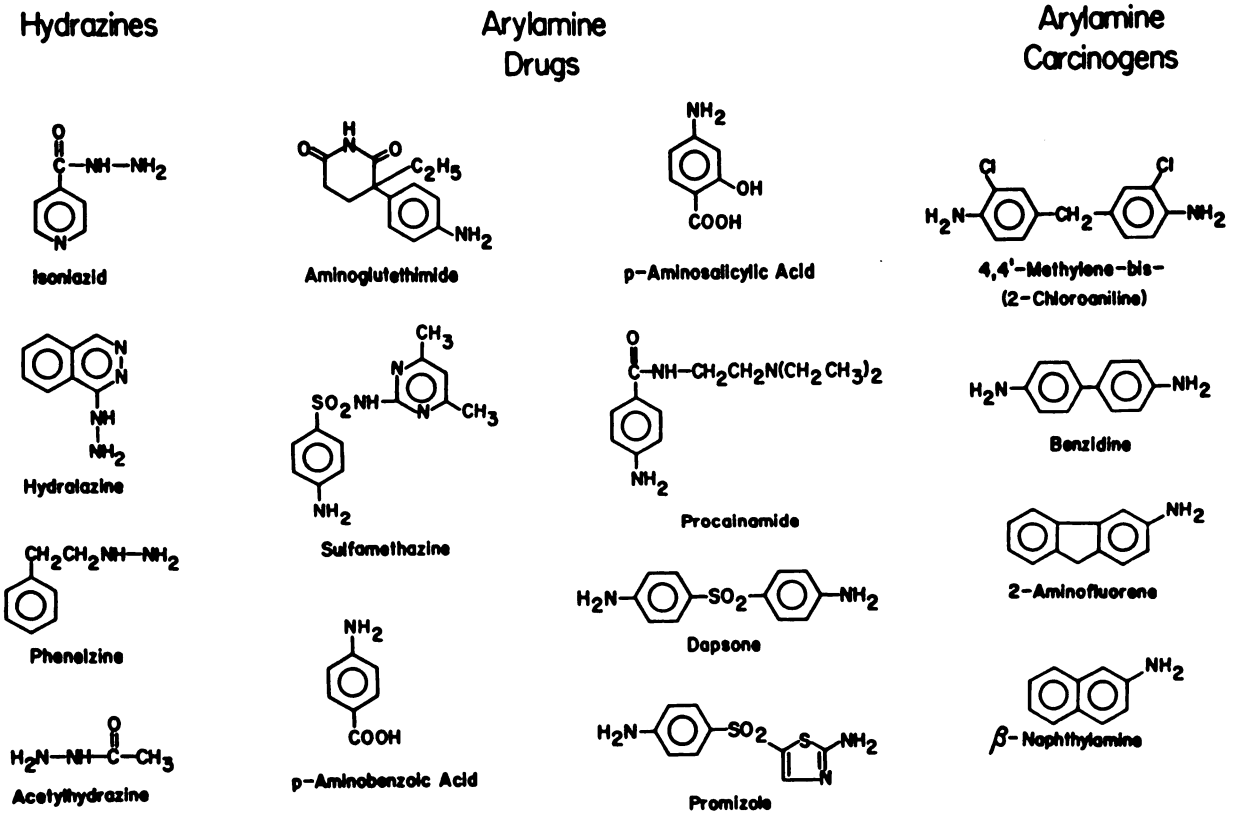


FIG. 1. Structures of drugs and other environmental chemicals that are polymorphically acetylated.

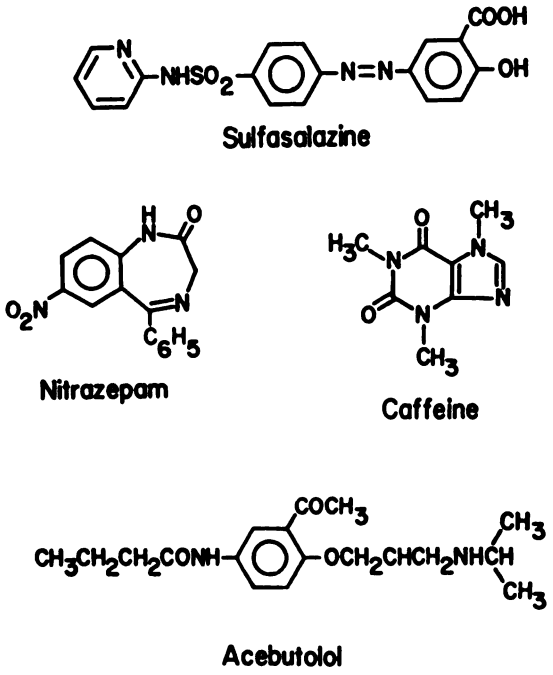


FIG. 2. Drugs metabolized to amines which are polymorphically acetylated.

cancer, diabetes, and Gilbert's disease are discussed. Section IV focuses on the genetics of the human acetylation polymorphism and methodology for acetylator phenotype determination. Section V discusses kinetic mechanisms of enzymatic acyltransfer by liver N-acetyltransferase (E.C. 2.3.1.5) (NAT) and by N,O-arylhydroxamic acid acyltransferase (AHAT), an enzyme that appears to be the same as the genetically polymorphic NAT, or closely linked to it. Section VI discusses acetylation polymorphisms in inbred rabbits, mice, hamsters, and rats; the genetic and biochemical basis for them; and their pharmacological and toxicological significance.

II. Acetylation of Drugs and Other Foreign Compounds

A. Arylamine Drugs

1. Procainamide. Procainamide (PA) has been widely used as an effective agent for prevention and therapy of cardiac arrhythmias since it was synthesized in 1951 (260). Extensive studies have shown that plasma concentrations of procainamide are correlated with its therapeutic actions and toxicity in man, but N-acetylation was not identified as the major pathway of its metabolism until 1972 (104).

Since then, various effects of the acetylator status on the pharmacology of procainamide have been studied in healthy volunteers (144, 220, 106, 137, 143, 359, 360, 365, 347). The influence of acetylator status on procainamide disposition in healthy subjects is best evidenced by a significantly greater excretion of N-acetylprocainamide (NAPA) in the urine of rapid acetylators compared to slow acetylators. In one study of 33 healthy persons, NAPA accounted for 19% ± 4% (mean ± S.D.) of a single fixed dose (50 mg) of procainamide ingested by rapid acetylators while the corresponding percentage in slow acetylators was 9% ± 1% ($P < 0.001$) during 1 to 4 hr after administration (220). In another study of 20 persons ingesting a single dose of 20 mg/kg of body weight the concentrations of NAPA and of procainamide in urine and plasma were distinctly bimodal with rapid acetylators excreting 28% and slow acetylators excreting 14% within 2 to 6 hr after administration of the drug (137). Qualitatively similar results were obtained by other investigators after healthy volunteers had ingested a single dose of procainamide (144, 106, 359) and after ingestion of multiple doses of the drug (137). Two groups of investigators found that there was no difference in the plasma half-life of unchanged procainamide, a measure of the whole body clearance of the drug (137, 143), although another group found that the plasma half-life of procainamide was slightly shorter in the rapid acetylators (2.2 ± 0.4 hr) compared to slow acetylators (3.9 ± 0.5 hr; $P < 0.005$) (106). These studies in healthy subjects show further that acetylator phenotype can be determined with procainamide as the test drug, but because the separation between rapid and slow acetylators is less pronounced for procainamide than it is for drugs such as sulfamethazine or isoniazid, precise criteria for the test with procainamide must be followed and the influence of diseases such as renal impairment, or congestive heart failure must be taken into consideration to avoid erroneous classification.

Observations on different patient groups have provided confirmation of these findings in healthy subjects and have provided additional insights toward the relation of acetylator status to the disposition of procainamide. For example, several groups of investigators (107, 108, 337, 220, 144, 365) have shown that the NAPA/PA ratios in plasma and in urine are bimodally distributed and are significantly greater in rapid than in slow acetylators. Individuals with NAPA/PA ratios less than approximately 0.85 appeared to be slow acetylators and those with ratios greater than 0.95 appeared to be rapid (337). Renal clearance of procainamide characteristically exceeded glomerular filtration indicating that procainamide is actively secreted. The clearance of NAPA was about half that of procainamide, but there were no differences in the renal clearance of procainamide or of NAPA associated with acetylator status (337, 245). However, because of its marked dependence on renal function, the steady state NAPA/PA ratio is not a reliable index of acetylator phenotype in patients with renal impairment.

The relationship of acetylator status at different daily doses of procainamide to its plasma concentrations was clearly demonstrated in a study of arrhythmic patients (56): slow acetylators ingesting 1.5 g of procainamide during an 8-hr period had mean plasma levels in the toxic range (16 ± 2.1 μg/ml) whereas those ingesting a

lower dose of 1.0 g during an 8-hr period had mean trough levels that were sub-therapeutic. Furthermore, toxic levels were more common in slow acetylators ingesting high doses of the drug, and ineffective levels were more common in rapid acetylators ingesting low doses. A comprehensive analysis of procainamide pharmacokinetics in which procainamide was infused for an hour indicated that acetylator status had no effect on tissue distribution volumes either initially or at the steady state (268).

Although N-acetylation is the major metabolic pathway of procainamide in man and several other species (except the dog), additional studies have shown that other metabolites are formed in man. These include *p*-aminobenzoic acid, N-acetyl-*p*-aminobenzoic acid (106), desethyl-procainamide (PADE), and desethyl-N-acetyl-procainamide (NAPADE) (104, 351, 352).

In contrast to the bimodal excretion of NAPA by rapid and slow acetylators, no difference is seen between these two groups in the total urinary N-acetyl-*p*-aminobenzoic acid, and the percentages are distributed unimodally (106). The disposition of desethyl-N-procainamide and its N-acetyl derivative is more complex, however, as shown by studies of procainamide metabolism in cardiac patients (351, 352). Plasma levels of procainamide, NAPA, and NAPADE showed no consistent relation of NAPADE to either procainamide or NAPA, but kinetic analysis of the data suggests that the principal route of formation of NAPADE from procainamide occurs by initial dealkylation of procainamide to PADE, an hypothetical metabolite that has yet to be identified; smaller amounts of NAPADE are presumably formed from NAPA. Thus it might be anticipated that a large difference in NAPADE values might occur in rapid and slow acetylators because of differences in the rate of acetylation of PADE to NAPADE. When this hypothesis was tested by taking account of differences in NAPA/PA ratios, a correlation of 0.74 ($P < 0.02$) was found between measured and predicted NAPADE concentrations. Also, presumably the rate of acetylation of PADE to NAPADE is mediated by the genetically polymorphic N-acetyltransferase and thus is dependent on acetylator status. The exact significance of the observations on NAPADE in relation to acetylator status is unclear as documentation of the pharmacological activity of NAPADE is lacking, but Ruo et al. (352) point out that such activity would be expected because antiarrhythmic and anticonvulsant activity of lidocaine and its N-dealkylated metabolite are comparable.

The pathways of metabolism described above account for approximately 85% of procainamide metabolism, but the remainder is eliminated by unknown pathways (143, 106). Elucidation of these pathways has taken on greater significance since evidence has been obtained for the formation of reactive metabolites from procainamide (136, 416, 135) and since evidence relating PA toxicity to acetylator status has been obtained in an animal model of the hereditary acetylation polymorphism (402, 403, 436) (see section III B and section VI C).

2. Dapsone. For the past 30 years, dapsone (DDS) has been the principal drug for leprosy chemotherapy and is effective treatment for chloroquine-resistant malaria (355). Dapsone is also a dramatically effective drug therapy for dermatitis herpetiformis (24).

The development of sufficiently selective and sensitive colorimetric (113) and fluorimetric methods for measurement of DDS in biological tissues (147, 114) and their modification (313) enabled investigators to determine DDS and its acetylated metabolites, MADDS, and diacetyldapsone (DADDS). The demonstration in rabbits that DDS was polymorphically acetylated had suggested that it might also be polymorphically acetylated in man (313). However, prior studies in animals and in man demonstrating that DDS was formed by deacetylation of DADDS caused investigators to anticipate that the metabolism of DDS might differ from that of isoniazid or sulfamethazine, which do not undergo deacetylation in man, and that the criteria for demonstrating the hereditary acetylation polymorphism with DDS might also differ.

Polymorphic acetylation of DDS in man was first clearly shown by the study of the acetylator characteristics of the drug in 19 healthy subjects and leprosy patients whose acetylator phenotype had been determined with both isoniazid and sulfamethazine (142). DDS acetylation capacity was measured from the ratio of MADDS to DDS in plasma 4 hr after ingestion of 100 mg of DDS. The mean values of the MADDS/DDS plasma ratio in rapid acetylators were 3 to 5 times greater than in slow acetylators, and there was no overlap of individual values for any of the tests used. The ratio was found to be a stable individual characteristic as for isoniazid and sulfamethazine. The MADDS/DDS ratio rapidly attains a constant value within 30 to 60 min after ingesting DDS (142) which persists for at least 8 hr (314). Although rapid acetylators excreted significantly more MADDS (and its acid-labile conjugates) than slow acetylators, the acetylator phenotype could not be determined from the urine data because these compounds accounted for such a small fraction of the dose of DDS.

Unusually high plasma levels of MADDS were found within 15 min after the ingestion of this compound, and comparison of these levels with MADDS levels shortly after DDS ingestion suggested that DDS acetylation and MADDS deacetylation occur simultaneously, but that the latter reaction was much slower. The MADDS/DDS ratios observed were relatively constant after DDS ingestion indicating that a steady-state of acetylation of DDS and deacetylation of MADDS was attained very soon whereas after MADDS administration approximately 4 hr elapsed before a steady-state was achieved. These results suggested that the MADDS/DDS ratios which

differentiate rapid and slow acetylators result primarily from differences in the capacity for acetylation rather than deacetylation.

During the past 10 years the DDS acetylation characteristics of various other human volunteer and patient populations have been determined by Peters and his colleagues (319), and by several other groups of investigators (102, 117, 58, 166, 237) with results similar to those described in the original study (142).

Peters et al. (315, 319) have shown that the half-life of elimination of DDS and of MADDS are the same, or nearly the same, in rapid and slow acetylators despite clear differences in the MADDS/DDS ratio. For instance, a study of DDS pharmacokinetics in two slow and two rapid acetylators after ingesting 100 mg of DDS showed that the half-life of DDS varied from 17 to 21 hr, while that of MADDS varied from 15 to 23 hr. Furthermore, the values were essentially the same in all subjects regardless of acetylator status (142). Similar results were obtained following ingestion of 117 mg of MADDS, and no differences in half-life for either compound were observed. Thus, it was concluded that MADDS was deacetylated in man. A larger study of Phillipine subjects involving eight slow and 31 rapid acetylators led to the same conclusion, but the half-lives in the Phillipine study were highly variable, ranging from 14 to 53 hr, and the group mean of approximately 28 to 31 hr, was much higher. A more limited study performed to assess the effect of dosage on the percentage of acetylation of DDS, or the elimination half-life of DDS, showed that these characteristics were not affected by changes in schedule from a dose of 25 mg 2 or 3 times weekly to 5 mg 2 to 3 times weekly.

DDS and MADDS are both strongly bound to plasma proteins, with the latter being somewhat more strongly bound (142). While their binding characteristics are unaffected by acetylator status, they nevertheless may provide an explanation for some of the pharmacokinetic behavior observed for MADDS. Thus, the very high initial levels of MADDS and the slow attainment of a constant MADDS/DDS ratio after MADDS administration may be a consequence of its slow release from plasma proteins. The excretion of negligible quantities of MADDS in urine is perhaps also attributable to this factor.

Several additional observations concerning the human metabolism of DDS can be made. In summary, these include the finding that the extent of DDS acetylation is directly correlated with MADDS levels, but is unrelated to DDS levels; that the age, or body weight of the patients is unrelated to either DDS acetylation or its half-life of elimination; that the half-lives of DDS and MADDS are directly related to each other; and that DDS provides a poorer discriminator of acetylator phenotypes than sulfamethazine (142, 315–319).

The possibility that measurements of DDS and MADDS in saliva might provide a useful, non-invasive means of determining DDS pharmacokinetics and the acetylator phenotype was also explored. Measurements by Lammintausta et al. (237) of DDS and its acetylated derivatives by a fluorimetric procedure in 10 healthy male subjects after they had ingested 200 mg of the drug showed mean MADDS/DDS ratios of 1.0 in six rapid acetylators and 0.19 in four slow acetylators. Elimination half-lives averaged 20 to 21 hr for both DDS and MADDS and differences in the pharmacokinetics were absent. Salivary concentrations of DDS averaged 49% of total serum concentrations throughout the entire study period of 72 hr, and the elimination half-life of DDS and MADDS in saliva did not differ from that in serum. A highly significant correlation ($P < 0.001$) existed between salivary and serum protein-free concentrations. Lammintausta et al. concluded that clinical monitoring of DDS and MADDS was possible from measurements on saliva, and that the acetylator phenotype determined from saliva was more reliable than from serum. In contrast, a study by Peters et al. (320) on six healthy human volunteers who had ingested 50 mg of DDS found that decay rates for DDS from saliva and plasma were identical or nearly identical, but that salivary DDS levels were only 15% to 20% of plasma levels. Concentrations of DDS in dialysates of plasma were nearly identical to those in saliva levels. Saliva levels of MADDS ranged from 0.8% to 2.0% of their plasma levels and were so low that a reliable definition of acetylator phenotype could not be made from saliva. Peters et al. noted that the amounts of DDS and MADDS secreted into saliva could be used to monitor compliance to DDS intake, or for preliminary assessment of possible abnormalities in DDS binding, or disposition, but they strongly disagreed with Lammintausta et al. (237) that saliva affords a simple, reliable method of acetylator determination. Peters et al. suggested that the discrepancy between the two studies may be attributable to use of an older, somewhat less reliable fluorimetric method for DDS and MADDS determination (313) by Lammintausta et al. than methods currently in use (292).

3. Aminoglutethimide. Aminoglutethimide was introduced as an anticonvulsant drug in the 1950s but was withdrawn because it caused signs and symptoms of adrenal insufficiency. Subsequently it was shown to suppress the synthesis of adrenal steroids and to be effective as a medical adrenalectomizing agent in treatment of mammary carcinoma in postmenopausal women (101, 380).

Initial studies of the metabolic fate of aminoglutethimide in healthy volunteers showed that 34% to 50% of the dose ingested was excreted in the urine unchanged and that an appreciable portion was excreted as N-acetylaminoglutethimide. Further studies in healthy male volunteers revealed an array of urinary metabolites, two of which were unusual, N-formylaminoglutethimide

and nitroglutethimide, and both were excreted in small quantities (15). N-acetylaminoglutethimide was found to be the major urinary metabolite, but its amount varied six-fold from one person to another, accounting for only 4% of the dose in certain persons and as much as 25% in others. Results of a previous study of 21 breast cancer patients had also shown that the N-acetyl metabolite varied greatly between individuals, accounting for 9% to 48% of the drug in circulation (73). More recently, Coombes et al. (74) reported that a bimodal distribution of urinary metabolites occurred in 10 healthy individuals. Slow acetylators excreted more aminoglutethimide (mean 28% of a single 250-mg dose) than rapid acetylators (12%), but the rapid acetylators excreted more as N-acetylaminoglutethimide (8.8%) than slow acetylators (3.9%). Urinary levels of aminoglutethimide overlapped between the acetylator phenotypes, and the difference between acetylator phenotypes fell short of statistical significance ($P < 0.074$).

In addition, a bimodal distribution in the excretion of the minor metabolite, nitroglutethimide, was observed. Rapid acetylators excreted less of the dose (0.047%) than slow acetylators (0.10%). The mechanism of formation of nitroglutethimide was not investigated, but was considered to have arisen non-enzymatically from an unstable intermediate hydroxylamine derivative. The bimodality was attributed to differences in the size of the pool of aminoglutethimide being smaller in the rapid acetylators than in the slow acetylators and thus was considered to be a reflection of the acetylator phenotype. In contrast, excretion of the N-formylaminoglutethimide was virtually identical for the slow (0.475%) and the rapid acetylators (0.465%) through the range of values was greater for the rapid acetylators.

The capacity of aminoglutethimide to induce its own metabolism (290) was also observed. Jackson et al. (199) previously reported a similar observation for glutethimide. Jarman et al. (202) identified hydroxylaminoglutethimide as an induced metabolite on chronic dosing with the parent drug. They suggested that the formation of this metabolite was principally responsible for the decreased half-life of aminoglutethimide observed in chronic therapy. Acetylaminoglutethimide was almost absent from the urine after 6 weeks of treatment with aminoglutethimide (202).

Auto-induction of aminoglutethimide metabolism and its effect on the pharmacokinetics of the drug have recently been studied in relation to the acetylator phenotype by Adam et al. (1). The renal clearances of aminoglutethimide and acetylaminoglutethimide were found to be unchanged by induction, but their metabolic clearances were increased. The decreased half-life of the drug was accompanied by a reduction in the acetylaminoglutethimide/aminoglutethimide ratio. The altered ratio reflected a significant ($P < 0.05$) decrease in the AUCs of both aminoglutethimide and acetylaminoglutethimide. Thus, induction appears to activate oxidative pathways with higher affinity for aminoglutethimide than N-acetyltransferase. The small amount of the acetyl urinary metabolite is consistent with this interpretation but stands in contrast to the earlier observation of Douglas and Nicholls (101).

Pharmacokinetic studies of aminoglutethimide performed by Adam et al. (1) indicated that the half-life of aminoglutethimide was 19.5 ± 7.7 hr in seven rapid acetylators and 12.6 ± 2.3 hr in five slow acetylators. These observations are paradoxical because rapid acetylators have a longer half-life and lower total body clearance for aminoglutethimide than slow acetylators despite having a higher acetylaminoglutethimide/aminoglutethimide ratio. With the possible exception of phenelzine, this paradox does not appear to have been noted with other drugs. Caddy et al. (52) found that the half-life of phenelzine in two rapid acetylators (1.03 and 1.61 hr) was longer than in three slow acetylators (0.40, 0.58, and 0.72 hr). Coombes et al. (74) showed that slow acetylators excreted more nitroglutethimide in the urine than rapid acetylators, but this metabolite is unlikely to account for these observations since it constituted only a minor part (0.1%) of the dose. Adam et al. (1) speculated that acetylaminoglutethimide may act as a reservoir for the drug, like protein binding, before it is eventually eliminated by the oxidative mechanisms. However, this eventuality would require that acetylaminoglutethimide undergo deacetylation, a reaction that has not been demonstrated with this drug in man. Further explanation of this paradox must await more complete assessment of the metabolic fate of aminoglutethimide in man.

B. Hydrazine Drugs

1. Isoniazid. INH (fig. 2) is the drug used most widely for specific treatment of tuberculosis in children and adults, either alone in patients who have had a skin test conversion, or in combination with other drugs for treatment of all active forms of the disease.

Initial metabolic studies clearly showed that the human hereditary acetylation polymorphism is a major determinant of INH disposition and elimination. Much is known about its metabolism and pharmacology from additional studies which take account of acetylator status (433).

The metabolic pathways proposed for INH in man as shown in figure 3 differ quantitatively but not qualitatively. After ingestion of INH the urine excreted contains INH, pyruvic acid hydrazone, alpha-ketoglutaric acid hydrazone, acetylisoniazid, isonicotinic acid, isonicotinyl glycine, MAH and diacetylhydrazine. In humans acetylation is the most important pathway in INH elimination (312, 282), and differences in the rates of INH acetylation account for the phenotypic difference between rapid and slow acetylators. More recent pharmacological studies confirm this (39, 118). After ingestion INH is converted to acetylisoniazid more rapidly in rapid acetylators than

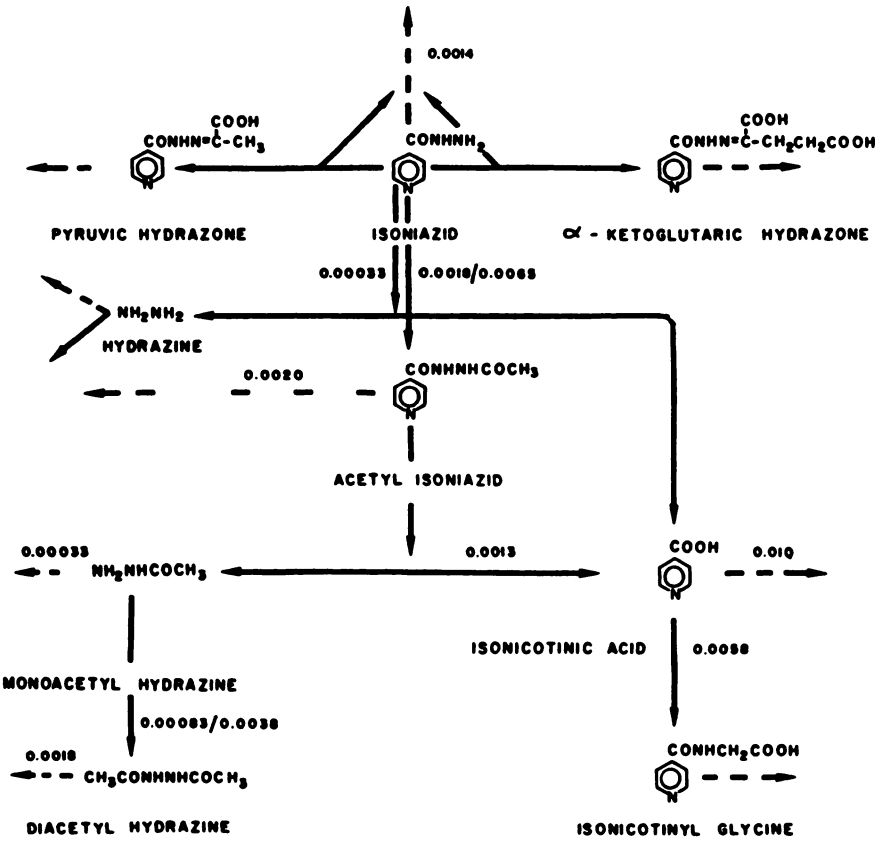


FIG. 3. Pathways for the metabolism of isoniazid in man. First order rate constants (min^{-1}) for the polymorphic acetylation steps were determined for one slow and one rapid acetylator, respectively (—). Renal elimination (- - -) modified from Ellard and Gammon (118). Reproduced with permission from Weber and Hein (433).

TABLE 1

*Apparent first-order rate constants for elimination of isoniazid and its metabolites in urine**

Compound	Dose (mg)	Process	First-Order Rate Constant (min^{-1})	
			Slow acetylator	Rapid acetylator
INH	250	Acetylation†	0.0018	0.0065
Acetylisoniazid	500	Excretion	0.0023	0.0018
		Hydrolysis to isonicotinic acid	0.0011	0.0015
Isonicotinic acid	25	Excretion	0.0080	0.0082
	25	Conjugation with glycine	0.0060	0.0065
Monoacetylhydrazine	74	Excretion and hydrazone formation	0.00033	0.00033
	74	Acetylation†	0.00083	0.0038
Diacetylhydrazine	116	Excretion	0.0017	0.0020

* Modified from Ellard and Gammon (118).
† Polymorphic acetylation steps.

TABLE 2

*Plasma concentration and urinary excretion of INH and acetylisoniazid by rapid and slow INH acetylators after an oral INH dose**

	Time (h)	Acetylator Phenotype			
		Slow (12 subjects)		Rapid (5 subjects)	
		Mean	Range	Mean	Range
plasma concentrations		μg/ml			
Isoniazid	3	5.83	3.49–8.06	2.10	1.50–3.34
Acetylisoniazid	3	1.88	1.50–2.49	4.32	3.36–5.16
Acetylisoniazid/isoniazid	3	0.24	0.19–0.36	1.57	1.18–2.26
Urinary excretion		Molar Ratios			
Acetylisoniazid/acid-labile isoniazid†	2.5–3.5	0.57	0.39–0.73	3.29	2.79–3.89

* 20 mg isoniazid/kg, 0.7; modified from Ellard and Gammon (118).
† Isoniazid plus acid labile hydrazones.

in slow acetylators (fig. 3 and table 1). Pronounced differences in the plasma concentration and urinary excretion of both INH and acetylisoniazid occur in rapid and slow acetylators (table 2).

