*Journal of Pharmacokinetics and Biopharmaceutics, Vol. 4, No. 2, 1976*

# Pharmacokinetics of Isoniazid Metabolism in Man

**Gordon A. Ellard**[1] **and Patricia T. Gammon**[1]

Received May 20, 1975—Final Sept. 15, 1975

*Detailed pharmacokinetic studies undertaken on a slow and a rapid acetylator of isoniazid enabled approximate first-order rate constants to be calculated for the metabolic processes involved in the conversion of isoniazid to acetylisoniazid, isonicotinic acid, isonicotinylglycine, monoacetylhydrazine, and diacetylhydrazine, and their excretion in the urine. Further studies of the metabolism of isoniazid were carried out on another 17 slow and 11 rapid acetylators. The major pathway of isoniazid metabolism was acetylation. The rapid acetylators acetylated isoniazid 5–6 times more rapidly than the slow acetylators. Acid-labile hydrazones were also formed, and some isoniazid was hydrolyzed directly to isonicotinic acid. The major metabolic route for the formation of isonicotinic acid from isoniazid was via acetylisoniazid. Cleavage of acetylisoniazid in the body resulted in the formation of monoacetylhydrazine, which was then acetylated polymorphically to diacetylhydrazine in a manner analogous to the acetylation of isoniazid. Individuals differed in their ability to conjugate isonicotinic acid with glycine, and these differences were unrelated to the rates at which isoniazid was acetylated. The conjugation of isonicotinic acid with glycine and the acetylation of isoniazid appeared to be partially saturated* in vivo *after the administration of doses of as little as 250 mg of either compound.*

**KEY WORDS:** isoniazid; acetylisoniazid; acetylhydrazine; isonicotinic acid; acetylation; polymorphism; glycine conjugation; saturable metabolic processes.

## INTRODUCTION

Isoniazid (isonicotinyl hydrazine) continues to be the most widely used chemotherapeutic agent for the treatment of tuberculosis (1,2). Its pharmacology has been extensively investigated (3–5). There are large differences between individuals in the rates at which isoniazid is metabolized to compounds devoid of antituberculosis activity, and studies on tuberculosis patients treated for many months with isoniazid-containing regimens have demonstrated that its metabolism is noninducible. The great majority of individuals can be clearly characterized as either "slow inactivators" or

[1]Medical Research Council's Unit for Laboratory Studies of Tuberculosis, Royal Postgraduate Medical School, London W12 0HS, England.

"rapid inactivators" of isoniazid. Other investigations have revealed that the polymorphic metabolism of isoniazid is genetically determined in a simple Mendelian fashion and that the frequencies of the genes controlling slow or rapid metabolism of isoniazid vary among different racial populations. Evidence has been obtained that rapid inactivators acetylate isoniazid and certain sulfonamides more rapidly than do slow inactivators, and similar conclusions have been drawn from *in vitro* studies using liver biopsies from previously phenotyped individuals. Although the acetylator phenotype of tuberculosis patients is of no prognostic significance when daily isoniazid-containing regimens are employed, it is very important if treatment is given once weekly (6).

This article describes an investigation of the kinetics of the conversion of isoniazid to acetylisoniazid, isonicotinic acid, isonicotinylglycine, monoacetylhydrazine, and diacetylhydrazine in slow and rapid acetylators of isoniazid, and of the excretion of these compounds in the urine. A brief report of part of the investigation has been presented elsewhere (7).

## MATERIALS AND METHODS

### Compounds Employed

Isoniazid, acetylisoniazid, isonicotinic acid, and diacetylhydrazine were prepared and purified as described previously (8). Monoacetylhydrazinium fumarate, synthesized by the method of McKennis *et al.* (9) (m.p. uncorr. 112°C, effervescing 122–125°C), was a gift from Mr. R. A. Selway, and contained the theoretical amount of acid-hydrolyzable hydrazine and less than 4% free hydrazine or 2% diacetylhydrazine.

### Subjects Studied, Methods of Treatment, and Collection of Samples

The volunteers in these studies were healthy students and staff from the Royal Postgraduate Medical School, London. The investigation was divided into three major parts. In Study I, the authors, who had been classified using sulfadimidine (10) as a slow acetylator (G. A. E.) and a rapid acetylator (P. T. G.), took, on successive occasions separated by at least a week, oral doses of 10, 50, and 250 mg isoniazid, 50 and 500 mg acetylisoniazid, 10 mg isoniazid plus 50 mg acetylisoniazid, 25 mg isonicotinic acid, 116 mg diacetylhydrazine, 74 mg monoacetylhydrazine (as 190 mg monoacetylhydrazinium fumarate), and 750 mg sulfadimidine, respectively. Additional doses of 900 mg isoniazid and 250 mg isonicotinic acid were also taken by the slow acetylator. The weights of the two subjects were 63 and 62 kg, respectively. Urine was collected from both subjects at repeated intervals

extending up to 48 hr, and blood samples were obtained from the slow acetylator after ingestion of the highest doses of isoniazid, acetylisoniazid, and isonicotinic acid.

In Study II, oral doses of 20 mg isoniazid/kg$^{0.7}$ were taken by 17 subjects, blood samples were obtained at 3 and 6 hr, and urine was collected at 0–2.5, 2.5–3.5, 3.5–5.5, and 5.5–6.5 hr, respectively. This dosage was chosen in order to improve the discrimination obtained between slow and rapid acetylators in their 6-hr isoniazid plasma concentrations (11). The results obtained in the study (Table IV) demonstrated that 12 of the subjects were slow acetylators and five were rapid acetylators. In Study III, which was carried out in connection with an investigation of the pharmacology of some slow-release preparations of isoniazid of potential use in the intermittent treatment of tuberculosis (12), urine was collected for 48 hr from 13 subjects, including the two individuals investigated in Study I, after 600 mg of isoniazid had been given in a matrix formulation (Smith and Nephew HS 82). The subjects were classified as six slow and seven rapid acetylators from the ratio of acetylisoniazid to acid-labile isoniazid excreted in the urine at 23–24 hr (13).

Twenty-four hour urine collections were also made on 2 consecutive days from each of 25 tuberculosis patients in Singapore during daily treatment with 300 mg isoniazid plus 150 mg thiacetazone (samples by courtesy of Dr. J. M. J. Supramaniam).

All the doses were taken on an empty stomach with a glass of water, and during the course of the investigations no other medicaments were taken. The isoniazid metabolites (Study I) were taken dissolved in water. Blood samples were taken into heparin tubes, and the plasma was separated and extracted into butan-1-ol within 2 hr to minimize potential *in vitro* conversion of acetylisoniazid to isonicotinic acid and monoacetylhydrazine (8). All urine samples were diluted to the equivalent of 100 ml/hr and stored at −20°C until analysis.

## Analytical Methods

The concentrations of isoniazid, isoniazid together with its acid-labile hydrazones ("acid-labile isoniazid"), isonicotinic acid, and isonicotinylglycine in plasma and urine were determined colorimetrically (8). Isoniazid, acid-labile isoniazid, and acetylisoniazid were determined fluorometrically by a slight modification of the methods described previously (8). In this modification, which gave more reproducible results than the original procedure, 0.1 ml 20% (w/v) sodium metabisulfite was added after *N*-isonicotinyl-*N'*-(salicylidene)hydrazine had been reduced with 2-mercaptoethanol, instead of before the reduction. Since the results obtained by the colorimetric

and fluorometric methods for isoniazid did not differ significantly, only those obtained with the more sensitive fluorometric procedure are reported.

Monoacetylhydrazine was determined fluorometrically by reacting 1 ml of the 0.1 M HCl extract by the procedure used for the fluorometric determination of isoniazid and measuring the fluorescence of the reaction product directly at 370/430 nm, without extraction into butan-1-ol. Although this method is specific for monoacetylhydrazine, it could not be used to determine monoacetylhydrazine in the presence of considerably higher concentrations of isoniazid, apparently because the reaction product formed from isoniazid quenches the fluorescence of the monoacetylhydrazine fluorophore.

Sulfadimidine and acetylsulfadimidine were determined by a modification (14) of the Bratton and Marshall procedure.

### Calculation of Pharmacokinetic Parameters

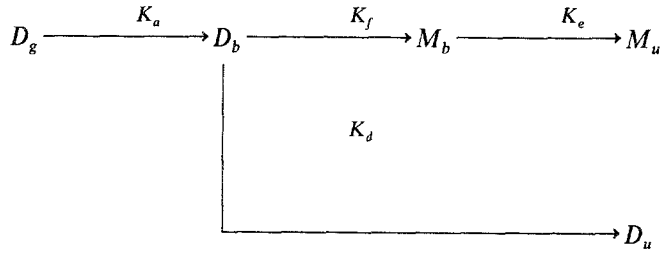
The half-lives for the overall elimination of isonicotinic acid, acetylisoniazid, monoacetylhydrazine, diacetylhydrazine, and isoniazid by the two subjects investigated in Study I were calculated from the terminal log linear slopes of the fall in their plasma concentrations or rates of urinary excretion (Table I). Virtually all of the administered doses of isoniazid, acetylisoniazid, and isonicotinic acid were recovered in the urine of both subjects as isonicotinyl compounds (Tables II and V), while the doses of monoacetylhydrazine and diacetylhydrazine given to the rapid acetylator were recovered as these hydrazine derivatives (Table II). It was therefore assumed that the absorption of all these compounds was effectively complete. The rapidity with which exponentially falling plasma concentrations and urinary excretion rates of isoniazid, acetylisoniazid (Figs. 4 and 5), isonicotinic acid (Figs. 2 and 3), monoacetylhydrazine, and diacetylhydrazine (Fig. 8) were achieved also indicated that all the compounds were very rapidly absorbed and distributed throughout the body. The rapidity of isoniazid's absorption is well established (15). The first-order rate constant for the urinary excretion of each compound could therefore be calculated by multiplying the rate constant for its overall elimination (0.693/half-life in hr) by the proportion of the dose that was excreted unchanged. Similarly, when metabolites were formed via a single metabolic pathway, the first-order rate constant for the pathway was calculated by multiplying the overall elimination rate constant by the proportion of the dose excreted as the metabolite. When the primary metabolite was further metabolized to secondary metabolites, the rate constant for the metabolic pathway was calculated by multiplying the overall elimination rate constant by the proportion of the dose excreted by all the metabolites formed via the metabolic pathway.

The plasma renal clearances of isonicotinic acid, isonicotinylglycine, and acetylisoniazid in the slow acetylator investigated in Study I were calculated from their plasma concentrations at the midpoints of the urine collected periods and the concomitant rates of urinary excretion. Their apparent distribution volumes (in liters) could then be calculated by dividing the plasma renal clearances (in liters/hr) by the rate constants ($hr^{-1}$) for urinary excretion.

