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Pharmacokinetics of Isoniazid and Some Metabolites in Man[1,2,7]

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The pharmacokinetics of isoniazid and its metabolites acetylisoniazid, isonicotinic acid, and isonicotinuric acid were investigated in man by administering each compound intravenously to a rapid and slow isoniazid acetylator. Isoniazid was measured in blood, and the metabolites were determined in urine. Appropriate models are formulated, with the overall model describing isoniazid and metabolite disposition consisting of 12 compartments.

KEY WORDS: isoniazid; acetylation polymorphism; pharmacogenetics.

INTRODUCTION

The synthesis of isonicotinic acid hydrazide (isoniazid) was first reported in 1912 by Meyer and Mally (1), but it was not until the early 1950s that three independent research groups simultaneously discovered the marked antituberculosis activity of this compound (2). Presently, isoniazid is considered to be the most effective of the commonly employed antituberculosis drugs (3).

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[1]This article is dedicated to the memory of Dr. L. Strait, teacher and friend.

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The major urinary excretion products resulting from isoniazid (INH) administration to humans are intact INH, pyruvic acid isonicotinoylhydrazone (INH-PA), α-ketoglutaric acid isonicotinoylhydrazone (INH-KA), acetylisoniazid (AcINH), isonicotinic acid (INA), isonicotinuric acid (INU), acetylhydrazine (AcHy), and 1,2-diacetylhydrazine (DiAcHy) (4–6). The metabolic scheme proposed by Ellard and Gammon (5) for the metabolism of INH in humans is shown in Fig. 1. Hydrazine (Hy) was presumed to be formed from the hydrolysis of INH to INA, but this compound was not detected. Examination of the literature indicates other metabolic routes may exist. Ziporin *et al.* (7,8) reported an elevation of blood ammonia following INH administration. Additionally, *in vitro* studies in which INH was converted by animal tissue preparations into INA and Hy indicated that the Hy was subsequently converted to ammonia (9,10). Schmidt (11) reported that INH-PA and INH-KA were cleaved promptly in *p*H 6–6.5 buffer to free INH and the corresponding ketoacids. Taking cognizance of the lability of the hydrazones, Schmidt suggested that their cleavage back to INH in the body is possible. The direct conversion of INH to INU was suggested by the experiments of Wenzel (12–14). Incubation of INH with glycine with

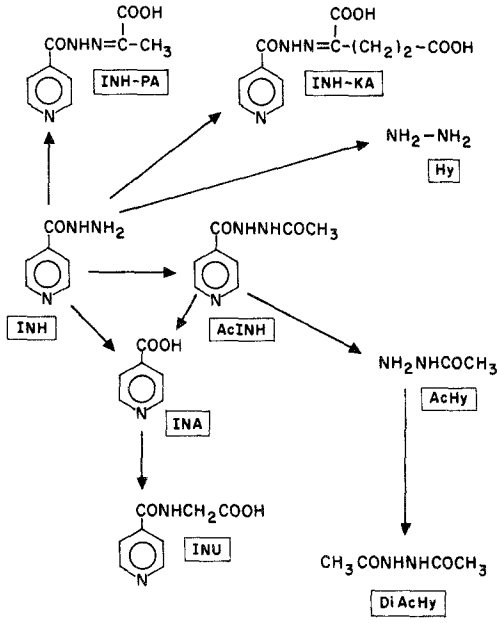


Fig. 1. Scheme for the metabolism of INH in man.

or without human serum resulted in the production of INU. Incubation of INA with glycine under the same conditions did not produce INU.

In humans, individuals have been classified as either rapid or slow acetylators of INH, and this capacity to acetylate INH is a permanent hereditary characteristic (4,5,15,16). Approximately half of the White and American Black populations are rapid INH acetylators, and the other half are slow acetylators. Plasma half-lives in rapid acetylators range from 45 to 80 min and in slow acetylators from 140 to 200 min.

Ellard and Gammon (5) studied the pharmacokinetics of INH, AcINH, INA, INU, AcHy, and DiAcHy in human subjects. With the exception of INU, each of the aforementioned compounds was administered orally, and parameter estimates for one-compartment open-system models were reported. It was the purpose of this investigation to supplement the work of Ellard and Gammon (5) and to develop sophisticated pharmacokinetic models to describe the disposition of INH, AcINH, INA, and INU. Each of these compounds was therefore administered intravenously to both a rapid and a slow acetylator subject. Urinary excretion data were obtained for AcINH, INA, and INU, and blood data were obtained for INH; appropriate pharmacokinetic analyses led to the disposition models reported herein.

MATERIALS AND METHODS

Chemicals

INH, AcINH, INA, and INU have been described previously (17,18).

Assay Procedures

AcINH, INA, INU, and the sum of INH plus hydrazones were determined in urine, and INH was determined in blood; the methods, which are specific and accurate, have previously been described (17). The sum of INH and its hydrazones in urine was determined, since it was demonstrated that the amount of free INH in urine most probably does not reflect the amount of free INH excreted by the kidney (17). This is a consequence of the extreme lability of hydrazones in urine (forming INH), as well as the spontaneous formation of hydrazones from INH and α-ketoacids normally found in urine. The concentrations of INH and hydrazones in urine therefore probably represent an equilibrium condition.

In a preliminary study in which INH was administered orally, the method of Scott and Wright (19) was used to measure INH serum levels. When serum INH levels exceeded 5 μg/ml, samples were diluted with blank serum to achieve concentrations below 5 μg/ml.

Subjects

Two adult, male volunteers in apparent good health served as subjects. Subject A was a White male, age 30 years, weight 69.0 kg, and height 165 cm. Subject B was a White male, age 50 years, weight 80.0 kg, and height 181 cm. Subject A was a slow INH acetylator, and subject B was a rapid INH acetylator. Subjects were phenotyped on the basis of terminal exponential half-lives, according to the procedure used by Scott and Wright (19). Neither subject smoked tobacco, and neither had received other drugs for several months prior to these experiments or during them. Experiments in any one subject were conducted at least 2 weeks apart.

Test Procedures

A preliminary study was conducted with subject A. A 1.00-g dose of INH dissolved in 100 ml water was ingested after an overnight fast, and food and water were subsequently withheld for 2 hr; blood and urine were collected at frequent intervals. The INA rate of excretion curve (see Results and Discussion) appeared to be affected by changes in urinary *p*H, and consequently all other studies were conducted with controlled urinary *p*H.