Acetylisoniazid is metabolized to isonicotinic acid and MAH (115). This pathway is the main route of formation of isonicotinic acid which is conjugated with glycine and excreted. Individuals differ in their glycine conjugating

capacity, but these differences are unrelated to acetylator status. MAH is acetylated to diacetylhydrazine. Rapid acetylators acetylate a much greater fraction of MAH (27.6% ± 1.5%) than slow acetylators (7.9% ± 0.9%). Comparison of a rapid and a slow acetylator shows that the rate of acetylation of MAH was at least 4 times greater for the rapid acetylator than the slow acetylator. Thus, MAH like INH, is polymorphically acetylated in man. These observations are consistent with observations made in the rabbit model of the human acetylation polymorphism (see section VI B).

Hydrazine has been measured as a metabolite of INH (283). It has been detected in plasma of rapid and slow acetylators within an hour after ingesting ordinary doses (300 mg) of the drug, and, in addition, accumulation occurred in slow acetylators after a dosing period of 2 weeks. Although free hydrazine is produced during INH metabolism further studies are needed to determine its significance and mechanism of formation.

Ingested INH is rapidly and completely absorbed and appreciable first pass metabolism occurs. This results in two- to four-fold differences in the concentration of INH and acetylisoniazid in plasma of rapid and slow acetylators. For many drugs which are ingested, first pass effects are usually attributed to their rapid uptake or metabolism by liver. For INH this effect may be primarily due to acetylation of the drug by the mucosal cells of the small intestine, the main site of INH absorption, and differences in acetylating capacity are expressed there. This is supported by the fact that serum INH concentrations are identical in rapid and slow acetylators after intravenous administration of the drug. For instance, in one study serum INH concentrations were 8.0 ± 1.9 μg/ml vs 8.0 ± 1.7 μg/ml in 20 slow and 23 rapid acetylators (204).

Whole body clearance studies conducted on patients from different races and different countries showed that INH elimination rates are more than 10 times faster in rapid compared to slow acetylators. The half-lives of INH for rapid acetylators ranged from 35 to 110 min, whereas they ranged from 110 to more than 400 min in slow acetylators.

The distribution of INH is unrelated to acetylator status; the drug is distributed into total body water, and the mean apparent distribution volumes in 36 rapid and 44 slow acetylators were nearly identical in yielding a combined estimate of 61% ± 11% body weight (205, 116).

The rate of plasma renal clearance of "acid-labile" INH (INH plus hydrazones that decompose to INH in mildly acidic solution) was 46 ± 3 ml in 12 slow and in five rapid acetylators, and was nearly proportional to INH plasma concentration. The renal clearance was unaffected by the time of measurement after dosing (3 or 6 hr) or by acetylator status. Renal clearance of a similar magnitude had been observed in another study (205). Because INH and its acid-labile hydrazones are interconvertible, an unambiguous value for the plasma renal clearance of unchanged INH could not be calculated. The clearance of isonicotinic acid and isonicotinyl glycine were 457 and 493 ml/min, indicating that they are actively secreted while that for acetylisoniazid is similar to the glomerular filtration rate (111 ml/min), but data are too few to determine a relation between these metabolites and acetylator status.

Estimates of the relative contributions of acetylation and renal excretion to the total body clearance of INH have also been made. Assuming a distribution volume of 43 liters (calculated for a 70-kg person with a distribution volume for INH of 61%) and a renal clearance of 46 ml/min, the renal clearance rate is calculated to be 0.00107 min^{-1}. Further, if the total body clearance rates for INH in slow and rapid acetylators are taken as 0.004 min^{-1} and 0.011 min^{-1}, respectively, it can be shown that renal excretion contributes an average of about one-fourth (27%) to total body clearance of INH in slow acetylators and about one-tenth (11%) in rapid acetylators. Estimates of the same kind for another polymorphically acetylated drug, sulfamethazine, gave virtually identical results (26% and 11% respectively) (303), indicating that the process of renal excretion is much less important than acetylation in eliminating these drugs, even in the slow acetylators.

2. *Hydralazine.* Hydralazine (HP) (fig. 2) is a vasodilator substance that has been widely adopted as an antihypertensive drug since its properties were first recognized in the early 1950s (309).

Shortly after its introduction as an antihypertensive agent, HP was shown to be rapidly and extensively metabolized in humans (309), and to be metabolized by acetylation (265). The presence of the hydrazine group had suggested that HP might be subject to the human acetylation polymorphism (125), but major analytical problems and problems associated with instability of HP and its metabolites cast doubt on the early observations (253). A more complete picture of the fate of HP that took account of differences in human acetylator status was obtained only recently after these problems were resolved (358, 412, 130).

Biochemical studies of HP showed that it disappeared more rapidly in homogenates of fresh human liver biopsies from rapid acetylators compared to slow acetylators. This was the first evidence that the disposition of the drug depended on acetylator status (125). Later, blood concentrations of the drug were found to be higher in slow acetylator patients ingesting the drug than in rapid acetylators (465). Since then, much additional information about the disposition of HP in rapid and slow acetylators has been obtained in support of this concept (253, 372, 373).

Pathways proposed for HP metabolism in human rapid and slow acetylators are complex (fig. 4). Urinary excretion products include HP, N-acetylhydrazinophthalazine

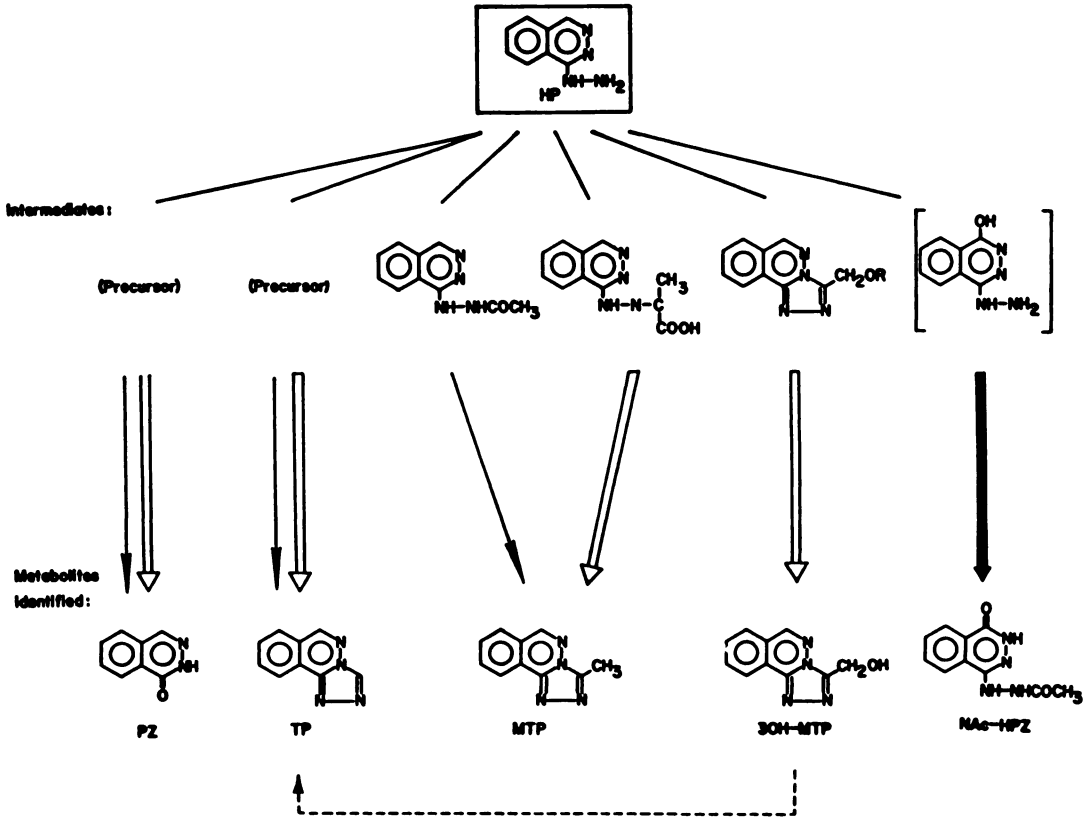


FIG. 4. Pathways for the metabolism of hydralazine in man. Metabolites of hydralazine as identified prior to (↓) and after (⟹) treatment with glucuronidase-sulfatase in the 0- to 24-hr urine of hypertensive patients. Redrawn with permission from Schmid (358).

(NAcHPZ), phthalazin-one (PZ), *s*-triazolo-(3,4-a), phthalazine (TP), 3-methyl-*s*-triazolo-(3,4-a) phthalazine (MTP), and 3-hydroxy-*s*-triazolo-(3,4-a) phthalazine (3-OH-MTP). Uncertainties surround the details of the formation of these metabolites, but several findings seem to be reasonably well established:

1. HP is extensively metabolized with only 1% to 15% of the unchanged drug undergoing urinary excretion, and large variation occurs from one person to another (165, 309, 366, 398, 412, 425, 466). The major circulating metabolite is hydralazine pyruvic acid hydrazone (HPH), but it is converted to another metabolite prior to excretion as only a small fraction of HP is found in this form in the urine.

2. The percentage of the dose excreted as HP metabolites is related to acetylator phenotype, and is significantly higher in rapid acetylators (358).

3. After HP is ingested it undergoes saturable "first pass" metabolism that depends on acetylator status. The fraction of HP that is available in rapid acetylators is only 10% to 16% of the dose compared to 31% to 35% in slow acetylators. This is consistent with observations that slow acetylators have significantly higher peak concentrations, AUCs and steady state concentrations of HP than rapid acetylators after oral doses of the drug in the therapeutic range (465, 214, 372, 373, 396, 397). In contrast, acetylator status has no appreciable influence on the rate of HP elimination after intravenous administration. This is because the formation of HPH contributes so significantly to extrahepatic, acetylator phenotype-independent clearance.

4. Two pathways, both involving acetylation, are postulated for HP metabolism: in one pathway, HP is oxidized to an unstable intermediate which is acetylated to yield NAcHPZ; in the other, HP is acetylated without undergoing oxidation, and the product cyclizes spontaneously to MTP. An oxidation product of MTP, 3-OH-MTP, is also produced.

NAcHPZ is the main metabolite excreted by both acetylator phenotypes, and rapid acetylators eliminate about twice as much NAcHPZ and 3-OH-MTP as slow acetylators. In contrast, excretion of MTP (which has been regarded as the major metabolite of the primary acetylation pathway) is excreted in equally low amounts in both phenotypes. This was an unexpected result because MTP should reflect the acetylator phenotypic difference as 3-OH-MTP does, and this puzzling observation is not yet explained. It has been suggested that it may occur as a result of MTP being involved in another pathway, or pathways, that are acetylator phenotype independent (332, 466). For example, an increase in MTP has been observed from HPH under mildly acidic conditions such as normally occur in the urine (332).

5. Acetylator phenotype related differences in HP me-

tabolism are reflected in the patterns of metabolites arising from pathways other than acetylation. For example, TP amounted to 0.9% of the dose excreted by slow acetylators and 6% of the dose excreted by rapid acetylators, but after enzymatic hydrolysis of urinary products, the pattern was reversed resulting in higher values for TP in slow acetylators (10.9%) than in rapid acetylators (8.4%) (358). In another study (140), qualitatively similar results were obtained.

Hydrazine is a potential metabolite of HP that accumulates in urine. Studies of urinary excretion products of hypertensive patients after ingestion of HP (50 to 200 mg/day) showed that the amount and the percent of the dose at 100- to 200-mg doses that accumulated was significantly greater in slow acetylators than in rapid acetylators. The significance of this observation remains unclear, however, because its concentrations were close to the limit of detection (410).

Thus, acetylator phenotype-related differences in human HP metabolism have been found in acetylation pathways and in pathways other than acetylation. These metabolic differences are reflected in differences of therapeutic importance to rapid and slow acetylators resulting in plasma concentrations of HP which vary by at least 15-fold among individuals with lower concentrations in rapid than in slow acetylators (372). As blood pressure responses correlate directly with plasma HP concentrations and inversely with acetylator indices, the concentration differences appear to be an important factor accounting for poor responses of some patients to conventional oral doses of HP (373). The urinary output of several metabolites, as well as the ratios of certain metabolites, also differ in the two acetylator phenotypes. Thus, measurement of NAcHPZ and the determination of the PZ/TP ratio have both been proposed as a basis for differentiating rapid and slow acetylators that may be more relevant to HP treatment than conventional phenotype tests (358, 130).

3. Phenelzine. Phenelzine (fig. 2) is a monosubstituted derivative of hydrazine that has been used in treatment of neurotic depression because of its monoamine oxidase inhibitor properties.

Since Evans et al. (126) suggested that phenelzine might demonstrate the acetylation polymorphism because of its chemical resemblance to INH and HP, the relation of its metabolic and pharmacological disposition to acetylator status has been of interest to basic (298–300) and clinical investigators (346, 350, 415, 306, 348, 354, 448). Almost all of the early studies assessed the relation of acetylator status to the antidepressant response side effects or the degree of monoamine oxidase inhibition produced by phenelzine in depressed patients without correlating them with drug concentrations because phenelzine and its metabolites could not be measured quantitatively in biological fluids.

In 1976, Johnstone et al. (212, 50) reported that urinary excretion of free phenelzine measured as phenelzine acetonide by a gas liquid chromatography method was significantly greater in 12 slow acetylators compared to 15 rapid acetylators (1575 ± 1138 vs 698 ± 483 μg/24 hr; $P < 0.01$). An oral dose of 1% to 2% (30 mg t.i.d.) was eliminated unchanged in urine. These data were obtained 13 days after starting administration to permit drug levels to reach pharmacokinetic equilibrium, but analytical difficulties were encountered with the acetonide procedure, and attempts to measure phenelzine in plasma of these patients failed. Later these investigators obtained drug excretion profiles in three slow and two rapid acetylator patients (52) which showed a marked change in pharmacokinetics during the course of treatment. The average plasma half-life of the drug in these four patients was 0.87 ± 0.47 hr which was significantly different ($P < 0.05$) on day 1 of treatment from that at the end of treatment 13 days later (3.11 ± 2.49 hr). In addition, the amount of phenelzine eliminated in urine at day 13 (4.67% ± 1.22%) expressed as a percentage of the ingested dose was significantly greater than that eliminated on day 1 (1.14% ± 0.26%) ($P < 0.05$). The prolongation of the half-life and the decrease in urinary excretion of the drug observed was attributed to irreversible inhibition of monoamine oxidase. Whole body clearance of the drug was rapid in all patients studied but the half-lives on day 1 in 3 slow acetylators were faster (ranging from 0.40 to 0.72 hr) than in two rapid acetylators (ranging from 1.03 and 1.61 hr). However, the difference in half-lives associated with acetylator status was not always maintained with the prolongation of the half-lives that occurred by day 13.

Further examination of the relation of acetylator status to the disposition of phenelzine by Tilstone et al. (407) showed a significant correlation ($P < 0.01$) between the amounts of phenelzine and sulfamethazine in urine in 11 rapid and 16 slow acetylators. In contrast, other investigators found that plasma and urinary levels of acetylphenelzine are below the level of detection by a specific gas liquid chromatography-mass spectrophotometric method on patients ingesting a therapeutic dose of phenelzine (60 mg/day). They have concluded that acetylation is not a major pathway in phenelzine metabolism (394, 295).

Pharmacokinetic observations suggest that another reaction, one that may change with phenelzine administration, may compete with acetylation for elimination of phenelzine as has been observed for aminoglutethimide (1). Unfortunately, the phenelzine assay procedure used in these studies did not differentiate the drug from metabolites which retain the hydrazine function (51). Further studies with an improved assay procedure may be warranted to assess this possibility.

C. Secondary Arylamine and Hydrazine Metabolites

1. Sulfasalazine. Sulfasalazine (salicylazosulfapyridine) was introduced into medical practice in 1941 for

treatment of chronic inflammatory disorders (392). It continues to be widely used as a clinically effective drug for ulcerative colitis (395). The drug combines two other agents with anti-inflammatory properties, 5-aminosalicylic acid and sulfapyridine, into a single molecule. The clinical pharmacology of sulfasalazine has been comprehensively reviewed by Das and Dubin (81) and its fate in patients with chronic inflammatory bowel disease has been further examined more recently in Sharp et al. (369) and by Azad Khan et al. (13).

Most of an ingested dose of sulfasalazine transverses the small bowel and approximately 70% reaches the cecum unchanged (81, 13). Absorption of intact sulfasalazine is poor (< 12%) and it is excreted with low renal and biliary clearances; approximately two-thirds of that which is absorbed intact is excreted in urine and one-third in bile. Sulfasalazine serves mainly as a vehicle to deliver its two active constituents to the colon in higher concentrations than could be achieved by ingestion of both separately. In the colon, it is split at the azo-link by bacterial azoreductase into sulfapyridine and 5-aminosalicylic acid. The fate of these two compounds is complex and quite different (fig. 5). Sulfapyridine acetylsulfapyridine-O-glucuronide, 5′-OH-sulfapyridine-O-glucuronide, 5-aminosalicylic acid, and acetyl-5-aminosalicylic acid have been identified as metabolites in humans (363, 81, 83, 84).

Sulfapyridine derived from sulfasalazine is almost completely absorbed. Acetylator status has a pronounced effect on its disposition as was first shown in healthy persons by Schroder and Evans (361, 362). Sulfapyridine also undergoes ring hydroxylation followed by glucuronide conjugation. It is excreted almost entirely (approximately 95%) in the urine as unchanged drug, acetylsulfapyridine, and as the O-glucuronides of these two compounds (361, 362). Acetylator status affects the degree of acetylation of sulfapyridine in serum and urine, though the difference between acetylator phenotypes is largely compensated by another mechanism since the average serum concentration of total sulfapyridine observed is about the same for both phenotypes after ingestion of a single dose of sulfasalazine (table 3, line 6). This compensatory effect was explained by the fact that substantial portions of the sulfapyridine and acetylsulfapyridine undergo ring hydroxylation. The hydroxylated metabolites appear in the blood and urine as O-glucuronides. Both acetylator phenotypes hydroxylated and glucuronidated sulfapyridine to about the same extent (table 3, line 9). The rapid acetylator transforms most of the poorly excreted sulfapyridine into acetylsulfapyridine and acetylsulfapyridine-O-glucuronide, whereas the slow acetylator excretes a greater proportion of unacetylated sulfapyridine and sulfapyridine-O-glucuronide in approximately equal quantities. However, the renal clearances for these compounds differ greatly as follows: sulfapyridine, 10 ml/min; acetylsulfapyridine, 30 ml/min; sulfapyridine-O-glucuronide, 200 ml/min; and acetylsulfapyridine-O-glucuronide, 64 ml/min. The high concentration of sulfapyridine accumulated in the slow acetylator is presumably decreased by more extensive formation and rapid excretion of the sulfapyridine-O-glucuronide.

These effects of acetylator status on the single-dose pharmacokinetics of sulfasalazine in healthy subjects have been confirmed in several other studies (21, 89, 370). Additional observations have shown that the average half-life of sulfapyridine after a 2.0-g dose of sulfasalazine is approximately twice as long in slow acetylators (10.6 to 16.7 hr) as in rapid acetylators (6.0 to 6.3 hr) (21). It has also been shown that acetylsulfapyridine is cleared by the kidney about twice as fast as sulfapyridine, irrespective of acetylator phenotype, and that the total body and extrarenal plasma clearances of sulfapyridine are substantially higher in rapid acetylators than in slow acetylators (21).

Studies in patients with chronic inflammatory bowel disease show that the absorption, metabolism, and excretion of sulfasalazine is similar to that in healthy persons (83–85, 364). Studies carried out in these patients under steady state conditions show further that the influence of acetylator status on sulfasalazine disposition closely resembles the disposition after ingestion of a single dose of the drug (89, 370). In a study of acetylator phenotype and serum concentration relationships, acetylator phenotype (and percent acetylation) influenced sulfapyridine concentrations but not total serum sulfapyridine concentrations. For instance, 19 slow acetylators had a significantly higher average serum concentration of sulfapyridine (21.9 ± 14.0 μg/ml) than nine rapid acetylators (8.8 ± 4.3 μg/ml) receiving comparable oral doses of sulfasalazine, but the average serum concentrations of total sulfapyridine (sulfapyridine + acetylsulfapyridine) were not significantly different in the slow acetylators (29.9 ± 18.5 μg/ml) compared to the rapid acetylators (21.2 ± 10.0 μg/ml) (one-way analysis of variance, $P < 0.05$). These findings are in agreement with two previous studies on healthy volunteers (362, 363) but differ from results of a study of 27 patients with chronic inflammatory bowel disease (82–84). Das et al. (82–84) reported that both total sulfapyridine and sulfapyridine metabolites were higher in slow compared to rapid acetylators. Statistical analysis of the data from these patients suggested that there were relationships between drug concentrations and certain study variables (percent of acetylation, daily dose, time elapsed since the previous sulfasalazine dose, brand of sulfasalazine ingested, and clinical state of inflammatory bowel disease). The most important determinant of serum sulfapyridine and total sulfapyridine concentrations was the daily sulfasalazine dosage whether all patients were considered together or whether slow and rapid acetylators were considered separately (370). Further analysis suggested

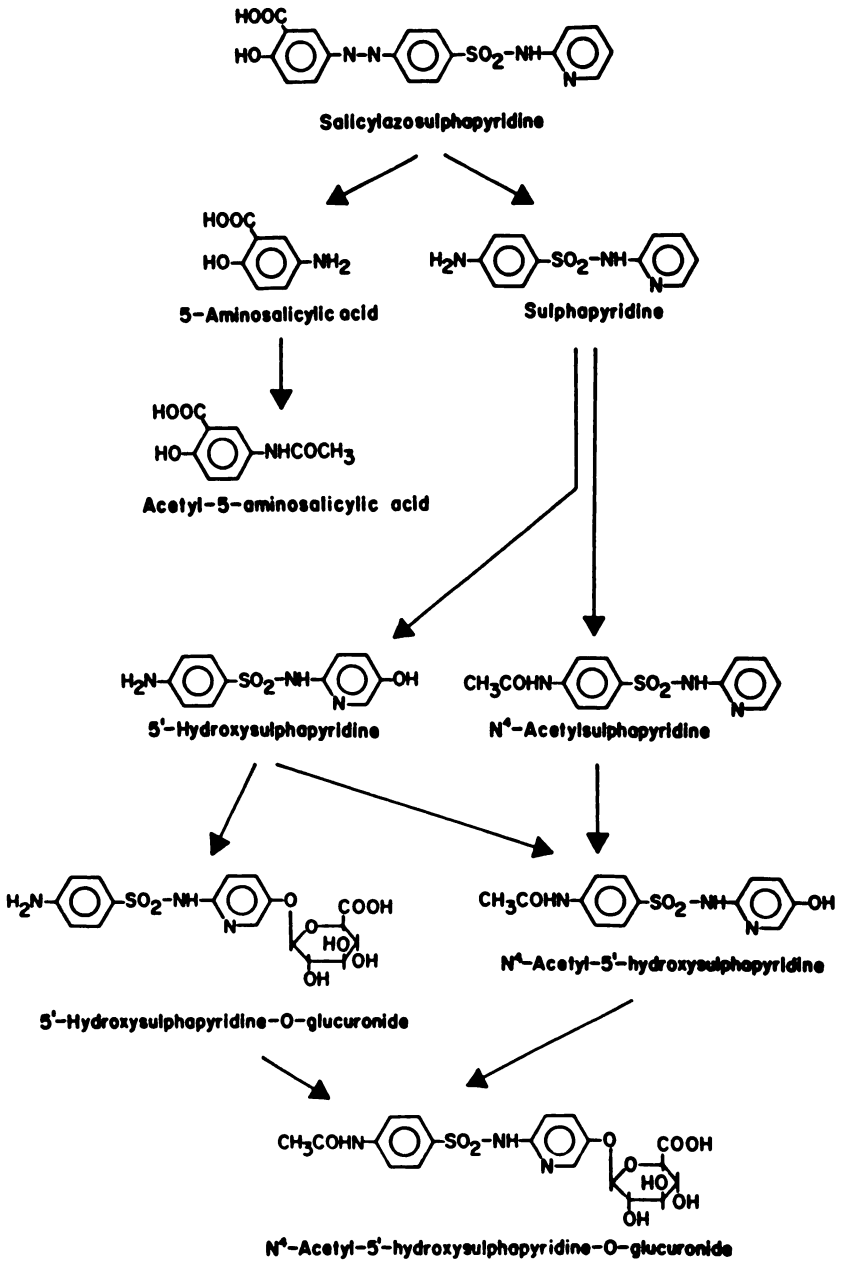


FIG. 5. Pathways for the metabolism of sulfasalazine in man. Redrawn with permission from Das and Dubin (81).

that these relationships differed with acetylator status. Thus, Cowan et al. (76) had shown that, in active chronic inflammatory bowel disease, an equal number of patients had serum levels of total sulfapyridine metabolites above and below 20 μg/ml. Sharp et al. (370) found that total serum sulfapyridine concentrations ranged from 5.7 to 95.1 μg/ml in patients with controlled disease and from 14.0 to 54.7 μg/ml in patients with active disease. Furthermore, Sharp found that the clinical disease state correlated with serum drug concentrations only in rapid acetylators ($P < 0.05$)—i.e., those rapid acetylator patients with controlled disease generally had higher serum sulfapyridine and total sulfapyridine concentrations than those with active disease. A definite relation between serum sulfapyridine levels and therapeutic response was thus not unequivocally established.

Several groups of investigators have found that salivary excretion and pharmacokinetics of sulfasalazine and sulfapyridine correlate well with those in plasma of healthy volunteers (21, 89) and in patients with chronic inflammatory bowel disease (89, 370). In a study of three slow and two rapid acetylator healthy male subjects who had ingested 2.0 g of sulfasalazine, the half-life and total body clearance of sulfapyridine differed appreciably (21). The saliva/plasma concentration ratio for sulfapyridine was 0.559 ± 0.027 (mean of five subjects ± S.E.) and was independent of plasma concentration and saliva pH. The mean saliva/plasma concentration ratio for acetylsulfapyridine was lower (0.246 ± 0.056), and showed consid-

TABLE 3

*Comparison of mean values for slow and rapid acetylators for sulfamethazine (sulfadimidine) and sulfapyridine**

Line	Experimental Data	Mean and Standard Error of the Mean for: Slow acetylators (n = 28)	Rapid acetylators (n = 22)	t†	p*
	Sulfadimidine				
1	Percentage acetylation in serum	18.8 ± 0.8	64.8 ± 1.5		
2	Serum concentration of total sulfonamide (μg/ml)	12.9 ± 0.5	8.6 ± 0.4		
3	Percentage acetylation in urine	58.6 ± 1.5	89.7 ± 0.8		
4	Urinary excretion, percentage of the dose	10.8 ± 1.0	15.5 ± 1.2		
	Sulfapyridine				
5	Percentage acetylation in serum	24.3 ± 0.8	58.0 ± 0.9	25.22	<0.0005
6	Serum concentration of total sulfonamide (μg/ml)	4.7 ± 0.1	4.7 ± 0.2	0.25	NS
7	Percentage acetylation in urine	42.8 ± 1.1	76.5 ± 1.0	21.53	<0.0005
8	Urinary excretion of total sulfapyridine metabolites, percentage of the dose	9.2 ± 1.0	12.5 ± 0.9	2.28	0.027
9	Percentage hydroxylation in urine‡	56.7 ± 1.6	60.6 ± 1.9	1.56	NS

* Modified from Schroder and Evans (361).