Alternative methods were used to estimate the relative rates at which the groups of slow and rapid acetylators investigated in Studies II and III acetylated isoniazid. In Study III, the relative rates at which isoniazid was acetylated were estimated by comparing the ratios of the cumulative excretion of acetylisoniazid to those of acid-labile isoniazid for the two phenotypes. The validity of this method rests on the demonstration in this and previous investigations that slow and rapid acetylators metabolize acetylisoniazid in a quantitatively similar fashion (16) and that the plasma renal clearances of acid-labile isoniazid of the two phenotypes do not differ significantly (17).

The relative rates at which isoniazid was acetylated by the subjects investigated in Study II were estimated by calculating the initial rates of increase with time of the ratios of the excretion rate of acetylisoniazid to that of acid-labile isoniazid for the two phenotypes during the period in which these ratios increased linearly with time. The mathematical basis for this method will now be described.

First, consider the situation whereby a "drug" $D$ is absorbed by a first-order process (rate constant $K_a$), is metabolized by a first-order process to a metabolite $M$ (rate constant $K_f$), and where both drug and metabolite are excreted in the urine by first-order processes (rate constants $K_d$ and $K_e$, respectively):




Scheme I

This one-compartment open model is identical to that considered by Wagner (18) in his Scheme 38.4. The amounts of drug ($D_b$) and metabolite ($M_b$)

present in the body at time $t$ are given by the following equations (18):

$$D_b = D_g^0 \frac{K_a}{(K_a - K)}(e^{-Kt} - e^{-K_a t}) \tag{1}$$

$$M_b = K_a K_f D_g^0 \times \left[\frac{e^{-Kt}}{(K_a-K)(K_e-K)} - \frac{e^{-K_a t}}{(K_a-K)(K_e-K_a)} + \frac{e^{-K_e t}}{(K_e-K_a)(K_e-K)}\right] \tag{2}$$

where $K$, the first-order rate constant for the elimination of drug $D$, is equal to $K_f + K_d$, and provided that $K_a \neq K \neq K_e$.

If the ratio of the excretion rate of metabolite $M$ to that of drug $D$ at time $t$ is denoted by $Q$,

$$Q = K_e M_b / K_d D_b \tag{3}$$

Substituting for $D_b$ and $M_b$ from equations 1 and 2,

$$Q = \frac{K_e K_f (K_a - K)}{K_d} \times \frac{\dfrac{e^{-Kt}}{(K_a - K)(K_e - K)} - \dfrac{e^{-K_a t}}{(K_a - K)(K_e - K_a)} + \dfrac{e^{-K_e t}}{(K_e - K_a)(K_e - K)}}{e^{-Kt} - e^{-K_a t}} \tag{4}$$

Multiplying both the denominator and numerator by $(K_a - K)(K_e - K) \times (K_e - K_a)\, e^{Kt}$, the equation can be arranged in the form

$$Q = \frac{K_e K_f}{K_d (K_e - K)(K_e - K_a)} \times \left[\frac{(K_e - K_a) - (K_e - K)\, e^{-(K_a - K)t} + (K_a - K)\, e^{-(K_e - K)t}}{1 - e^{-(K_a - K)t}}\right] \tag{5}$$

and further rearranged to give

$$Q = F\left[\frac{(K_e - K)(1 - e^{-(K_a - K)t}) - (K_a - K)(1 - e^{-(K_e - K)t})}{1 - e^{-(K_a - K)t}}\right] \tag{6}$$

where $F$ is a constant

$$F = K_e K_f / [K_d (K_e - K)(K_e - K_a)] \tag{7}$$

Expanding the exponential terms,

$$Q = F\left\{\frac{(K_e-K)\left[(K_a-K)t - (K_a-K)^2\frac{t^2}{2!} + (K_a-K)^3\frac{t^3}{3!}\ldots\right] - (K_a-K)\left[(K_e-K)t - (K_e-K)^2\frac{t^2}{2!} + (K_e-K)^3\frac{t^3}{3!}\ldots\right]}{(K_a-K)t - (K_a-K)^2(t^2/2!) + (K_a-K)^3(t^3/3!)\ldots}\right. \tag{8}$$

Dividing both the denominator and numerator by $t$ leads to an equation of the form

$$Q = (a_0 + a_1 t + a_2 t^2 + a_3 t^3 + \cdots)/(b_0 + b_1 t + b_2 t^2 + b_3 t^3 + \cdots) \quad (9)$$

Suppose this function is identical to the following polynomial in $t$:

$$Q = c_0 + c_1 t + c_2 t^2 + c_3 t^3 + \cdots \quad (10)$$

Equating coefficients after cross-multiplying gives

$$a_0 = c_0 b_0 \quad (11)$$

$$a_1 = c_0 b_1 + c_1 b_0 \quad (12)$$

$$a_2 = c_0 b_2 + c_1 b_1 + c_2 b_0 \qquad \text{etc.} \quad (13)$$

Hence

$$c_0 = a_0/b_0$$

$$= [F/(K_a - K)][(K_e - K)(K_a - K) - (K_a - K)(K_e - K)] = 0 \quad (14)$$

Substituting for $c_0$ in equation 12 leads to

$$c_1 = a_1/b_0 = K_e K_f/2K_d \quad (15)$$

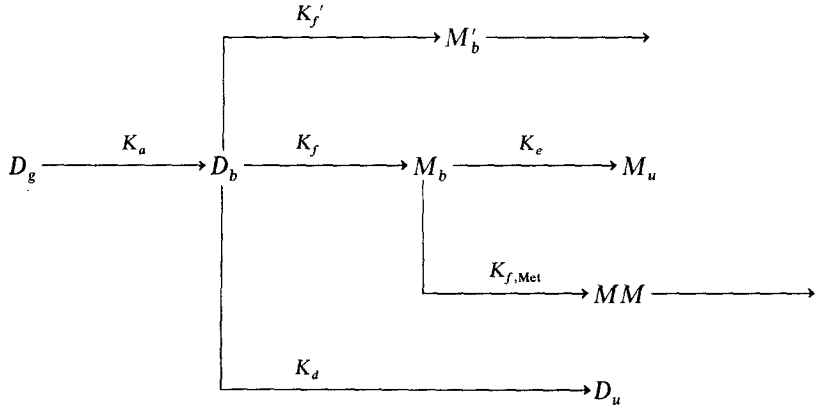
Thus at the earliest times, when values of $t$ are such that terms involving $t^2$ and higher powers of $t$ become negligible, $Q$, the ratio of the rates of excretion of metabolite $M$ to that of drug $D$, tends to the function

$$(K_e K_f/2K_d)t \quad (16)$$

Hence in the initial period during which the ratio of the excretion rate of metabolite $M$ to that of drug $D$ increases approximately linearly with time, the slope of such plots is equal to $K_e K_f/2K_d$. It will be noted that the magnitude of the slope is not a function of $K_a$ and is therefore independent of the rate of absorption of the drug. Among healthy subjects with normal renal function, it may be anticipated that interindividual differences in the ratio of the rate constants for the excretion of the metabolite and drug ($K_e/K_d$) are likely to be much smaller than differences between individual metabolic rate constants ($K_f$). In these circumstances, approximate estimates of the relative rates at which the drug is metabolized by different individuals can be obtained by comparing the slopes of plots of the change with time in the ratios of the excretion rate of metabolite to that of drug during the initial period when such plots are linear.

Although the model illustrated in Scheme I was directly applicable in this investigation only to the case where isonicotinic acid was given and then quantitatively eliminated in the urine as isonicotinic acid plus isonicotinylglycine, it can be shown that this method can be applied to more complex situations such as the case of an orally administered drug being metabolized

to two metabolites $M$ and $M'$, where metabolite $M$ is further metabolized to metabolite $MM$:




Scheme II

The equations describing the pharmacokinetics of drug $D$ and metabolite $M$ in Scheme II can be simply obtained by replacing the rate constants $K$ and $K_e$ in equations 1 and 2 by $K'$ and $K_e'$, respectively, where

$$K' = K_f + K_f' + K_d \tag{17}$$

and

$$K_e' = K_e + K_{f,\text{Met}} \tag{18}$$

to give new equations for $D_b$ ($D_b'$) and $M_b$ ($M_b'$), respectively. If the ratio of the excretion rate of metabolite $M$ to that of drug $D$ at time $t$ is now denoted by $Q'$,

$$Q' = K_e M_b'/K_d D_b' \tag{19}$$

Carrying through the mathematical procedures described above reveals that at the earliest times such that $Q'$ increases linearly with time its slope is still equal to $K_e K_f/2K_d$. Identical conclusions still apply when yet more complex metabolic schemes are considered, such as the case of isoniazid, which is initially metabolized by acetylation, hydrazone formation, and direct hydrolysis to isonicotinic acid, and where its principal metabolite, acetylisoniazid, is metabolized to further metabolites (isonicotinic acid and monoacetylhydrazine), which in their turn are also metabolized (Fig. 1). Hence the relative rates at which slow and rapid acetylators acetylate isoniazid can be determined by comparing the initial rates of increase with time of the ratios of the excretion rate of acetylisoniazid to that of isoniazid. Since it was shown that similar proportions of unchanged and acid-labile isoniazid were excreted in the urine at all times by both slow and rapid acetylators,

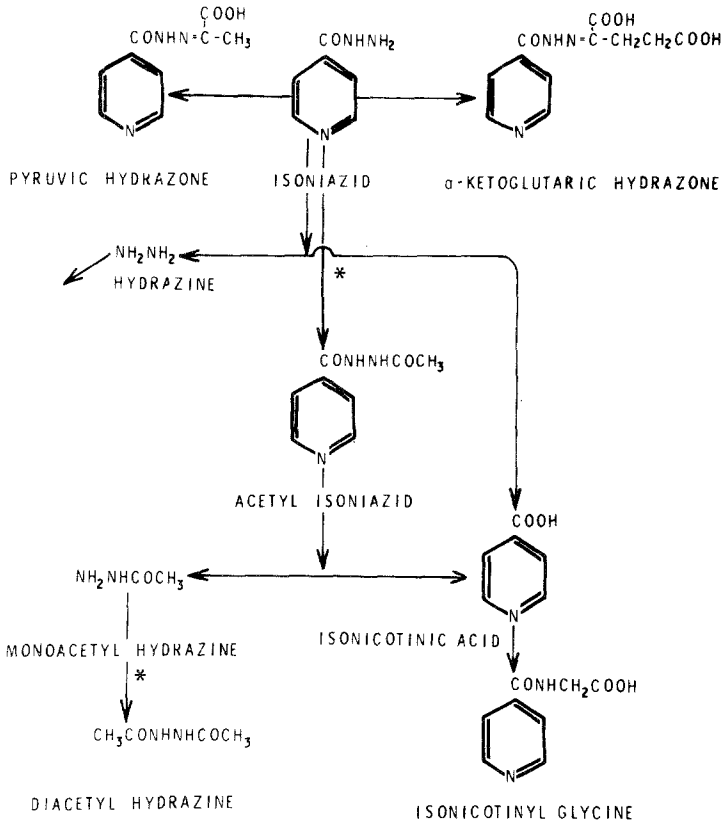
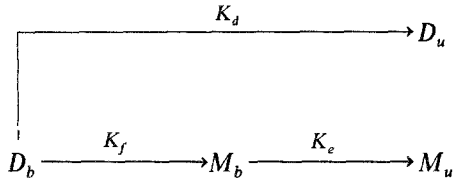



**Fig. 1.** Scheme proposed for the metabolism of isoniazid in man, showing polymorphic acetylation steps (*).

valid comparisons could also be made from plots of the ratios of the excretion rate of acetylisoniazid to that of acid-labile isoniazid.