Each of the two subjects received intravenous injections of INH, AcINH, INA, and INU. With the exception of INH, all other solutions for injection were prepared in the laboratory. Isoniazid injection U.S.P. (Nydrazid injection, Squibb Laboratories) contained 100 mg/ml. The other solutions were prepared by dissolving the requisite amount of compound in 60 ml sodium chloride injection U.S.P. and adding sufficient NaOH to bring the *p*H to 7.4; all solutions were sterilized by micropore filtration.

Each injection was administered intravenously in a vein on the dorsum of the forearm at a zero-order rate; the duration of infusion for INH was approximately 5.3 min, whereas AcINH, INA, and INU were infused for exactly 60.0 min. This lengthy infusion time was elected to order to minimize risks. The approximate doses were (1) INH, 675 mg; (2) AcINH, 580 mg; (3) INA, 200 mg; and (4) INU, 120 mg. Because the preliminary study with INH indicated that the pattern of INA excretion was affected by changes in urinary *p*H, sodium bicarbonate was administered orally to maintain urine *p*H at 8.0 ± 0.2 in all intravenous experiments. The bicarbonate dosing regimen was modified from Kostenbauder *et al.* (20) and utilized 325 mg sodium bicarbonate tablets U.S.P. administered every 30 min. In most cases, after initial alkalinization was achieved, four tablets every 30 min maintained the urine *p*H close to 8.0.

No restrictions were placed on food consumption during the experiments, except that alcoholic beverages were not permitted; no attempt was made to control urine volume.

In the INH studies, blood was collected at approximate 2–5 min intervals during and for some time after the infusion. Blood collections were later reduced to approximate 30-min intervals. Urine was collected at approximate 30-min intervals for about 12 hr, and then *ad libitum* for a total of 48 hr postinfusion.

For the AcINH, INA, and INU studies, urine was generally collected at 15-min intervals during and for 1 hr postinfusion (total of eight collections). Urine collections were subsequently made at approximate 30-min intervals for about 11 hr postinfusion, and then *ad libitum* for 48 hr total.

In each study, urine *p*H was measured within 5–10 min after voiding, and all samples were frozen at −18°C until assay. INH in blood was measured within 24 hr from time of filtrate preparation. Urine assays were performed within 2 weeks after collection. Under these conditions, all compounds were stable (17).

Mathematical, Statistical, and Computer Methods

Equations were derived by the method of Benet (21), and were expressed in terms of macroscopic (hybrid) constants indicated by Greek letters, the sum of exit rate constants from peripheral compartments, and volume of the central compartment (when appropriate). In fitting equations to urinary excretion data, integrated rate equations were employed.

Equations were fitted to observed data using the NONLIN program. This program (22) uses an adaptation of Hartley's modification (23) of the Gauss-Newton method for the fitting of nonlinear regression functions by least squares. Initial parameter estimates were determined by graphical analysis (24,25). Microscopic parameter estimates and associated statistical information were derived from appropriate equations (24,26). Comparisons between parameter estimates were made using the appropriate t test (26); the results from this test apply only to the parameters from the data being evaluated, and may not be extrapolated *per se* to any other studies, other subjects, or the populations of rapid and slow acetylator subjects as a whole.

In analyzing urine for metabolite concentrations, urine was diluted so that all measurements for a particular metabolite were made at similar concentrations. Consequently, for a particular metabolite, the percent error in urinary levels was approximately the same for all data. Accordingly (26), equation 1 was used to weight urinary excretion rate data:

$$\frac{1/Y_i^2}{\sum_{i=1}^{N} 1/Y_i^2} \times N \tag{1}$$

where Y_i is the observed value of the ith measurement and N is the number of data points.

In the case of INH blood concentrations, it was also felt that all measurements had approximately the same percent error (27). Consequently, weights for INH blood concentration data were also determined from equation 1.

RESULTS AND DISCUSSION

Oral Isoniazid Study

The INH serum levels and rate of excretion data for AcINH, INA, and INU following a 1.00-g oral dose of INH in aqueous solution to subject A are illustrated in Fig. 2. Unfortunately, early blood sample specimens were

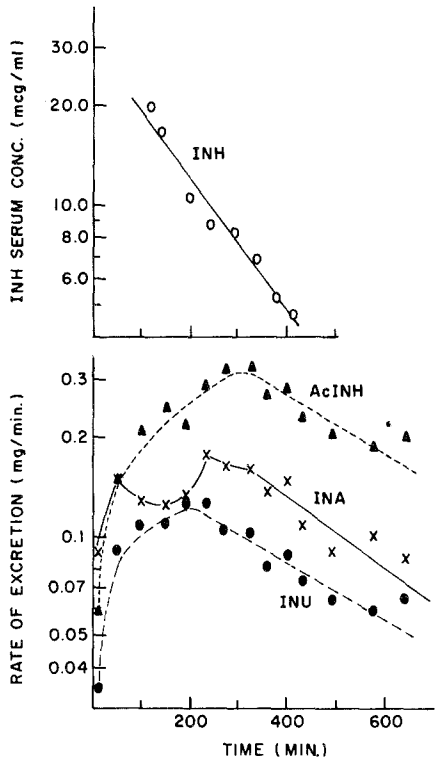


Fig. 2. Serum levels of INH and rates of excretion of AcINH, INA, and INU after oral administration of 1.00 g INH to subject A on an empty stomach (curves were empirically drawn).

accidentally lost. The rate of excretion of INA was unusually low between 60 and 230 min, and this appeared to be related to urine *p*H. As the urine *p*H dropped from values of approximately 6–7 to a value of approximately 5, the rate of excretion dropped. A decision was therefore made to conduct all other studies under conditions of controlled urinary *p*H. For this purpose, sodium bicarbonate was administered orally so as to maintain urine *p*H at 8.0 ± 0.2.

The INH serum concentration–time curve in this initial study declined exponentially with a half-life of 148 min; 96.5% of the oral dose was recovered in urine as INH plus hydrazones, AcINH, INA, and INU.