† Differences between slow and rapid acetylators were tested by Student's *t* test.

‡ Two extremely poor hydroxylators (one of each acetylator phenotype) were omitted from the calculations. When they were included the *t* value was found to be 1.11.

erably more intrasubject variation than the ratio for sulfapyridine. Thus, while assessment of acetylator status according to the percentage of acetylated sulfapyridine in serum provides a clear distinction between rapid and slow acetylators, the percentage of acetylsulfapyridine in saliva is a less precise index of acetylator phenotype. Support was obtained for these results in another study involving 13 slow and 15 rapid acetylator bowel disease patients (21, 89). The mean saliva/serum concentration ratio for sulfapyridine (0.55 ± 0.09) and for acetylsulfapyridine (0.18 ± 0.12) found in 28 patients were also similar to those reported for healthy subjects (370), but sulfapyridine and acetylsulfapyridine concentrations in saliva did not provide a reliable estimate of serum concentrations. The uncertainties associated with results obtained in different laboratories has left the validity of monitoring serum sulfasalazine concentrations via measurements of sulfapyridine concentrations in saliva in doubt.

When sulfasalazine therapy is initiated in patients with chronic inflammatory bowel disease and a steady state (3 to 5 days) has been achieved, knowledge of acetylator status provides a more reliable prediction of serum sulfapyridine concentrations than would be possible from expectation based only on sulfasalazine dosage. Once the acetylator phenotype is known, knowledge of the specific percentage of acetylation does not increase the accuracy of prediction of serum sulfapyridine concentrations further. Also, as sulfapyridine-O-glucuronide and acetylsulfapyridine-O-glucuronide make up only a relatively small part of the serum total sulfapyridine, these need not be ascertained in determining the acetylator phenotype. A specific HPLC method of measuring sulfapyridine and acetylsulfapyridine derived from sulfasalazine provides a rapid and reliable method of establishing acetylator phenotype (369).

The metabolism and disposition of 5-aminosalicylic acid, has been investigated much less extensively than that of sulfapyridine, even though 5-aminosalicylic acid is believed to be the effective moiety in treatment of chronic inflammatory bowel disease with sulfasalazine (11, 420). It is subject to acetylation (329, 133) (fig. 6). Studies in rats (368) have shown that 5-aminosalicylic acid is absorbed from the upper small bowel and that it is predominantly excreted in urine and bile. In the study by Rasmussen et al. (331) of 14 healthy volunteers who took 1500 mg of slowly released 5-aminosalicylic acid per day for 6 days, acetyl-5-aminosalicylic acid reached a steady state concentration in plasma (1.1 to 2.9 μg/ml), but 5-aminosalicylic acid was not detected. At steady state, 93% of the 24-hr dose was recovered in feces (40%) and urine (53%). Neither the plasma concentrations nor their excretion patterns at steady state differed in seven rapid and seven slow acetylators. The lack of any difference in acetylation of 5-aminosalicylic acid in human rapid and slow acetylators agrees with predictions made from observations with the monomorphic substrates in

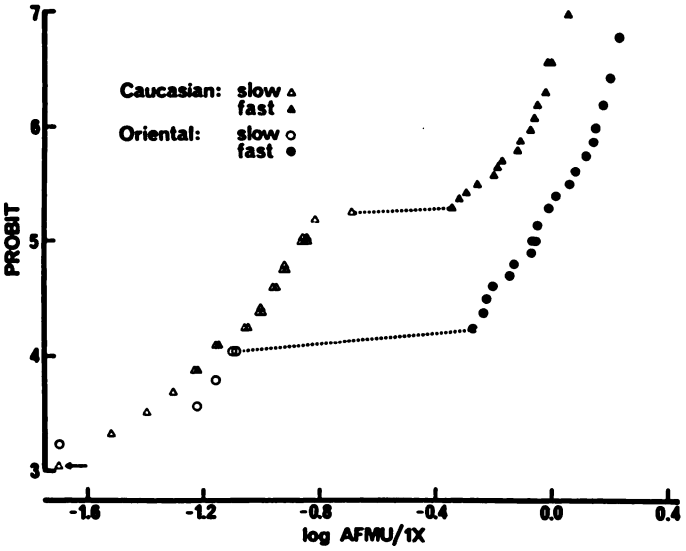


FIG. 6. Probit transformation of human caffeine metabolic data in Caucasian and Oriental subjects. Reproduced with permission from (160).

the rabbit model for the human polymorphism (see section VI B).

2. Nitrazepam and Clonazepam. Nitrazepam (1,3-dihydro-7-nitro-5-phenyl-2H-1,4-benzodiazepin-2-one) (fig. 2) is a drug that is widely used in treatment of insomnia and anxiety. It is metabolized in man in part by enzymic nitroreduction to an amine and followed by acetylation of the amine to the amide (28). Studies in a group of 25 healthy volunteers (eight slow and 17 rapid acetylators) showed that the latter step is controlled by the acetylation polymorphism (218). Whether there is an effect of polymorphic acetylation on the hypnotic effects of nitrazepam or its adverse effects does not seem to be reported.

Clonazepam is an anticonvulsant drug effective in treatment of infantile spasms and petit mal, myoclonic and atonic seizures. It is also effective in treatment of status epilepticus if given intravenously. Chemically, clonazepam is similar to nitrazepam (fig. 2). It is metabolized in man by nitroreduction, acetylation, and hydroxylation (123). In 12 healthy volunteers (six rapid and six slow acetylators) clonazepam was polymorphically acetylated (278). Recovery of urinary metabolites after ingestion of 2 mg of clonazepam showed that less than 1% of the drug was excreted unchanged irrespective of acetylator phenotype in confirmation of previous studies (130, 406). Recovery of the N-acetyl metabolite from the six slow acetylators was 22.7% ± 5.0% compared to 13.6 ± 4.1% ($P < 0.001$) from six rapid acetylators. Recovery of this metabolite was also significantly less in the slow acetylator (1.5% ± 0.4%) than in the rapid acetylator (3.9% ± 1.8%) ($P < 0.01$). The mean ratio of the amine/acetyl metabolite was 15.4 ± 4.2 in the slow acetylators compared to 4.1 ± 2.0 in the rapid acetylators.

Peng et al. (307) confirmed the polymorphic acetylation of 7-amino-clonazepam through studies in cytosolic preparations from human adult livers. Mean N-acetyltransferase activities in livers from 11 rapid and three slow acetylator subjects were 117 ± 11 and 27 ± 16 pmol/min/mg of protein, respectively, ($P < 0.01$). In further studies with 10 fetal human liver specimens, N-acetyltransferase activities varied between 0 and 1.1 pmol/min/mg of protein and there was no evidence of a bimodal distribution.

3. Acebutolol. Acebutolol (fig. 2) [1-(2-acetyl-4-butyramido-phenoxy)-2-hydroxy-3-isopropylaminopropane] is a competitive beta-adrenergic receptor antagonist effective in treatment of cardiac arrhythmias. A sensitive, specific HPLC method of analysis for acebutolol (271) revealed that the N-acetylated derivative was a major metabolite in man.

Upon prolonged acebutolol ingestion by seven cardiac patients, the N-acetyl metabolite was present in concentrations several times larger than the concentrations of acebutolol (452). Under steady state conditions, the mean value of the acebutolol acetyl metabolite/acebutolol ratio was 2.7 ± 1.0 (range 1.7 to 4.1). In contrast to the high plasma concentration of the N-acetyl metabolite after ingestion of the drug, the plasma concentration of the N-acetyl metabolite after its intravenous administration was lower than the unchanged drug (270). This pattern corresponds to a plasma clearance of acebutolol of approximately 300 ml/min, or about 40% of the hepatic plasma flow. Because only about 15% of the drug is excreted unchanged in the urine (270), most of the clearance is extrarenal and probably hepatic in origin.

The effect of acetylator status on the production of the acetyl metabolite was assessed in eight healthy volunteers (five slow and three rapid acetylators) (163). After ingestion of a single dose of acebutolol (400 mg), the total amounts of N-acetyl metabolite measured in the 0- to 12-hr urine ranged from 24.6 to 44.1 mg in the five slow acetylators and from 24.6 to 37.8 mg in the three rapid acetylators. The ratio of the N-acetyl metabolite/acebutolol ranged from 0.69 to 1.33 and from 0.82 to 1.06 in slow and rapid acetylators respectively. Thus, in the only study which has been reported, no correlation between acetylator status and the metabolic fate of acebutolol was found.

4. Caffeine. The sources and uses of caffeine (1,3,7methylxanthine) (fig. 2) are many and varied. It is a natural constituent of various foodstuffs and a common additive in others. It is used in various prescription drugs and in "over-the-counter" preparations, and is reputed to be the most widely consumed drug in the United States and Europe (79). Until recently, progress in investigating caffeine metabolism has been slow because of its complexity and because caffeine is converted to a large number of metabolites, many of which were inseparable by available analytical techniques. The fact that caffeine metabolism was found to exhibit an unusually large maturational component stimulated much new investigation of the drug during the past decade. Newborn infants are relatively incapable of metabolizing caffeine, eliminating as much as 85% of the dose unchanged in the urine, but their metabolic capacity has matured sufficiently by 7 to 9 months of age so that the amount of the unchanged drug excreted in the urine (1% to 2%) and the pattern of metabolites excreted both closely resemble that in adults (5).

Several reports have verified that caffeine undergoes oxidative demethylation and hydroxylation reactions mediated by microsomal mono-oxygenases containing P-450 enzymes, and that dimethylxanthine also undergoes ring cleavage yielding substituted uracil metabolites (5, 10, 36, 53, 240). Recently, Yesair and his colleagues (54) found that more than 90% of an administered dose of radiolabeled caffeine could be recovered in the urine. More than 95% of the urinary radioactivity could be identified as specific metabolites, a substantial improvement over prior studies which had accounted for less than 70% of the dose (75, 393). Partial resolution and quantitative measurement of two radiolabeled polar me-

tabolites, (A_1 and A_2), which together accounted for 10% to 40% of the radiolabeled caffeine administered, was a particularly interesting observation from Yesair's investigations. The percentage of A_1, the more abundant of these two metabolites, varied remarkably from one subject to another (7% to 35%). It was also a highly reproducible characteristic of the individual. A_1 was an N-acetylated derivative of caffeine with a structure corresponding to 5-acetylamino-6-amino-3-methyluracil (AAMU). The latter compound was also a component of human urine long known to be associated with ingestion of diets containing caffeine and also with caffeine-free diets, but this appeared to be the first report establishing caffeine as one of its primary sources.

Complete structural identification of metabolite A_2 was not possible because of its lability during purification, but it appeared to be closely related to AAMU (54). Kalow's laboratory (400) reported UV, NMR, and mass spectral data from which they suggested the A_2 metabolite was a formylated intermediate, having the structure of 5-acetyl-6-formylamino-3-methyluracil (AFMU). They also found that AFMU underwent deformylation under certain conditions to AAMU.

Knowledge of human caffeine metabolism has thus advanced at a rapid pace during the past few years, but recent reports indicate that there are additional pathways in man not yet completely known. During the course of Kalow's studies (159–161) to assess the extent of variability in human caffeine metabolism, 1-methyluric acid (1U), 1-methylxanthine (1X), 1,7 dimethyluric acid (17U), and 1,7 dimethylxanthine (17X) were the chief metabolites of caffeine recovered in the urine. Lesser amounts were recovered as 7-methylxanthine (7X), 3-methylxanthine (3X), 1,3 dimethyluric acid (13U), 3,7-dimethylxanthine (37X), and 1,3-dimethyluric acid (13X). Other metabolites [3-methyluric acid (3U), 7-methyluric acid (7U), 3,7-dimethyluric acid (37U) and caffeine (137U)] were generally below the limits of detection.

Urinary excretion of the new uracilic acid metabolite, AFMU (400), was highly variable in healthy subjects and followed a bimodal distribution pattern. In a study of 68 healthy persons including 42 Caucasians and 26 Orientals (159), the mole ratio of AFMU/(1U + 1X + 17U + 17X + AFMU) was used as an index to differentiate high and low AFMU excretors—persons producing less than 8% of AFMU were classified as low excretors and those producing more than 8% were classified as high excretors. Low excretors made up 19.2% of the Oriental sample and 59.5% of the Caucasian sample. Both the high degree of variability associated with AFMU excretion and its bimodal intraethnic variability are illustrated by the probit plot in figure 6. The modal frequencies observed corresponded closely to those derived from acetylator allele frequencies previously reported for various Caucasian and Oriental populations (see section IV C). The average percentage recovery of AFMU with each excretor subgroup did not differ ($P < 0.05$) as recoveries were 3.2% ± 1.4% and 2.2% ± 0.9% for low excretors and 17.9% ± 4.6% and 20.2% ± 4.2% for high excretors. The segregation of 20 subjects as high and low AFMU excretors after a single 300-mg dose of caffeine was identical to that for rapid and slow sulfamethazine acetylation (χ-squared analysis = 16.20, $P \ll 0.001$, for n = 20). The correlation between the plasma indices for sulfamethazine acetylation and AFMU excretion was also highly significant with a non-parametric (Spearman) rank correlation coefficient of 0.881 ($P < 0.001$, n = 20). The high degree of concordance between AFMU excretion and sulfamethazine acetylation in all subjects tested strongly suggests that the acetylation polymorphism is influential in the formation of AFMU. Results consistent with those obtained in Kalow's laboratory have been reported for a few subjects by Yesair's laboratory (54).

The pathway(s) of formation of the N-acetylated metabolite AFMU have not yet been completely elucidated but some clues to the origin of this substance have been obtained from metabolite ingestion studies performed in Kalow's laboratory with a single rapid acetylator (160). The results indicate that AFMU was formed after ingestion of caffeine and of 17X, but not after several other metabolites (37X, 13X, 1X, or 17U) (fig. 7). Since the structure of AFMU suggests that demethylation of 17X must occur, it was proposed that AFMU is formed from an unstable intermediate (possibly ring-opened) arising from 7-demethylation. In rapid acetylators the intermediate is presumably acetylated quickly and thus stabilized in an open-ring structure. In a slow acetylator, however, the unknown intermediate tends to reclose to form metabolite 1X. Finding that slow acetylators excreted more 1X + 1U (27.4% ± 7.6% of dose) than did rapid acetylators (17.7% ± 7%, $P < 0.001$, Student's t test) tends to support their proposal although the overall excretion of AFMU + 1X + 1U did not differ between the groups. (* See footnote added in proof on p. 79.)

D. Arylamine Mutagens and Carcinogens

Occupational exposure to arylamines has long been associated with the development of cancer in man. Many arylamines have been shown to be carcinogenic, and benzidine, beta-naphthylamine, 4-aminobiphenyl, and methylene bis-chloroaniline (fig. 2) are some examples of these compounds that are widely used in the manufacture of plastics and dyes. Since these chemicals usually induce tumors in excretory tissues such as the liver, urinary bladder, and intestinal tract, rather than at sites of administration, their carcinogenic activity was considered to be largely dependent on metabolic activation by enzymes of the host (275). Now the role of metabolic factors as important determinants of species and individual susceptibility to arylamine-induced tumors is generally accepted.

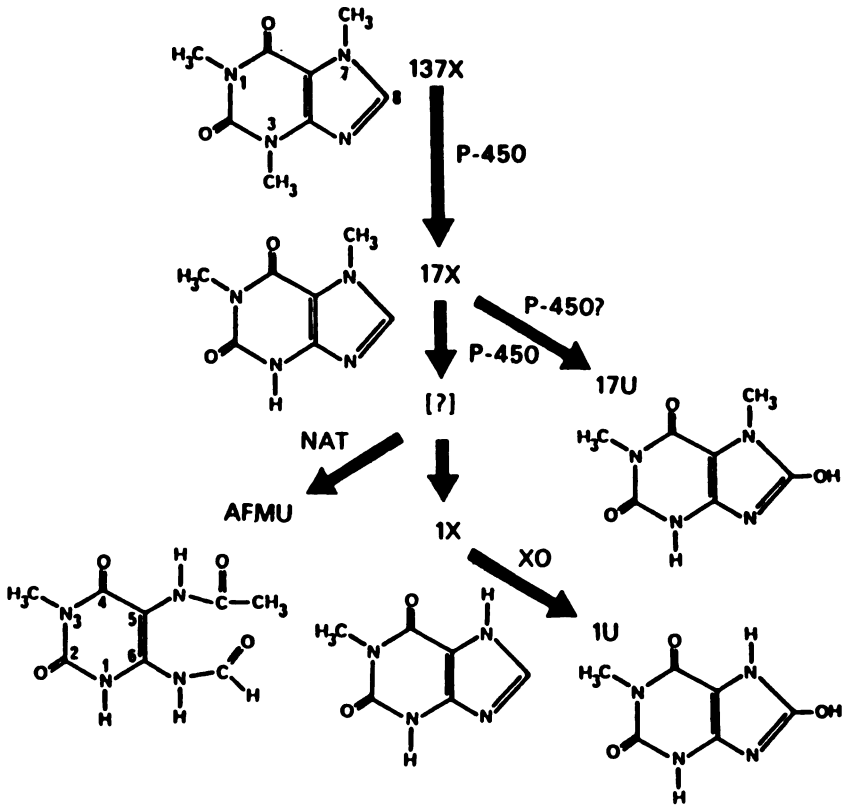


FIG. 7. Proposed pathways of formation of major human caffeine metabolites. Reproduced with permission from Grant et al. (161).

Benzidine and beta-naphthylamine have provided much of the evidence implicating aromatic amines as causes of occupational cancer in man, but they have been relatively less used in fundamental studies. In contrast, 2-aminofluorene (AF) and its N-acetyl derivative, which have no commercial application, have been extensively studied in many laboratories (442). Many different enzymatic reactions contribute to the metabolic activation of arylamines in mammalian species (387). Among these, N-acetylation, N-deacetylation, N-hydroxylation, and conjugation are the chief contributors to the activation of the arylamine to toxic (carcinogenic) substances (68, 273), while others such as ring hydroxylation result in the formation of relatively less toxic substances (441).

Differences in susceptibility of tissues to arylamine carcinogenesis are related to differences in metabolic activation by these pathways. Cramer et al. (78) had demonstrated that enzymatic N-hydroxylation yielded N-hydroxyarylacetamides (arylhydroxamic acids) that were more potent than the parent carcinogens, but even so, were still relatively unreactive with tissue macromolecules (78, 274). The concept that enzymatic acetylation might be an important modulator of tissue susceptibility differences emerged from a series of investigations in animals that began in the 1960s. The studies of Poirier et al. (323) showed that dogs develop tumors of both liver and urinary bladder when they are administered acetylated arylamines. However, they develop only urinary bladder tumors when they are adminstered unacetylated arylamines. Since the dog, unlike most other mammalian species, including man, is incapable of acetylating the amino group of AF and other arylamine bladder carcinogens (451, 249), these observations suggested that N-acetylation might be required for liver carcinogenesis, but not for urinary bladder carcinogenesis. Observations by several other investigators provided further support for this concept (90, 277, 198, 91, 249, 250). Subsequently, King and Phillips (225, 226) reported that enzymatic conjugation of the arylhydroxamic acids with acetate, sulfate, or phosphate groups yielded very reactive electrophiles that were capable of binding covalently to proteins and nucleic acids. Then Bartsch et al. (18, 19) and King and his associates obtained evidence that an N,O-arylhydroxamic acid acyltransferase (AHAT) mediated the rearrangement of the arylhydroxamic acid (N-OH-AAF) to an N-acetoxyarylamine (N-OAc-AF). The latter metabolite was highly reactive and capable of reacting spontaneously with macromolecules of the liver and mammary gland (224, 227).

At present, a preponderance of evidence suggests that metabolic activation by acetyl transfer accounts for much of the adduct formation between arylhydroxamic acids and the nucleic acids of liver, gastrointestinal tract, and mammary gland. The evidence for this concept is largely indirect and is based mainly on the extensive loss of the acetyl group on adduct formation in these tissues with

N-OH-4-acetylaminobiphenyl and the N-OH-acetylaminofluorene; structural identification of the products formed has been hindered by instability of the arylamine-substituted nucleic acid adducts. Evidence implicating AHAT in tumor formation is strongest for the mammary gland. This tissue has no activity for deacylase or for sulfate conjugation. Furthermore, a single direct application of N-OH-AAF results in tumor formation at the site of application (257, 258). It also receives support from evidence that AHAT catalyzes the formation of reactive derivatives that form nucleic acid adducts, that AHAT is distributed in tissues that develop tumors from arylamines, and that there is a direct correlation between these two sets of observations (222). However, AHAT cannot account for all arylamine-induced tumors because it is found in organs that do not respond to arylamines, and it is undetectable in the dog which does develop bladder and liver tumors on administration of AAF.

A more comprehensive assessment of the activation of arylamines to bladder carcinogens was provided by Radomski and coworkers (327) who demonstrated the urinary occurrence of N-glucuronides of non-acetylated N-hydroxylamines, and by Kadlubar et al. (216, 217) and by Poupko et al.(325) who demonstrated the formation of this hydroxylamine conjugate in liver. The glucuronide conjugate, which is relatively stable at neutral and alkaline pHs, hydrolyzes at weakly acidic pHs encountered in the urinary bladder of some species. The hydroxylamine which is reformed presumably loses water spontaneously to yield an electrophilic arylnitrenium ion that binds DNA and initiates carcinogenesis.

A lucid summary of the conceptual foundation of the activation process based upon these observations has been presented by Lower (252). He suggests that activation by which arylamines produce cancer of the liver and mammary gland versus that which produces urinary bladder cancer proceeds by interrelated but separate parallel pathways as shown in figure 8. One of these pathways results in the formation of the hepatic carcinogen, and possibly also the mammary carcinogen, while the other results in the formation of the bladder carcinogen. In the former pathway, an arylamine such as AF is N-acetylated to AAF by cytosolic liver NAT. This is followed by enzymatic hydroxylation by a microsomal P_1-450 (P-448) mixed-function oxidase. The hydroxamic acid (N-OH-AAF) that is produced can be conjugated with sulfate to yield a potent hepatic carcinogen. It can be deacetylated by AHAT, which has been implicated in both liver and mammary gland cancer, to a reactive N-acetoxyarylamine that can react with nucleic acid in these target tissues. In the latter pathway, microsomal N-hydroxylation is followed by glucuronide conjugation to form N-hydroxylamine-N-glucuronide. This metabolite is believed to decompose in the bladder to a reactive arylnitrenium ion which combines with nucleic acid as described previously.

These investigations have gone far toward identifying the nature of the ultimate carcinogen and in discerning how the carcinogenic process induced by arylamines is initiated. No doubt our knowledge of this process is yet incomplete, but a consensus appears to have been reached about the main events and the general scheme of activation. Investigators have thus been able to focus more intensively on other factors affecting the outcome. Only for the past decade or a little longer has the significance of heredity been demonstrated as a factor capable of introducing remarkable variability into this process. Several reactions occur in the metabolism of arylamines where hereditary variation would not be surprising. Animal studies have shown that all of the major metabolic steps in this pathway—N-acetylation (434), N-hydroxylation (406, 131), sulfation (255, 33), and deacetylation (193)—have been identified with variability that is to a greater or lesser extent genetically conditioned.

Animal models are essential research tools for such studies. Selection of the most appropriate species in which to evaluate the effect of heredity on mechanisms of activation of arylamine carcinogens poses a problem because the metabolism of a foreign compound in any one species does not faithfully mimic its metabolism in man (145). The dog has been used advantageously but the dog is a species that consists entirely of slow acetylators. It thus cannot be used to assess the effects of individual variation in N-acetylation. The rabbit expresses a genetic polymorphism for the acetylation of numerous drugs and other foreign compounds including the arylamine carcinogens. It confers large, predictable differences in acetylating capacity on individual rabbits. In addition, rabbits are susceptible to cancer from arylamines (37, 195, 455). This suggests that the rabbit might be a better species than the dog for investigating the metabolic activation of arylamines.

A few investigations of the effects of heredity on arylamine carcinogenesis have been performed in the rabbit model (148, 173, 175). Initial rates of acetylation of several typical arylamine carcinogens such as AF, benzidine, and beta-naphthylamine, determined with fresh liver specimens from rabbits of both acetylator phenotypes are shown in table 4. Activity ratios calculated from initial rates among rabbits of both acetylator phenotypes showed that the range of ratios obtained for the carcinogens (190 to 580) overlapped those for a standard substrate of the genetically polymorphic NAT, sulfamethazine. Similar studies have also been performed with fresh human liver biopsies (see table 4) and the activity ratios for human liver showed a trend similar to that for rabbits. Initial rate data and the activity ratios clearly show that the carcinogens are substrates for the polymorphic liver NAT in both humans and rabbits. Apparent K_m values determined for these arylamines were strongly correlated with their octanol-water partition coefficients.

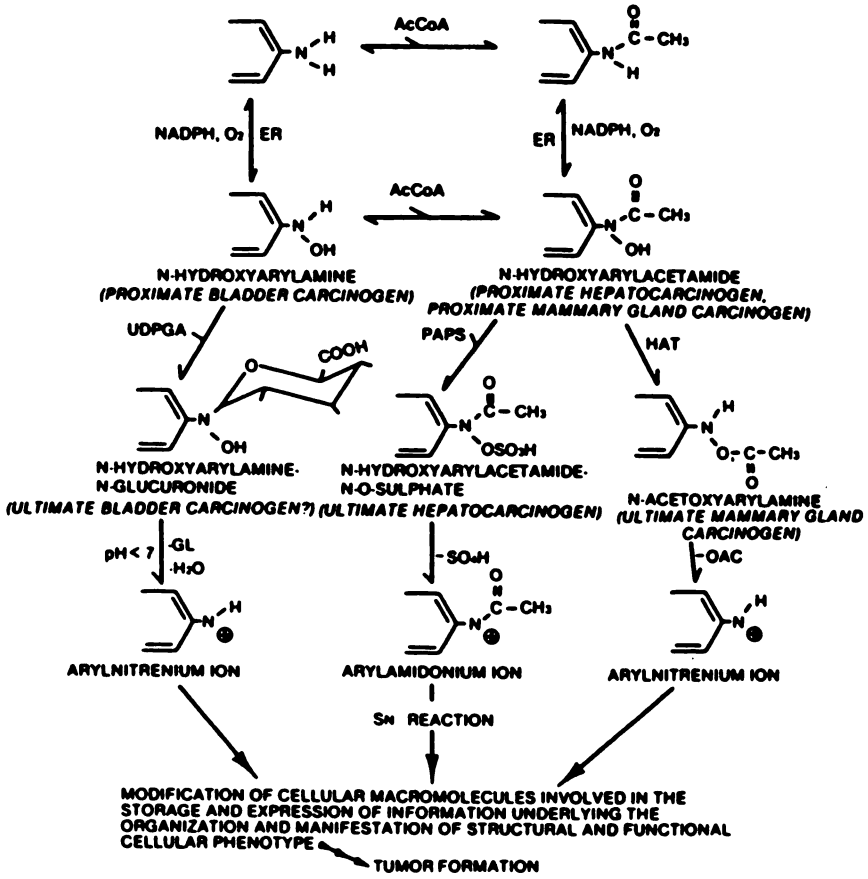


FIG. 8. Parallel pathways proposed for the metabolic activation of arylamines and arylacetamides. Reproduced with permission from Lower (252).