If one considers the case of a drug $D$ delivered by "instantaneous" intravenous infusion into a one-compartment system and eliminated by metabolism to metabolite $M$ and excretion




Scheme III

as in the model considered by Wagner (18) in his Scheme 38.3, the amounts of drug ($D_b$) and metabolite ($M_b$) present in the body are given by the following equations (18):

$$D_b = D_b^0 e^{-Kt} \qquad \text{where } K = K_f + K_d \tag{20}$$

$$M_b = D_b^0[K_f/(K_e - K)](e^{-Kt} - e^{-K_e t}) \tag{21}$$

Hence $Q$, the ratio of the excretion rate of metabolite $M$ to that of drug $D$, is given by

$$Q = \frac{K_e K_f}{K_d} \frac{(1 - e^{-(K_e - K)t})}{(K_e - K)} \tag{22}$$

Expanding the exponential term,

$$Q = (K_e K_f/K_d)[t - (K_e - K)(t^2/2!) + (K_e - K)^2(t^3/3!) \cdots] \tag{23}$$

As will be seen from inspection of equation 22, if the metabolite is eliminated much more rapidly than the drug ($K_e \gg K$), $Q$ will tend to a constant value $K_e K_f/K_d(K_e - K)$ with increasing time (convex curve), and Cummings *et al.* (19) have shown that an identical pattern would be expected in the post-absorption period after oral administration of the drug. If the metabolite is eliminated more slowly from the body than its precursor, a concave curve is to be expected. In the limiting situation where the rate constants for the elimination of the metabolite and its precursor are identical ($K_e = K$), a consideration of equation 23 reveals that $Q$, the ratio of the excretion of the metabolite to that of its precursor, will continue to increase linearly with time (slope $K_e K_f/K_d$). Certain other pharmacokinetic aspects of this special situation have also been considered by Cummings *et al.* (19). Since equation 2 cannot be employed in the situation where $K$ and $K_e$ are equal, it is difficult to predict the pattern of $Q$ in this special situation following oral administration of a drug. However, it would be anticipated that after absorption was complete the ratio of the excretion rate of metabolite to that of drug would eventually tend to increase linearly with time with a slope given by $K_e K_f/K_d$.

## RESULTS

The metabolic pathways demonstrated in this investigation whereby isoniazid is converted in man to acetylisoniazid, isonicotinic acid, isonicotinylglycine, hydrazine, monoacetylhydrazine, and diacetylhydrazine are shown in Fig. 1. The kinetics of the elimination of isoniazid, acetylisoniazid, isonicotinic acid, monoacetylhydrazine, and diacetylhydrazine by the slow and the rapid acetylator investigated in Study I are summarized in Table I. The linearity of the decrease with time of the logarithms of the plasma concentrations or rates of urinary excretion of these compounds

**Table I.** Kinetics of the Elimination of Isoniazid, Acetylisoniazid, Isonicotinic Acid, Monoacetylhydrazine, and Diacetylhydrazine by a Slow and a Rapid Acetylator (Study I)

| Compound administered | Dose (mg) | Acetylator | Fluid | Period after dose[a] (hr) | Elimination equation[b] | Half-life ($hr^{-1}$) |
|---|---|---|---|---|---|---|
| Isoniazid | 900 | Slow | Plasma | 2.0– 6.0 (5)[c] | $1.26 - 0.090t$ | $3.3 \pm 0.3$ |
| Acetylisoniazid | 50 | Slow | Urine | 1.5– 6.5 (6) | $1.11 - 0.084t$ | $3.6 \pm 0.6$ |
| | 50 | Rapid | Urine | 1.5– 6.5 (6) | $1.18 - 0.075t$ | $4.0 \pm 0.5$ |
| | 500 | Slow | Urine | 2.0–10.0 (5) | $1.09 - 0.089t$ | $3.4 \pm 0.3$ |
| | 500 | Rapid | Urine | 2.0–10.0 (5) | $1.05 - 0.101t$ | $3.0 \pm 1.6$ |
| | 500 | Slow | Plasma | 2.0–10.0 (5) | $0.896 - 0.073t$ | $4.1 \pm 0.9$ |
| Isonicotinic acid | 25 | Slow | Urine | 1.5– 5.5 (5) | $1.62 - 0.386t$ | $0.78 \pm 0.16$ |
| | 25 | Rapid | Urine | 1.5– 5.5 (5) | $1.71 - 0.411t$ | $0.73 \pm 0.10$ |
| | 250 | Slow | Urine | 1.0– 6.0 (6) | $1.83 - 0.454t$ | $0.66 \pm 0.11$ |
| | 250 | Slow | Plasma | 1.0– 4.0 (4) | $0.854 - 0.447t$ | $0.67 \pm 0.11$ |
| Monoacetylhydrazinium fumarate | 190 | Slow | Urine | 3.0–30.0 (6) | $0.34 - 0.050t$ | $6.0 \pm 0.9$ |
| | 190 | Rapid | Urine | 3.0–18.0 (5) | $0.42 - 0.094t$ | $3.2 \pm 1.1$ |
| Diacetylhydrazine | 116 | Slow | Urine | 1.5–30.0 (7) | $1.02 - 0.058t$ | $5.2 \pm 0.3$ |
| | 116 | Rapid | Urine | 1.5–30.0 (7) | $1.08 - 0.061t$ | $4.9 \pm 0.5$ |

[a]Times shown for urine samples are the midpoints for their collection periods.
[b]Equation relating the change with time ($t$) in hours of the $\log_{10}$ of the concentration of the administered compound in the plasma ($\mu$g/ml) or its rate of elimination in the urine (percent dose excreted/hr).
[c]Number of samples.

**Table II.** Cumulative Urinary Excretion of Isoniazid and Its Metabolites[a] by a Slow and a Rapid Acetylator Receiving Oral Doses of Isoniazid, Acetylisoniazid, Isonicotinic Acid, Monoacetylhydrazine, or Diacetylhydrazine (Study I)

| Compound administered: | Isoniazid | | | Acetylisoniazid | | Isonicotinic acid | | | Monoacetyl-hydrazinium fumarate | | Diacetylhydrazine | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dose (mg): | 250 | | 900 | 500 | | 25 | | 250 | 190 | | 116 | |
| Collection period (hr): | 48 | | 48 | 48 | | 7 | | 24 | 48 | | 48 | |
| Acetylator status[b] | S | R | S | S | R | S | R | S | S | R | S | R |
| Compound recovered | | | | | | | | | | | | |
| Acid-labile isoniazid | 34.0 | 16.5 | 39.9 | —[c] | — | — | — | — | — | — | — | — |
| Acetylisoniazid | 28.5 | 40.7 | 28.5 | 74.8 | 46.9 | — | — | — | — | — | — | — |
| Diacetylhydrazine | 5.7 | 26.8 | 4.5 | 17.4 | 42.3 | — | — | — | 44.5 | 103.9 | 76.8 | 86.1 |
| Acid-labile monoacetylhydrazine | | | | | | — | — | — | 18.8 | 8.8 | | |
| Total hydrazides[d] | 68.2 | 84.0 | 72.9 | 92.2 | 89.2 | — | — | — | 63.3 | 112.7 | 76.8 | 86.1 |
| Isonicotinic acid | 14.7 | 24.5 | 16.6 | 24.0 | 25.3 | 54.1 | 51.8 | 67.3 | — | — | — | — |
| Isonicotinylglycine | 9.8 | 14.1 | 12.2 | 13.3 | 14.9 | 41.0 | 40.8 | 28.9 | — | — | — | — |
| Total isonicotinyl compounds[e] | 87.0 | 95.8 | 97.2 | 112.1 | 87.1 | 95.1 | 92.6 | 96.2 | — | — | — | — |

[a]As percent administered dose.
[b]S, Slow; R, rapid.
[c]Nil.
[d]Sum of acid-labile isoniazid, acetylisoniazid, diacetylhydrazine, and acid-labile monoacetylhydrazine.
[e]Sum of acid-labile isoniazid, acetylisoniazid, isonicotinic acid, and isonicotinylglycine.

**Table III.** Urinary Excretion of Isoniazid and Its Metabolites[a] by a Slow and a Rapid Acetylator Receiving Oral Doses of 10, 50, and 250 mg Isoniazid (Study I)

| | Dose of isoniazid (mg) | | | | | |
|---|---|---|---|---|---|---|
| | 10 | | 50 | | 250 | |
| Compound recovered | S[b] | R[b] | S | R | S | R |
| Acid-labile isoniazid | 13.2 | 8.4 | 16.9 | 9.9 | 14.7 | 13.8 |
| Acetylisoniazid | 23.5 | 37.5 | 19.4 | 37.0 | 13.0 | 32.9 |
| Isonicotinic acid | 13.2 | 16.3 | 10.0 | 15.6 | 7.3 | 13.0 |
| Isonicotinylglycine | 7.8 | 12.1 | 4.5 | 8.6 | 2.7 | 7.4 |
| Total isonicotinyl compounds | 57.7 | 74.3 | 50.8 | 71.1 | 37.7 | 67.1 |
| Ratio acetylisoniazid/acid-labile isoniazid | 1.78 | 4.46 | 1.15 | 3.74 | 0.88 | 2.38 |

[a]As percent administered dose excreted within 7 hr.
[b]Acetylator status: S, slow; R, rapid.

indicated that during the period indicated they could be considered to be effectively distributed throughout a single compartment. The cumulative excretion of these compounds and their metabolites is recorded in Tables II and III. The plasma concentrations and urinary excretion of isoniazid and acetylisoniazid determined in Study II after administration of isoniazid are

**Table IV.** Plasma Concentrations and Urinary Excretion of Isoniazid and Acetylisoniazid by Slow and Rapid Acetylators Receiving Isoniazid Orally (Study II)

| | | Acetylator status | | | |
|---|---|---|---|---|---|
| | | Slow (12 subjects) | | Rapid (5 subjects) | |
| | Time (hr) | Mean | Range | Mean | Range |
| Plasma concentrations ($\mu$g/ml) | | | | | |
| Isoniazid | 3 | 5.83 | 3.49–8.06 | 2.10 | 1.50–3.34 |
| | 6 | 3.48 | 2.66–4.38 | 0.84 | 0.55–1.38 |
| Acetylisoniazid | 3 | 1.88 | 1.50–2.49 | 4.32 | 3.36–5.16 |
| | 6 | 2.06 | 1.34–2.91 | 3.58 | 3.02–4.17 |
| Acetylisoniazid/isoniazid[a] | 3 | 0.24 | 0.19–0.36 | 1.57 | 1.18–2.26 |
| | 6 | 0.42 | 0.31–0.67 | 3.27 | 1.92–5.23 |
| Urinary excretion | | | | | |
| Acetylisoniazid/acid-labile isoniazid[a] | 0–2½ | 0.47 | 0.32–0.73 | 2.39 | 2.01–2.95 |
| | 2½–3½ | 0.57 | 0.39–0.73 | 3.29 | 2.79–3.89 |
| | 3½–5½ | 0.64 | 0.43–0.94 | 3.72 | 2.64–5.29 |
| | 5½–6½ | 0.84 | 0.60–1.16 | 4.56 | 3.70–5.34 |

[a]Molar ratios.