Isonicotinuric Acid Administration Studies

Following intravenous infusion of INU in the two test subjects, only intact compound was detected in urine; urinary recovery, within experimental error, was equivalent to the administered dose (27). Postinfusion rate of excretion–time curves were biexponential, and the model illustrated in Fig. 3 was used to describe INU disposition. A single equation describing the rate of urinary excretion of INU during and after the zero-order infusion is presented in the Appendix. The equation was fitted to the data by NONLIN, and the estimated parameters are presented in Table I. The percent coefficients of variation (% CVs) of the estimated parameters are relatively small (maximum of 17.3); consequently, the approximate 90% confidence intervals are relatively narrow. The *t* test indicates that some differences in parameters could be detected between the two subjects.

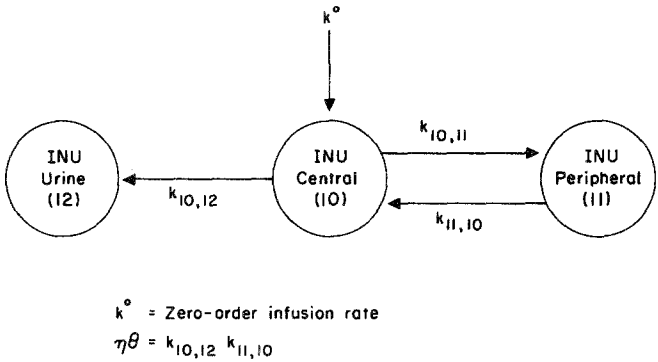


Fig. 3. Model used to describe INU disposition, including zero-order input (with the exception of the zero-order input, arrows represent first-order processes).

Table I. Parameter Estimates from INU Administration Studies (η, θ, and $k_{11,10}$ Were Fitted by NONLIN)

Parameter	Parameter estimate (min^{-1}) Subject A	Subject B	t test[a]
η	0.0675 (2.42)[b] (0.0625–0.0725)[c]	0.0473 (6.81) (0.0380–0.0566)	–
θ	0.0114 (1.73) (0.0108–0.0120)	0.0129 (6.55) (0.0105–0.0153)	+
$k_{11,10}$	0.0133 (1.65) (0.0126–0.0140)	0.0158 (6.01) (0.0130–0.0186)	–
$k_{10,11}$	0.00787 (4.04) (0.00690–0.00884)	0.00579 (17.3) (0.00289–0.00869)	+
$k_{10,12}$	0.0577 (2.44) (0.0534–0.0620)	0.0386 (6.30) (0.0315–0.0457)	–

[a]Plus sign indicates acceptance of the null hypothesis, meaning that differences between "true" parameters from the two subjects cannot be detected; minus sign indicates rejection (5% level of significance) (26).
[b]Percent coefficient of variation (26).
[c]Approximate S-plane 90% confidence interval (26).

Theoretical rate of excretion–time curves were generated using the estimated values of the parameters, and this is shown for subject A in Fig. 4

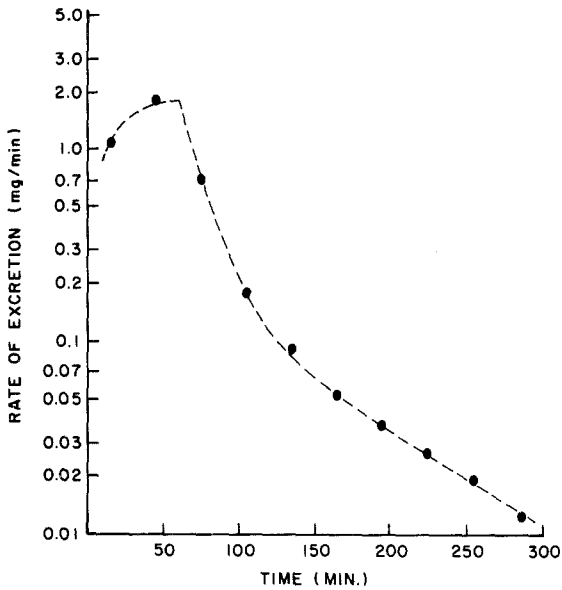


Fig. 4. Rate of excretion of INU in subject A; curve was generated from parameter estimates (INU was infused over a period of 60 min).

with the experimental data points. An examination of the scatter of the data points about the fitted line indicates satisfactory randomness of scatter.

Ellard and Gammon (5) reported the renal clearance of INU in one subject to be 493 ml/min. This value exceeds glomerular filtration rate (approximately 123 ml/min in man) (28), and indicates active tubular secretion of INU by the renal tubules. Since INU renal clearance is equal to $k_{10,12}V_{10}$, the value of V_{10} may be estimated (V_{10} is the volume of compartment 10). This value was calculated to be approximately 10 liters, which is almost twice the estimated blood volumes of 5.21 and 5.50 liters for subjects A and B, respectively (29).

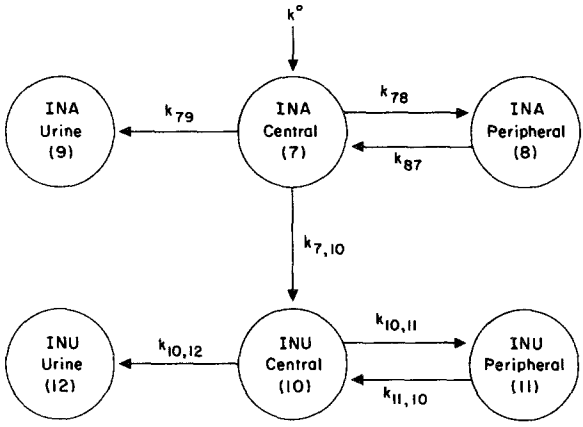
Ellard and Gammon (5) applied a one-compartment model to their data and reported an approximate value of $0.014\,min^{-1}$ for the INU elimination constant. This is in good agreement with the estimated values of the terminal hybrid exponential rate constant (θ) reported herein ($0.0114\,min^{-1}$ and $0.0129\,min^{-1}$ in subjects A and B, respectively).

Isonicotinic Acid Administration Studies

The two test subjects were administered approximately 200 mg INA by a 1-hr intravenous infusion. Subsequently, INA and INU appeared in urine; subjects A and B excreted 24.3 and 26.2% of the dose, respectively, as INU. The recovery of INA and INU in urine, within experimental error, was equivalent to the administered dose (27). Peters *et al.* (4) administered INA orally to seven subjects (4.49 mg/kg body weight, 314 mg for a 70-kg man). An average of 34.9% of the recovered dose was excreted as INU. Wide variations existed between subjects in the percentage of the dose recovered as INU (range of 19.3 to 48.3%). Ellard and Gammon (5) administered 25 and 250 mg INA orally to a subject; at the lower dosage, 43.1% of the recovered dose was excreted as INU, whereas 30% of the recovered dose was excreted as INU after the higher dose. The possible partial saturation of the INA glycine-conjugating system has been discussed previously (4,5,18). Both Peters *et al.* (4) and Ellard and Gammon (5) found no correlation between an individual's acetylation phenotype and his capacity to conjugate INA with glycine.