TABLE 4

*N-Acetyltransferase activity in the livers of rapid and slow acetylators among humans and rabbits**

Compounds	Rabbit†			Human		
	Activity, nmol/min/mg		Ratio	Activity, nmol/min/mg		Ratio
	Rapid	Slow		Rapid	Slow	
Test drugs						
Sulfamethazine	6.4	0.037	173	0.84	0.23	3.7
p-Aminobenzoic acid	1.2	0.50	2.4	0.15	0.16	0.94
Arylamine carcinogens						
α-Naphthylamine	12.4	0.023	540	1.2	0.23	5.2
Benzidine	6.5	0.019	315	0.17	0.019	8.9
β-Napthylamine	8.7	0.028	310	0.23	0.026	8.8
2-Aminofluorene	7.5	0.013	580	0.28	0.021	13
Methylene-bis-2-chloroaniline	—†	—	—	0.14	0.015	9.3
Mean			436			9.0

* Modified from Glowinski et al. (148)

† Dashes = not determined.

Enzymatic transfer of the acetyl group occurs twice in the metabolic activation of arylamines such as AF. Acetyl coenzyme A-dependent N-acetylation of the unacetylated arylamine is the first, and the intramolecular transfer of the acetyl group of the hydroxamic acid from the amine nitrogen to the oxygen attached to this nitrogen is the second. In the latter instance, acetyl transfer is independent of acetyl coenzyme A and is mediated by AHAT (see section V B). There are several similarities in the properties of NAT and AHAT and no obvious differences (149). Species variation was remarkably similar since both activities were present in the rabbit, monkey, rat, and mouse. Both were absent in the dog. Distribution of both activities was widespread in tissues and regionally similar. NAT and AHAT activities could be partially purified from liver by procedures with the same steps. Both activities were associated with a cytosolic liver protein that contained essential sulfhydryl groups and were protected by dithiothreitol. Neither was sensitive to inhibition by organophosphates and neither was inducible by barbiturates.

Characterization of the NAT and AHAT activities from rabbit liver (in collaboration with C.M. King) showed that both activities were high in rapid acetylators and both were low in slow acetylators (table 5) and that the difference between these activities was highly significant. These activities and the two acetyl transfer steps thus appeared to be under the same genetic control in the rabbit model. Further evidence for this concept came from a study of inbred rabbit strains in which 11 of 17 strains were rapid and six were slow acetylators for both activities (437). Bartsch et al.(18) had observed a

TABLE 5

*Liver N-acetyltransferase (NAT) and arylhydroxamic acid N,O-acyltransferase (AHAT) from rabbits of specified acetylator phenotype**

Rabbit phenotype	AHAT	NAT	AF‡
	N-OH-AAF	SMZ†	
	nmol/min/mg	*nmol/min/mg*	
Rapid			
	0.802	4.62	1.74
	0.626	3.93	2.64
	0.343	2.85	3.20
	0.330	3.97	2.33
	0.669	3.58	3.65
Slow			
	0.00040	0.019	0.075
	0.00033	0.019	
	0.00021	0.019	0.071

* Modified from Glowinski et al. (149).
† SMZ, sulfamethazine.
‡ AF, aminofluorene.

TABLE 6

*Relationship of acetylator status to chemically induced DNA repair in rabbit hepatocytes**

Chemical	Concentration (μM)	Net Grains/Nucleus of Hepatocytes†	
		Rapid acetylators	Slow acetylators
Benzidine	1	35.4	0.9
	10	>150	2.8
2-Aminofluorene	100	46.8	17.8
	1000	Toxic	41.0
Hydralazine	1000	2.7	34.7

* Adapted from McQueen et al. (267, 268).
† Mean of triplicate determinations in each of three animals.

sixfold variation in AHAT activities in their studies of rabbits. Perhaps this was a reflection of the hereditary difference that we had observed. Further evidence for the relationship of NAT and AHAT came from experiments with preparations of rat tissues that catalyze the acetyl transfer from arylhydroxamic acids to arylamines (35, 18, 221). The capacity of rat tissues to carry out this reaction tended to parallel the levels of AHAT (221), though the differences in transfer rates were smaller than those in rapid and slow rabbits. In addition, NAT and AHAT activities could not be resolved by sequential centrifugation, fractional precipitation with ammonium sulfate, ion exchange chromatography, gel filtration on Sephacryl, or on purification to homogeneity on polyacrylamide gel electrophoresis. Both activities migrated as a single symmetrical protein band with a molecular weight of 33,000 after sodium dodecylsulfate-polyacrylamide electrophoresis. Furthermore, the ability of rabbit liver to catalyze the transfer of the N-acetyl group from N-OH-AAF to 4-aminobenzene was found to be subject to the same genetic determinant as NAT and AHAT. Thus arylhydroxyamic acids are recognized as, and can substitute for, acetyl coenzyme A as an acetyl donor in the acetylation of arylamines. The acetyl coenzyme A-dependent NAT reaction and the intramolecular, N,O-acetyltransferase AHAT reaction thus appeared to be properties of the same enzyme protein in the rabbit, apparently involving the same gene or closely linked genes.

Since the genetic difference in acetylator status reflects a difference in AHAT activity as well as in NAT activity, and since arylamine-nucleic acid adduct formation results directly from the spontaneous reaction between the acetoxyarylamine and nucleic acid, a difference in DNA damage in rapid and slow acetylators might reasonably be expected. An experimental cell system was thus devised (in collaboration with G.M. Williams and C.A. McQueen) by using hepatocytes isolated in primary culture (449) from rapid and slow acetylator rabbits so that measurements of NAT activity and DNA damage could be made in the same cells. Exposure of the hepatocytes to different concentrations of AF showed that DNA damage induced by AF, measured as unscheduled DNA repair, was dose-dependent. In addition, hepatocytes from rapid acetylators were more sensitive to damage than hepatocytes from slow acetylators (table 6). These studies were the first to demonstrate that DNA damage resulting from DNA exposure was related to acetylator status (268). Additional studies have since shown that benzidine is also capable of inducing DNA damage that is related to the acetylator status (table 6) (269). The latter studies also showed that 4-aminobiphenyl and 4,4′methylene-bis-2-chloroaniline were both weakly damaging to DNA in the rabbit hepatocyte system, and under the conditions used no clear correlation with acetylator status could be established. Beta-naphthylamine failed to produce any DNA damage in the same system (269), but AHAT has been observed to be essentially inactive with beta-naphthylamine (18).

The explanation for the difference in extent of DNA damage that is associated with acetylator status in hepatocytes probably lies at least in part in qualitative or quantitative differences in the pattern of reactive metabolites that are formed. However, this remains to be determined.

III. Human Acetylator Status, Drug Toxicity, and Disease

The acetylation polymorphism has important consequences in drug therapy and has made the unwanted effects of certain other environmental chemicals more intelligible. On ordinary therapeutic drug doses, slow acetylators usually have higher serum concentrations of drugs such as INH or HP for any specified period of time, an important consideration in toxicity of many arylamine and hydrazine drugs. As expected, toxic effects of these drugs are more common and are more pronounced in slow acetylators. One can readily see how this hereditary polymorphism might also be important

in affecting individual susceptibility to carcinogenic arylamines and their metabolites.

Biological differences in acetylating capacity have remarkable effects on the metabolic and pharmacological profile of certain drugs and the clinical importance of individual acetylator status is thus clear-cut. Effects that are widely known and are now regarded as textbook examples are taken from the end of the spectrum of responses where the hereditary constitution predominates. The expression of a peculiar response to a drug and another chemical is, however, likely to be due to more than one individual characteristic, and to be influenced appreciably by extrinsic factors, too. In such instances, unequivocal assessment of the influence of any single factor (such as acetylator status) is difficult. For some disorders associated with prolonged drug intake, or occupational exposure to these hazards, the relationship to acetylator status is just emerging or is controversial. In certain additional conditions, an association with acetylator status affords a convenient and rational explanation for the peculiar response, but evidence in hand is incomplete and/or insufficient to establish a definite connection. For a few disease syndromes, no rationale for a dependence on acetylator status exists, but anecdotal reports alluding to the acetylator polymorphism as a predisposing host factor of possible importance have appeared. In the assessment which follows, we have attempted to categorize clinically relevant toxicities, or diseases, in relation to the acetylation polymorphism according to the extent and significance of the information which is available.

A. Disorders for Which Acetylator Status is an Established Predisposing Factor

1. Hydrazine- and arylamine-induced neurotoxicity. INH-induced neuritis was the first instance of drug toxicity to be associated with slow acetylation (191, 192). The occurrence and severity of this toxicity appeared to be related to the total dose of INH ingested (28), but the small number of patients investigated in these initial studies left the conclusion in doubt. Subsequently peripheral neuropathy associated with chronic ingestion of INH was demonstrated to be more common in slow acetylators and was dose-related (93). INH-induced peripheral neuropathy is unusual nowadays but still may be seen and is more pronounced in severely malnourished patients. It is attributed to vitamin B_6 (pyridoxine) deficiency resulting from loss of the pyridoxal hydrazone of INH (29). The neuropathy can be effectively prevented by daily administration of supplementary pyridoxine (232).

For other drugs which undergo acetylation, few accounts of drug-induced peripheral neuropathies are reported, or are difficult to evaluate (8). Among the few cases of polyneuritis occurring during HP therapy (229, 330, 414), only one suggested that the origin of the neuropathy may have been related to acetylator status. Tsujimoto et al. (414) described a slow acetylator Japanese patient who developed a peripheral neuropathy after the ingestion of HP for 6 months. The medical history showed that this patient took INH for treatment of tuberculosis but did not experience any sign of nerve damage.

Peripheral neuropathy may also be a rare complication of dapsone therapy (reviewed in ref. 426). A total of 17 cases have been published associated with a variety of skin diseases. Acetylator status was determined in only three of them, and all were found to be slow acetylators (230, 349, 426). Since Batchelor et al. (20) found a significant association between HP-induced lupus, slow acetylation and DR4 positivity, Waldinger et al. (426) determined the HLA DR4 phenotype in their affected patient and found that he was also DR4 positive. Additional studies of polyneuritis occurring during dapsone therapy are necessary to establish whether a pattern of association with slow acetylation, and possibly DR4 positivity, exists.

INH is also a central nervous system irritant whose acute neurotoxic effects have been documented (94, 122). The occurrence of INH overdose may be becoming more widespread because of ready availability of INH for prophylaxis and treatment of tuberculosis. Brown (45) reviewed 62 reports of acute INH overdose and also reported on cases of acute central nervous system toxicity in 42 native Americans in Alaska. Instances of acute central nervous system toxicity associated with INH ingestion by children have also been reported (276). The severity and extent of this problem is exemplified in southwestern native Americans in whom INH was implicated in 7% of all suicide attempts (375). Acute central nervous system toxicity, like INH-induced peripheral neuropathy, is believed to be related to reduction in pyridoxine (vitamin B_6) tissue levels, partly because pyridoxine administration has been shown to be protective to animals and humans against this disorder (66, 77, 155, 178, 340).

Acute encephalopathy manifested by convulsions usually occurs within 1 or 2 hours after taking large doses of INH. Such toxicity is rare if INH doses do not exceed about 17 mg/kg, and hence would not be expected to occur at ordinary doses of the drug. No human reports of a relationship between acetylator status and acute central nervous system toxicity have been reported, but several animal studies suggest that central nervous system toxicity induced by hydrazines could be influenced by the acetylator status. For example, studies in rats have shown that hydrazines, monomethylhydrazine or 1,1-dimethylhydrazine, cause central nervous systems toxicity at 100 mg/kg or less, whereas 1,2 dimethylhydrazine is non-toxic at doses up to 500 mg/kg. These results suggest that a free NH_2 group favors the occurrence of such toxicity (301). Furthermore, the dog, a species with no demonstrable capacity to acetylate INH,

is particularly sensitive to INH-induced toxicity (450, 31).

Genetically determined differences in acetylation of hydrazines are important in the metabolism of these compounds (215, 176). This led us to investigate the effect of INH and MAH in the rabbit model of the acetylation polymorphism (178). Both of these compounds produced dose-dependent lethal occurrences of central nervous system toxicity in rabbits. In most instances, after administration there was a lag period of at least 1 hr before the onset of irritability and frank seizures. Onset was marked by intermittent seizures and enhanced sensitivity to audiogenic stimuli. This was followed by frantic running episodes and repetitive grand mal seizures during which the rabbits died of respiratory arrest, usually within 24 hr of the onset of this phase. It was clear that the animals died from acute central nervous system toxicity and that signs of hepatotoxicity were absent.

The significance of acetylator status in hydrazine-induced toxicity is best illustrated by the survival times of rapid and slow acetylator rabbits studied (178). All rapid acetylator rabbits receiving daily injections of 30 mg/kg of INH survived the entire experiment of 12 weeks duration, whereas all the slow acetylators died from the same dosage regimen in an average of 3.4 weeks (range 1 to 10 weeks). Pyridoxine was useful in protecting rabbits against hydrazine-induced convulsions. Rapid acetylator rabbits were also more resistant to convulsions of INH in various other dosage regimens tested, but the differences observed between acetylation phenotypes were less pronounced. The results of the experiments with pyridoxine suggest that N-acetylation of the hydrazine is an important detoxication step in the prevention of hydrazine-induced central nervous system toxicity in rabbits. They further suggest that differences in N-acetylation capacity are important in susceptibility to hydrazine-induced central neurotoxicity.

2. Drug-related lupus erythematosus. The occurrence of lupus erythematosus has been linked to at least 35 drugs, many of which contain a primary (unsubstituted) amine group (184). The most important of these are the aromatic amines such as PA, and the hydrazines such as HP. The first case of drug-related lupus, which was attributable to sulfadiazine, was described in 1945 (186). HP was introduced as an antihypertensive drug in 1952 and the first case of HP-induced lupus erythematosus was documented a year later (288, 309). Reports of cases associated with PA (235) and INH (467), both of which were introduced in 1951, were reported subsequently. The lupus syndrome induced by HP is a relatively common complication of low-dose HP therapy (55). Since the mid-1970s, however, PA has become the most common cause of drug-induced lupus in adults.

In the 1960s, studies in twins and in families indicated a genetic etiology for systemic lupus erythematosus, but specific genetic elements were not identified (188, 238). Alarcon-Segovia et al. (2) examined 50 hypertensive patients with the HP-lupus syndrome and their families. They found signs that antedated the complete clinical picture, and inferred that affected persons were predisposed. Family histories revealed a relation to the "lupus diathesis" in 34% of the 50 patients but in only 8% of 100 controls. In 1967, some 13 years after the first reported case of lupus related to drug ingestion, Perry and his associates documented the connection between slow acetylation and HP-induced lupus (310, 311). Wider acceptance of the concept of genetic predisposition of patients with abnormal serology, arthritis, and other signs of drug-related lupus stimulated new investigation of this disorder and confirmed the prevalence of slow acetylators among HP-lupus patients (386) and PA-lupus patients (183, 458).

The onset, incidence, clinical features, and laboratory characteristics of drug-induced lupus have been reviewed recently by Uetrecht and Woosley (417). Generally speaking, the signs and symptoms in patients with drug-related lupus are similar to and must fulfull at least four of 11 criteria set forth for idiopathic lupus (399). In addition, the patient must have received the drug before the onset of the syndrome, and the symptoms should abate promptly when the drug is discontinued. On average, about a year of drug ingestion is required before symptoms develop, but onset varies greatly depending on the drug used, the dose selected, and the acetylator status of the patient. HP and PA are the only drugs which produce drug-induced lupus sufficiently frequently to obtain a picture of this disorder for assessment with respect to acetylator status. In therapy with both of these drugs, the development of antinuclear antibodies (ANA) has always been observed to occur before the onset of symptoms.

In the first report of drug-related lupus, Perry et al. (310) found a 54% incidence of ANA among 57 patients on HP. The incidence was 38% among rapid acetylators and 67% among slow acetylators. In a sample consisting of 371 patients, the overall incidence of lupus attributed to HP was 12% (311). The average daily HP dosage in the latter group was high (555 mg) for those who developed overt disease, and for patients administered a daily dose of less than 200 mg, symptoms were unusual. In another study of 31 patients administered lower daily doses of HP (100 to 200 mg), 28 were slow acetylators who developed signs and symptoms of lupus after a mean duration of treatment of approximately 2½ years (388). Litwin et al. (247) have prospectively examined the characteristics of ANA development in 27 patients administered HP. Autoantibodies to various polynucleotides such as poly(A), poly(A-U), and single-stranded DNA were detected within a few months after initiation of treatment and the time of initial detection was dose-dependent. Litwin also found that the extent of ANA

development was profoundly influenced by the acetylator phenotype with slow acetylators evincing a higher incidence and larger amounts of autoantibodies of the IgM type.

Mansilla-Tinoco et al. (259) have investigated further the significance of ANA positivity in relation to the occurrence of overt disease. Their 3-year study in 221 patients indicated that rapid acetylators have a significantly lower incidence of ANA positivity than slow acetylators ($P < 0.001$), but the main difference was in the rate of development of ANA positivity, confirming the results of others. ANA positivity occurred in slow acetylators soon after starting treatment and about half developed ANA titers of 1/20 after 50 g of HP, but thereafter little change in the proportion of positive test results occurred. Rapid acetylators developed ANA positivity more slowly, though after a cumulative dosage of 200 g, half had positive results at a titer of 1/20. In 32 patients (16 slow and 16 rapid acetylators) whose ANA tests became positive during the study period, the mean duration of treatment was 15 months for slow acetylators and 23 months for rapid acetylators. No significant difference in the incidence of ANA positivity between the sexes was observed. There were important differences between the factors that influenced the development of ANAs and the development of the lupus syndrome. A few ANA titers were very high, but large and unpredictable fluctuation in titers occurred which far exceeded the error in the test system. ANA positivity alone appeared to be of little predictive value in relation to the lupus syndrome because most positive results occurred in patients with a low risk of developing the lupus syndrome. The incidence of ANA positivity was 30% to 60% after 3 years of treatment, but that of lupus was only 1% to 3%. These findings are not inconsistent with the observations of Batchelor et al. (20), who found that several genetic factors such as sex, acetylator status, and HLA type determine which patients develop lupus but do not influence the occurrence of ANA positivity. Mansillo-Tinoco and coworkers conclude that the presence of ANAs does not require any change in treatment unless symptomatology persists or there are other features that place the patient in a high risk group.

In PA-induced lupus, the acetylator status of the patient has effects which are qualitatively similar to those seen with HP, but quantitatively they are more pronounced. The incidence of ANA positivity in PA therapy was initially estimated to be approximately 50% (32), but when longer therapy was instituted, the incidence rose toward 100% (231, 183, 458). In contrast, judging from a prospective study of therapy in 42 patients, the incidence of PA-related lupus was 29% (183), but with longer term therapy, this also appeared to increase. In the latter study of 11 patients with overt disease, eight were slow acetylators and three were rapid, whereas among 12 patients who had not developed any signs of the syndrome, two were slow acetylators and 10 were rapid. The duration of therapy required to induce ANA in 50% of 11 slow and nine rapid acetylators was 2.9 and 7.3 months respectively ($P < 0.002$). The median total PA dose at the time ANAs were detected was 1.5 and 6.1/kg of body weight in slow and rapid acetylators, respectively. After 1 year of therapy, antibodies had developed in 18 of 20 patients studied. Retrospective evaluation of the relationship between acetylator status and the rate of development of lupus symptoms by Woosley et al. (458) yielded a mean duration of therapy at onset of 12 ± 5 months for 14 slow acetylators and 48 ± 22 months for seven rapid acetylators ($P < 0.002$). Thus, ANA and the clinical manifestations of lupus appear to develop preferentially in slow acetylators at lower cumulative doses of PA and after shorter periods of PA therapy. At least one report to the contrary has appeared (464).

Contradictory data have been reported regarding the influence of acetylator status on susceptibility to PA-induced lupus (88, 183, 383). Nevertheless, rapid acetylation appears to exert a protective effect against the development of ANA and the lupus syndrome. Moreover, this action appears to afford somewhat greater protection against the HP-related syndrome. It seems reasonable, as Woosley et al. (458) have suggested, that the differences in pharmacokinetics and metabolic disposition of these two drugs may account in large part for the difference in protection afforded by acetylator status. Thus, the high first pass metabolism of ingested HP by the gut mucosa and liver contribute to a much greater degree to the elimination of HP (see section II B) than they do to the elimination of PA. HP elimination depends almost completely on metabolism while PA elimination depends to a greater extent on renal excretion. Hence, it is not surprising that the rate of acetylation is a more important determinant in the development of ANAs and the lupus syndrome from HP than from PA.

The prevalence of slow acetylators in patient populations at risk to drug-induced lupus syndromes is obviously appreciably greater than the proportion that ultimately develop the clinical disorder so that additional determinants must be at work. Batchelor et al. (20) have provided new information about additional genetic factors that determine individual predisposition to HP-induced lupus. Their study saw a striking connection with a specific HLA antigen and with a sex factor. Among 26 HP lupus patients, 25 were slow acetylators and the ratio of women to men was 4:1. The frequency of HLA-DR4 was 73% in lupus-affected persons and 32.7% in healthy control subjects, a highly significant difference. They found that all slow acetylator, DR4 positive women administered HP had developed lupus, but the only men affected were those who were DR4 positive and had received a rather high dose, 200 mg or more daily, of HP. The association of DR4 positivity appears relevant to

the susceptibility to the lupus syndrome because it was not secondary to either a putative association between hypertension and HLA, or to linkage between the loci for the acetylator and DR4 genes. About 8% of hypertensive patients treated with HP developed the lupus complications. In this population about one-quarter are DR4, and half are female, and almost half are slow acetylators giving an expected complication rate of about 6%. Perhaps the occasional DR4-positive male patient on high doses of HP, DR4-positive rapid acetylator female patients, and DR4-negative slow acetylator female patients who develop the complication account for the small discrepancy between the observed and expected estimates (445). These findings suggest that most cases of HP-induced lupus might be avoided by withholding the drug from female subjects who are both slow acetylators and HLA-DR4 positive. Failure to find an association of HP-induced lupus with HLA-DR4 positivity in 18 patients has recently been reported by Brand et al. (40).

Prolonged ingestion of INH can also induce premonitory signs of lupus though the fraction of patients who manifest the complete disorder is small. Approximately 20% of INH-treated tuberculous patients develop ANA, usually without concurrent clinical symptoms (57, 3). In a study by Evans et al. (127) of 103 tuberculosis patients undergoing INH treatment, the presence of ANA positivity was found in 17, but was unassociated with acetylator phenotype or with other parameters such as age, sex, dosage of INH, and other disease concomitant with tuberculosis.

3. Phenytoin-INH interaction. The acetylation polymorphism provides a rational explanation for the occurrence of phenytoin toxicity in patients treated with both phenytoin and INH. Observations on the interaction of these two drugs by Miller et al. (280) showed that six (27%) of 22 hospitalized patients who received phenytoin and INH concurrently for at least 5 days manifested central nervous system toxicity. Symptoms varied from typical toxicity of phenytoin such as disorientation, ataxia, nystagmus, and dysarthria to more unusual symptoms including psychotic behavior, convulsions, and coma. In contrast, only 30 (3%) of 1093 patients administered phenytoin without INH had adverse effects involving the central nervous system. The toxicities observed among the latter patients were qualitatively similar to those receiving combined therapy with phenytoin and INH and distinctly different from the toxic effects of INH alone on the central nervous system. The difference in frequency of phenytoin toxicities between the two groups was highly significant ($P < 0.001$).

The occurrence of such toxicity was first recognized by Murray (291) who found that 70 (11%) of 637 institutionalized epileptic patients who had taken phenytoin for years rapidly developed central nervous system toxicity when a trial of prophylactic antituberculosis therapy with INH (5 mg/kg) was commenced. In a control group of 845 patients adminstered a placebo, 23 (2.7%) suffered phenytoin-like adverse reactions. Attempts to find the source of these reactions did not reveal any difference in the free and total concentrations of INH in blood. Women were much more susceptible (4 to 5 times) to these reactions than men, and the incidence of reactions of women increased with age while those of men were essentially unaffected by age.

Kutt and his colleagues (42) have also observed that 11% of persons concurrently administered ordinary doses of phenytoin and INH are likely to develop phenytoin toxicity. Kutt determined the acetylator phenotype of 36 patients who had received combined therapy with these two drugs and found that phenytoin toxicity was confined to slow acetylators. Furthermore, toxic signs were accompanied by elevations of the phenytoin concentration in blood of slow acetylators with the greatest elevations occurring in the slowest of the slow acetylators, i.e., in those with the highest concentrations of INH in the blood. Kutt et al. (233, 234) examined this peculiar response to INH further in rats in an attempt to determine its pharmacological basis and found that INH is a non-competitive inhibitor of the rat liver microsomal enzyme which metabolizes phenytoin to its biologically inactive hydroxylated metabolite and enables its excretion.

Muakkassah et al. (289) have reexamined the mechanism of the inhibitory action of isoniazid in microsomal drug metabolism. In their studies addition of INH to rat liver microsomes produced a biphasic response resulting in a decrease in carbon monoxide binding to reduced cytochrome P-450 and by analogy, a decrease in oxygen binding to cytochrome P-450. They explained that this may be, in part, the mechanism by which INH inhibits cytochrome P-450-dependent oxidations. Examinations of compounds structurally related to INH indicated that both the hydrazine moiety and the aromatic ring were needed to produce the effects observed. This decrease in availability of cytochrome P-450 may account for the inhibition of the mixed-function oxidases by INH observed by Kutt and his coworkers for phenytoin.

4. Sulfasalazine-induced side effects. Sulfasalazine is effective as long-term drug treatment of ulcerative colitis and may be beneficial in treatment of Crohn's disease (281, 98, 7). Unfortunately, its use is limited by side effects which occur with frequencies varying from 5% to 55% in patients with inflammatory bowel disease (82, 395). Adverse effects of sulfasalazine are divisible into a set that is non-dose-related, is allergic in nature, and essentially unpredictable, and another major set that is dose-related, tends to occur early in therapy, and is dependent on acetylator status. Skin rash, aplastic anemia, liver and lung disorders, and autoimmune hemolysis belong to first set. No predilection of the allergic side effects to one or the other acetylator phenotypes been reported (326). The dose-related adverse reactions to

sulfasalazine are the most widely reported, are usually predictable, and can be decreased or avoided by reducing the dose of sulfasalazine. Non-allergic intolerance includes hematological side effects such as hemolytic anemia, leukopenia, and methemoglobinemia is well as generalized side effects such as nausea, vomiting, abdominal discomfort, malaise, headache, and vertigo (82, 157, 395).

5-Aminosalicylic acid appears to be the therapeutically active moiety (11, 133, 420), but most of the dose-related side effects of sulfasalazine in patients with inflammatory bowel disease are attributable to elevated serum total sulfapyridine levels that occur in slow acetylators. An early study noted that 13 of 28 patients experienced one or more of these side effects, and 10 of these patients were slow acetylators. A significant association of the occurrence of toxicity with serum total sulfapyridine concentrations greater than 50 μg/ml was also noted (82). Additional evidence for the predilection of slow acetylators to the dose-dependent side effects related to sulfasalazine has been reported more recently by others (12). Hemolytic anemia, another well recognized complication of sulfasalazine therapy, is also more prevalent among slow acetylators. Pounder et al. (324) found that morphological red cell abnormalities and reticulocytosis in sulfasalsazine-treated patients occurred primarily in slow acetylator patients. Goodacre et al. (157) and van Hees et al. (419) both noted that hemolysis was significantly correlated with an elevated serum sulfapyridine level, and van Hees et al. found that 18 of 19 patients exhibiting hemolysis were slow acetylators.

A bluish discoloration of the skin and mucosa, a phenomenon long known to occur in a high proportion of patients administered sulfasalazine, was observed in 10 of 28 patients studied by Das et al. (82). Of these, nine were slow acetylators and one was rapid. The cause of the cyanotic appearance was unclear because neither methemoglobin nor sulfhemoglobin was detected in the blood of these patients but the "cyanosis" was related to high serum total sulfapyridine concentrations.