**Table V.** Urinary Excretion of Isoniazid and Its Metabolites by Slow and Rapid Acetylators Receiving Isoniazid Orally (Study III)

| Compound recovered | Acetylator status | |
|---|---|---|
| | Slow (6 subjects) (mean ± SEM)[a] | Rapid (7 subjects) (mean ± SEM) |
| Acid-labile isoniazid | 29.0 ± 2.1 | 7.5 ± 0.8 |
| Acetylisoniazid | 26.3 ± 1.1 | 45.2 ± 2.1 |
| Diacetylhydrazine | 7.9 ± 0.9 | 27.6 ± 1.5 |
| Total hydrazides[b] | 63.1 ± 2.3 | 80.3 ± 2.5 |
| Isonicotinic acid | 19.7 ± 1.0 | 28.2 ± 1.7 |
| Isonicotinylglycine | 14.5 ± 1.8 | 13.7 ± 1.5 |
| Total isonicotinyl compounds[c] | 89.4 ± 2.9 | 94.5 ± 1.7 |

[a]Mean percent administered dose excreted within 48 hr ± standard error of mean.
[b]Sum of acid-labile isoniazid, acetylisoniazid, and diacetylhydrazine.
[c]Sum of acid-labile isoniazid, acetylisoniazid, isonicotinic acid, and isonicotinylglycine.

summarized in Table IV, and the cumulative excretion of isoniazid and its metabolites determined in Study III is shown in Table V.

The evidence obtained for the relative importance of each metabolic pathway in slow and rapid acetylators is described below. After calculation

**Table VI.** Apparent First-Order Rate Constants Calculated for the Conversion of Isoniazid to Acetylisoniazid, Isonicotinic Acid, Isonicotinylglycine, Monoacetylhydrazine, and Diacetylhydrazine, and Their Excretion in the Urine[a] (Study I)

| Compound | Dose (mg) | Process | First-order rate constant ($hr^{-1}$) | |
|---|---|---|---|---|
| | | | Slow acetylator | Rapid acetylator |
| Isonicotinic acid | 25 | Excretion | 0.48 | 0.49 |
| | 250 | | 0.70 | —[b] |
| | 25 | Conjugation with glycine | 0.36 | 0.39 |
| | 250 | | 0.30 | — |
| Acetylisoniazid | 500 | Excretion | 0.14 | 0.11 |
| | 500 | Hydrolysis to isonicotinic acid | 0.07 | 0.09 |
| Isoniazid | 250 | Acetylation | 0.11[c] | 0.39[c] |
| | 900 | | 0.09 | — |
| | 900 | Excretion and hydrazone formation | 0.085 | — |
| | 900 | Hydrolysis to isonicotinic acid | 0.02 | — |
| Diacetylhydrazine | 116 | Excretion | 0.10 | 0.12 |
| Monoacetylhydrazine[d] | 74 | Excretion and hydrazone formation | 0.02 | 0.02 |
| | 74 | Acetylation | 0.05[c] | 0.23[c] |

[a]Metabolic scheme shown in Fig. 1; details of calculations given in the text.
[b]Not determined.
[c]Differences between the two subjects characteristic of slow and rapid acetylators.
[d]Given as 190 mg monoacetylhydrazinium fumarate.

of the rate constants for the metabolism and urinary excretion of each compound by the two subjects investigated in Study I, appropriate evidence is then drawn from Studies II and III to indicate whether the results obtained in this Study I were typical of slow and rapid acetylators in general. The estimated rate constants are summarized in Table VI.

Comparisons between the results obtained for slow and rapid acetylators have been analyzed by *t* tests, using logarithmically transformed data when these gave more similar variances. Unless otherwise stated, when "significant" differences between results are reported, *P* values are less than 0.001, and means are given together with their standard errors.

## Conjugation of Isonicotinic Acid with Glycine and Urinary Excretion of Isonicotinic Acid and Isonicotinylglycine

After administration of isonicotinic acid, the rates of urinary excretion and the plasma concentrations fell exponentially from 1 hr or even earlier (Figs. 2 and 3), suggesting that absorption was extremely rapid. The plasma renal clearance of isonicotinic acid and isonicotinylglycine calculated from the plasma concentrations and concomitant urinary excretion of the compounds 1–3 hr after administration of 250 mg isonicotinic acid to the slow acetylator investigated in Study I were 453 ± 31 and 493 ± 54 ml/min,

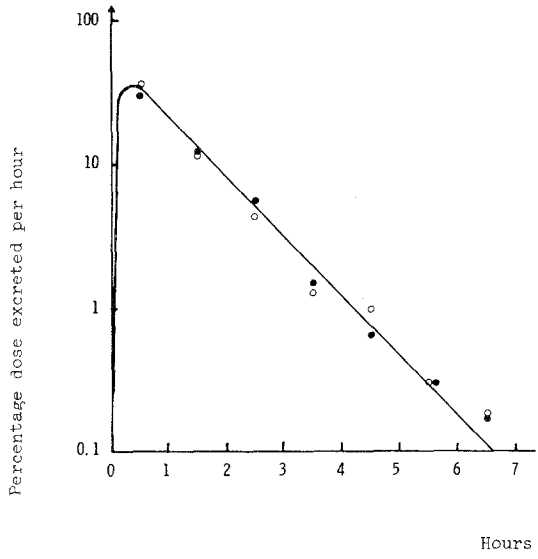



**Fig. 2.** Urinary excretion of isonicotinic acid following oral administration of 25 mg isonicotinic acid. ○, Slow acetylator; ●, rapid acetylator.

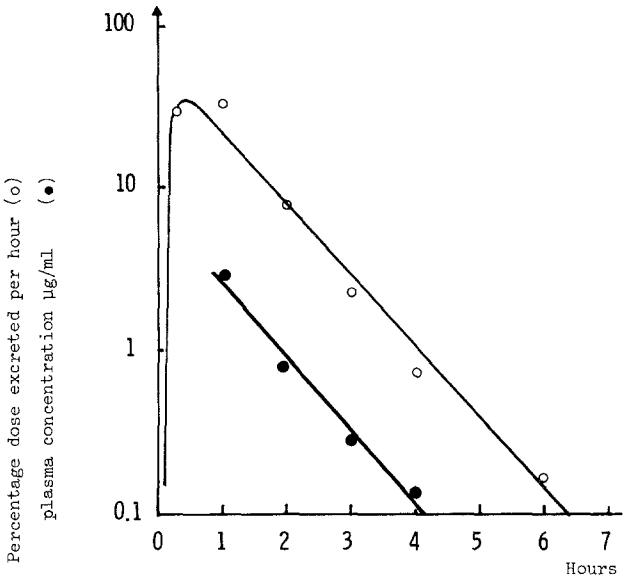



**Fig. 3.** Plasma concentrations and urinary excretion of isonicotinic acid following oral administration of 250 mg isonicotinic acid (slow acetylator).

respectively, indicating that both compounds were actively secreted by the kidneys. Isonicotinic acid was rapidly eliminated by both subjects (half-life about 0.7 hr) (Table I), and over 90% of the dose was recovered in the urine as isonicotinic acid plus isonicotinylglycine (Table II). The first-order rate constants for the excretion of isonicotinic acid and for its conjugation with glycine were calculated by multiplying the rate constants for the overall elimination of isonicotinic acid (0.693 divided by the half-lives for isonicotinic acid summarized in Table I) by the proportions of the doses excreted as unchanged isonicotinic acid and as isonicotinylglycine, respectively (summarized in Table II). They are shown in Table VI. The apparent volume of distribution of isonicotinic acid calculated for the slow acetylator was 39 liters.

The two subjects investigated in Study I conjugated isonicotinic acid with glycine at very similar rates (rate constants 0.36 and 0.39 $hr^{-1}$, respectively, after administration of 25 mg isonicotinic acid) and both eliminated 41% of the dose as isonicotinylglycine (Table II). The similarity in the rates at which they conjugated isonicotinic acid with glycine was also demonstrated by plotting the ratios of the rates of excretion of isonicotinylglycine to isonicotinic acid. During the period of observation (0–7 hr), these plots were linear and superimposable. By contrast, a two-way analysis of variance

of the ratios of isonicotinylglycine/isonicotinic acid excreted by the 17 subjects investigated in Study II indicated that there were significant differences between the subjects in the extent to which they conjugated isonicotinic acid with glycine. The extent of glycine conjugation was unrelated to their isoniazid acetylator status. Significant differences were also demonstrated among the 25 tuberculosis patients from Singapore in the extent to which they conjugated isonicotinic acid with glycine after receiving 300 mg isoniazid daily (ratios of isonicotinylglycine/isonicotinic acid excreted ranging from 0.24 to 1.36, mean 0.72). The ratios of isonicotinylglycine/isonicotinic acid excreted by the 13 subjects investigated in Study III after administration of 600 mg matrix isoniazid ranged from 0.20 to 0.96 (mean 0.57).

There appeared to be a substantial dosage effect on the conjugation of isonicotinic acid with glycine. Thus the proportion of the dose excreted as isonicotinylglycine fell from 41 % when 25 mg isonicotinic acid was given to 29 % when 250 mg was given (Table II).