In the present analysis, a linear pharmacokinetic model was employed (i.e., all disposition processes were treated as being first order). The inconsistency of this model will be discussed subsequently. Following intravenous infusion of INA, postinfusion INA rate of excretion–time curves appeared to be biexponential for each subject. As is customary (24), a two-compartment body model for INA disposition was formulated; metabolism and excretion were assumed to occur from the central compartment. This assumption that metabolism of INA to INU occurs solely from the central

compartment will be demonstrated subsequently. The formation of INU was assumed to occur in its central compartment, and distribution and excretion of this metabolite were also described by a two-compartment body model; such a model for INU seems justifiable from the analysis of data after INU administration. The overall model is shown in Fig. 5, and the equations for the rates of excretion of INA and INU are in the Appendix. The two equations for INA and INU were fitted simultaneously to the data by computer, and the estimated parameters and statistical data are presented in Table II. Before discussing these data, a number of important points need to be mentioned. The equations fitted to the data contain the term f_7 (see Fig. 5); the value of this term was determined from urinary excretion data [$f_7 = A_9^{\infty}/(A_9 + A_{12})^{\infty}$] and subsequently fixed in the computer analysis (i.e., it was made a constant). An alternative approach was tried in which f_7 was put into the program as a parameter; this increased the number of parameters from six to seven. For a given subject, statistical analysis by the t and F tests (26) indicated that the values of f_7 and the weighted sums of



k° = Zero-order infusion rate

$\epsilon\zeta = k_{70}\, k_{87}$

$\eta\theta = k_{10,12}\, k_{11,10}$

$k_{70} = k_{79} + k_{7,10}$

$f_7 = \dfrac{k_{79}}{k_{70}}$

Fig. 5. Model used to describe INA disposition, including zero-order input (with the exception of the zero-order input, arrows represent first-order processes).

Table II. Parameter Estimates from INA Administration Studies (ε, ζ, k_{87}, η, θ, and $k_{11,10}$ Were Fitted by NONLIN)

Parameter	Parameter estimate (min^{-1})		t test[a]
	Subject A	Subject B	
ε	0.0621 (9.03)[b]	0.0416 (8.82)	–
	(0.0425–0.0817)[c]	(0.0286–0.0546)	
ζ	0.0184 (8.09)	0.0118 (18.7)	–
	(0.0128–0.0240)	(0.00401–0.0196)	
k_{87}	0.0228 (12.7)	0.0136 (22.8)	–
	(0.0127–0.0329)	(0.00262)–0.0246)	
k_{78}	0.00769 (24.1)	0.00380 (29.0)	+
	(0.00118–0.01420)	(−0.000095–0.00770)	
k_{70}	0.0499 (5.58)	0.0359 (5.49)	–
	(0.0401–0.0597)	(0.0289–0.0429)	
k_{79}	0.0378 (5.58)	0.0265 (5.49)	–
	(0.0304–0.0452)	(0.0213–0.0317)	
$k_{7,10}$	0.0121 (5.58)	0.00941 (5.49)	–
	(0.00973–0.0145)	(0.00758–0.0112)	
η	0.0991 (12.5) [−][d]	0.102 (23.1) [−]	+
	(0.0557–0.1425)	(0.0184–0.186)	
θ	0.0113 (19.5) [+]	0.0157 (14.9) [+]	+
	(0.00360–0.0190)	(0.00738–0.0240)	
$k_{11,10}$	0.0138 (21.3) [+]	0.0204 (20.9 [+]	+
	(0.00348–0.02412)	(0.00528–0.0355)	
$k_{10,11}$	0.0158 (21.6) [−]	0.0187 (45.0) [+]	+
	(0.00383–0.0278)	(−0.0112–0.0486)	
$k_{10,12}$	0.0807 (11.0) [−]	0.0786 (19.3) [−]	+
	(0.0498–0.112)	(0.0248–0.132)	

[a]Plus sign indicates acceptance of the null hypothesis, meaning that differences between "true" parameters from the two subjects cannot be detected; minus sign indicates rejection (5% level of significance) (26).
[b]Percent coefficient of variation (26).
[c]Approximate S-plane 90% confidence intervals (26).
[d]Plus sign in square brackets next to % CV indicates acceptance of the null hypothesis, meaning that differences between "true" parameters from this study and the INU study cannot be detected; minus sign indicates rejection (5% level of significance) (26).

squared deviations were not significantly different when fitting the equations by the two methods. Since it is possible to calculate f_7, it was concluded that its value should be fixed in the computer analyses. Another point to be mentioned is the influence of the order in which the parameters are listed in defining the function on the parameter standard deviations (SDs). When parameters 1–6 were defined as ε, ζ, k_{87}, η θ, and $k_{11,10}$, respectively, different SDs of all parameters were calculated than when parameters 1–6 were defined as ε, ζ, k_{87}, θ, η, and $k_{11,10}$, respectively; this occurred despite the fact that identical parameter estimates were reported. The reason for this

anomalous behavior was thought possibly to result from "rounding off" errors in the calculation of the SDs (30). Because listing the parameters in the normal sequence (i.e., ε, ζ, k_{87}, η, θ, and $k_{11,10}$) resulted in lower estimates of the SDs, this order was adopted in these and subsequent studies. Last, the influence of fitting two or more curves on the SDs of the parameter estimates need be mentioned. For the data reported here, INA and INU curves were fitted simultaneously. One could, however, just fit the INA curve with three parameters; when this approach was taken with the data from subject A, slightly different estimates of the three parameters were computed, but the % CVs were substantially lower (approximately 1.7–10-fold). This finding suggests that the INA rate of excretion–time curve is more sensitive to changes in INA disposition parameters than the INU rate of excretion–time curve. Hence fitting only the INA rate of excretion equation to the data results in more precise estimates of the parameters.