The occurrence of a mild degree of methemoglobinemia related to the administration of sulfasalazine has also been reported (12). While the symptomatic side effects and the other dose-dependent hematological abnormalities accompanying sulfasalazine administration occurred more frequently in slow acetylators, methemoglobinemia occurred chiefly in rapid acetylator patients. In addition to its occurrence in connection with high levels of acetylated sulfapyridine, it also occurred primarily at high doses of sulfasalazine.

Even though slow acetylator patients are especially prone to adverse effects from administered sulfasalazine, recent clinical trials suggest that the onset of a side effect should not preclude the use of sulfasalazine as commonly practiced (395). Such patients should be identified and treated with small doses according to specific recommendations which have been developed. They should be followed closely during the initial 8 to 12 weeks, since side effects usually occur during this period. Under these circumstances, the beneficial effects of the drug may be realized in most patients.

B. Disorders for Which Acetylator Status Has Been Claimed to Be a Predisposing Factor

1. INH-induced hepatitis. The relationship of acetylator status to INH-induced hepatitis presents a complex problem in clinical pharmacology that has been investigated and debated at length for more than 10 years (438, 164). Initially, Mitchell and colleagues had explained that INH might be more hepatotoxic for rapid than slow acetylators because rapid acetylators might be expected to form MAH more rapidly than slow acetylators. MAH could then be converted to potent reactive electrophiles that bind covalently to hepatic macromolecules causing hepatic necrosis. This hypothesis was questioned on the grounds that rapid acetylators acetylate MAH more rapidly than slow acetylators to the non-toxic diacetylhydrazine, and that the exposure of rapid acetylators is thus similar to that of slow acetylators (118, 408, 176).

There is no doubt that prophylactic and therapeutic use of INH alone or in combination with other antituberculosis drugs carries an appreciable risk of hepatotoxicity. Observations of Black et al. (30) on 13,838 patients administered 300 mg of INH daily for prophylaxis of tuberculosis bear this out. Frank hepatic disease occurred in 114 (0.82%) of these patients, and 13 of them died. The relative risk of developing INH-induced hepatotoxicity was age-dependent: in no patients under 20 years of age, in 0.24% of patients aged 20 to 34, in 0.92% of patients aged 35 to 49, and 1.92% of patients aged 50 to 64. Clinically, biochemically, and pathohistologically it was manifestly different from viral hepatitis. Liver necrosis was chiefly hepatocellular, and there was no evidence that an allergic reaction had occurred.

The hypothesis of Mitchell and colleagues was supported directly and indirectly by human epidemiological investigations. Retrospective analysis of 13,838 patients found the incidence of probable INH-induced liver toxicity was more frequent in Orientals (1.8%) than in Caucasians (1.1%) or Blacks (0.7%) (283). This was a noteworthy observation because 90% or more of Orientals are rapid acetylators compared to approximately 45% rapid acetylators among Caucasian and Black populations. These findings provided indirect support for the hypothesis that rapid acetylators were at increased risk of liver injury from INH. This epidemiological study did not control for ages of the subjects, but it is pertinent that the proportion of individuals over 35 years of age was higher in the Oriental group than in either the Caucasian or Black groups. Mitchell and colleagues subsequently concluded that of 21 non-Oriental patients with probable INH-induced hepatitis, 18 (86%) were rapid acetylators in contrast to an expected proportion of 45% rapid acetylators in the population at risk (282).

Publication of this hypothesis brought forth disagreement from several sources (25, 121, 120) because of conflicting and complex epidemiological data. Definitive data were lacking for several reasons: First, INH hepatotoxicity is often used in a vague manner; some investigators include subclinical elevation of serum transaminases under this label (even when reversible), while others do not. Similarly, threshold values for transaminase elevation and the time interval that this elevation must be maintained to define the hepatotoxic state are inconsistent across studies. Second, comparisons are usually made against historical controls rather than directly generated on matched populations. Unfortunately, historical controls differ from experimental groups more frequently and to a greater extent than controls which have been generated at the time of the study (353). Finally, reliable comparisons are difficult to make because studies differ in INH dosage regimens, and some studies involve combination therapy that includes other hepatotoxic drugs such as rifampicin.

Several additional investigations over the last decade have also examined the role of acetylator status in INH hepatotoxicity and found that slow acetylators were more susceptible. In 1972 Lal and coworkers (236) reported that of nine patients experiencing elevated serum aminotransferase (SGOT) (EC 2.6.1.1) levels after combined INH, rifampicin, and streptomycin therapy, eight (89%) were slow acetylators, whereas of seven patients exposed to the same dosage regimen with no signs of hepatotoxicity, only two (29%) were slow acetylators. Smith and coworkers (379) reported that of 14 patients treated with INH and rifampicin who showed elevated serum transaminase and bilirubin levels, 13 (93%) were slow acetylators. In 1974, Beaudry and coworkers (22) reported that 25 of 369 children treated with INH experienced elevated SGOT levels. They determined the acetylator phenotypes of seven of these children, and all (100%) were slow acetylators. In 1978 Gronhagen-Riska and coworkers (162) determined the acetylator phenotypes of 45 patients with high SGOT and/or serum alanine aminotransaminase (EC 2.6.1.2) elevations after combined INH, rifampicin, and either ethambutol, streptomycin, or capreomycin therapy and reported that 33 (73%) were slow acetylators. However, no significant differences were noted in the proportions of rapid and slow acetylators in a group experiencing mild serum transaminase elevations. In 1977, Dickinson and coworkers (95) reported that of seven patients experiencing hepatotoxicity from INH therapy, six (86%) were slow acetylators. In a more recent prospective study of multifactorial risk factors for INH-induced liver dysfunction in 113 patients taking INH for at least 8 weeks, Dickinson et al. (96) described three distinct risk levels: rapid acetylators younger than 35 have very little risk (4%) of developing significant liver abnormality; slow acetylators younger than 35 and rapid acetylators older than 35 have a moderate risk (13%) or about the population average; slow acetylators older than 35 have a very high risk (37%) and represent a readily identifiable group where the risk versus benefits of giving INH should be most carefully weighed. Musch and coworkers (293) determined the acetylator phenotypes of 30 patients with hepatotoxicity produced by an antituberculous regimen including INH, rifampicin, and ethambutol. Hepatotoxicity was evident in 26 of 56 slow acetylators (46.6%), whereas it occurred in only four of 30 rapid acetylators (13.3%). Furthermore, the 12 patients with the most severe hepatotoxicity were all slow acetylators. These observations suggest that rapid acetylation does not carry increased risk of developing INH-induced liver dysfunction; indeed, the contrary seemed more likely.

It is perhaps not surprising that other studies found no significant differences in susceptibility to the INH-induced hepatotoxicity between the two acetylator phenotypes. In a large study (377) reported in 1977 by the Singapore Tuberculosis Service, 422 rapid and slow acetylators were treated with INH and rifampicin; no significant differences in serum transaminase elevations were noted between the two phenotypes. Similar findings were also reported in 1977 by the Hong Kong Chest Service (189) in which 113 rapid and 32 slow acetylators, all of them Chinese, were treated with INH and either streptomycin or pyrazinamide. Recently, the results of a retrospective analysis by Gurumurthy et al. (164) of the incidence of hepatitis with jaundice was reported for 3,000 patients treated in a series of controlled clinical trials at the Tuberculosis Research Center, Madras over the past 20 years. The overall incidence of jaundice among 1757 slow acetylators was 1.9% as compared with an incidence of 1.2% among 1238 rapid acetylators, a non-significant difference. The incidence of jaundice among the two phenotypes was between 1% and 2% whether INH was given alone or in combination with PAS, ethambutol, or pyrazinamide, but there was a strong suggestion ($P < 0.01$) that the incidence increased to about 4% with the addition of rifampicin plus pyrazinamide. Gurumurthy et al. (164) concluded that the incidence of clinically significant hepatic toxicity is unrelated to the acetylator phenotype. In addition, Riska (345) reported in 1976 that no differences in the incidence of hepatotoxicity were noted in 20,838 rapid and slow acetylators treated with INH.

Thus, epidemiological support can be found for the hypotheses that rapid acetylators are more susceptible, that slow acetylators are more susceptible, or that neither phenotype is more susceptible. Consequently, basic scientific studies in humans and animal models have been carried out to provide useful information toward more fully understanding the complex role of acetylator differences in INH hepatotoxicity. For example, the initial hypothesis that rapid acetylators should have higher INH-induced hepatotoxicity because of greater forma-

tion of MAH has been challenged by more complete INH pharmacokinetic data. Timbrell and coworkers (408) examined the percentages of metabolites formed and excreted after a 300-mg oral dose of INH and confirmed that higher amounts of acetyl-INH are excreted in rapid acetylators than slow acetylators. Consequently, estimated amounts of acetyl-INH (87.4% vs 54.5%) and MAH (41.1% vs 25.6%) formed also are higher in rapid than in slow acetylators (fig. 3). However, MAH is further metabolized in at least three ways: it can be excreted unchanged or as a hydrazone; it can be acetylated to diacetylhydrazine, a nontoxic metabolite; or it can undergo N-hydroxylation through the cytochrome P-450 mixed-function oxidase system to a highly electrophilic intermediate. The last pathway is reputed to be responsible for the hepatotoxicity, whereas the other two are detoxication pathways (283, 411). Ellard and Gammon (118) were the first to provide evidence that acetylation of MAH to diacetylhydrazine was also under genetic control. The first-order rate constants for MAH acetylation was 0.23 h^{-1} for a rapid acetylator, but only 0.05 h^{-1} for a slow acetylator. After INH ingestion, 27.6% was excreted as diacetylhydrazine in rapid acetylators vs 7.9% in slow acetylators. Similarly, Timbrell and coworkers (408) reported that 23.0% was excreted as diacetylhydrazine in rapid acetylators vs 4.9% in slow acetylators. Horai et al. (190) provide still more evidence that the rate of acetylation of monoacetylhydrazine is dependent on acetylator status. Thus, rapid acetylators not only form greater quantities of hepatotoxic MAH than slow acetylators, but also detoxify more MAH than slow acetylators. Based on these measurements, Timbrell and coworkers estimated that the amount of MAH that should undergo metabolic activation through the cytochrome P-450 mixed-function oxidase pathway to hepatotoxicity in the rapid acetylator (16.3%) should not be significantly different from the slow acetylator (18.2%).

Questions arise as to whether MAH is acetylated by the same liver N-acetyltransferase that acetylates INH or by a different one, because MAH is markedly different in structure from any known substrate for the enzyme. Hein and Weber (176) recently demonstrated in a rapid and slow acetylator rabbit model that MAH and INH are both acetylated by the same enzyme. The apparent K_m values were 0.89 mM for INH and 1.3 mM for MAH. Despite the relatively low affinities, there is an indication that they would be higher with human liver N-acetyltransferase (428, 430). Furthermore, both INH and MAH produce more frequent incidences of lethal CNS toxocity in slow than in rapid acetylator rabbits (178), which illustrates the significance of the acetylator differences in the metabolism and toxicity of these substrates.

Since INH and MAH are acetylated by the same NAT, inhibitory interactions between them could shunt the metabolism of INH and/or MAH through non-acetylation pathways. In vitro studies with rats by Timbrell and Wright (409, 460) showed that INH inhibits microsomal activation of MAH through the cytochrome P-450 mixed-function oxidase system, but in vivo, INH inhibits the acetylation of MAH to diacetylhydrazine. They suggest from these results that the predominant interaction after normal therapeutic doses of INH in humans would be INH inhibition of MAH acetylation. Inasmuch as higher amounts of INH remain for longer periods of time in slow than in rapid acetylators (118, 433), one might expect that slow acetylators would have greater inhibition of the MAH acetylation (detoxification) pathway, and consequently have higher amounts of MAH shunted through the microsomal pathway leading to the hepatotoxicity, i.e., a greater toxicity in slow acetylators. Investigation of this hypothesis in rats (14) showed that INH inhibited MAH-induced hepatotoxicity. In addition, the microsomal activation for MAH was a relatively minor pathway that could be greatly induced by phenobarbital pretreatment, but not by INH or rifampicin. The latter finding is pertinent because rifampicin is often prescribed in combination with INH for antituberculous therapy, and several studies have reported higher and more severe incidences of hepatotoxicity for combined therapy than for INH therapy alone, supposedly because of rifampicin's induction of cytochrome P-450 microsomal pathways. Because of conflicting epidemiological results, Girling (146) has reviewed these studies and concluded that there is no consistent evidence that combination rifampicin-INH therapy increases the risk of hepatotoxicity. Bahri et al. (14) found that rifampicin did not increase or decrease MAH-induced hepatotoxicity in rats, but rifampicin inhibited MAH acetylation and caused a reduction in hepatic cytochrome P-450 enzyme levels. Thus, the effects of rifampicin on INH metabolism and hepatotoxicity are complex, and human epidemiological studies of hepatotoxicity in rapid and slow acetylators on combined INH-rifampicin therapy should be interpreted with these complex interactions in mind.

2. *Aromatic amine-induced bladder cancer.* The epidemiology of aromatic amine bladder cancers is, for practical purposes, synonymous with the epidemiology of industrial bladder cancer (304). In English and Welsh populations, bladder cancer produces an annual morbidity ranging from 140 to 195 per million population and ranks as the sixth most common cause of all male cancer deaths. In the larger population of the United States, major regional differences in bladder cancer occur. In Texas, the annual morbidity is as low as 44 bladder cancer cases per million male population, but in Connecticut it is 235 per million. Figures published in 1970 (99) drawn from four regional cancer registries in California, Connecticut, Nevada, and Texas indicate that bladder cancer ranks as the fourth most common cancer affecting white males and is rising. For the 1950 to 1970 period, the crude death rates per million population in

England and Wales rose from 79 males in 1950 to 97 in 1960 to 113 in 1970; a similar though less pronounced trend was observed among females from 34 to 39 to 45 for 1950, 1960, and 1970, respectively.

Epidemiological investigation of industrial bladder cancer started with the report of Rehn, a German physician, who reported in 1895 the unusual occurrence of urinary bladder cancer among fuchsin dye workers (334) and today is a matter of historical record (335, 243, 210). By the late 1940s, a great deal of basic clinical and epidemiological evidence collected in humans and in animals indicated that aromatic amines, particularly beta-naphthylamine and benzidine, constituted a grave bladder cancer hazard. Ultimate proof of the association and estimation of risks to particular groups of English industrial workers engaged in the manufacture of certain dyes was provided, however, in the classic epidemiological investigation performed by Case and his associates (62). Case assessed the incidence and causes of industrial bladder cancer by compiling a list of 341 cases of bladder cancer derived from records of 4622 men who had been employed in the dyestuffs industry and had been in contact with one or more of the aromatic amines for longer than 6 months and compared them with bladder cancer cases in local populations at low risk of exposure to aromatic amines. The data were analyzed by "comparative composite cohort analysis," a technique whereby the occurrence of the event is observed and the result compared with the expectation in a general population unexposed to specific risk and observed for the same length of time, to arrive at an estimate of an expected frequency of the event with exposure to specific risk. The study of Case and coworkers showed that the risk of contracting bladder cancer was 30 times as great for the exposed dyestuff worker as for the general population. The latency for bladder cancer development averaged 18 years, but varied widely from less than 5 years to more than 45 years. This study also revealed that beta-naphthylamine was the principal carcinogenic amine with the greatest relative potency compared to benzidine, 1-naphthylamine, and mixed exposure to dyestuffs. Case's study was also the first to demonstrate, contrary to expert opinion in vogue, that the hazard posed by aromatic amines extended beyond the limits of the dyestuff industry and involved the rubber industry, the electric cable industry, and other diverse occupations (reviewed in refs. 304 and 252). Perhaps the most important consequence of Case's study was the decision taken by the British within the decade to cease production of beta-naphthylamine and benzidine.

More recent epidemiology studies indicate that 15% to 30% of bladder cancer cases in the Boston area are occupation-related (72), whereas the estimate may be as high as 30% in the metropolitan area of Leeds, England (71). In view of the association of cigarette smoking as a cause of bladder cancer (285), it may reasonably be suggested that a major portion of human bladder cancer is attributable, at least in some regions of the United States, to occupational and cultural sources which involve exposure to aromatic amines.

Six studies provide information on the acetylator status of bladder cancer patients. The first of these performed on a low risk population of urban bladder cancer patients showed a 13% excess ($P = 0.065$) of slow acetylator persons (46/71 = 64.8%) compared to a Danish control population (38/74 = 51.4%) suggesting that slow acetylators may be slightly more susceptible (odds ratio = 1.74) than rapid acetylators (453). One additional study has shown an association of slow acetylator status with the occurrence of bladder cancer in low risk populations (128), while three others failed to observe a significant trend (251, 284, 456, 279). Cartwright et al. (59) have extended these studies to high risk populations and have recently described a population of 23 persons in Yorkshire, England, with documented exposure to benzidine from employment as chemical dye workers. This group displayed a 40% excess ($P = 0.00005$) of slow acetylator persons (22/23 = 96%) compared to Huddersfield controls, a highly significant increased susceptibility (odds ratio = 16.7) of slow acetylators to aromatic amine-induced cancer. By further stratifying their data on this subset of patients according to standard histopathological criteria for bladder tumor classification, Cartwright and coworkers also found an excess of slow acetylators among those presenting with more invasive forms of bladder cancer (T3 or T4 disease or carinoma in situ).

The data of Cartwright and coworkers also indicate the lack of correlation between bladder cancer among individuals with histories of tobacco smoking and slow acetylator status. As Lower observes (438), this suggests either that aromatic amines are not the bladder carcinogenic components of tobacco smoke, or that a dose response phenomenon is in operation that masks the consequences of differences in acetylator status, i.e., at very low doses of aromatic amines, such as accompany cigarette smoking (189), they may be detoxified nearly as well by slow acetylators as by rapid acetylators, but at higher doses the lower detoxifying capacity of slow acetylators becomes apparent. To our knowledge, there are no data at present which differentiate these alternatives.

Another unanswered question concerns the influence acetylator status on the longevity of survival of bladder cancer patients. Evans et al. (128) suggested that the association of slow acetylator status with bladder cancer might signify that rapid acetylators were protected because they are more capable of rendering aromatic amines non-carcinogenic by acetylation, or that slow acetylators have greater survival with bladder cancer than rapid acetylators. Whether acetylator status has an appreciable effect on the survival of patients with occu-

pational bladder cancer is a question that will require additional prospective analysis.

The observation of Cuzick et al. (80) and Cartwright and Glashan (61) may have a bearing on the pathological heterogeneity and etiology of bladder cancers. Cuzick and colleagues (80) reported a remarkably high incidence of palmar keratoses in lung and bladder cancer, particularly in bladder cancer patients. In their case-control study of 69 bladder cancer cases, keratotic lesions were present in 87% (odds ratio 11.7, $\chi^2 = 49.1$, $P < 0.0001$). Cuzick's observations receive confirmation from those of Cartwright and Glashan (61) in their Yorkshire series of 15 bladder cancer patients and matched controls. Cuzick et al. speculated that this lesion might be a marker for increased susceptibility to certain cancers, possibly reflecting a genetically conditioned inability to metabolize efficiently or neutralize certain chemical carcinogens, but they did not determine the acetylator status of their patient cohort nor did they examine the patients. The only bladder cancer patient examined by Cartwright and Glashan who had occupational aromatic amine exposure, however, had no palmar keratotic lesions, while all other cases had "papillomavirus" types of bladder cancer lesions. The full significance of these observations is unclear, but Cartwright and Glashan suggest that these lesions may represent a type of human papilloma virus infection, and that this virus could be a causal link between the bladder tumors and the palmar lesion.

3. Spontaneous (idiopathic) lupus erythematosus. Godeau et al. (153) first observed higher serum levels of INH after a test dose of the drug in 47 lupus patients, six of whom were drug-induced, than in control subjects, and proposed that both forms of lupus included a preponderance of slow acetylators. Reidenberg and Martin (336) found 10 slow acetylators and four rapid acetylators in a sample of patients with idiopathic lupus and inferred that some cases of this disorder might result from exposure to unknown environmental aromatic amines or hydrazines that persist in affected patients because of slow acetylation. The observations of Fritzler and Tan (138) may be pertinent in connection with this concept, since they reported that 35% of sera from idiopathic lupus patients were immunologically similar to those obtained from drug-induced lupus patients. In a summary of the world literature (338), Reidenberg identified 150 slow acetylators and 77 rapid acetylators among patients with idiopathic lupus. In six studies (336, 239, 209, 132, 134, 338), there were more idiopathic lupus patients than would have been expected from the acetylator phenotype distribution in the normal population of the regions studied. Three studies (421, 287, 241) failed to confirm this association. Chi square analysis performed on the combined data suggested that the prevalence of slow vs rapid acetylators observed among idiopathic lupus patients differed significantly ($P < 0.001$) from an expected ratio of 122 slow to 105 rapid acetylators. Since 1980, at least one additional study has failed to find a preponderance of slow acetylators among a sample population of Japanese patients afflicted with idiopathic lupus (197, 190). This report of Japanese patients is particularly interesting because only 11.5% of the Japanese are slow acetylators, though the prevalence of idiopathic lupus in Japan is similar to that observed in Europe and the United States (141).

The concept that Reidenberg and Martin (336) put forward is not implausible. A subset of cases of lupus-like disorders that might have been labeled as idiopathic may in fact be attributable to environmental chemicals. For example, a case of lupus in a slow acetylator female patient has been attributed to occupational exposure to hydrazine (339). The patient had an HLA-DR2,3 phenotype which is characteristic of idiopathic lupus and not hydralazine-induced lupus (20). Attention is drawn to another patient in remission from procainamide-induced lupus who developed signs and symptoms of lupus after ingesting another hydrazine derivative, the food dye, tartrazine (308). Reidenberg notes that hydrazines are present naturally in tobacco and tobacco smoke, in mushrooms, and penicillin while aromatic amines are present in permanent hair dye preparations which may be absorbed in appreciable quantities through the skin. He suggests that since the number of these compounds produced industrially and the opportunity for exposure to them is becoming more widespread that the incidence of lupus from such sources might also be increasing.

An important question evoked by the studies of acetylator phenotype in both drug-related and idiopathic lupus concerns the pathophysiological mechanism(s) involved. Thus far the origin of these disorders remains unknown despite many studies. Litwin has recently evaluated several reasonable immunopathological mechanisms for these disorders (436). The evidence so far suggests that the inciting agent is probably a non-acetylated reactive metabolite rather than the parent compound. The studies of Freeman et al. (135, 136) indicated that an active non-acetylated metabolite of procainamide binds covalently to liver macromolecules and is capable of causing a positive Ames test. This metabolite which has recently been suggested to be a hydroxylamine (418), may act immunologically by one or more of several mechanisms. 1) The metabolite could be an antigen that is cross-reactive with nucleic acid. This was at first seen as an attractive idea because there is a chemical resemblance between hydralazine and adenine, and because antibodies to adenine could be inhibited by hydralazine. However, Litwin and coworkers found that while rabbits produced high titers of antibodies to hydralazine that were entirely cross-reactive with DNA, rabbits did not acquire the disease (461). Furthermore, in a prospective study of hydralazine-treated hypertensive patients, no antibodies to hydralazine were detected, nor were titers of antibodies to single-stranded DNA, poly(A), or

poly(A-U) inhibited by hydralazine or hydralazine-human serum albumin conjugates (247). Significant lymphocyte transformation to hydralazine-human serum albumin conjugates was observed in some patients, implying the presence of sensitized T cells in the hydralazine-treated patients. On the whole, however, evidence is lacking for the cross-reactive antigen theory. 2) The reactive metabolite could alter lymphocyte subpopulations such as T suppressor cells, suppressor monocytes, or suppressor B cells, or could interfere with T helper cells. To date, there appear to be no studies to support such a mechanism. Lymphocytotoxic antibodies have been observed in some patients with drug-related lupus, but their significance is unknown. 3) The reactive metabolite could interact directly with the nucleic acid. Should adducts be formed between lupus-inducing chemicals, or their metabolites and nuclear components, this could afford a potential mechanism which could alter the DNA of recipient cells to increase its antigenicity, as shown by Stollar (386, 387) or to induce immune nonresponsiveness. Either of these mechanisms might account for the profile of clinical and immunological effects associated with drug-related or idiopathic lupus. Observations of McQueen et al. (268) who found that DNA damage induced by hydralazine was more extensive in hepatocytes of slow acetylator rabbits than in rapid acetylator hepatocytes (table 6), is of interest in connection with this concept. 4) The reactive metabolite might act as an adjuvant, particularly a polyclonal B cell activator. The fact that poly I-C can act both as an adjuvant and an immunogen is of interest in this connection (200). Such a mechanism might account for the variety of antibodies that are produced in drug-related and idiopathic lupus.

None of the immunopathological mechanisms listed above excludes any other one, and more than one of them could be operating simultaneously. Furthermore, each of these mechanisms would probably require an additional genetically conditioned factor for the expression of an autoimmune response and the disease. This possibility is consistent with observations implicating HLA DR antigens as susceptibility factors in both drug-related and idiopathic forms of lupus as already mentioned.

4. Phenelzine toxicity. Evans et al. (126) initially reported from a retrospective analysis that adverse effects of phenelzine in 47 depressed patients were more common among slow acetylators. The evidence from a number of later clinical studies that attempted to quantify the influence of acetylator phenotype on phenelzine responses has been conflicting. Among six studies which have been reported since 1973, three have found greater therapeutic effects in slow acetylators (213, 211, 350) while three have not found any association with the acetylator phenotype (87, 262, 415). Johnstone and Marsh (213) found that therapeutic responses to phenelzine in two separate studies of patients with neurotic depression was greater in slow acetylators. They also found that acetylator status was unrelated to the severity or frequency of side effects. In a second study, Johnstone (211) showed that the antidepressant effect, the degree of MAO inhibition and the amount of free phenelzine excreted in the urine were all significantly greater in slow acetylators than in rapid acetylators. In another study Rowan et al. (350) chose 11 measures of depression (four global, four depression, and three anxiety measures) for evaluating the effects of acetylator phenotype on therapeutic responses to phenelzine. After 6 weeks of phenelzine therapy, stronger therapeutic effects were observed in slow acetylators, reaching significance in eight of 11 outcome measures as compared with only three in rapid acetylators. However, the size of the differences due to acetylator phenotype was small, and on practical grounds was judged to be unimportant. In care of patients, rather than routine determination of the acetylator phenotype, Rowan suggested that an increase in daily dose of phenelzine would be better in patients not responding and who do not show major side effects, particularly the usual dose-limiting one of dizziness. Preliminary results obtained in a study of 21 slow and eight rapid acetylators by Robinson et al. (346) did not reveal significant differences in improvement on Standard Depression Interview subscale scores, global response, side effects or percent of MAO inhibition between rapid and slow acetylators. A trend toward greater improvement was discernible when the standard interview items were compared to the ratio of acetylated/free sulfapyridine (the drug used to phenotype patients) rather than to the less precise categorical discrimination of rapid and slow acetylators. The studies of Davidson et al. (87) and Tyrer et al. (415) both concluded that the acetylator status is not a good predictor of clinical response to phenelzine. Tyrer et al. (415) actually observed a tendency toward greater improvement among rapid acetylators than in slow acetylators, although the difference between acetylator phenotypes did not reach statistical significance.

The question of whether phenelzine undergoes acetylation (261) is thus no longer an issue (176). In addition, the therapeutic responses of depressive patients to phenelzine are influenced to no more than a minor extent by differences in acetylator status, and the difference tends to favor a greater response of slow acetylators. To date, the only report which indicates a differential effect in the frequency of side effects is the original report of Evans et al. (126).