## Conversion of Acetylisoniazid to Isonicotinic Acid and Urinary Excretion of Acetylisoniazid

The rapidity and completeness of the absorption of acetylisoniazid were indicated by the fact that maximal urinary excretion and plasma concentrations were attained within 1–2 hr (Figs. 4 and 5) and that almost

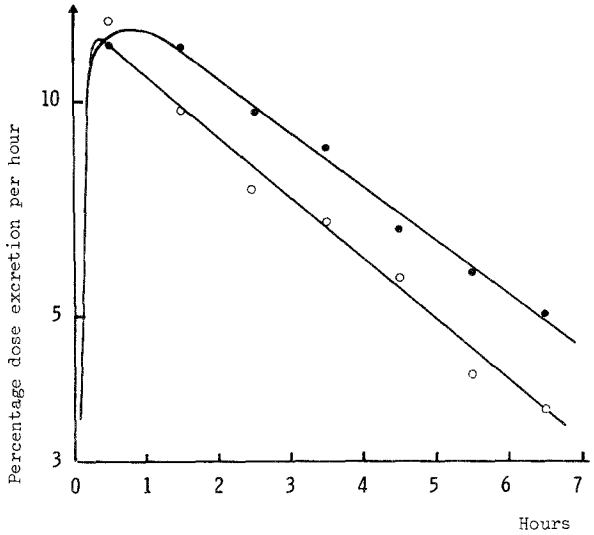



**Fig. 4.** Urinary excretion of acetylisoniazid following oral administration of 50 mg acetylisoniazid. ○, Slow acetylator; ●, rapid acetylator.

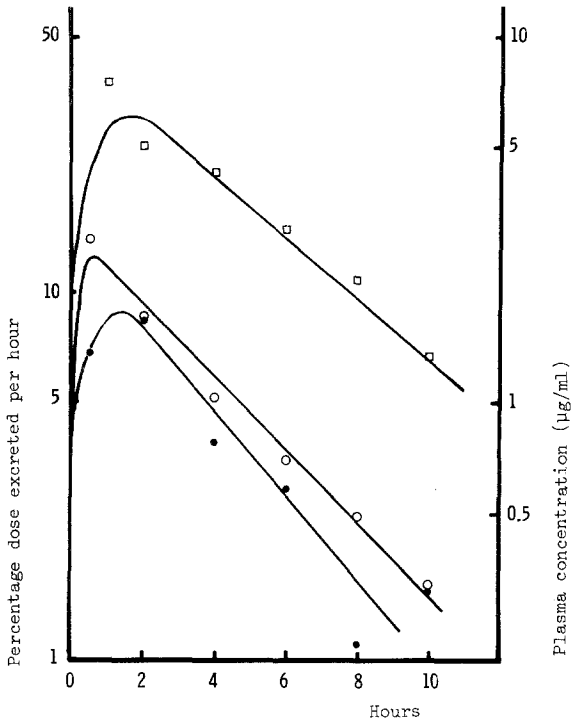



**Fig. 5.** Plasma concentrations and urinary excretion of acetylisoniazid following oral administration of 500 mg acetylisoniazid. □, Plasma concentrations slow acetylator; ○, urinary excretion slow acetylator; ●, urinary excretion rapid acetylator.

all of the acetylisoniazid given to the two subjects in Study I was recovered in urine as the isonicotinyl derivatives acetylisoniazid, isonicotinic acid, and isonicotinylglycine (Table II). The half-life of acetylisoniazid in both subjects was estimated to be between 3 and 4 hr (Table I), and appeared to be unaffected by the size of the dose given (50 or 500 mg). Rate constants for the excretion of acetylisoniazid and for its hydrolysis to isonicotinic acid were calculated by multiplying the rate constants for the overall elimination of acetylisoniazid by the proportions of the doses excreted unchanged and as isonicotinic acid plus isonicotinylglycine, respectively (Table II). They are shown in Table VI. The plasma renal clearance of acetylisoniazid estimated from the plasma concentrations and urinary excretion of acetylisoniazid after 500 mg of the compound had been given to the slow acetylator was 111 ± 10 ml/min, giving an apparent distribution volume of 48 liters. The change with time in the ratios of the elimination rate of isonicotinic

acid plus isonicotinylglycine to that of acetylisoniazid after administration of 50 mg acetylisoniazid is shown in Fig. 6. The ratios rose most rapidly within the first 2 hr and had reached almost constant values by about 6 hr of 0.6 and 0.7 in the slow and the rapid acetylator, respectively. Very similar results were obtained after 500 mg acetylisoniazid was given to the same subjects, the ratios of the excretion rates being 0.6 and 1.0, respectively, at

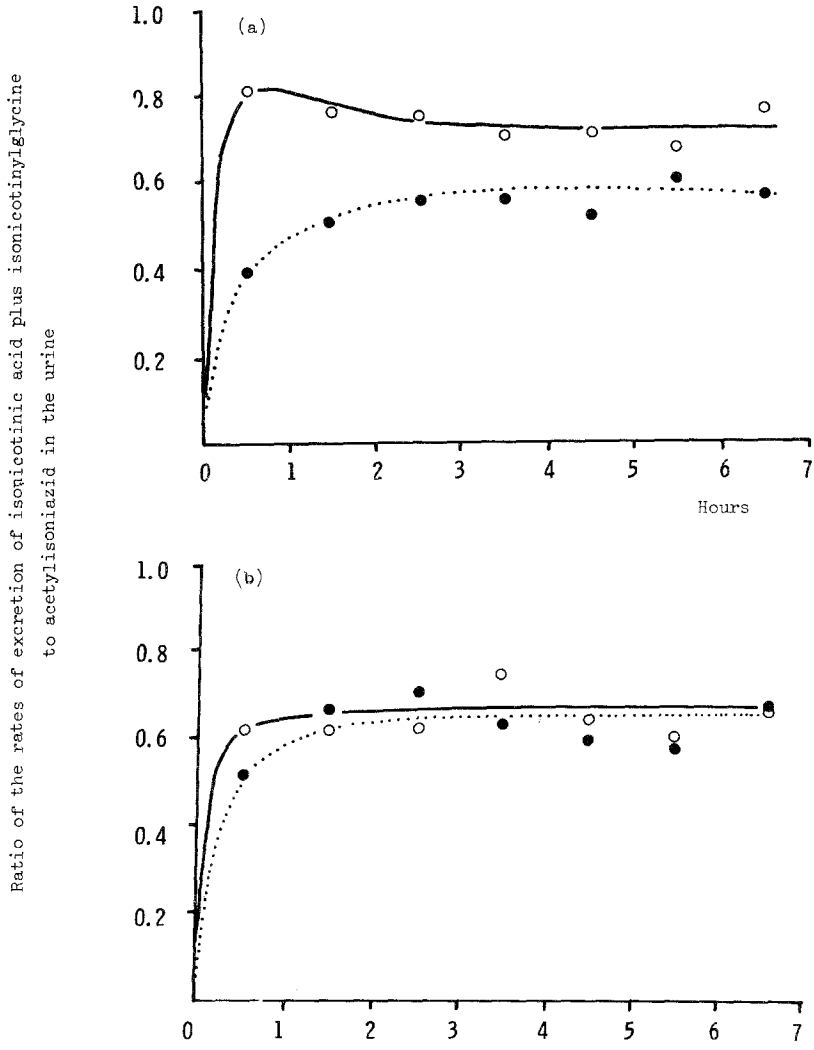



**Fig. 6.** Urinary excretion of isonicotinic acid, isonicotinylglycine, and acetylisoniazid following oral administration of 50 mg isoniazid (○) or 50 mg acetylisoniazid (●). (a) Slow acetylator. (b) Rapid acetylator.

6 hr and rising only slightly thereafter (to 0.7 and 1.1 at 24 hr), suggesting that such a dose had not saturated the enzyme responsible for hydrolyzing acetylisoniazid in the body.

**Direct Conversion of Isoniazid to Isonicotinic Acid**

The kinetics of the relative excretion rate of isonicotinic acid plus isonicotinylglycine to that of acetylisoniazid by the rapid acetylator investigated in Study I after administration of either isoniazid or acetylisoniazid were extremely similar (Fig. 6). The ratios of the cumulative excretion of isonicotinic acid plus isonicotinylglycine to that of acetylisoniazid by the rapid acetylator were also similar after administration of isoniazid or acetylisoniazid (0.95 and 0.86, respectively, calculated from the data summarized in Table II). These findings demonstrated that in the rapid acetylator isonicotinic acid must be formed from isoniazid almost exclusively via acetylisoniazid. Rather different results were, however, obtained in the slow acetylator. Thus in the first hour after administration of isoniazid the ratio of the excretion rate of isonicotinic acid plus isonicotinylglycine to that of acetylisoniazid was approximately double the ratio found after administration of acetylisoniazid. Furthermore, after administration of isoniazid the ratio fell slightly during the next 5 hr, in contrast to the significant increase when acetylisoniazid was given. The ratio of the cumulative excretion of isonicotinic acid plus isonicotinylglycine to that of acetylisoniazid after administration of isoniazid (0.86) was also higher than when acetylisoniazid was given (0.50). Two-way analyses of variance of the ratios of excretion rate of isonicotinic acid plus isonicotinylglycine to that of acetylisoniazid after administration of 10, 50, and 250 mg isoniazid to the slow and the rapid acetylator investigated in Study I, or 20 mg isoniazid/$kg^{0.7}$ to the 12 slow and five rapid acetylators in Study II, confirmed the conclusion that the ratio falls significantly in slow acetylators from 1 to 6 hr but remains virtually constant in rapid acetylators. This indicates that isoniazid can be hydrolyzed directly to isonicotinic acid and that this pathway is most readily apparent at the earliest times in slow acetylators, when the formation of isonicotinic acid via acetylisoniazid is minimal.

Evidence for this pathway is also provided by the higher ratios of the cumulative excretion of isonicotinic acid plus isonicotinylglycine to that of acetylisoniazid in the slow acetylators given 600 mg matrix isoniazid in Study III (slow acetylators $1.30 \pm 0.08$, rapid acetylators $0.94 \pm 0.06$), and the similar ratios obtained in this study and those of Peters *et al.* (16) and Mitchell *et al.* (20) after administration of acetylisoniazid to both slow and rapid acetylators ($0.93 \pm 0.09$ and $0.89 \pm 0.08$, respectively). It was estimated that of the 34% of the matrix isoniazid dose excreted as isonicotinic acid

plus isonicotinylglycine by the six slow acetylators investigated in Study III (Table V), approximately 24% of the dose [0.91 times the excretion of acetylisoniazid (26% of the dose)] had probably been formed via acetylisoniazid. It is therefore concluded that in slow acetylators about 10% of the dose of isoniazid could be converted by direct hydrolysis to isonicotinic acid. The rate constant for this process calculated for the slow acetylator investigated in Study I (isoniazid half-life 3.3 hr) would therefore be about 0.02 $hr^{-1}$.