Referring to the data in Table II, the t test indicates that one cannot detect differences in the "true" values of all but one of the INA disposition parameters for the two subjects; differences between the "true" values of all the INU disposition parameters cannot be detected between subjects. In this and subsequent t tests, small values for t result as a consequence of large values for the SDs; this makes acceptance of the null hypothesis more likely. Acceptance of the null hypothesis occurs when parameter estimates differ from each other no more than is reasonable to expect from the SDs. Comparing the INU disposition parameters in these studies to those determined after INU administration reveals that differences between some of the parameter values cannot be detected by the t test whereas others can. A trend begins here which continues in subsequent studies. The % CVs of the INU disposition parameters are large when estimated from the INA study; this means that in a relatively large region of INU parameter spaces about an equally good fit to the data may be obtained (22). The large % CVs consequently result in wide 90% confidence intervals, and therefore the t tests result in the acceptance of all null hypotheses comparing INU parameters between subjects in the INA studies even though these were not all accepted from the INU studies.

Figure 6 shows the theoretical rate of excretion–time curves for subject A generated from the computer-estimated parameters together with the experimental data points. The fits of the generated curves to the data points are excellent; scatter of the data points about the fitted lines appears random.

Ellard and Gammon (5) reported the renal clearance of INA in one subject to be 453 ml/min; because renal clearance is equal to $k_{79}V_7$, the value of V_7 may be estimated (V_7 is the volume of compartment 7). The calculated value is approximately 14 liters, and this is almost three times the

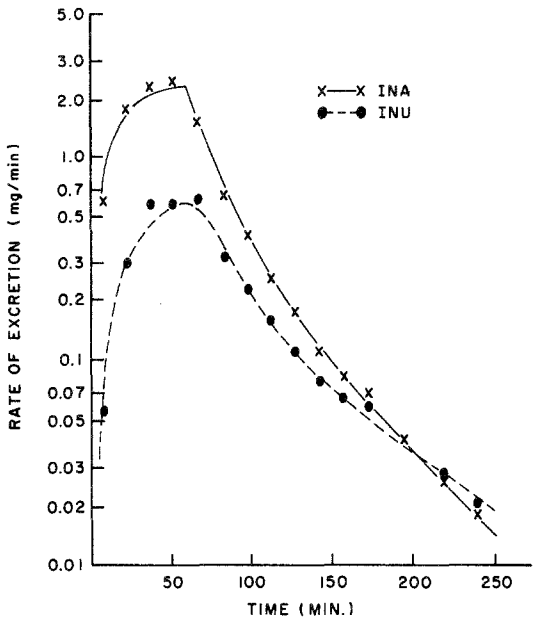


Fig. 6. Rate of excretion of INA and INU in subject A following INA administration; curves were generated from parameter estimates (INA was infused over a period of 60 min).

estimated blood volumes. As with INU, INA is actively secreted by the renal tubules.

Ellard and Gammon (5) also reported disposition parameters for INA using a one-compartment model. The excretion rate constant reported was 0.0125 min^{-1}, and this is in good agreement with the values calculated from the data reported herein (0.0139 min^{-1} and 0.00871 min^{-1} for subjects A and B, respectively). The metabolism rate constant reported was 0.0050 min^{-1}, and this also is in good agreement with the values calculated here (0.00447 min^{-1} and 0.00309 min^{-1} for subjects A and B, respectively). These metabolic and excretion rate constants, for each respective subject, add up to ζ, the terminal hybride exponential rate constant.

It was mentioned previously that metabolism of INA to INU occurs solely from the central compartment. This determination was made employing the method of Rowland *et al.* (31). Equation 12 of that article was modified to accommodate urinary excretion data resulting from a zero-order infusion.

The appropriate equation for INA and INU, derived by the method of Benet (21), is

$$\frac{dA_{12}/dt}{k^0 \sum_{i=\varepsilon}^{\theta} [(1 - e^{ib})\, e^{-it}]/[-i(j - i)(k - i)(l - i)]} = \frac{k_{7,10}k_{10,12} \sum_{i=\varepsilon}^{\theta} [(1 - e^{ib})(E_8 - i)(E_{11} - i)\, e^{-it}]/[-i(j - i)(k - i)(l - i)]}{\sum_{i=\varepsilon}^{\theta} [(i - e^{ib})\, e^{-it}]/[-i(j - i)(k - i)(l - i)]} + k_{78}k_{8,11}k_{11,10}k_{10,12} \quad (2)$$

where dA_{12}/dt is the rate of INU excretion when INA is infused intravenously; k^0 is the zero-order infusion rate; i takes on values of ε, ζ, η, and θ, while j, k, and l are the superscripted values not equal to i; t is time; b is equal to t while the infusion is continuing and when the infusion ceases b becomes a constant corresponding to the time infusion is stopped; $k_{8,11}$ is the peripheral metabolism rate constant for conversion of INA to INU; E_8 is $k_{87} + k_{8,11}$; and E_{11} is $k_{11,10}$.

A plot of the appropriate functions gives a slope of $k_{7,10}k_{10,12}$ and y intercept of $k_{78}k_{8,11}k_{11,10}k_{10,12}$. Therefore, the rate constant $k_{8,11}$ may be evaluated (31). Values of the y function varied by a magnitude of 10^4-fold; therefore, they were weighted by $1/y^2$ (26). Analysis of the plots (31) indicated that less than 1% of INA was eliminated from the peripheral compartment; this was considered quantitatively negligible, and most probably differed from zero only as a result of experimental error.

Acetylisoniazid Administration Studies

Following intravenous infusion of AcINH in the two test subjects, AcINH, INA, and INU were detected in urine. The recovery of these three compounds in urine, within experimental error, was equivalent to the administered dose. Average cumulative urinary recoveries of AcINH, INA, and INU in urine were 63.2, 24.8, and 12.0%, respectively.

Following intravenous infusion of AcINH in these studies, postinfusion AcINH rate of excretion–time curves appeared to be biexponential. A two-compartment body model for AcINH was formulated, with metabolism and excretion occurring from the central compartment. This assumption that metabolism of AcINH to INA occurs solely from the central compartment was demonstrated by the method previously discussed. Two-compartment body models were also used to describe both INA and INU disposition; metabolism and excretion of these metabolites were also taken to occur from their central compartments. The overall model used to describe AcINH disposition is shown in Fig. 7, and the equations for the rates of excretion of AcINH, INA, and INU are in the Appendix.