5. Promizole-induced hemolysis in glucose-6-phosphate dehydrogenase deficiency. A peculiar drug response may be influenced by more than one metabolic element in the hereditary make-up of an individual. This principle has been clearly documented by Magon et al. (256) who found that slow acetylators, who were also deficient in glucose-6-phosphate dehydrogenase of red cells, are especially

prone to hemolysis from certain sulfone and sulfonamide drugs.

Deficiency in glucose-6-phosphate dehydrogenase activity is one of the most common genetically determined enzyme effects of clinical significance, affecting approximately 11% of Black American males. Red cells of subjects that have an intrinsic deficiency of this enzyme are especially susceptible to hemolysis induced by a wide variety of drugs. There is, however, only an approximate relationship between the quantitative level of glucose-6-phosphate dehydrogenase activity and the apparent sensitivity to hemolysis from one affected person to another, which suggests that other factors influence this response. In the landmark paper of Dern et al. (92), the results clearly showed for the first time that several drugs like primaquine were hemolytic while others including some arylamines were not. One drug, thiozalsulfone (Promizole) (fig. 2), was outstanding because it elicited hemolysis in five recipients but not in four others. The fact that promizole-induced hemolysis was bimodal, occurring in approximately half of the recipients and that promizole is an arylamine, suggested that the acetylation polymorphism might be an etiological factor which could explain, at least in part, the hemolytic response of glucose-6-phosphate deficient subjects to promizole. Magon et al. (256) developed a mouse model to test whether genetically determined differences in acetylation contributed to this response. In addition, they tested whether hydroxylation might contribute to the variability in hemolytic response by incubating glucose-6-phosphate dehydrogenase-deficient and normal red cells together with mouse liver microsomes at two states of hydroxylase activity (uninduced and induced), an NADPH-generating system, and acetylated or unacetylated promizole. They found that the highest level of glutathione depletion, a sensitive measure of the tendency to hemolysis, occurred in glucose-6-phosphate dehydrogenase-deficient red cells in the presence of induced (high hydroxylase activity) microsomes and unacetylated promizole. In contrast, acetylation of promizole protected against glutathione depletion. The importance of hydroxylation in producing marked glutathione depletion was shown further by the protection afforded by the specific hydroxylation inhibitor, SKF-525A. In a parallel set of experiments with dapsone, a similar pattern of results was obtained. The results suggested that glucose-6-phosphate dehydrogenase-deficient, genetically slow acetylators, having high microsomal activity would be most susceptible to promizole-, or dapsone-induced hemolysis compared to other metabolic phenotypes.

The report of a human trial by Woolhouse and Atu-Taylor (457) provides further support for this concept. Woolhouse and Atu-Taylor (457) found that when 16 healthy Ghanian men, eight of whom were glucose-6-phosphate dehydrogenase-deficient, were administered a single oral dose (1.5 g) of sulfamethazine that red cell glutathione concentrations in all subjects decreased, but the greatest decrease occurred in subjects who were both glucose-6-phosphate dehydrogenase-deficient and slow acetylators. Slow acetylators were exposed to greater amounts of free sulfamethazine as evidenced by higher plasma concentrations, longer half-lives and greater areas under the plasma concentration-time curves. In slow acetylator subjects with normal glucose-6-phosphate dehydrogenase-deficient activity, the mean decrease in red cell glutathione concentration (12.7% ± 0.6%) was significantly greater ($P < 0.005$) than in rapid acetylators (7.6% ± 0.8%). In glucose-6-phosphate dehydrogenase-deficient subjects, the mean decrease in glutathione concentration was also somewhat greater in slow acetylators (20.3%) than in rapid acetylators (17.7%) but not significantly so. In these subjects the effects of the double genetic polymorphism were approximately additive. Even though the effects of sulfamethazine reported by Woolhouse and Atu-Taylor are marginal, it should be noted that sulfamethazine is a relatively non-toxic sulfonamide that has not been associated with frank hemolysis.

C. Disorders for Which Anecdotal Accounts of a Relationship to Acetylator Status Have Been Reported

1. Diabetes. The increased prevalence of rapid acetylators among a small series of diabetics reported by Mattila and Tiitinen (263) prompted other investigators to ask whether acetylator status was an index of dimorphism in diabetes, and might also be related to complications of diabetes. Mattila and Tiitinen (263) found that the mean half-life for INH elimination in 13 diabetic rapid acetylator subjects was 84 ± 5 min while the corresponding value for 15 slow acetylators was 199 ± 18 min. Those values did not differ from values obtained on non-diabetic subjects (83 ± 3 min and 187 ± 8 min), but rapid acetylators occurred more frequently among insulin-dependent (type I) diabetic children (seven rapid acetylators among nine patients under 16 years). Drawing on the studies of Nikkila and Kerppola (296) who showed that acetylation could be accelerated in severe diabetes, Mattila and Tiitinen explained that the rate of INH acetylation might be accelerated in diabetic children (17). Unfortunately, Mattila and Tiitinen did not study a non-diabetic group of controls to determine the validity of their explanation.

McLaren et al. (266) subsequently investigated the proportions of rapid and slow acetylators in two groups of diabetic patients and a group of historical controls. Sixty-six diabetic patients with symptomatic peripheral neuropathy were thus compared with 64 diabetic patients who had had the disease for at least 10 years without developing neuropathy, but who were both comparable in sex distribution, type of diabetes and type of treatment. Those without neuropathy were significantly older and had been treated significantly longer than those with neuropathy. A significantly lower proportion of slow

acetylators was found among diabetics with neuropathy than among historical control subjects ($\chi^2 = 6.71$; $P < 0.01$) or among diabetics without neuropathy ($\chi^2 = 5.17$; $P < 0.05$). When the two diabetic groups were combined, the overall prevalence of slow acetylators was 47%, which, though lower than the 57% in the control series, was not significantly so ($P < 0.1$).

In a study by Burrows et al. (49), two groups of diabetic patients and 19 normal control subjects provided additional information on the possible relationship between diabetes and acetylator status. Among 47 patients with maturity-onset diabetes in the first group, 29 (62%) were rapid acetylators compared to 50% among controls. The 29 rapid acetylators were significantly older at diagnosis, and also had higher pretreatment fasting insulin concentrations at a given glucose concentration than slow acetylators. Moreover, they also had a larger first-phase insulin secretion in response to intravenous glucose than slow acetylators. Among the second group of diabetics subjects, consisting of 38 patients who had suffered diabetes-induced tissue damage, the proportion of rapid acetylators (47%) was not different from that among normal control subjects.

Burrow's results confirmed McLaren's postulate (266) that diabetic persons differ in the nature of their disease, but on different grounds. McLaren et al. (266) had proposed that rapid acetylators might be protected against diabetic neuropathy. Burrows et al. (49) point out that diabetes may involve different patterns of beta cell response in keeping with the study of Lindsten et al. (246) which indicated a genetic influence on insulin response to glucose, and with Cerasi and Luft's (63) description of "low insulin responders." The data of McLaren and of Burrows thus might indicate that rapid acetylators are more susceptible to diabetes than slow acetylators. In contrast, McLaren has suggested that slow acetylators with diabetes are more susceptible to peripheral neuropathy.

The relationship of acetylator status to type I diabetes and its microvascular complications was further explored by Bodansky et al. (34). They found that the frequency of the rapid phenotype (49%) in 55 type I caucasoid diabetics was higher than in the general Northern European population (37%). This difference was not significant ($\chi^2 = 2.64$; $P = 0.10$), but the combination of this data with data from two other studies (263, 266) yielded a total of 113 type I diabetics. The prevalence of rapid acetylators in the combined data (54%) compared with 37% in control subjects indicated there was a significant correlation between rapid acetylator status and type I diabetes in the Northern European population ($P = 0.00013$ one-tailed Fisher Irwin exact test; relative risk 2.0, 95% confidence limits 1.3 to 3.0). In contrast, none of the groups of patients with diabetic complications including severe retinopathy, neuropathy, and impaired renal function differed significantly in their phenotype distribution from the group without complications. Bodansky also observed no difference in the mean levels of glycosylated hemoglobin A between rapid (12.33 ± 2.27%) and slow acetylators (11.55 ± 1.65%), and concluded that the acetylator phenotype was unlikely to affect the control of diabetes or conversely, that glycemia affects the phenotyping procedures. Strong evidence for an association between type I diabetes and a gene or genes of the HLA complex that conferred a several-fold increase in the susceptibility to this disorder in subjects with HLA-D3 or D4 positivity was also observed by these investigators.

Burrow's claim (49) of an association between rapid acetylator status and type 2 (maturity onset or non-insulin dependent) diabetes led Bodansky et al. (34) to suggest that a second susceptibility gene might exist which is common to both types of diabetes associated with rapid acetylation. This possibility was not studied, though it could be assessed by conducting studies of diabetic families with two or more affected siblings to see whether acetylator status segregates with type 2 diabetes.

Shenfield et al. (371) investigated the association between acetylator status and the prevalence of peripheral neuropathy in groups of type 1 and type 2 diabetic patients. Acetylator status determinations in 116 diabetic subjects indicated a significantly greater proportion of rapid acetylators with type 1 diabetes (74%) and in type 2 diabetes with poor diabetic control. On the other hand, there was no difference in the prevalence of rapid acetylators of patients with type 2 diabetes (54%) and control subjects (48%). The mean glycosylated hemoglobin levels were not significantly different in either group of diabetic subjects. In contrast to the results of McLaren et al. (266), no significant association between acetylator status and diabetic neuropathy was found.

Shenfield et al. (371) say that acetylator status could be a genetic marker for type 1 diabetes, but observe that the increased proportion of rapid acetylators in both types of diabetes suggest the possibility of an artifact due to high glucose levels. The rate of acetylation of sulfamethazine, the drug used for phenotype determination, was significantly greater in both rapid and slow acetylators with diabetes compared to control subjects. There was also a significant association between high plasma glucose and acetylator status. It should also be noted that INH elimination rates are significantly enhanced by administration of oral glucose (405) and decreased by starvation (46). Thus, while acetylator status may have etiological significance in individual susceptibility to diabetes and its neuropathic complications, neither the extent or significance of these associations is clear. Prospective studies will probably be necessary to determine whether acetylator status is a true genetic marker for diabetes.

2. *Other disorders.* The literature contains numerous

citations on the relationship of acetylator status, or lack of it, to various other pathophysiological states, but most of them have been investigated only to a limited extent. Yet some of them are of sufficient general interest to bear mention while others, in our opinion, are sufficiently novel or intriguing as to justify their inclusion.

A. BREAST CANCER. References to a putative relationship between acetylator status and breast cancer in women have been reported in the Russian literature (97, 47, 48) during the past decade. Bulovskaya's study (47) of 41 patients with invasive breast cancer compared to 38 healthy female subjects showed the group of cancer patients included a large excess of rapid acetylators (68% of rapid vs 32% of slow acetylators), while the proportions of rapid and slow acetylators among age-matched control subjects were 37% and 63% respectively. The levels of acetylating capacity in cancer patients, however, were relatively higher, in both rapid and slow phenotypes than in the corresponding groups of controls. Thus, individual rates of sulfamethazine acetylation measured in blood serum by Evan's method (124) ranged from 13.9% to 34.1% in control subjects, and from 21.2% to 47.4% in cancer patients classified as slow acetylators. Among individuals classified as rapid acetylators, values ranged from 54.8% to 79.0% in control subjects and from 63.6% to 89.5% in cancer patients, respectively. In 16 of 22 patients treated for their disease, the level of acetylation was unchanged, but among the other six patients, three of the slow phenotype converted to the rapid phenotype, while change in the opposite direction was observed for the three remaining cases. No such changes in acetylator phenotype were observed with repeated observation in controls. These studies suggest that the pathophysiological changes which accompany breast cancer are able to influence the level of aromatic amine acetylating capacity, but the mechanism of this effect is not understood. Conceivably, it could be due to a disturbance in metabolism as may accompany preneoplastic changes and tumor growth to promote a shift toward intensified utilization of fatty acids and greater supplies of the cofactor acetyl coenzyme A, for the N-acetyltransferase. On the other hand, the influence of breast cancer may be capable of altering synthesis or expression of polymorphic N-acetyltransferase in affected individuals.

Cartwright (60) presents results on acetylator status and breast disease that broadly support Bulovskaya's observations (47). Those results indicate a deficit of slow acetylator phenotypes in both malignant and benign (fibrocystic) breast disease; this was statistically significant only in the benign breast disease group ($P < 0.01$).

These observations suggest that a search for, and a more detailed study of metabolic pathways are of interest in breast cancer, and that specific chemical agents may be implicated.

B. ISONIAZID-INDUCED ENFLURANE DEFLUORINATION. A survey of 20 surgical patients treated with INH (300 mg daily) for periods up to a year prior to anesthesia with enflurane compared to 36 patients anesthesized by enflurane but taking no other drugs showed that INH treatment resulted in microsomal enzyme induction in nine patients with high peak fluoride levels (264). Three- to fourfold enhancement of defluorination by INH was of special interest because such a pattern of induction did not resemble that of inducing agents including phenobarbital, phenytoin, 3-methylcholanthrene, beta-naphthoflavone, or ethanol (100). Although the acetylator phenotype of these patients was not determined, the occurrence of induction in approximately half of them led Mazze and coworkers to speculate that induction might be related to acetylator status (264). In vitro studies by Mazze et al. demonstrated that INH significantly increased the hepatic microsomal defluorination of enflurane by 370% and that of several other flurane analogs by 168% to 283% (342, 341). Enhanced enflurane defluorination was attributable to a subspecies of P-450, P-450$_1$, in rats (342). Furthermore, Rice and Fish (341) found that rats treated with INH metabolites, acetyl-INH and hydrazine (sulfate), but not with the non-hydrazine moiety, isonicotinic acid, significantly increased defluorination of the flurane analogs. It might be expected that flurane toxicity, which is attributed to the fluoride metabolite, might be enhanced in certain patients administered INH, perhaps preferentially in rapid acetylators, but this remains to be demonstrated.

C. ISONIAZID-PERTURBED VITAMIN D METABOLISM. Studies of the effects of INH (300 mg) used daily in combination with rifampicin (600 mg) on vitamin D metabolism demonstrated a reduction of both 25-hydroxyvitamin D and 1-alpha,25dihydroxyvitamin D concentrations in healthy persons (43, 44). Depression of serum calcium and phosphate levels as well as increased levels of circulating parathyroid hormone were also observed. The vitamin D metabolites were both greatly reduced ($P < 0.001$), compared to base line levels, in four rapid acetylators after 4 weeks of treatment, but not in slow acetylators. No significant differences in parathyroid hormone levels were observed between slow and rapid acetylators. The pattern of change induced in the vitamin D metabolites in healthy rapid acetylators persisted as long as 2 weeks after the drugs were discontinued. This provides evidence that INH metabolites such as MAH may be responsible for the reduction in circulating vitamin D metabolites by irreversibly inactivating rather than merely reversibly inhibiting hepatic 25-hydroxylase and renal 1-alpha-hydroxylase. Among nine tuberculosis patients, none of whose acetylator phenotype had been assessed, there were consistent decreases in the concentrations of 25-hydroxyvitamin D and 1-alpha,25-dihydroxyvitamin D together with a rise in parathyroid levels (all $P < 0.05$) after 1 month's therapy of INH combined with rifampicin. After 6 months, 25-hydroxyvitamin D was further reduced ($P < 0.01$) but 1-

alpha,25-dihydroxyvitamin D and parathyroid levels did not differ significantly from pretreatment values.

These observations leave little doubt that INH and rifampicin in combination perturb vitamin D metabolism, and that the extent of the effect may differ in rapid and slow acetylators. To decide whether or not these effects are sufficient to disturb calcium homeostasis in tuberculosis patients requires longer term studies in patients whose acetylator status has been determined.

D. GILBERT'S SYNDROME. A survey (322) of polymorphic acetylation in 27 patients with Gilbert's syndrome (mild chronic unconjugated hyperbilirubinemia) showed a large excess of slow acetylators (78% compared to 51% in 78 control subjects). This suggests an association between slow acetylation and Gilbert's syndrome could lead to a deficiency in acetylation of aromatic amines such as sulfamethazine, but no pathophysiological relationship between bilirubin metabolism or disposition and N-acetyltransferase appears to have been identified. Combined evaluation of bilirubin disposition and acetylator phenotype in families with Gilbert's syndrome might help reveal such a connection.

IV. The Human Acetylation Polymorphism

A. Acetylator Phenotype Determination

A large number of drugs and procedures are available for the determination of acetylator phenotype (table 7). The first assessments of acetylator phenotype were carried out by various methods with INH as the test drug. They include chemical, microbiological, or fluorometric determinations of INH and/or acetyl-INH in blood or urine. Among these, the fluorometric method is the most sensitive and currently preferred.

The first procedure for determination of acetylator status with INH was reported by Evans and coworkers (124) who chemically assayed the concentration of unacetylated INH in plasma 6 hr following oral ingestion. Eidus and coworkers (111, 112, 208) modified this method by determining the ratio of acetylated to nonacetylated INH in a 6- to 8-hr urine sample following oral ingestion. Subsequently, the procedure was automated (422). Ellard and coworkers (119) further altered this procedure for analysis of a 24- to 25-hr urine sample in patients maintained on an oral slow-release matrix preparation of INH.

Acetylator status also has been determined from measurement of the first-order half-life or rate constant for the elimination of INH from plasma after intravenous (204, 263) and oral (367) administration. Scott's procedure (367) involved a more sensitive fluorometric measurement. Miceli and coworkers (272) modified this fluorometric method permitting the use of as little as 25 μl of serum to potentially enable the assessment of acetylator status in newborn infants.

Until recently, determinations of acetylator status were frequently carried out with SMZ as a test agent rather than INH. A few of the reasons for this include its stable storage properties (INH is unstable in some preparations) (38), relative ease of chemical analysis, absence of extraction procedures, and rapidity of the Bratton and Marshall (41) assay to measure SMZ. The procedures of Evans (124) and Rao et al. (329) use the Bratton-Marshall assay method and they are the simplest methods for acetylator phenotype determinations. They also have been automated (129). Unfortunately, SMZ approved for human use has become difficult to obtain in the United States, and this has much reduced the popularity of this method. Rapid and slow acetylators are clearly differentiated by determination of the ratio of acetyl-SMZ to SMZ in venous blood and/or urine 6 hr after SMZ ingestion. Olson and coworkers (303) and du Souich and coworkers (109) have shown that SMZ in a dose of 40 mg/kg produced non-linear disposition kinetics, whereas an oral dose of 10 mg/kg resulted in linear disposition kinetics and a more accurate determination of acetylator status. Evans (124) first demonstrated that the SMZ to acetyl-SMZ ratio was a clearer method for acetylator phenotype determination in blood than in urine. This is partially due to the fact that variation in renal function can alter measurement of SMZ and acetyl-SMZ elimination in urine. For example, the renal clearance of SMZ and acetyl-SMZ can be affected by changes in urine pH because their pK_a values differ significantly from each other; yet both are similar enough to urine pH to critically influence the ratio of acetyl-SMZ to SMZ. Furthermore, high urine flow rates also affect renal clearance, potentially causing dilution of sample. Computer simulation of kinetic data showed that many commonly used SMZ acetylation markers (i.e., elimination half-life, excretion of SMZ in urine, urinary acetyl-SMZ to SMZ ratio, etc.) are susceptible to environmental alterations such as changes in absorption or urinary elimination rate constants (109). Consequently, they concluded that the acetyl-SMZ to SMZ ratio in blood was the least sensitive to such changes, and hence minimized misclassification.

With the procedures of Evans (124) and Rao et al. (329), "total drug" (diazotizable after acid and heat hydrolysis) and "directly reacting drug" (diazotizable without hydrolysis) are measured and their difference is considered to be due to acetylated drug. Two problems may arise with this method: 1) the "directly reacting drug" may contain other metabolites in addition to parent SMZ, and 2) SMZ decomposes under conditions needed for hydrolysis of acetyl-SMZ, potentially leading to inaccurate values for the acetyl-SMZ/SMZ ratio as has been reported by Whelpton et al. (446). To avoid these problems, Whelpton et al. (446) and Vree et al. (423, 424) have developed HPLC methods which measure both SMZ and acetyl-SMZ directly. Neither method requires hydrolysis, and both are applicable to as little as 10 μl of capillary blood, making them suitable for

TABLE 7
Methods for determination of acetylator status in humans

Drug	Method	Measurement	References
Caffeine	HPLC	Ratio of 5-acetylamine-6-formylamino-3-methyluracil to 1-methylxanthine in 2- to 6-hr urine	(159) (160) (161)
Dapsone	Fluorometric	Ratio of monoacetylated dapsone to dapsone in 4-hr plasma	(142) (321)
Dapsone	HPLC	Ratio of monoacetylated dapsone to dapsone in 3-hr blood	(58) (168)
Hydralazine	GC and HPLC	Ratio of 3-hydroxymethyl triazolophthalazine to acid-labile hydralazine in 0- to 24-hr urine	(412) (413)
Isoniazid	Chemical	Isoniazid concentration in 6-hr plasma	(124a)
Isoniazid	Chemical	Ratio of acetylated isoniazid to isoniazid in 6- to 8-hr urine	(208) (111) (112)
Isoniazid	Chemical (automated)	Ratio of acetylated isoniazid to isoniazid in 6- to 8-hr urine	(422)
Isoniazid	Chemical	Ratio of acetylated isoniazid in 24- to 25-hr urine after oral administration of slow-release, matrix isoniazid	(116)
Isoniazid	Chemical	First-order rate constant for isoniazid elimination in serum	(204) (263)
Isoniazid	Fluorometric	First-order rate constant for isoniazid elimination from serum	(367) (272)
Isoniazid	Microbiological	Microbiological active levels in blood	(390) (110)
Procainamide	TLC	Ratio of acetylprocainamide in 3-hr plasma	(337)
Procainamide	GLC	Ratio of acetylprocainamide in 6-hr plasma	(137)
Procainamide	HPLC	Ratio of acetylprocainamide in 24-hr urine or acetylation clearance of procainamide	(245) (244)
Sulfamerazine	HPLC	First-order elimination of sulfamerazine in blood	(424)
Sulfamethazine	Chemical	Ratio of acetylsulfamethazine in 6-hr plasma and/or urine	(124) (329) (303) (109)
Sulfamethazine	Chemical (automated)	Ratio of acetylsulfamethazine in 6-hr plasma and/or urine	(129)
Sulfamethazine	HPLC	Ratio of acetylsulfamethazine in 5- to 9-hr plasma	(446)
Sulfamethazine	Chemical	First-order elimination rate or metabolic clearance	(62) (242)
Sulfamethazine	HPLC		
Sulfadiazine	HPLC	Renal clearance of N-acetylmetabolite	(423)
Sulfapyridine	HPLC		
Sulfapyridine	Chemical	Ratio of acetylsulfapyridine in 6-hr plasma and/or urine	(361)
Sulphasalazine	Chemical	Ratio of acetylsulfapyridine in 6-hr blood or 5- to 6-hr urine	(86)
Sulphasalazine	Chemical	First-order elimination of sulfapyridine in saliva or plasma	(21)
Sulphasalazine	HPLC	Ratio of acetylsulfapyridine and other pharmacokinetic parameters	(67) (370)

acetylator phenotype determination of infants and children.

Schroder and Evans (361) found that sulfapyridine was equivalent to SMZ for the determination of acetylator status according to the percent of acetylation of sulfapyridine in plasma or urine. An HPLC method of acetylator phenotype classification with sulfapyridine has also been reported by Vree et al. (423).

Grant and coworkers (159–161) have used urinary metabolites of caffeine to develop a noninvasive method for acetylator phenotype determination (see section II C). Subjects ingested caffeine in coffee, tea, or cola soft drinks, and urine was collected during a period 2 to 6 hr after administration. Although caffeine itself is not acetylated directly, the AFMU metabolite is polymorphically acetylated (159–161). The mole ratio of AFMU to 1X is determined by HPLC.

For patients who are maintained on therapy with a drug that is subject to polymorphic acetylation, assessment of their acetylator status may be performed without using another drug such as SMZ or INH. For example, acetylator status has been determined with DDS by using either a fluorometric (142) or an HPLC method (58, 168, 321). HP can be used for acetylator phenotype determination with a combination of GC and HPLC methods of analysis (412, 413). Rapid and slow acetylators can also be classified with PA by using a TLC-densitometry procedure (337), a GLC procedure (137), or an HPLC procedure (244, 245). Sulfasalazine can be used for acetylator phenotype determinations by direct measurement of acetylsulfapyridine and sulfapyridine in plasma and/or

urine (86, 370, 67). Bates and coworkers (21) have adapted the technique to measure the concentration of sulfapyridine in saliva, eliminating the need for venipuncture(s) or urine collection (see section II C).

B. Determination of Acetylator Genotype

Each of the procedures listed in Table 7 discriminate rapid (RR, Rr) and slow acetylator (rr) phenotypes. However, most of them do not discriminate homozygous (RR) from heterozygous rapid (Rr) acetylator genotypes. Early reports by Sunahara et al. (389–391) and Dufour et al. (110) utilizing a vertical diffusion microbiological assay yielded trimodal distributions in acetylation capacity. Excellent agreement was obtained between the number of subjects within each mode and the expected frequency of each acetylator genotype. The microbiological method is relatively imprecise, however, and requires about 10 days to perform.

Methods have been recently developed that appear to distinguish between the two rapid acetylator genotypes. Chapron and coworkers (64) reported a trimodal frequency histogram when plotting the distribution of overall elimination rate constant, acetylation clearance, or metabolic clearance of SMZ. Chapron et al. (65) noted more recently that acetylation clearance was significantly dependent upon body weight and suggested that acetylation clearance values should be corrected for this variable. Family studies to confirm acetylator genotype have not been carried out to determine whether the frequency of each acetylator genotype observed is consistent with the expected value. The procedure that Chapron et al. used to determine acetylation clearance is a modification of that of Evan's procedure (124). Lee and Lee (242) used essentially the same procedure and found trimodal frequency distributions in acetylation clearance and metabolic clearance. The overall elimination rate failed to yield a trimodal distribution. Grant and coworkers (159–161) also have observed trimodal distributions of acetylation capacity by measuring mole ratios of caffeine metabolites. This method may be useful for discriminating homozygous and heterozygous acetylators, though this remains to be more firmly established.

Procedures which measure the ratio of acetyl metabolite to parent drug in a single plasma or urine sample usually give reliable acetylator phenotype classifications in healthy persons or in persons with unimpaired renal function. However, they must be interpreted cautiously in patients in whom the elimination pathway of the drug or its acetylated metabolite is hindered. For example, neither ratios of NAPA to PA in serum or urine provide a clear classification of acetylator status in patients with variable renal function (244). Iselius and Evans (196) reported that INH plasma concentrations were affected significantly by the age and gender of the patient. They suggested that these variables should also be considered in acetylator genotype classifications.

A simple, noninvasive in vitro method is needed for the determination of acetylator status in all individuals. Each of the in vivo methods listed in table 7 require administration of a test drug, and rely upon measurements of parent drug and/or acetyl metabolite in patient samples collected over a period of time. Large epidemiological studies relating acetylator status to toxicity in infants, patients with impaired renal function, elderly patients, diseased patients, or others who should not receive a test drug have been compromised by the lack of such a simple and noninvasive method. McQueen and Weber (267) found a heat stability difference in lymphocyte N-acetyltransferase activity between rapid and slow acetylators, but the distinction was not clear enough for use as an in vitro method for determination of acetylator status.