### Urinary Excretion of Acid-Labile Isoniazid

The plasma half-life of isoniazid in the slow acetylator investigated in Study I after administration of 900 mg isoniazid was 3.3 hr, and 40% of the dose was eliminated in the urine as acid-labile isoniazid, giving a rate constant for the excretion of isoniazid together with the formation of acid-labile derivatives of 0.085 $hr^{-1}$. The results set out in Table IV unequivocally demonstrated that the 17 subjects investigated in Study II consisted of 12 slow and five rapid acetylators. The rapid acetylators could be distinguished on the basis of lower plasma isoniazid concentrations at 6 hr (11), higher plasma acetylisoniazid concentrations at 3 and 6 hr, and higher ratios of acetylisoniazid to isoniazid in the plasma and urine at all times. The rates of excretion of acid-labile isoniazid by the 17 subjects were approximately proportional to the concomitant isoniazid plasma concentrations and gave a mean plasma renal clearance rate for the elimination of acid-labile isoniazid of 46 $\pm$ 3 ml/min. Estimates of the plasma renal clearance of acid-labile isoniazid were unaffected by the time of measurement (3 or 6 hr after dosage) or by the acetylator phenotype of the subjects. Parallel analyses in which free and acid-labile isoniazid were determined using a modification (21) of Dymond and Russell's picryl sulfonic acid method (22) demonstrated that approximately a third of the acid-labile isoniazid excreted consisted of the unchanged drug.

### Acetylation of Isoniazid

After 900 mg isoniazid was given to the slow acetylator investigated in Study I (plasma half-life 3.3 hr), 28.5% of the dose was eliminated in the urine as acetylisoniazid. When acetylisoniazid was taken by the same subject, the ratio of the cumulative elimination of isonicotinic acid plus isonicotinylglycine to that of acetylisoniazid averaged 0.50 (data in Table II). It was therefore concluded that when isoniazid was given 43% of the dose (28.5% $\times$ 1.5) was metabolized via acetylisoniazid. Using this value, the rate constant calculated for the acetylation of isoniazid was 0.09 $hr^{-1}$. Similar calculations indicated that when 250 mg isoniazid was given the proportions of the dose metabolized via acetylisoniazid in the slow and the

rapid acetylator investigated in Study I were 43% and 76%, respectively. Since isoniazid half-lives were not determined at that dosage, approximate estimates of the rate constants for the acetylation of isoniazid were obtained by multiplying the rate constant calculated for the excretion of isoniazid together with the formation of acid-labile derivatives ($0.085\ hr^{-1}$) by the ratio of the proportion of the dose eliminated via acetylisoniazid to the proportion eliminated via acid-labile isoniazid (0.43/0.34 and 0.76/0.165 for the slow and the rapid acetylator, respectively). The values calculated (Table VI) indicated that the rapid acetylator acetylated isoniazid 3–4 times more rapidly than the slow acetylator. The ratios of the initial excretion rate of acetylisoniazid to that of acid-labile isoniazid for the rapid acetylator after administration of 10, 50, and 250 mg isoniazid were also approximately 3 times those for the slow acetylator (Fig. 7).

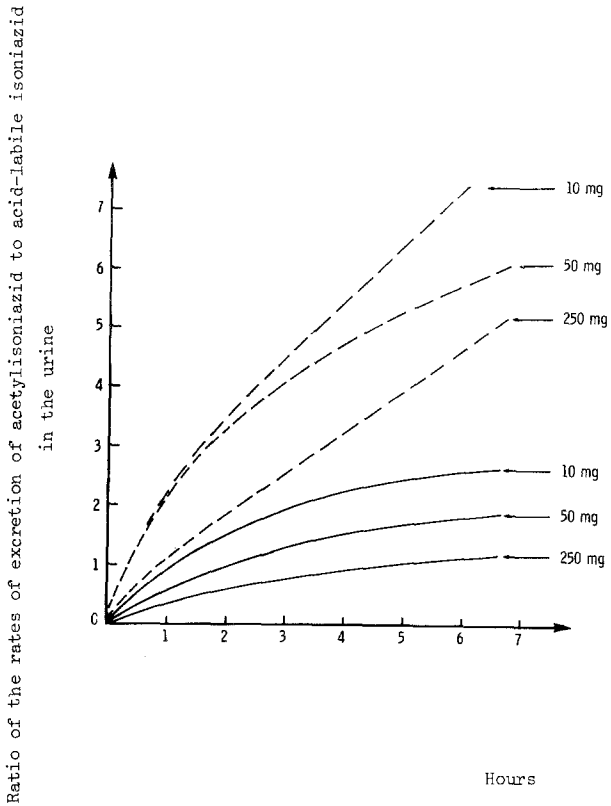



**Fig. 7.** Urinary excretion of acetylisoniazid and acid-labile isoniazid following oral administration of 10, 50, or 250 mg isoniazid. ——, Slow acetylator; – – –, rapid acetylator.

Increasing the isoniazid dosage from 10 to 250 mg appeared to result in progressive saturation of the enzyme system responsible for the acetylation of isoniazid in both subjects since proportionately less of the dose was excreted as acetylisoniazid and its metabolites, isonicotinic acid and isonicotinylglycine, at the higher isoniazid doses (Table III). Giving 50 mg acetylisoniazid together with 10 mg isoniazid to the two subjects did not alter the pattern of the excretion of acid-labile isoniazid, supporting *in vitro* evidence that acetylisoniazid is only a weak inhibitor of human acetyltransferase (W. W. Weber, personal communication).

The ratios of the initial excretion rate of acetylisoniazid to that of acid-labile isoniazid for the five rapid acetylators investigated in Study II after receiving 20 mg isoniazid/$kg^{0.7}$ averaged approximately 5 times those for the 12 slow acetylators (Table IV), suggesting that they acetylated isoniazid 5 times more rapidly than the slow acetylators. The ratios of acetylisoniazid to isoniazid in the plasma at 3 hr supported this conclusion. The ratios of the cumulative excretion of acetylisoniazid to that of acid-labile isoniazid by the seven rapid and six slow acetylators investigated in Study III derived from the data summarized in Table V ($6.74 \pm 1.12$ and $0.93 \pm 0.08$, respectively) indicated that the rapid acetylators acetylated isoniazid approximately 6.5 times more rapidly than the slow acetylators.

## Acetylation of Monoacetylhydrazine and Urinary Excretion of Monoacetylhydrazine and Diacetylhydrazine

After administration of 116 mg diacetylhydrazine to the two subjects investigated in Study I, maximal rates of urinary excretion occurred within 1 hr. Thereafter the urinary excretion fell exponentially (Fig. 8) at rates equivalent to a half-life of about 5 hr in both subjects (Table I). The slow acetylator excreted 77% of the dose unchanged and the rapid acetylator 86% (Table II), giving rate constants for the excretion of diacetylhydrazine of 0.10 $hr^{-1}$ and 0.12 $hr^{-1}$ in the two subjects. These values were similar to those calculated for the excretion of acetylisoniazid (Table VI).

Monoacetylhydrazine also appeared to be absorbed rapidly, maximal rates of urinary excretion of acid-labile monoacetylhydrazine occurring in both subjects within 1 hr. In the slow acetylator, the rate of excretion of acid-labile monoacetylhydrazine then fell exponentially with a half-life of 6 hr (Table I). About 20% of the dose was excreted as acid-labile monoacetylhydrazine and 45% as diacetylhydrazine, but about 35% of the dose was not accounted for (Table II). In the rapid acetylator, the rate of urinary excretion of acid-labile monoacetylhydrazine fell considerably more rapidly, although the results enabled only a very approximate estimate of its half-life to be obtained ($3.2 \pm 1.1$ hr). The rapid acetylator excreted about 10%

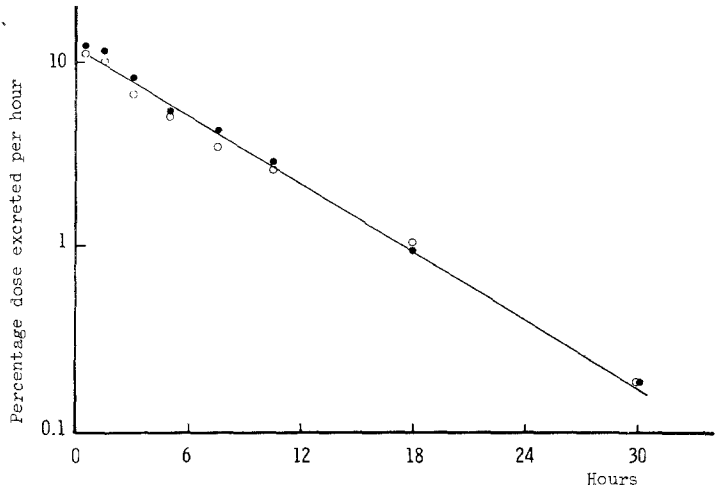



**Fig. 8.** Urinary excretion of diacetylhydrazine following oral administration of 116 mg diacetylhydrazine. ○, Slow acetylator; ●, rapid acetylator.

of the dose as acid-labile monoacetylhydrazine and the rest as diacetylhydrazine. These data enabled rate constants for the excretion of acid-labile monoacetylhydrazine (0.02 $hr^{-1}$ in both subjects) and for the acetylation of monoacetylhydrazine (0.05 $hr^{-1}$ and 0.23 $hr^{-1}$, in the slow and rapid acetylator, respectively) to be calculated. Calculation of the initial ratios of the excretion rate of diacetylhydrazine to that of acid-labile monoacetylhydrazine also suggested that the rapid acetylator acetylated monoacetylhydrazine 4–5 times more rapidly than the slow acetylator. Analyses using a colorimetric method employing picryl sulfonic acid to determine free and acid-labile monoacetylhydrazine separately (G. A. Ellard and P. T. Gammon, unpublished work), demonstrated that only about a third of the acid-labile monoacetylhydrazine excreted consisted of the unchanged compound.