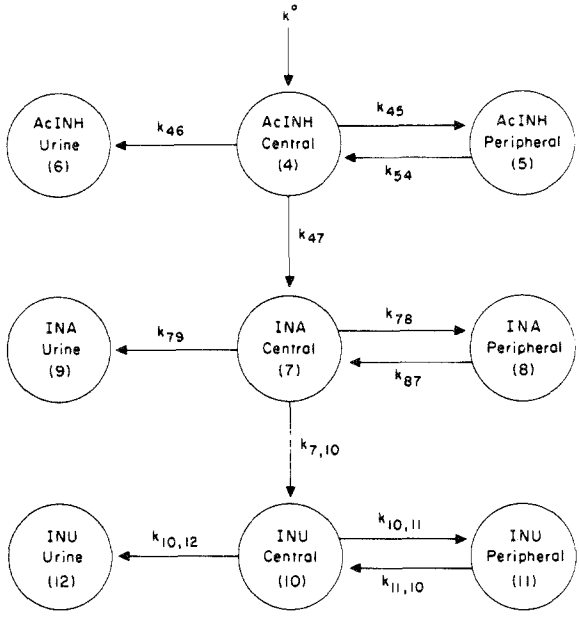


Fig. 7. Model used to describe AcINH disposition, including zero-order input (with the exception of the zero-order input, arrows represent first-order rate constants).

All three equations for AcINH, INA, and INU were fitted to the data simultaneously, and the estimated parameters and statistical data are presented in Table III. The values of f_4 and f_7 (see Fig. 7) were determined by standard methods (32,33) and were held constant in the computer analyses. The t tests indicate that differences between most parameters from the two subjects cannot be detected. Acceptance of the null hypotheses in many of these tests resulted as a consequence of large values for the SDs. With SD values as large as these, values for the parameters are not well determined. This is reflected by the rather wide 90% confidence intervals. The large values for the SDs are not a consequence of poor fit of the equations to the data or inadequate numbers of data points. On the contrary, the equations

Table III. Parameter Estimates from AcINH Administration Studies (γ, δ, k_{54}, ε, ζ, k_{87}, η, θ, and $k_{11,10}$ Were Fitted by NONLIN)

Parameter	Parameter estimate (min^{-1})		t test[a]
	Subject A	Subject B	
γ	0.0456 (9.76)[b] (0.0281–0.0631)[c]	0.0324 (11.9) (0.0172–0.0476)	–
δ	0.00389 (1.83) (0.00361–0.00417)	0.00389 (5.19) (0.00309–0.00469)	+
k_{54}	0.0171 (8.15) (0.0116–0.0226)	0.0122 (12.3) (0.00629–0.0181)	–
k_{45}	0.0220 (13.2) (0.0106–0.0334)	0.0137 (16.3) (0.00481–0.0226)	–
k_{40}	0.0104 (4.55) (0.00855–0.0123)	0.0104 (5.54) (0.00812–0.0127)	+
k_{46}	0.00692 (4.55) (0.00568–0.00816)	0.00617 (5.54) (0.00481–0.00753)	+
k_{47}	0.00345 (4.55) (0.00283–0.00407)	0.00418 (5.54) (0.00326–0.00510)	–
ε	0.0934 (48.8) (−0.0855–0.272) [+][d]	0.0643 (34.1) (−0.0226–0.151) [+]	+
ζ	0.0141 (51.7) (−0.0145–0.0427) [+]	0.00738 (86.0) (−0.0178–0.0326) [+]	+
k_{87}	0.0216 (79.7) (−0.0459)–0.0891) [+]	0.00943 (99.9) (−0.0280–0.0468) [+]	+
k_{78}	0.0251 (98.5) (−0.0718–0.122) [+]	0.0120 (87.7) (−0.0297–0.0537) [+]	+
k_{70}	0.0608 (22.8) (0.00627–0.115) [+]	0.0503 (21.6) (0.00704–0.0936) [+]	+
k_{79}	0.0385 (22.8) (0.00402–0.0730) [+]	0.0357 (21.6) (0.00510–0.0663) [+]	+
$k_{7,10}$	0.0223 (22.8) (0.00233–0.0423) [−]	0.0147 (21.6) (0.00216–0.0272) [+]	+
η	0.111 (85.0) (−0.259–0.481) [+]	0.614 (264) (−6.07–7.35) [+]	+
θ	0.00720 (96.4) (−0.0200–0.0344) [+]	0.0184 (97.5) (−0.0526–0.0894) [+]	+
$k_{11,10}$	0.00872 (103) (−0.0265–0.0439) [+]	0.0485 (132) (−0.205–0.302) [+]	+
$k_{10,11}$	0.0178 (201) (−0.123–0.158) [+]	0.368 (292) (−3.88–4.61) [+]	+
$k_{10,0}$	0.0915 (74.4) (−0.176–0.359) [+]	0.243 (275) (−2.41–2.89) [+]	+

[a]Plus sign indicates acceptance of the null hypothesis, meaning that differences between "true" parameters from the two subjects cannot be detected; minus sign indicates rejection (5% level of significance) (26).

[b]Percent coefficient of variation (26).

[c]Approximate S-plane 90% confidence intervals (26).

[d]Plus sign in square brackets next to % CV indicates acceptance of the null hypothesis meaning that differences between "true" parameters from this study and the INA study cannot be detected; minus sign indicates rejection (5% level of significance) (26).

fitted the data extremely well, and the numbers of data points are substantial (e.g., see Fig. 8). As was pointed out by Metzler (22) and Westlake (34), the large SDs result from parameter estimates which may be widely varied, yet give rise to only minute changes in the goodness of fit of the theoretical curves to the experimental data.

When the parameters from a particular subject in the AcINH study were compared to those same parameters from the INA study, the t test indicated that differences could not be detected for most values of these parameters (again, acceptance of null hypotheses in these tests resulted as a consequence of large SDs).

Figure 8 shows the theoretical rate of excretion–time curves for subject A generated from the computer-estimated parameters together with the experimental data points. For both subjects, the fit of the generated curves to the data points was excellent; scatter of the data points about the fitted lines appeared random.