C. Ethnic and Geographic Variation in N-Acetylation Capacity

The proportions of rapid and slow acetylators vary remarkably in different ethnic and/or geographic origin populations. The percentage of slow acetylators is quite low in many Far-Eastern populations whereas it is considerably higher in some Mid-Eastern populations. For example, the percentage of slow acetylators among Canadian Eskimos is 5% (9) whereas it rises to 83% among Egyptians (169) and 90% among Moroccans (185). Most populations of European origin are approximately equally divided between rapid and slow acetylators (219).

Sunahara (389) first suggested a relationship between geographic latitude and frequency of the slow acetylator allele (q). Karim and coworkers (219) assembled data from a number of studies and reviewed this hypothesis. They concluded that there was a suggestion of such a relationship along the Asian-Pacific coast, but no clear pattern could as yet be demonstrated from data in Europe, Africa, Asia (excluding the Pacific Coast), or America. Karim et al. (219) mentioned, however, that much of the data should be interpreted with caution in light of the diverse nature of both the procedures used to gather the data and the populations studied. For example, the ethnic origin of many of the populations was either not clearly defined or included people of diverse origins (i.e., North American Blacks, Indians, and Eskimos). In other instances, the population moved to its present location within the past few generations (i.e., most North American Caucasians). In addition, many studies include both healthy subjects and patients with a variety of health disorders, particularly tuberculosis.

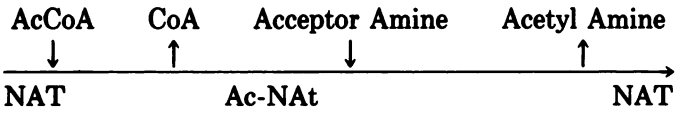
Although the populations analyzed by Karim et al. (219) are diverse, a number of important indigenous populations of third world countries need to be assessed to complete our knowledge of ethnic and geographic variation in N-acetylation capacity.

V. Mechanisms of Acetyl Transfer

A. Acetyl Coenzyme-A-Dependent N-Acetyl Transfer

Enzymatic N-acetylation of drugs proceeds according to a ping pong Bi-Bi reaction mechanism. This is a

double displacement reaction in which the enzyme oscillates between free and acetylated forms as follows:



The overall reaction can be written in conventional terms as two sequential half reactions, i.e.:

$$\text{AcCoA} + \text{NAT} \leftrightarrow \text{CoA} + \text{Ac-NAT}$$
$$\text{Ac-NAT} + \text{Amine} \leftrightarrow \text{Acetyl-amine} + \text{NAT}$$

This reaction is one of a class of two-substrate, two-product reactions in which the enzyme releases the first product produced, CoA, before the second substrate, the acceptor amine, enters the reaction (70).

Conceptually, a ping-pong mechanism was implicit in the mechanism initially suggested by Bessman and Lipmann (26) though that terminology had not yet been proposed at the time of their report. Bessman and Lipmann found that pigeon liver preparations reversibly catalyzed the low energy exchange of the acetyl group between 4-acetaminoazobenzene-4′-sulfonic acid and various acceptor arylamines including aniline, benzidine, PABA, sulfanilamide, hydroxylamine, and hydrazine. They postulated that an acetyl-enzyme intermediate was formed from a study of the exchange of isotopically labeled acetyl in each of the half reactions. They proposed further that the enzymatic transfer of the acetyl group from acetyl coenzyme A to aromatic amines occurred by the same mechanism. Jenne and Boyer (206) extended the study of pigeon liver NAT by using INH as the acceptor amine. Their double reciprocal (Lineweaver-Burk) plots of initial velocities for the acetylation of INH at various fixed concentrations of acetyl coenzyme A, and vice versa, gave sets of parallel lines, suggesting that acetylation occurred via an acetyl-enzyme intermediate.

We subsequently performed a more comprehensive study of the mechanism of acetylation of arylamines and hydrazines by using more highly purified liver NAT preparations from several species of mammals including rabbit, man, monkey, and rat (reviewed in ref. 430). Detailed studies of the mechanism were performed with enzyme purified from livers of rapid and slow acetylator rabbits and man with acetyl coenzyme A and various arylamines and hydrazines (427, 428, 384, 385, 430). Double reciprocal plots of initial velocity data with acetyl coenzyme A as the acetyl donor and INH as the acetyl acceptor yielded sets of parallel lines. Product inhibition patterns of INH acetylation were consistent with a "one-site" or classical ping pong pathway. Similar studies with human liver NAT showed the same type of product inhibition patterns. Thus, coenzyme A was found to be a non-competitive inhibitor of INH acetylation with respect to acetyl coenzyme A and a competitive inhibitor with respect to INH while acetyl-INH (or acetylsulfamethazine as an alternate product inhibitor) was competitive with respect to acetyl coenzyme A. These product inhibition patterns eliminated a mechanism containing an isomerization step as an alternative to the simple "one-site" ping pong mechanism.

Product inhibition patterns observed by Andres et al. (6) with homogeneous pigeon liver NAT differed from those for the mammalian liver enzyme. They reported product inhibition patterns of coenzyme A with respect to *p*-nitroaniline as the acceptor amine and for 3′,5′-ADP and AMP as analogs of coenzyme A with respect to acetyl coenzyme A. Coenzyme A was a non-competitive inhibitor of transacetylation of *p*-nitroaniline with a K_I of 70 μM while the coenzyme analogs were both competitive inhibitors with respect to acetyl coenzyme A. The respective K_I values for 3′,5′-ADP and AMP were 1.7 and 10 mM. These product inhibition patterns are exactly the opposite of those observed with rabbit liver NAT. The pigeon liver NAT patterns are consistent with the "two-site" or "hybrid" pathways originally described by Northrop (297). The same patterns have also been reported recently for N-acetylation of histones by nuclear NAT from calf thymus (454). Thus, while arylamine:acetyl coenzyme A NAT of pigeon liver shares certain mechanistic features with its opposite number in humans and rabbits, the product inhibition patterns of the avian enzyme distinguish it from the mammalian enzyme.

Isotope exchange studies with the rabbit and human liver enzymes showed interchange of the acetyl group between acetyl coenzyme A and coenzyme A, and between the amine, aniline, and the acetylated derivatives, acetanilide or acetyl-INH. An acetyl-NAT intermediate was isolated and shown to be catalytically competent (385).

In addition, the rate-determining step in the overall N-acetylation reaction for the mammalian enzyme was identified. Studies of pigeon liver NAT had demonstrated that the rate of the second half reaction was highly sensitive to the structure of the amine (201) and to its basicity or polarity (343, 203). With weakly basic acceptor amines, the maximum velocity differed for different acetyl acceptor amines, and correlated with the pK_a of the amines. This suggested that the transfer of the acetyl group from the acetyl-enzyme intermediate to the acceptor amine was rate-determining. It also suggested that the saturation with increasing amine concentration represented binding of the acceptor amine to the acetyl-enzyme. In contrast to weakly basic amines, the maximum velocities with strongly basic amines were identical. This indicated that the rate-determining step had changed from the second to the first half reaction, i.e., to the formation of the acetyl-enzyme, a common step in the acetylation of all amines.

Most of the hydrazines and arylamines that were first investigated such as INH, hydralazine, and sulfamethazine have pKa's greater than 4. We had found with acetyl coenzyme A as the acetyl donor that the maximum

velocities were identical within experimental error with all of these acceptor amines even though they are structurally dissimilar and their apparent K_m's differed by almost a thousandfold. This suggested that the formation of the acetyl-enzyme was the rate-determining step for the mammalian enzyme for more basic acceptor amines (430). These studies have recently been extended by investigating a series of para substituted anilines with pK_a's that differ by small increments over the range of 1.1 to 5.3, i.e., with pK_a's at or below the pK_a region where the change in the rate-limiting step occurs. Preliminary observations indicate that the acetylation of these amines with the rapid NAT rabbit liver isozyme is rate-determining when the pK_a of the amine is greater than 1.1 to 1.74 (Andres, Vogel, and Weber, unpublished observations). Comparative studies of this type with the slow acetylator isozyme should tell whether a pK_a-dependent difference of the rate-determining step between rapid and slow NAT isozymes is discernible.

B. Acetyl Coenzyme A Independent Acetyl Transfer

Bartsch et al. (18, 19) proposed that metabolic activation of arylamine carcinogens occurs by intramolecular transfer of the acetyl group of the arylhydroxamic acid from N to O mediated by an N,O-arylhydroxamic acid acyltransferase (AHAT). A two-step mechanism (18, 221) similar to the one described above for acetyl coenzyme-A-dependent acetyl transfer was proposed, but without participation of acetyl coenzyme A in the AHAT activation process. The first step was postulated to be the transfer of the acetyl group from the arylhydroxamic acid (N-OH-AAF) to the enzyme from an acetyl enzyme intermediate while the second step was the transfer of the acetyl group from the intermediate to the hydroxylamine to yield an N-acetoxyarylamine; in the presence of an arylamine, the acetyl group would be transferred to the arylamine to form a stable amide. This mechanism was supported indirectly by showing that the enzyme transferred the acetyl group from the arylhydroxamic acid to the amino group of 4-aminobenzene as described by Booth (35). Complete block of the formation of nucleic acid adducts from the arylhydroxamic acid by the substitution of the oxygen of the hydroxamic acid with a methyl group (221) provided additional support for this mechanism.

Determination of complex enzyme reaction mechanisms ordinarily includes the determination of initial velocity patterns, product inhibition patterns, and isotope exchange studies with substrates and products of each of the putative half reactions. These techniques could not be readily applied to this reaction because of the instability of the N-acetoxyarylamine. The existence of this transient product was inferred instead from adducts formed when nucleophiles (e.g., acetylmethionine, nucleic acid, protein) were added to incubation mixtures containing arylhydroxamic acids and AHAT (18, 221, 23), presumably following its ionization to the reactive arylnitrenium ion.

Studies performed by Hanna and his associates serve to illustrate the difficulties attending the mechanistic analysis of the AHAT reaction (16, 167). From studies of rat and hamster liver preparations with the arylhydroxamic acid of AF, Hanna showed that the arylhydroxamic acid was not only a substrate for AHAT, but also was a suicide inhibitor of AHAT. He suggested that suicide inactivation of AHAT by arylhydroxamic acids resulted from their conversion to electrophilic species that reacted directly with nucleophilic groups of amino acids at or near the active site of the enzyme, or, alternatively, escaped from the enzyme to form adducts with tRNA, amino acids, or other biological nucleophiles, including AHAT.

Several pieces of evidence for the ping pong mechanism proposed for the AHAT reaction rest on the similarities of NAT and AHAT. Booth (35) suggested that an NAT-like mechanism was involved in the activation of arylamines to carcinogens but the precise relationship of that system to activation was unclear. Bartsch et al. (18) obtained evidence for the possible identity of AHAT with the NAT system studied by Booth. Subsequently, our studies with the rabbit model of the acetylation polymorphism indicated that NAT and AHAT are probably mediated by the same enzyme (149, 437) (see section VI B). Hanna's (167) observation that suicide inactivation of hepatic AHAT activity by AF was accompanied by concurrent inactivation of some NAT activities provided additional evidence for an intimate relationship between these activities.

VI. Hereditary Acetylation Polymorphisms in Animals

A. Genetic Animal Models in Acetylation Pharmacogenetics

Investigations in several species have sought to develop experimental animal models of the acetylation polymorphism for elucidation of its biochemical and genetic basis, and its toxicological consequences in man. Extensive characterizations of NAT have been carried out with pigeon liver, though the pigeon does not express a genetic acetylation polymorphism. Until recently, the acetylator polymorphism in rabbits was the only animal model for this genetic trait. The rabbit has some disadvantages as a model because of a relative lack of published genetic information, a lengthy breeding time, and the problems associated with maintenance of a rabbit colony. Consequently, other species of animals have been screened for the acetylation polymorphism, including rhesus monkey (154, 356, 344), baboon (328), deer mouse (*Peromyscus maniculatus*) (401), laboratory mouse (*Mus musculus*) (401–403, 150, 151), rat (401), and Syrian hamster (174). Each of these animals, with the exception of the baboon (328), expresses the N-acetylation polymorphism,

though data in the rhesus monkey (344) and rat are incomplete. Together with the rabbit model, the mouse model and the hamster model have provided valuable biochemical, genetic, and toxicological information concerning N-acetylation, much of which could not have been derived from human studies alone.

B. The Rabbit Model

1. Identification and inheritance studies. Rabbits were the first mammalian species after humans in which genetically rapid and slow acetylators were identified. Frymoyer and Jacox (139) found a bimodal distribution in the elimination half-life of sulfadiazine administered intravenously to intact rabbits (fig. 9). Gordon et al. (158) reported a similar finding with sulfamethazine. Weber and coworkers (431, 178) reported bimodal distributions in both sulfadiazine and INH acetylation half-lives. In addition, they observed a unimodal distribution in PABA N-acetylation half-lives (431), similar to observations of Frymoyer and Jacox (139) with PAS.

Rapid and slow acetylators also have been identified in outbred rabbits by measurement of hepatic N-acetyltransferase activity in vitro. Frymoyer and Jacox (140) first described a trimodal distribution in N-acetyltransferase activity and suggested that it corresponded to the RR, Rr, and rr acetylator genotypes. Subsequently, bimodal distributions in hepatic acetylation capacity in vitro have been reported in numerous studies with various substrates (170, 394, 148, 305, 149, 171, 173). Homozygous rapid and slow acetylators have also been identified among established inbred rabbit lines (Table 8) (437, 175).

Gene dose-response relationships in hepatic N-acetyltransferase activity have also been shown for many substrates including PABA, PAS, SMZ, INH, AF, and PA (175). However, the magnitude of the genetically determined activity differences between acetylator genotypes depends upon the substrate tested, with PABA and PAS exhibiting the smallest differences and PA, SMZ, INH, and AF exhibiting progressively larger differences in hepatic N-acetyltransferase activity across all three acetylator genotypes.

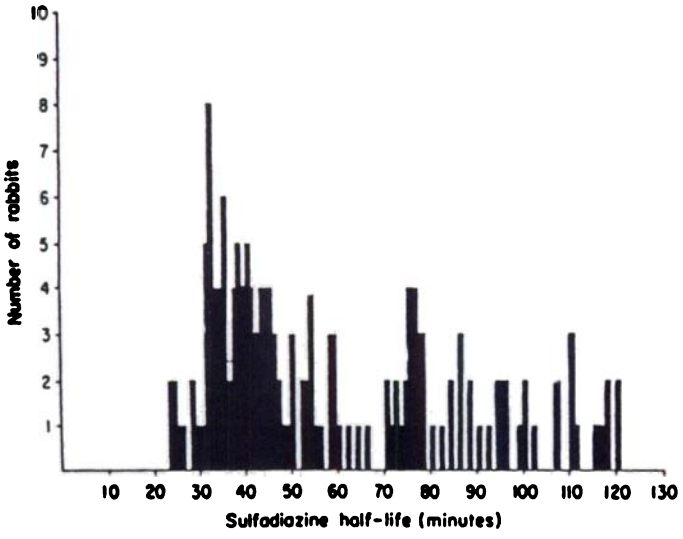


FIG. 9. Frequency distribution of sulfadiazine N-acetylation half-lives for rabbits. Reproduced with permission from Frymoyer and Jacox (139).

2. Mechanism of inheritance. It has been well documented from pedigree analysis of rabbit families that acetylator differences in rabbits are controlled by a simple autosomal Mendelian gene with two major alleles. Rabbits are divisible into phenotypically rapid (RR, Rr) or slow (rr) acetylators according to the rate in vivo at which they acetylate drugs such as sulfadiazine, SMZ, or INH (139, 158, 431). They are also divisible into homozygous rapid (RR), heterozygous rapid (Rr), or homozygous slow (rr) by in vitro measurements of hepatic NAT activity for these substrates (175).

3. Biochemical basis of monomorphic and polymorphic expression of hepatic NAT activity. Sulfadiazine N-acetylation half-lives in rabbits yield bimodal frequency distribution histograms whereas PABA and PAS yield unimodal (or nearly unimodal) histograms, as described above (139, 431). Substrates have thus been categorized as either "monomorphic" if they yield a unimodal distribution pattern in vivo or "polymorphic" if they yield bimodal and trimodal distributions.

Monomorphic and polymorphic substrates may exhibit dissimilar patterns of hepatic NAT activity in vitro. For example, hepatic PABA NAT activity is about 2- to 2.5-fold higher in rapid acetylator than slow acetylator rabbits, whereas hepatic NAT activity measurements of polymorphic substrates such as SMZ, INH, or AF range from approximately 10 to more than 100-fold higher in rapid versus slow acetylators (148, 305, 171, 173, 175). Investigators initially suggested that separate forms of hepatic NAT were responsible for catalyzing the N-acetylation of monomorphic and polymorphic substrates (207, 170). Recent studies, however, provide convincing arguments against this hypothesis. Thus, monomorphic and polymorphic NAT activities could not be separated by extensive purification and gave indistinguishable heat inactivation patterns at each of two different temperatures (173). In addition, monomorphic substrates exhibited a gene dose-response relationship in liver and gut NAT activity similar to the polymorphic substrates (175). These findings support the hypothesis that monomorphic and polymorphic expression of NAT activity is catalyzed by a single major NAT rather than two or more separate ones in rabbit liver.

4. Qualitative differences between rapid and slow acetylator hepatic NAT. Frymoyer and Jacox (140) reported a direct relationship between N-acetylation capacity in vivo and hepatic NAT activity in vitro for rabbits, but they did not investigate the molecular basis for these heritable metabolic differences in N-acetylation capacity

TABLE 8

*Liver N-acetyltransferase activities in inbred strains of rabbit, mouse, hamster, and rat**

Acetylator Phenotype	Inbred Strain	PABA NAT (nmol/min/mg)	PAA NAT (nmol/min/mg)
Rabbit			
Rapid	B/J	4.23	52.1
	III/J	7.23	34.9
	IIIC/J	4.28	48.6
	III *Dw*/J	4.08	36.2
	III EP/J	3.43	52.2
	III VO/J	3.85	52.1
Slow	ACEP/J	1.26	0.094
	III*cd*/J	1.86	0.109
	IIIVO/*ahJ*	2.98	0.189
	IIIVO/*vptJ*	1.97	0.112
Mouse			
Rapid	AuSs/J	30.3 ± 4.2	0.361 ± 0.042
	Castaneous	35.4 ± 8.3	0.238
	ST/bJ	18.8 ± 1.1	0.155
	C57BL/6J	24.7 ± 2.4	0.364 ± 0.075
	Molossinus	24.4 ± 1.3	0.207
	SF	14.7 ± 2.4	0.215 ± 0.033
	SWR/J	23.8 ± 3.4	0.294 ± 0.042
	129/SV	26.7 ± 4.7	0.233
	RF/J	20.8 ± 1.8	0.338 ± 0.051
	RIII/2J	22.3 ± 4.2	0.151
	IsCam	27.8	0.411
	SJL/J	24.3 ± 2.9	0.247
	BALB/cJ	25.3 ± 4.9	0.314 ± 0.114
	C3H/HeJ	23.1 ± 3.6	0.362 ± 0.026
	CBA/J	24.4 ± 1.7	0.314 ± 0.090
	AKR/J	21.0 ± 3.4	0.345 ± 0.085
	DBA/J	24.4 ± 2.8	0.320 ± 0.070
Slow	A/J	17.9 ± 4.0	0.058 ± 0.024
	AHe/J	17.3 ± 1.6	0.032 ± 0.032
	X/Gf	19.4 ± 2.2	0.178 ± 0.033
Hamster			
Rapid	Bio. 1.26	47.0 ± 3.44	12.7 ± 0.8
	Bio. 4.22	44.4 ± 3.53	14.2 ± 1.3
	Bio. 4.24	55.1 ± 2.19	10.6 ± 0.9
	Bio. 7.88	43.1 ± 3.97	11.4 ± 0.8
	Bio. 10.37	37.2 ± 2.89	15.7 ± 1.3
	Bio. 11.10	42.6 ± 2.27	16.1 ± 1.2
	Bio. 12.14	48.0 ± 3.17	16.7 ± 2.8
	Bio. 14.6	36.2 ± 1.69	12.6 ± 0.9
	Bio. 15.16	41.6 ± 3.42	17.4 ± 2.1
	Bio. 40.54	34.8 ± 4.61	14.4 ± 1.7
	Bio. 41.56	37.5 ± 3.38	13.2 ± 0.8
	Bio. 45.5	45.1 ± 1.74	15.3 ± 1.2
	Bio. 53.58	51.6 ± 5.27	19.0 ± 1.6
	Bio. 65.67	53.0 ± 2.32	16.7 ± 2.3
	Bio. 72.29A	40.0 ± 4.10	23.5 ± 2.9
	Bio. 72.29T	40.4 ± 4.48	14.9 ± 0.6
	Bio. 82.62	36.2 ± 2.95	13.5 ± 1.6
	Bio. 84.9	42.9 ± 3.07	18.3 ± 2.2
	Bio. 86.93	55.5 ± 8.36	12.7 ± 1.4
	Bio. 87.20	53.5 ± 4.98	16.6 ± 3.0
	Bio. IPI	51.4 ± 4.29	16.9 ± 2.0
	Bio. PD4	46.0 ± 2.67	21.9 ± 1.4
	Bio. XXB	43.0 ± 3.32	13.2 ± 0.8
Slow	Bio. 1.5	0.141 ± 0.012	11.9 ± 1.2
	Bio. 82.73	0.176 ± 0.021	10.3 ± 0.7
Rat			
Not designated	NSD/N	0.09 ± 0.03	2.33 ± 0.06
	WKY/N	0.24 ± 0.04	1.65 ± 0.27
	SHR/N	0.32 ± 0.02	2.38 ± 0.15
	ODU/N	0.39 ± 0.03	1.81 ± 0.15
	WN/N	0.45 ± 0.09	2.20 ± 0.26
	CAR/N	0.62 ± 0.04	2.11 ± 0.40
	BN/S$_a$N	0.64 ± 0.04	1.94 ± 0.27
	BUF/N	0.91 ± 0.11	2.91 ± 0.32
	M520	1.03 ± 0.20	2.38 ± 0.58
	ACI/N	1.14 ± 0.25	3.67 ± 1.76
	F344/N	1.28 ± 0.59	3.24 ± 1.07
	LEW/S$_a$N	1.32 ± 0.43	3.88 ± 0.56
	LA/N	1.66 ± 0.15	2.88 ± 0.29
	MR/N	1.77 ± 0.05	1.79 ± 0.07
	OM/M	1.77 ± 0.19	3.16 ± 0.56
	PETH/N	1.81 ± 0.66	3.46 ± 1.57
	MNR/N	1.87 ± 0.10	2.07 ± 0.16
	RHA/N	1.89 ± 0.14	2.60 ± 0.25
	ALB/N	2.51 ± 1.63	2.34 ± 1.14

* Abbreviations used are: PABA NAT, *p*-aminobenzoic acid N-acetyltransferase activity; PAA NAT, polycyclic aromatic amine N-acetyltransferase activity (2-aminofluorene for rabbit and hamster, benzidine for mouse, and 4-aminobiphenyl for rat). All NAT determinations were conducted with 100,000 xg liver cytosol at 37°C. Variability represents standard deviation of NAT activity for mouse and rat, and standard error of NAT activity for hamster. Variability values are absent for determinations made in fewer than three animals. For further details, see original references for rabbit (175), mouse (150), hamster (174), and rat (286).

further. Jenne (207) suggested that rapid and slow acetylators had quantitative differences in the amount of an identical hepatic NAT enzyme from biochemical studies of enzyme prepared from human liver autopsy specimens. This hypothesis was inconsistent with evidence forthcoming later from studies in the rabbit model. Patterson et al. (305) undertook immunochemical studies to search for differences between rapid and slow acetylator hepatic NAT(s). Precipitin reactions between goat anti-NAT antibody and 100,000 g liver cytosol yielded equivalent amounts of precipitated protein at nearly identical equivalence points for rapid and slow acetylators, despite the several hundred-fold higher NAT activity in rapid acetylators. Consistent with these results, Hein et al. (175) reported large differences in apparent K_ms between rapid and slow acetylator NAT for PAS, suggesting a qualitative difference in their enzyme structure. Finally, studies have shown heat inactivation differences in hepatic PABA NAT activity between rapid and slow acetylators, a test capable of distinguishing enzymes with qualitative differences (432, 173).

These results obtained in studies of the rabbit NAT are best explained by a qualitative, structural difference in hepatic NAT between rapid and slow acetylators, rather than a quantitative difference in the amount of an identical enzyme.

5. Acetyl-CoA-dependent NAT and acetyl CoA-independent AHAT catalyzed by a common enzyme protein. Preliminary comparisons of AHAT and NAT properties across several species including rat and rabbit indicated a possible relationship between the two enzyme activities (149). Glowinski and coworkers (149) investigated this possibility further in rapid and slow acetylator rabbits as has been discussed above (section II D). The results obtained support the hypothesis that NAT and AHAT activities are associated with a common enzyme protein, or of closely linked enzymes, in rabbit liver.

6. Purification and substrate specificity of hepatic NAT. Initial characterization of rabbit liver NAT was carried out by Weber and Cohen (427), who achieved a 300- to 500-fold purification (15% recovery) following sequential centrifugation, ammonium sulfate precipitation, gel filtration on Sephadex G-100, and ion-exchange chromatography on DEAE-cellulose. Use of this procedure, slightly modified by Glowinski et al. (149, 435), yielded a single band of enzyme protein of 33,000 molecular weight after SDS slab gel electrophoresis.

Although large quantitative differences in catalytic activity are noted between rapid and slow acetylator NAT for polymorphic substrates, no significant qualitative differences in substrate specificity have been noted. The substrate specificity of rabbit liver NAT is broad, encompassing arylamines, biogenic amines, and hydrazines. Substrates include hydrazine drugs such as INH, hydralazine, and phenelzine (429, 176), arylamine drugs such as PAS, SMZ, sulfadiazine, PA, and dapsone (429, 173), various multicyclic arylamine carcinogens such as AF and benzidine (148, 172), various arylamine chemicals such as PABA and its derivatives (429, 172, 177), and various hydrazine derivatives of INH including MAH, a substrate whose structure is dissimilar to other substrates (172, 176). Evidence suggests that the substrate specificity of liver NAT does not include cyclohexylamine, glucosamine, pyridoxamine, phenylalanine, and glutamine (434).

7. Extrahepatic NAT. NAT activity is widely distributed among tissues with highest levels expressed in duodenum and lung (170). Lower levels (listed in order of decreasing activity) were found in thymus, ovary, spleen, uterus, adrenal gland, leukocytes, kidney, bone marrow, salivary gland, pancreas, pineal gland, erythrocytes, and brain. Recently, Smolen and Weber (381) reported NAT activity in bladder mucosa. NAT activity was undetectable in plasma, colon, esophagus, stomach, skeletal muscle, or fat (170). Rabbit spleen and kidney NAT could be purified 700-fold from 100,000 g cytosol by a procedure similar to that for liver NAT (170).

NAT activity is readily measured in many tissues, but genetically polymorphic activity is not expressed in every one. A gene dose-response relationship in NAT activity occurs in duodenal mucosa that is similar to that found in liver (175). However, NAT activity declines continuously throughout jejunum and ileum and is very low or undetectable in colon (170). In addition, SMZ NAT activity in spleen, kidney, and pineal gland was equivalent in rapid and slow acetylators (170).

The relationship between blood cell NATs and the genetically polymorphic liver NAT has been studied by Drummond and coworkers (105) who developed a procedure for purification of rabbit blood cell NAT and achieved up to 100-fold purification. No differences could be detected in substrate specificity, metal inhibition, stability, heat inactivation, and temperature and pH optima of blood NAT partially purified from rapid and slow acetylator rabbits. PABA gave the highest NAT activity of the various substrates tested. Activities for aniline, SMZ, anileridine, sulfadiazine, PA, and sulfanilamide were less than 5% of that for PABA. Activity was undetectable for sulfamerazine, sulfapyridine, PAS, or INH.