Serum contains an enzyme that catalyzes the hydrolysis of acetylisoniazid to isonicotinic acid and monoacetylhydrazine (8), and it was anticipated that the metabolism of acetylisoniazid would result in the formation *in vivo* of equimolar amounts of isonicotinic acid and monoacetylhydrazine, which would then be conjugated with glycine and acetic acid, respectively. A study of the kinetics and cumulative excretion of diacetylhydrazine, after administration of 500 mg of acetylisoniazid to the subjects investigated in Study I, indicated that the monoacetylhydrazine split from acetylisoniazid was acetylated in exactly the same fashion as when it had been administered as monoacetylhydrazinium fumarate. Thus the rapid acetylator initially excreted diacetylhydrazine at 4 times the rate of the slow acetylator when monoacetylhydrazinium fumurate was given, and at 3

times the rate of the slow acetylator after acetylisoniazid (Fig. 9). The cumulative excretion of diacetylhydrazine by the two subjects after administration of acetylisoniazid also paralleled the results obtained when monoacetylhydrazinium fumarate was given (Table II). Thus the rapid acetylator excreted similar proportions of the acetylisoniazid dose as diacetylhydrazine (42%) and as isonicotinic acid plus isonicotinylglycine (40%), suggesting

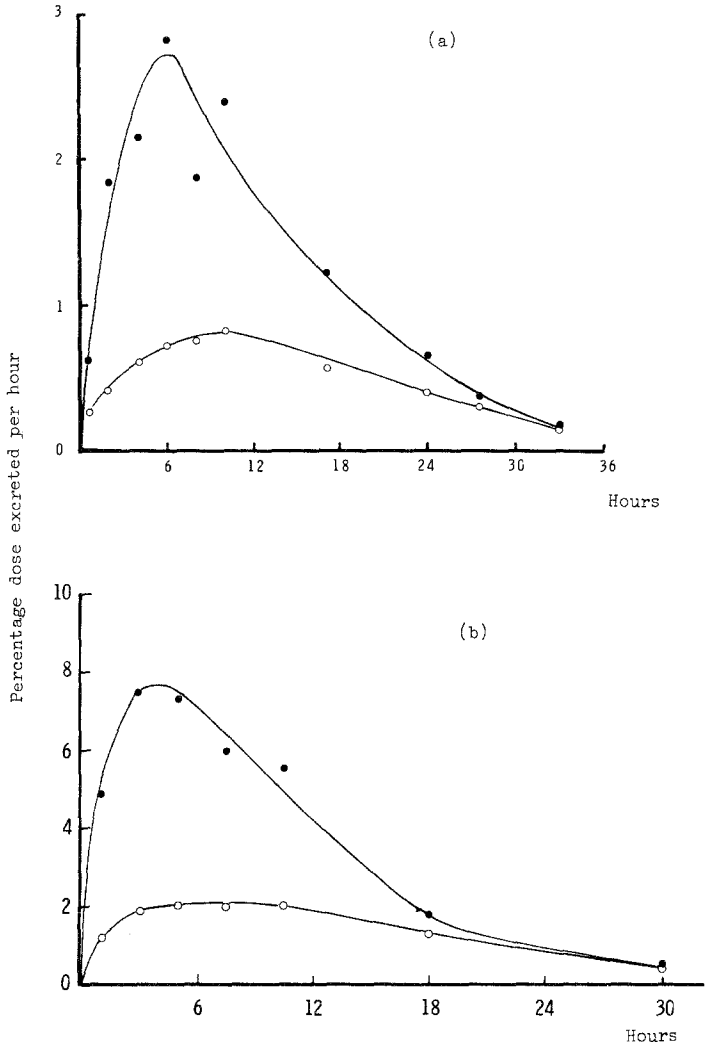



**Fig. 9.** Urinary excretion of diacetylhydrazine following oral administration of (a) 500 mg acetylisoniazid or (b) 190 mg monoacetylhydrazinium fumarate. ○, Slow acetylator; ●, rapid acetylator.

that almost all of the monoacetylhydrazine formed from acetylisoniazid had been acetylated. By contrast, the slow acetylator excreted only about 17% of the acetylisoniazid dose as diacetylhydrazine compared to 37% as isonicotinic acid plus isonicotinylglycine.

The more rapid acetylation of monoacetylhydrazine by the rapid acetylator suggested that monoacetylhydrazine might be polymorphically acetylated in a manner similar to isoniazid. Further evidence for this conclusion was obtained by demonstrating that the ratios of the cumulative excretion of diacetylhydrazine to that of acetylisoniazid for the seven rapid acetylators investigated in Study III calculated from the data summarized in Table V ($0.61 \pm 0.03$) were significantly greater than those for the six slow acetylators ($0.30 \pm 0.04$).

### Acetylation of Sulfadimidine

Determination of the ratios of the cumulative excretion (0–24 hr) of acetylsulfadimidine to that of sulfadimidine by the slow and the rapid acetylator investigated in Study I after administration of 750 mg sulfadimidine indicated that the rapid acetylator acetylated the drug 3 times more rapidly than the slow acetylator.

## DISCUSSION

The urinary recoveries of isoniazid and its metabolites summarized in Tables II and V indicate that the scheme shown in Fig. 1 describes all the quantitatively important pathways for the metabolism of isoniazid in rapid acetylators. However, the poorer recoveries of hydrazine compounds in the slow acetylators after administration of isoniazid or monoacetylhydrazine indicate that the metabolic fate of hydrazine and monoacetylhydrazine has not been fully accounted for. Further studies in tuberculosis patients with chronic renal failure have confirmed the conclusion that the terminal metabolites of isoniazid are isonicotinylglycine and diacetylhydrazine and have indicated that their elimination by extrarenal routes is minimal (G. A. Ellard and P. Sever, unpublished work).

The results obtained confirm the evidence obtained in the previous investigation of Peters *et al.* (16) and in the recent study of Mitchell *et al.* (20) that acetylation is the most important pathway for the metabolism of isoniazid, that differences in the rates of acetylation determine the isoniazid inactivator status of individuals, and that the most important route for the formation of isonicotinic acid from isoniazid is via acetylisoniazid. Our investigation has also confirmed the finding (16) that individuals differ in the extent to which and therefore in the rates at which they conjugate iso-

nicotinic acid with glycine, and that these differences are unrelated to their acetylator phenotype. The half-life of isonicotinic acid determined following oral dosage (0.7 hr) was similar to that determined after intravenous dosage by Boxenbaum *et al.* (23). Partial saturation of the enzymes involved in the formation of isonicotinylglycine from isonicotinic acid appeared to occur when the isonicotinic acid dose was increased from 25 to 250 mg. An analysis of the results of Peters *et al.* (16) suggested that the same phenomenon had occurred in their study. A somewhat similar dosage effect has also been found for the conjugation of salicylic acid with glycine in man (24).

Evidence was also obtained indicating that isonicotinic acid could be formed from isoniazid by direct hydrolysis, and it was estimated that in slow acetylators (Study I) about 30% of the isonicotinic acid and isonicotinylglycine excreted after administration of isoniazid had been formed by this route, whereas in the rapid acetylators the proportion was less than 10%. This metabolic route is of major importance in the dog, which is unable to acetylate isoniazid (25).

The shapes of the plots of the ratios of the excretion rate of metabolites to that of their precursors against time were in agreement with the theoretical predictions described in the Materials and Methods section. Thus convex curves were obtained when the half-life of a metabolite (isonicotinic acid) was much shorter than that of its precursor (acetylisoniazid) (Fig. 6). Furthermore, the near constant ratios of the excretion rate of isonicotinic acid plus isonicotinylglycine to that of acetylisoniazid demonstrated in the slow and the rapid acetylator by 24 hr (0.7 and 1.1, respectively) agree closely with those predicted (0.6 and 1.05, respectively) from equation 22 by substitution of appropriate rate constants from Table VI into the formula $K_eK_f/K_d(K_e - K)$.

The kinetics of the conjugation of isonicotinic acid with glycine by the slow acetylator investigated in Study I after administration of 250 mg isonicotinic acid were particularly noteworthy since the ratio of the excretion rate of isonicotinylglycine to that of isonicotinic acid increased almost linearly for 10 hr, or approximately 15 half-lives (Fig. 10). This finding suggested that in this subject the rate constants for the elimination of both compounds must be extremely similar. This hypothesis is supported by the recent report of Boxenbaum *et al.* (23) of similar half-lives for isonicotinic acid and isonicotinylglycine (0.76 and 0.95 hr, respectively) following intravenous dosage. The actual slope of the plot illustrated in Fig. 10 (0.33 unit/hr) ought according to equation 23 to tend to $K_eK_f/K_d$. Estimates for the rate constants for the conjugation ($K_f$) and excretion ($K_d$) of isonicotinic acid by this subject had already been obtained by standard methods ($0.30\,hr^{-1}$ and $0.70\,hr^{-1}$, respectively). The data illustrated in Fig. 10

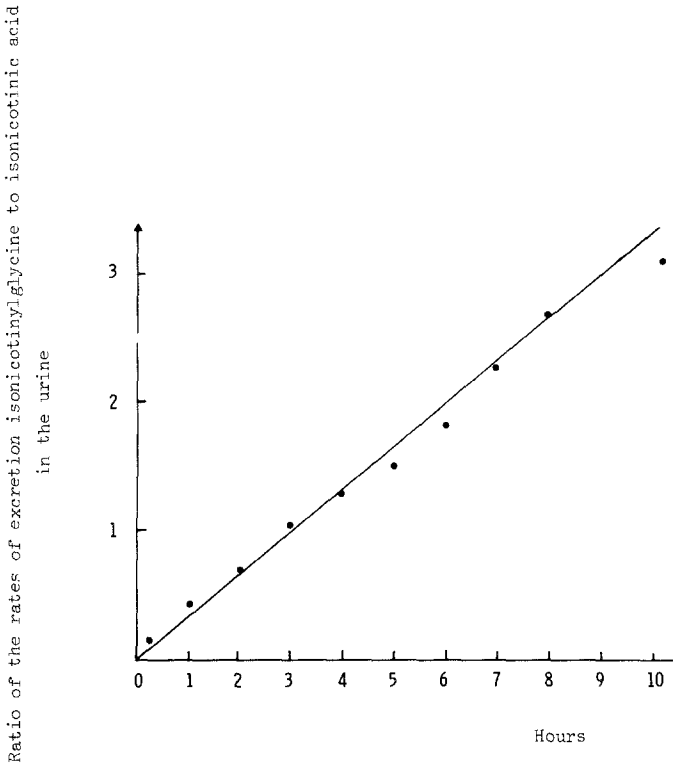



**Fig. 10.** Urinary excretion of isonicotinylglycine and isonicotinic acid following administration of 250 mg isonicotinic acid (slow acetylator).

therefore suggest a rate constant for the excretion of isonicotinylglycine ($K_e$) in this subject of about 0.8 $hr^{-1}$, which is equivalent to a half-life (0.9 hr) very similar to that determined intravenously by Boxenbaum *et al.* (23).

The plasma renal clearance calculated for the elimination of acid-labile isoniazid (46 ml/min) was similar to that determined by Jenne *et al.* (17) using a method that determined both isoniazid and its acid-labile hydrazones. Approximately a third of the acid-labile isoniazid excreted consisted of unchanged isoniazid, a finding in accord with other investigations (16,20). Although a previous study (21) demonstrated that the plasma concentrations of acid-labile hydrazones are less than a tenth of the concomitant concentrations of unchanged isoniazid, it was not possible to calculate an unambiguous value for the plasma renal clearance of unchanged isoniazid on account of the possibility of interconversion of isoniazid and its acid-labile hydrazones in the urine. The plasma renal clearance of isonicotinic acid and isonicotinylglycine (453 and 493 ml/min, respectively) indicates that they are actively

secreted. Although the plasma renal clearance of acetylisoniazid (111 ml/min) was similar to the glomerular filtration rate, the possibility of simultaneous active secretion and reabsorption cannot be excluded.