Ellard and Gammon (5) reported the renal clearance of AcINH in one subject to be 111 ml/min; since renal clearance is equal to $k_{46}V_4$, the value

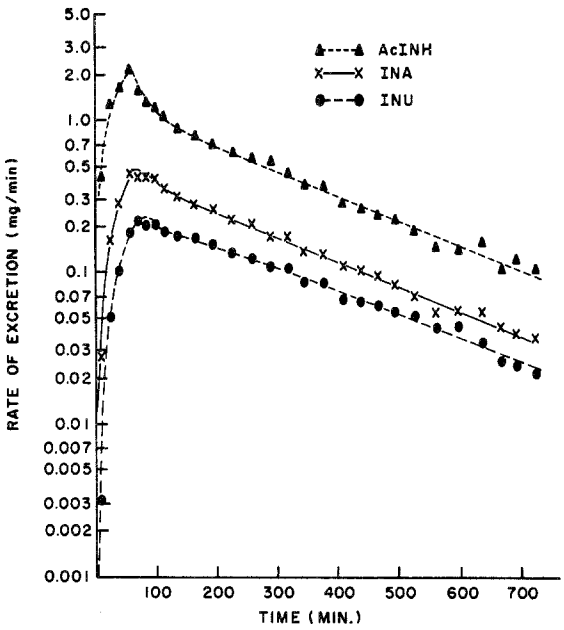


Fig. 8. Rate of excretion of AcINH, INA, and INU in subject A following AcINH administration; curves were generated from parameter estimates (AcINH was infused over a period of 60 min).

of V_4 may be estimated (V_4 is the volume of compartment 4). The calculated value is approximately 17 liters, and this is greater than three times the estimated blood volumes.

Ellard and Gammon (5) also reported disposition parameters for AcINH using a one-compartment model. The excretion rate constant reported was 0.0025 min^{-1}, and this is in excellent agreement with the values calculated from the data reported herein (0.00260 min^{-1} and 0.00232 min^{-1} for subjects A and B, respectively). The metabolism rate constant reported was 0.0017 min^{-1}, and this is in good agreement with the values calculated here (0.00129 min^{-1} and 0.00157 min^{-1} for subjects A and B, respectively). These metabolic and excretion rate constants, for each respective subject, add up to δ, the terminal exponential hybrid constant.

Equation 2 was appropriately modified and applied to the AcINH disposition model illustrated in Fig. 7. The purpose here was to solve for the rate constant k_{58} (i.e., the constant describing metabolism of AcINH to INA between compartments 5 and 8). The y function was weighted using the factor $1/y^2$. Analysis of the plots (31) indicated that less than 2.2% of AcINH was eliminated from the peripheral compartment; this was considered quantitatively negligible, and most probably differed from zero only as a result of experimental error.

Isoniazid Administration Studies

The virtual impossibility of separating INH from its hydrazones in urine has been discussed previously (17); the assay utilized in the urine determinations therefore pooled INH with its hydrazones.

After intravenous administration of INH to the two test subjects, INH plus hydrazones, AcINH, INA, and INU were detected in urine. For both subjects, within experimental error, the sum of INH and its metabolites recovered in urine was equivalent to the administered doses (27). The cumulative urinary recoveries of INH plus hydrazones, AcINH, INA, and INU were 54.7, 25.1, 12.7, and 7.57%, respectively, for subject A (slow acetylator). For subject B (rapid acetylator), these values were 23.0, 38.5, 25.0, and 13.5%, respectively. The percentage of the doses excreted as INH plus hydrazones was greater, and excretion of AcINH, INA, and INU was less than that reported by other groups (4,5,35). There is no obvious explanation for these differences.

One assumption inherent in all the mathematical analyses presented here is that drug is instantaneously and completely mixed within the central compartment after intravenous injection. As was pointed out by Ackerman *et al.* (36), such rapid mixing never occurs, but rather in practice one requires that the mixing within the central compartment be rapid compared to the

rates of efflux from that compartment. In subject A, the zero-order intravenous infusion of INH lasted 5.40 min; at 6.15 min and 7.85 min, INH blood levels were 16.8 and 21.1 μg/ml, respectively. In subject B, the infusion lasted 5.20 min; at 5.45 and 7.70 min, INH blood levels were 17.1 and 28.0 μg/ml, respectively. Thus there were short delays (at least 0.75 min) between the end of the infusions and achievement of maximum blood concentrations. Consequently, INH blood levels measured prior to attainment of the maximum blood levels were not used in the computer analyses.

For both subjects, postinfusion INH blood level–time curves appeared biexponential. Consequently, a two-compartment body model for INH was formulated as illustrated in Fig. 9. As will be discussed subsequently, it is necessary to include in the model an elimination pathway from the peripheral compartment. Initially, only the INH blood data were utilized in the computer analyses, and the appropriate equation (see Appendix) was fitted to the data. The model, blood data, and computer-generated curves are shown in Fig. 9. The terminal, linear logarithmic phases of the curves had

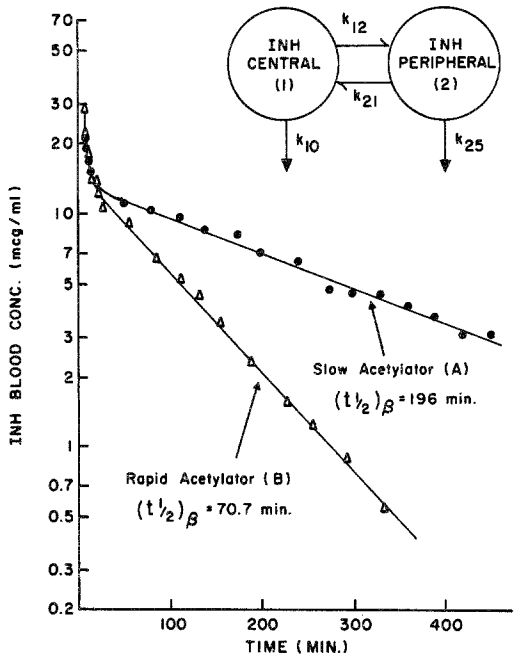


Fig. 9. Pharmacokinetic model, blood data, and fitted curves for INH disposition (arrows in model represent first-order processes).