Lymphocytes have levels of specific NAT activity which are about 100-fold higher than erythrocyte levels in both rapid and slow acetylator rabbits (394). However, slow acetylator rabbits have 2.5- to three-fold higher levels of lymphocyte and erythrocyte PABA NAT activity than rapid acetylators (431, 394).

8. Relationships of acetylator phenotype to toxicity. Genetically determined differences between rapid and slow acetylator rabbits demonstrated in vivo and in vitro, are also demonstrable in primary cultures of hepatocytes isolated from the two phenotypes. Differences in susceptibility to DNA damage produced by arylamines and hydrazines is also dependent on acetylator status (268, 269) (see section II D).

Other studies have shown that differences in acetylation capacity between rapid and slow acetylator rabbits also determine susceptibility towards neurotoxicity from hydrazine (178). These studies are discussed elsewhere in connection with hydrazine and arylamine-induced neurotoxicity in humans (see section III A).

C. The Mouse Model

1. Identification of the N-acetylation polymorphism. Initial surveys seeking genetic variability in NAT activity in inbred mouse strains indicated that SMZ NAT activity was undetectable in liver preparations from all strains surveyed, while liver PABA NAT activity varied approximately twofold from 7.61 to 16.4 nmol/min/mg across the strains (401). Interstrain variability of blood PABA NAT activity was much greater, ranging from 0.88 to 2.11 nmol/min/mg in eight of the strains, to undetectable levels in the A/J (A) inbred strain. Small but statistically significant differences in N-acetylation capacity of SMZ in vivo between the rapid (B6) and slow (A) acetylator strains were also found (402, 403).

A more extensive survey of inbred mouse strains (including the A and B6 strains) confirmed the polymorphic nature of blood PABA NAT activity, and provided additional data on blood benzidine NAT activity (150). Of

the 20 strains surveyed, 17 were classified as rapid acetylators and three were classified as slow acetylators (table 8). Rapid acetylator strains possessed severalfold higher levels of liver benzidine (or AF) NAT activity than the slow acetylator strains. Blood and liver benzidine NAT activities showed a significant correlation ($P < 0.001$) across the 20 strains. Liver PABA NAT activities in the strains exhibited a different pattern from that of liver benzidine NAT (table 8). In contrast to the polymorphic nature of blood PABA and benzidine NAT, and of liver benzidine NAT, The interstrain variation in liver PABA NAT was relatively small (about twofold). Liver SMZ NAT activities were measured, but the level of activity was close to the limit of detection of the assay.

In addition to differences in NAT activity observed in liver and blood, differences were observed in PABA and AF NAT activities in kidney and small intestine of A and B6 inbred mice. Differences in liver PABA NAT activities between A and B6 mice were small (up to twofold) (150, 151), and small intestine PABA NAT activity was approximately fourfold higher in rapid than in slow acetylators (166). Small intestine and kidney AF NAT activity also reflected differences in acetylator phenotype. Kidney NAT activities, in contrast to liver, blood, and small intestine were significantly higher in B6 males than females (151).

2. *Inheritance studies.* Tannen and Weber examined blood PABA (402, 403) and PA (403) NAT variability in rapid (B6) and slow (A) acetylator mice, and in their F_1, F_2, and backcross progeny. Blood PABA NAT activities were intermediate in F_1 hybrid animals and exhibited a trimodal distribution in the F_2 generation, not significantly different from the expected 1:2:1 ratio. Examination of backcross animals showed ratios (parental:intermediate) of approximately 1:1 in the $F_1 \times$ A mice and the $F_1 \times$ B6 mice. Genetic analyses of blood and liver AF NAT activities in F_1, F_2, and backcross generations yielded ratios consistent with simple autosomal Mendelian inheritance of two co-dominant alleles at a single locus for these activities. The gene symbol *Nat* was proposed, with Nat^r as the rapid acetylator allele and Nat^s as the slow acetylator allele (150).

Studies of blood and liver PABA and AF NAT activities were also performed in 11 recombinant inbred strains (obtained by inbreeding the F_2 generation of A and B6 crosses) (150). Blood PABA and AF NAT, and liver AF NAT activities in the recombinant inbred strain distribution pattern were bimodal. Five strains (BXA-1, AXB-2, AXB-5, AXB-6, and AXB-17) had high NAT activities resembling the B6 progenitor, and the other six strains (BXA-3, BXA-6, AXB-2, AXB-3, AXB-4, and AXB-9) had low NAT activities resembling the A progenitor. The hypothesis of single gene inheritance of the mouse acetylation polymorphism receives additional support from these observations (150). In addition, they suggest the existence of modifier genes that segregate independently of the major *Nat* gene. For example, liver AF NAT activity in the AXB-3 strain is approximately 50% of the A parental strain, suggesting a modifier gene which acts to enhance this activity. The homozygous Nat^r recombinant inbred strains (BXA-1, AXB-5, AXB-6, and AXB-17) exhibit higher blood AF NAT activities than the B6 progenitors, suggesting the existence of modifier genes in the B6 mouse which lower blood AF NAT activity (165). The twofold variation in blood PABA and AF NAT and liver AF NAT activity within the B6 parental rapid acetylator strain may be attributable to modifier genes that segregate independently of the major *Nat* gene. This possibility will be examined further through the development of congenic inbred lines to place the Nat^r allele onto the A background, and the Nat^s allele onto the B6 background.

3. *Evidence for intrinsically different rapid and slow acetylator liver NATs.* Apparent K_m differences between rapid and slow acetylator liver NAT were observed for PABA and AF, suggesting that there is a qualitative difference in structural gene products for NAT from A and B6 mouse strains (151).

4. *Evidence for coexistence of monomorphic (PABA) and polymorphic (AF) NAT activity on a common liver enzyme protein.* In both strains, liver PABA and AF NAT activities were inseparable by purification procedures that exploited differences in size and charge. The purification scheme was essentially identical to that described for purification of the rabbit liver enzyme (sequential centrifugation, ion-exchange chromatography on DEAE-cellulose, and gel filtration chromatography on Sephacryl S-200, and SDS PAGE). In addition, AF and PABA NAT activities from B6 mouse liver migrated together as a single, sharp symmetrical protein band during the final step in the procedure (151). Attempts to determine whether NAT and AHAT activities coexist on the mouse enzyme failed because the N-OH-AAF AHAT activities were at the limit of assay sensitivity for both B6 and A mouse strains, leaving this possibility to be further explored.

5. *Pharmacokinetic differences in N-acetylation capacity in vivo.* Tannen and Weber (403) found a higher rate of N-acetylation in vivo in B6 mice than A mice from measurements of urinary metabolites for both SMZ and INH, but not for PABA and PA. Hultin and Weber (193, 194, 440) measured elimination rates from blood in vivo of AF after intraperitoneal injection in A and B6 mice. AF elimination rates were dose-dependent and AF concentrations in blood decreased according to pseduo first order kinetics. AF elimination rate constants were approximately two- to fourfold higher in B6 than A mice. More detailed pharmacokinetic studies have been carried out in A, B6, F_1 hybrid mice. AF elimination rate constants in the F_1 hybrids were intermediate between those of B6 mice and A mice (193, 440). These results provide

added support for Mendelian inheritance of the acetylator trait in mice.

6. Pharmacokinetic differences in N-acetylation by isolated hepatocytes. Hultin (193) and Weber (440) demonstrated higher N-acetylation capacity in hepatocytes isolated from B6 mice compared to A mice and maintained in primary culture. At lower AF concentrations, its disappearance obeyed pseudo first order kinetics and was twofold higher in B6 than A mouse hepatocytes (193). At saturating concentrations of AF, the AAF rate of formation was threefold higher in B6 than A mouse hepatocytes.

Rates of AF disappearance and AAF formation were determined in the presence of the inhibitors. PABA and folic acid inhibited AAF formation 97% and 25% in B6 hepatocytes, respectively (193). This is consistent with K_i differences observed between PABA and folic acid in B6 liver cytosol (193). PABA and folic acid inhibited AF disappearance in B6 hepatocytes 83% and 26%, respectively, suggesting that the primary biotransformation of AF in B6 hepatocytes occurs chiefly via N-acetylation. In hepatocytes isolated from A mice, AF disappearance was inhibited 50% and 26% by PABA and folic acid, respectively, whereas AAF formation was inhibited 98% and 30%, respectively. Thus in A hepatocytes, PABA inhibits AAF formation more effectively than it inhibits AF disappearance, suggesting that other competing reactions, such as nitrogen or ring hydroxylation(s) may be relatively more important in the elimination of AF in A mice.

7. Relationship of acetylator status to toxicity. We have performed numerous studies (403, 436, 193, 286, 440) to investigate possible relationships between acetylator status and arylamine-induced toxicity in the mouse.

Preliminary observations on the rates of unscheduled DNA synthesis (indicative of DNA damage) in hepatocytes from both the rapid and slow acetylator strains suggest that AF-induced unscheduled DNA synthesis is two- three-fold higher in B6 hepatocytes than A hepatocytes with AF concentrations ranging from 1 μm to 1 mM (193, 440). Additional studies are necessary to confirm these observations though they are consistent with the effect of acetylator status on AF-induced DNA damage observed in rapid and slow acetylator rabbit hepatocytes (268, 269).

The occurrence of spontaneous and PA-induced antinuclear antibody development was investigated in B6 and A mice as a model for human drug-induced lupus erythematosus (403, 436). A mice are predisposed to both spontaneous and drug-induced antinuclear antibody formation (404), and to assess relationships between slow acetylator phenotype and antinuclear antibody formation further, both spontaneous and PA-induced antinuclear antibody formation were measured in sera obtained from parental A and B6 mice, F_1, F_2, and backcross animals exposed to PA and controls. These studies indicated that spontaneous and PA-induced antinuclear antibody development were dissociable processes. In addition, they indicated that the spontaneous occurrence of antinuclear antibodies was associated with the slow acetylator phenotype. The basis for this association has not been investigated further, but it suggested that slow acetylation might serve as a genetic marker for susceptibility to spontaneous antinuclear antibody formation in the mouse.

Several indices of hepatic damage were measured after administration of methylene dianiline to B6 and A mice in an attempt to understand the biochemical basis for jaundice induced by this arylamine (286, 439). Preliminary results suggested that the hepatic damage was higher in B6 males than in A males, but further findings indicated a more complex relationship, highly influenced by mouse gender. Examination of methylene dianiline-induced hepatic damage in recombinant inbred lines derived from B6 and A mice failed to demonstrate a correlation between strain distribution patterns of acetylator phenotype with extent of hepatic damage. These findings provide evidence against a critical role of acetylator status in methylene dianiline hepatotoxicity, but additional studies are needed to fully understand the complex effects of acetylation capacity, sex, and perhaps other factors, before a definite conclusion is reached.

D. The Hamster Model

1. Identification of inherited variation in inbred hamsters. Variation in N-acetylation capacity among inbred hamster strains was demonstrated in a series of 25 inbred hamster strains generously donated by the Bio Research Institute (174). Cytosolic liver PABA and PAS NAT activities showed high interstrain variation, whereas interstain variability for INH, PA, SMZ, or AF was about twofold (Table 8). INH NAT activities were higher in all hamster strains than had been found in rabbit, mouse, or rat and AF NAT activities were also high. PA and SMZ NAT activities were low across all strains.

The Bio. 1.5 and the Bio. 82.73 strains (slow acetylators) had remarkably lower PABA (and PAS) NAT activities than the other 24 inbred hamster strains (rapid acetylators). In the 24 rapid acetylator strains, PABA NAT activities were higher than in any species tested, ranging from 34.8 to 55.5 nmol/min/mg. In contrast, PABA NAT activity levels were more than 400-fold lower in the two slow acetylator strains (0.136 nmol/min/mg in Bio. 1.5 and 0.176 nmol/min/mg in Bio. 82.73). PAS NAT activities were also higher in rapid acetylator hamster strains than in any species tested and were about 20-fold lower in the two slow acetylator strains.

Thus, the substrate expression of the N-acetylation polymorphism in hamster is unique in comparison with humans, rabbits, and mice. It is interesting that features which characterize the hamster trait are essentially a mirror image of those which characterize the human and rabbit traits, i.e. the substrates that result in a polymor-

phic expression in hamster appear to be identical with those that result in monomorphic expression in human and rabbit, and vice versa.

2. Inheritance studies. Genetic analysis of liver PABA NAT activities carried out with slow acetylator (Bio. 1.5) and rapid acetylator (Bio. 4.24, Bio. 41.56, and Bio. 65.67) parental strains indicated that F_1 hybrids had intermediate PABA NAT activity levels, and F_2 generation progeny yielded trimodal distributions of PABA NAT activity in a ratio not significantly different from a 1:2:1 ratio (172, 180). No overlap was found between the three acetylator modes and no gender-related differences were apparent. Qualitatively similar distributions have been obtained for liver PAS NAT (172, 180) and blood PABA and PAS NAT activities (179). A gene dose-response relationship was apparent in both blood and liver for NAT activity. The hamster trait thus appears to be regulated by autosomal Mendelian inheritance of two codominant alleles at a single locus.

3. Tissue AHAT activity. The presence of AHAT activity is widespread in tissues of outbred Syrian hamsters (223). N-OH-AAF AHAT activities (reported in nmoles of AF bound to tRNA/20 mg of tissue/20 min) were in liver, 2.78; in small intestine, 1.18; in stomach, 0.36; in colon, 0.31; in lung, 0.18; in kidney, 0.11; in whole brain, 0.06; and in spleen, 0.04 (224, 223).

4. Biochemical basis for variability in N-acetylation. Monomorphic and polymorphic NAT activities are catalyzed by separate enzyme proteins in hamster. Preliminary suggestion of this hypothesis was reported by Olive and King (302) who noted two different AHAT proteins in small intestine of the Sprague-Dawley rat. In inbred hamster liver, two NAT enzyme proteins were separated by ion exchange chromatography on DEAE-cellulose (172, 181). In homozygous rapid acetylator inbred hamster liver preparations, the first NAT enzyme (identified as polymorphic) to elute from a DEAE-cellulose column exhibited a relative substrate specificity toward PABA and PAS. The second NAT enzyme to elute from the column (identified as monomorphic) catalyzed the acetylation of INH, PA, AF, and beta-naphthylamine, but did not catalyze the acetylation of PABA and PAS at appreciable rates. Thus, the relative capacity of the two NAT enzymes for catalyzing N-acetylation differed, with the first enzyme exhibiting a preference for polymorphic substrates, and the second exhibiting a preference for monomorphic substrates.

In homozygous slow acetylator inbred hamster liver preparations, only a single NAT enzyme eluted from a DEAE-cellulose ion exchange column (172, 181) at the same ionic strength as that for the second NAT enzyme in the homozygous rapid acetylator liver preparations. This single slow acetylator NAT enzyme catalyzed the N-acetylation of INH, PA, AF, and beta-naphthylamine at relatively rapid rates, but it had much less activity towards PABA and PAS, at rates analogous to those of the second enzyme from rapid acetylator liver. These observations explain the large differences in acetylation capacity expressed for the polymorphic substrates and the lack of appreciable activity differences observed with monomorphic substrates. Since both rapid and slow acetylators express the second NAT enzyme, substrates preferred by this enzyme are acetylated equally well in the two phenotypes. In contrast, substrates preferred by the first NAT enzyme such as PABA and PAS, express the phenotypic acetylator differences because the NAT enzyme is expressed in rapid acetylators but not in slow acetylators. Additional studies are necessary to explain the difference in enzyme expression in the two acetylator phenotypes in molecular terms.

5. Relationship between NAT and AHAT activities in hamster liver. The coexistence of NAT and AHAT activities on a common liver enzyme protein in rabbit liver (149) suggested they might coexist on the same enzyme in hamster liver. The expression of multiple forms of AHAT and NAT proteins is an added complication in hamster liver. Hanna and coworkers (167) have provided insight into this problem by the use of suicide inactivation inhibitors of NAT/AHAT activity. Hanna found that both SMZ NAT and AHAT hamster liver activities were irreversibly inactivated by incubation with N-OH-4-acetylamidobiphenyl. Neither N-OH-AAF AHAT activity nor SMZ NAT activity could be protected from inactivation by the low molecular weight nucleophile, cysteine, but PABA NAT activity was protected. They explained these results by proposing that N-OH-AAF AHAT and SMZ NAT activities were associated with a single enzyme protein, and that PABA NAT activity was dissociated from AHAT activity. Hanna's studies were performed with outbred golden Syrian hamsters (Charles River), but it is clear that they were rapid acetylators because their liver PABA NAT activities were in the range of rapid acetylator inbred hamsters. Since PABA NAT activity is polymorphic and SMZ NAT activity is monomorphic in hamster liver, it appears that the N-OH-AAF AHAT activity in hamster liver may be associated with the monomorphic enzyme. Recent studies have confirmed this hypothesis (182).

6. Pharmacogenetic differences in N-acetylation in vivo. Pharmacogenetic variation in N-acetylation capacity in vivo has been demonstrated with PABA (174). The percentage of acetyl-PABA to total drug excreted in a urine (12-hr) was determined in two homozygous rapid acetylator strains (Bio. 4.24 and Bio. 45.56) and two homozygous slow acetylator strains (Bio. 1.5 and Bio. 82.73). The percentage of acetyl-PABA excreted in the two rapid acetylator strains (83.7% and 92.3%, respectively) was significantly higher than in the two slow acetylator strains (59.5% and 63.6%, respectively). In contrast, the percentage of acetyl-SMZ to total drug in urine did not differ significantly between the same rapid and slow acetylator strains. The polymorphic pattern of

acetylation observed for PABA and monomorphic pattern observed for SMZ in vivo are thus consistent with those observed in vitro.

E. The Rat Model

1. N-Acetyltransferase. Many investigations of NATs in rat tissue have been carried out, though studies concerning genetic variation in N-acetylation in a rat model are only preliminary. Weissbach and coworkers (443, 443) were first to report that rat liver homogenates contained NAT activity toward a variety of amines, including serotonin, tryptamine, histamine, phenylethylamine, tyramine, octopamine, and normetanephrine (444). Schloot and coworkers (357) confirmed the acetylation of serotonin by rat liver NAT, and provided evidence that isoniazid and serotonin were acetylated by a single liver enzyme protein, in contrast to humans where serotonin and INH are reportedly acetylated by different liver NATs (447). In addition, both INH and serotonin NAT activities were inhibited by the monoamine oxidase inhibitors harmine, iproniazid, reserpine, imipramine, and opipramol in decreasing order of potency (357). The potent inhibitory properties of harmine on liver NAT have been confirmed by others in both rat and hamster (459), but not mouse (193).

Lower and Bryan (248) investigated additional substrates acetylated by rat liver NAT including arylamine carcinogens such as AF, 4-aminobiphenyl, and 2-aminonaphthalene. PABA and PA NAT activities have been reported in extrahepatic tissues such as lung, kidney, gut, and spleen (382, 359). HP, PABA, INH, and sulfapyridine were each competitive inhibitors of liver PA NAT activity, suggesting that a common NAT catalyzed the acetylation of each of the substrates (359). Yang and Neff (462) found the substrate specificity of brain NAT to be similar to that of liver NAT, including many of the indole- and phenylethylamine substrates identified by Weissbach and coworkers (444). Brain NAT is inhibited by several beta-carboline derivatives such as harmine (463), as had been observed in liver (357, 459). Tannen and Weber (401) examined blood PABA NAT activity in four outbred strains of rat and observed slight variability between strains. Blood PABA NAT activity was confirmed by Gollamudi and coworkers (156) who found that the substrate specificity of the blood NAT differed from that of the liver NAT.

2. N,O-Acyltransferase. Arylhydroxamic acid N,O-acyltransfer in rat tissues has been extensively studied. Highest AHAT activities in rat are found in liver, with significant activity in mammary tissue, gastrointestinal tract, kidney, stomach, spleen, and lung (35, 18, 19, 221, 224). King and Olive (224) found that N-OH-AAF AHAT activities in liver, stomach, small intestine, and colon were significantly higher (approximately twofold) in Sprague-Dawley rats than in comparable Fischer, Wistar, or Buffalo rats. Furthermore, F_1 hybrids of the Sprague-Dawley and Fischer rat strains had gastrointestinal tract AHAT activities intermediate between the parental strains.

Several studies have revealed multiple forms of AHAT activity that differ in substrate specificity. Bartsch and coworkers (19) presented evidence for multiple forms of AHAT activity in mammary tissue that differed in substrate specificity and pH optima. King and Olive (224) found two forms of AHAT activity in small intestine whose elution characteristics from Sephadex G-100 columns and substrate specificity differed. Several studies (23, 374, 152) have separated two different AHAT activities in rat liver which differ in substrate specificity: one had high specificity for N-OH-formyl-AF, the other for N-OH-AAF and N-OH-propionyl-AAF. Shirai and coworkers (374) also showed the presence of two forms of AHAT in mammary gland whose relative substrate specificities differed. As found for liver AHAT, one AHAT had a relative specificity for N-formyl derivatives of N-OH-AF, while the other has a relative specificity for N-acetyl and N-propionyl derivatives of N-OH-AF.

Although a relationship between NAT and AHAT activities has been established in rabbit liver (149, 437) and hamster liver (167, 182), such a relationship has not been established for rat liver. The possibility should be entertained in future pharmacogenetic investigation that "monomorphic" and "polymorphic" AHATs may occur in rat liver and mammary gland, analogous to those which occur in hamster.

3. Pharmacogenetic variation in N-acetylation. Attempts to measure variation in N-acetylation among outbred rat strains have shown monomorphic distribution patterns. Schneck and coworkers (359) reported that the capacity of male Sprague-Dawley rats to acetylate PA exhibited a monomorphic distribution pattern in vivo. Tannen and Weber (401) found no significant differences in liver SMZ NAT activity between Sprague-Dawley, Sherman, Wistar, and Long-Evans outbred rat strains. They reported, however, that Long-Evans rats possessed about two-fold higher PABA NAT activity in blood and liver than did Sprague-Dawley rats. More recent studies (Morgott, Torres, Hein, and Weber, unpublished observations) have found about a 25-fold variation in liver PABA NAT activity across inbred rat strains. As shown in Table 8 liver PABA NAT activities ranged from 0.09 nmol/min/mg in the NSD/N inbred strain to 2.51 nmol/min/mg in the ALB/N inbred strain. No significant variation was found in liver 4-aminobiphenyl NAT activity. These results, though preliminary, suggest that the inbred rat model may have characteristics in common with the inbred hamster model.

VII. Concluding Remarks

Evidence from human studies supported by studies in animal models which express genetic differences in the capacity to acetylate arylamine and hydrazine drugs and other environmental chemicals has emerged during the past 10 years to confirm the important role of the N-

acetylation polymorphism as a determinant of individual susceptibility to toxicity from these agents. Slow acetylator status predisposes the individual to hydrazine and arylamine neurotoxicity, drug-induced lupus erythematosus, and sulfasalazine-induced toxicity. Additional reports have claimed a role for the acetylation polymorphism as a susceptibility factor for several other drug-related and spontaneous disorders. Among these, INH-induced hepatitis, spontaneous (idiopathic) lupus, and arylamine-induced urinary bladder cancer have received most attention. Each of these disorders presents a complex problem in pharmacology and toxicology, and has been the target of numerous investigations at basic, clinical, and epidemiological levels. Conclusions reached have been vigorously contested, and are still not fully agreed upon. More recently, similar claims have appeared for diabetes of types I and II, benign and invasive malignant breast disease in women, and Gilbert's syndrome (mild chronic unconjugated hyperbilirubinemia). In the latter disorders, similar caution must be observed in accepting these conclusions until additional investigation has confirmed them. Perhaps more detailed examination of metabolic pathways in man and in experimental animal models relevant to the acetylation polymorphism may implicate specific chemical agents in the etiology of these spontaneous disorders. Clearly, more thorough and extensive assessments of the acetylator status as a susceptibility factor and determinant of biological effects are needed.

There are several reasons which may explain in part the failure of assessments to establish relationships between acetylator status and individual susceptibility to certain disorders. Part of the difficulty arises from the fact that polymorphic differences in acetylating capacity may act at at least two sites in the metabolism of arylamines and hydrazines. This has been demonstrated for drugs INH, HP, and PA, and for aromatic amine carcinogens such as aminofluorene and benzidine. For some chemicals, both acetyl transfer steps act in the same direction and tend to enhance toxicity (e.g. AF), whereas they oppose each other for certain others (e.g. INH). Until such steps are identified and their importance to the toxic (or therapeutic) outcome is determined, the overall effect of acetylator status on specific chemical toxicities may not be readily predicted.

Another source of difficulty may be due to inferences drawn from epidemiological studies which were performed, as, e.g., for INH hepatitis, spontaneous lupus erythematosus, and urinary bladder cancer. For these disorders, retrospective studies with potential, unknown biases were frequently used to support hypotheses proposed. In fairness, however, it should be said that unless such an approach had not been taken initially, no study would have been possible. The epidemiological investigations of urinary bladder cancer in high and low risk populations undertaken by necessity many years after exposure to arylamines are examples in point of this fact. In addition, however, the composition of some of the patient groups studied may have been too heterogeneous for comparison, or too small to permit reliable statistical analysis. In certain other studies, comparisons of experimental groups with historical control subjects rather than with randomly selected local control subjects have led to criticism. There now is an obvious need for the conduct of prospective epidemiological studies to better assess the relationship of acetylator status to these disorders.

Further characterization of NATs which are responsible for hereditary differences in acetylation of drugs and other environmental chemicals is also needed. Although, several reliable methods are available for acetylator phenotype determination, lack of appropriately simplified methodology for determination of the acetylator status has probably contributed to the difficulty in performance of appropriate epidemiological studies mentioned in the preceding paragraph, and to the underutilization of pharmacogenetic data and approaches to assess toxicity from arylamine and hydrazine chemicals. Currently available procedures for human acetylator phenotype determination are impractical to use on a large scale and on persons who are remote from medical facilities, in the sick or elderly, or in newborn infants and children. Some procedures are not wholly reliable for classification of patients with immature, or severely, impaired renal function. Furthermore, it may be of value to know the acetylator genotype, rather than merely the phenotype, in certain disorders and there is no procedure that enables the acetylator genotype to be determined without benefit of pedigree analysis. Recent studies of the characteristics of NAT variants in human and animal tissues which are readily available, such as peripheral blood cells of rapid and slow acetylators, indicate that such an approach is promising and should be pursued. Until an improved and simplified procedure for acetylator phenotype (and genotype) determination is available and used on a broader front by clinical and epidemiological investigators, it is unlikely that rapid progress in assessing the role of the acetylator polymorphism in drug-induced and spontaneous human illness will be made.

Acknowledgments. This work was partially supported by U.S. Public Health Service grants GM-27028, RR-08006, and CA-34627. The assistance of Gretchen Van Dam, Allie Clift, and Vereen Williams in preparation of the manuscript and of John Fellrath in preparation of figures is gratefully acknowledged.

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* *Footnote added in proof.* Dipyrone (noramidopyrine methanesulfonate) is an effective analgesic, antipyretic, and antiinflammatory drug used in Europe. Pharmacokinetic studies of dipyrone metabolites 4-methylaminoantipyrine, 4-aminoantipyrine (AA), 4-formylaminoantipyrine, and 4-acetylaminoantipyrine (AAA) performed on a panel of 12 slow and 13 rapid acetylator humans demonstrated that the AAA/AA ratio is an index of the acetylator phenotype (Levy, M., Flusser, D., Zylber-Katz, E., and Granit, L.: Plasma kinetics of dipyrone metabolites in rapid, and slow acetylators. Eur. J. Clin. Pharmacol. **27:** 453–458, 1984). The relevance of this finding to the toxicity and therapeutic effects of dipyrone is currently under study.