The apparent distribution volumes of acetylisoniazid and isonicotinic acid in the slow acetylator investigated in Study I (39 and 48 liters, respectively) were similar to that calculated (26) for the total body water of the subject (46 liters). Other studies have indicated that the apparent distribution volume of isoniazid is also similar to that of the total body water (17,27).

The demonstration that significant amounts of diacetylhydrazine are excreted in the urine after administration of acetylisoniazid (or isoniazid) confirms the report of Yard and McKennis (28). The failure of Peters *et al.* (16) to detect diacetylhydrazine after giving doses of acetylisoniazid and isoniazid similar to those employed in our investigations is difficult to understand, particularly in rapid acetylators. The results obtained in Studies I and III indicated that monoacetylhydrazine when given orally or released *in vivo* by hydrolysis of acetylisoniazid might be polymorphically acetylated in a similar fashion to isoniazid. Further studies undertaken in 30 slow and 19 rapid acetylators given 600 mg of matrix isoniazid confirmed this conclusion by demonstrating that the rapid acetylators excreted significantly higher ratios of diacetylhydrazine to acetylisoniazid in the urine at 23–34 hr than the slow acetylators (G. A. Ellard, P. T. Gammon, and H. Tiitinen, unpublished work).

It was calculated that the rapid acetylator acetylated all three substrates investigated in Study I (isoniazid, monoacetylhydrazine, and sulfadimidine) at between 3 and 5 times the rate of the slow acetylator. In view of the considerable differences in the chemical structures of these compounds, it would appear that the catalytic sites of the *N*-acetyltransferase from the slow and rapid acetylators were identical and that the rapid acetylator had about 4 times as many enzyme molecules with this catalytic site as did the slow acetylator. The apparently identical Michaelis constants found for the acetylation of isoniazid using purified enzyme preparations from the supernatant fraction of livers from either slow or rapid acetylators (29–31) are in accord with this hypothesis. The available evidence does not, however, rule out the possibility that there may be structural differences between the acetylase molecules from the two groups.

A comparison of the ratios of the concentration of acetylisoniazid to that of isoniazid in the plasma or urine of the subjects investigated in Studies II and III with those obtained after giving isoniazid intravenously to subjects from Finnish Lappland (27) indicated that all 12 rapid acetylators investigated in the current study were heterozygous rapid acetylators. It was estimated that they acetylated isoniazid approximately 5–6 times more rapidly than the slow acetylators.

The evidence obtained in Study I that human liver acetylase is partially saturated after giving doses of 250 mg isoniazid (approximately 4 mg/kg) is supported by results from previous investigations demonstrating that when isoniazid dosage was increased from 5 to 20 mg/kg the proportion of the dose excreted as acetylisoniazid decreased while that recovered as acid-labile isoniazid increased (16,32). This phenomenon has also been encountered in recent studies of the pharmacology of slow-release isoniazid formulations of potential use in the intermittent treatment of tuberculosis (21,33).

## ACKNOWLEDGMENTS

The authors wish to thank Professor D. A. Mitchison and Mr. V. R. Aber for their helpful advice and criticism.

## REFERENCES

1. W. Fox. General considerations in the choice and management of regimens of chemotherapy for pulmonary tuberculosis. *Bull. Int. Union Tuberc.* **47**:49–67 (1972).
2. W. Fox and D. A. Mitchison. Short course chemotherapy for pulmonary tuberculosis. *Am. Rev. Resp. Dis.* **111**:325–352 (1975).
3. D. A. P. Evans. Genetic variations in the acetylation of isoniazid and other drugs. *Ann. N.Y. Acad. Sci.* **151**:723–733 (1968).
4. B. N. La Du. Isoniazid and pseudocholinesterase polymorphisms. *Fed. Proc.* **31**:1276–1285 (1972).
5. J. H. Peters. Genetic factors in relation to drugs. *Ann. Rev. Pharmacol.* **8**:427–452 (1968).
6. G. A. Ellard. Variations between individuals and populations in the acetylation of isoniazid and its significance for the treatment of pulmonary tuberculosis. *Clin. Pharmacol. Ther.* (in press, 1976).
7. G. A. Ellard and P. T. Gammon. The pharmacokinetics of isoniazid metabolism in man. In M. Hejzlar, M. Semonsky, and S. Masak (eds.), *Advances in Antimicrobial and Antineoplastic Chemotherapy*, Urban and Schwarzenberg, Munich, 1972, pp. 45–46.
8. G. A. Ellard, P. T. Gammon, and S. M. Wallace. The determination of isoniazid and its metabolites acetylisoniazid, monoacetylhydrazine, diacetylhydrazine, isonicotinic acid and isonicotinylglycine in serum and urine. *Biochem. J.* **126**:449–458 (1972).
9. H. McKennis, Jr., A. S. Yard, J. H. Weatherby, and J. A. Hagy. Acetylation of hydrazine and the formation of 1,2-diacetylhydrazine *in vivo*. *J. Pharmacol. Exp. Ther.* **126**:109–116 (1959).
10. A. Viznerová, Z. Sláviková, and G. A. Ellard. The determination of the acetylator phenotype of tuberculosis patients in Czechoslovakia using sulphadimidine. *Tubercle* **54**:67–71 (1973).
11. D. A. P. Evans, P. B. Storey, and V. A. McKusick. Further observations on the determination of isoniazid inactivator phenotype. *Bull. Johns Hopkins Hosp.* **108**:60–66 (1961).
12. G. A. Ellard, V. R. Aber, P. T. Gammon, D. A. Mitchison, S. Lakshminarayan, K. M. Citron, W. Fox, and R. Tall. Pharmacology of some slow-release preparations of isoniazid of potential use in intermittent treatment of tuberculosis. *Lancet* **1**:340–343 (1972).
13. G. A. Ellard, P. T. Gammon, and H. Tiitinen. Determination of the acetylator phenotype using matrix isoniazid. *Tubercle* **56**:203–209 (1975).
14. H. Varley. *Practical Clinical Biochemistry*, 3rd ed., Heinemann, London, 1962, p. 634.
15. R. Gelber, P. Jacobsen, and L. Levy. A study of the availability of six commercial formulations of isoniazid. *Clin. Pharmacol. Ther.* **10**:841–848 (1969).

16. J. H. Peters, K. S. Miller, and P. Brown. Studies on the metabolic basis for the genetically determined capacities for isoniazid inactivation in man. *J. Pharmacol. Exp. Ther.* **150:** 298–304 (1965).
17. J. W. Jenne, F. M. MacDonald, and E. Mendoza. A study of the renal clearances, metabolic inactivation rates, and serum fall-off interaction of isoniazid and para-amino salicylic acid in man. *Am. Rev. Resp. Dis.* **84:**371–378 (1961).
18. J. G. Wagner. *Biopharmaceutics and Relevant Pharmacokinetics*, Drug Intelligence Publications, Hamilton, Ill., 1971, Chap. 38, pp. 292–296.
19. A. J. Cummings, B. K. Martin, and G. S. Park. Kinetic considerations relating to the accrual and elimination of drug metabolites. *Brit. J. Pharmacol. Chemother.* **29:**136–149 (1967).
20. J. R. Mitchell, U. P. Thorgeirsson, M. Black, J. A. Timbrell, W. R. Snodgrass, W. Z. Potter, D. J. Jollow, and H. R. Keiser. Increased incidence of isoniazid hepatitis in rapid acetylators: Possible relation to hydrazine metabolites. *Clin. Pharmacol. Ther.* **18:**70–79 (1975).
21. G. A. Ellard, P. T. Gammon, F. Polansky, A. Víznerová, I. Havlík, and W. Fox. Further studies on the pharmacology of a slow-release preparation of isoniazid (Smith and Nephew HS 82) of potential use in the intermittent treatment of tuberculosis. *Tubercle* **54:**57–66 (1973).
22. L. C. Dymond and D. W. Russell. Rapid determination of isonicotinic acid hydrazide in whole blood with 2,4,5-trinitrobenzenesulphonic acid. *Clin. Chim. Acta* **27:**513–520 (1970).
23. H. G. Boxenbaum, G. S. Jodhka, A. C. Ferguson, S. Riegelman, and T. R. MacGregor. The influence of bacterial gut hydrolysis on the fate of orally administered isonicotinuric acid in man. *J. Pharmacokin. Biopharm.* **2:**211–237 (1974).
24. G. Levy and L. P. Ansel. Kinetics of competitive inhibition of salicylic acid conjugation with glycine in man. *Biochem. Pharmacol.* **15:**1033–1038 (1966).
25. J. H. Peters and V. E. Hayes. Comparative studies on the metabolism of isoniazid and isoniazid hydrazones in the dog. *Arch. Int. Pharmacodyn. Ther.* **159:**328–339 (1966).
26. F. Skrabal, R. N. Arnot, and G. F. Joplin. Equations for the prediction of normal values for exchangeable sodium, exchangeable potassium, extracellular fluid volume, and total body water. *Brit. Med. J.* **2:**37–38 (1973).
27. G. A. Ellard, P. T. Gammon, and H. Tiitinen. Determination of the acetylator phenotype from the ratio of the urinary excretion of acetylisoniazid to acid-labile isoniazid: A study in Finnish Lappland. *Tubercle* **54:**201–210 (1973).
28. A. S. Yard and H. McKennis, Jr. Aspects of the metabolism of isoniazid and acetylisoniazid in the human and dog. *J. Med. Pharm. Chem.* **5:**196–203 (1962).
29. J. W. Jenne. Partial purification and properties of the isoniazid transacetylase in human liver: Its relationship to the acetylation of *p*-aminosalicylic acid. *J. Clin. Invest.* **44:**1992–2002 (1965).
30. W. W. Weber and S. N. Cohen. The mechanism of isoniazid acetylation by human *N*-acetyltransferase. *Biochim. Biophys. Acta* **151:**276–278 (1968).
31. W. W. Weber, S. N. Cohen, and M. S. Steinberg. Purification and properties of *N*-acetyltransferase from mammalian liver. *Ann. N.Y. Acad. Sci.* **151:**734–741 (1968).
32. L. H. Schmid. The problem of the rapid inactivator of isoniazid. *Bull. Int. Union Tuberc.* **32:**487–502 (1962).
33. L. Eidus, M. M. Hodgkin, A. H. E. Hsu, and O. Schaeffer. Pharmacokinetic studies with an isoniazid slow-releasing matrix preparation. *Am. Rev. Resp. Dis.* **110:**34–42 (1974).