Table IV. Pharmacokinetic Parameters Determined by Computer Analysis of INH Blood Data

Parameter	Parameter estimate (min^{-1} or ml)	
	Subject A	Subject B
α	0.195 (16.9)[a]	0.260 (18.6)
β	0.00354 (2.21)	0.00981 (1.88)
E_2 (i.e., $k_{21} + k_{25}$)	0.0819 (1.47)	0.0679 (3.61)
V_1	20,492 (16.1)	10,652 (20.0)

[a]Percent coefficient of variation (26).

half-lives of 70.7 and 196 min for the rapid and slow acetylator subjects, respectively. Table IV shows the estimated parameters and some statistical data. The volumes of the central compartments are quite different in the two subjects, and there is no apparent expianation for this.

Ellard and Gammon (5) reported one-compartment elimination constants for a rapid and slow acetylator of INH (0.0086 min^{-1} and 0.0036 min^{-1}, respectively). These are similar to those for subjects B and A (0.00981 min^{-1} and 0.00354 min^{-1}, respectively). Thus the two subjects used in these studies are representative of the two acetylation phenotypes.

It had previously been assumed that INH was eliminated entirely from the central compartment (27). In fact, such a pharmacokinetic analysis for subject A was published for illustrative purposes only (26). However, when it was assumed that INH was eliminated entirely from the central compartment, resultant AcINH and INA rate of excretion curves were very poorly fitted with disposition parameters obtained following intravenous administration of the latter two compounds (27). It was therefore decided to determine to what extent, if any, INH was eliminated from the peripheral compartment.

For the purpose of this determination, several assumptions were made:

1. Hydrazone formation and urinary excretion of intact INH occur only from within the central compartment.
2. INH conversion to AcINH may occur from the central and/or peripheral compartment.
3. The fractions of AcINH excreted intact and metabolized to INA following AcINH administration may be applied following INH administration.
4. INA formed in excess of what would be expected from assumption 3 is formed directly from INH solely within the central compartment.

The first assumption that hydrazone formation occurs only from within the central compartment may well not be valid. As discussed previously,

hydrazone formation is a spontaneous nonenzymatic reaction that may occur in aqueous solution between α-ketoacids and INH. Unfortunately, from a mathematical perspective, the inclusion into the model of hydrazone formation in the peripheral compartment would give rise to a nonunique model for which parameters could not be determined. This arises as a consequence of the fact that experimentally the individual hydrazones were not administered intravenously to the subjects, as well as the fact that the individual hydrazones could not be determined *per se* in blood or urine. Therefore, the strategy adopted herein was to assume that formation of hydrazones did *not* occur in the peripheral compartment. It must be emphasized that should this or any of the aforementioned assumptions not be valid, then the parameter estimates would not be valid. This is rather basic, for as has been pointed out previously (26) the fitting of any equation to data assumes and requires that the correct equation be chosen.

With regard to the acetylation of isoniazid in both the central and peripheral compartments, it is interesting to note that extrahepatic acetylation of INH has been reported to occur (37) *in vitro* with incubates from human ileum, jejunum, and spleen; the kidney showed no activity. Therefore, it seems reasonable to assume that acetylation may occur in more than one location in the human body.

The appropriate model is illustrated in Fig. 10. The direct conversion of INH to INA has never been unequivocally established for man; this is a consequence of not being able to distinguish between INA formed from AcINH and that formed from INH. Nonetheless, there is reason to believe that man may hydrolyze INH directly to INA. The dog, an animal which does not acetylate INH, converts INH directly to INA (11). Additionally, Ellard and Gammon (5) presented urinary excretion data in man consistent with the direct conversion of INH to INA.

To determine to what extent INH was metabolized to AcINH from the peripheral compartment, equation 2 was appropriately modified:

$$\frac{dA_6/dt}{k^0 \sum_{i=\alpha}^{\delta} [(1 - e^{ib})\, e^{-it}]/[-i(j-i)(k-i)(l-i)]} = \frac{k_{14}k_{46} \sum_{i=\alpha}^{\delta} [(1 - e^{ib})(E_2 - i)(E_5 - i)\, e^{-it}]/[-i(j-i)(k-i)(l-i)]}{\sum_{i=\alpha}^{\delta} [(1 - e^{ib})\, e^{-it}]/[-i(j-i)(k-i)(l-i)]} + k_{12}k_{25}k_{54}k_{46} \qquad (3)$$

where dA_6/dt is the rate of AcINH excretion when INH is infused intravenously; k^0 is the zero-order infusion rate; i takes on values of α, β, γ, and δ, while j, k, and l are the superscripted values not equal to i; t is time; b is equal to t while the infusion is continuing and when the infusion ceases b

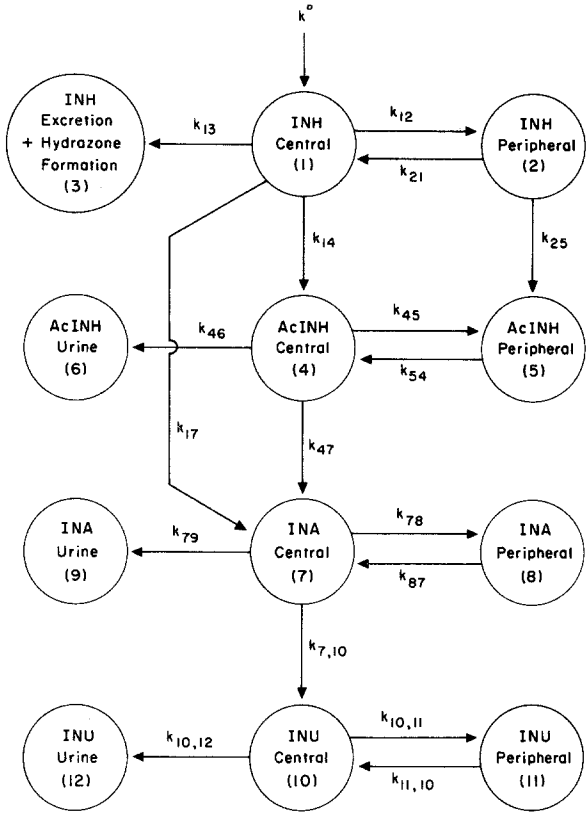


Fig. 10. Model used to describe INH disposition, including zero-order input (with the exception of the zero-order input, arrows represent first-order rate constants).

becomes a constant corresponding to the time infusion stops; $E_2 = k_{21} + k_{25}$; and $E_5 = k_{54}$.

Appropriate plots based on equation 3 were constructed weighting each y value by $1/y^2$. The results are summarized in Table V. It is readily apparent that peripheral elimination is significant; based on graphical analysis, subjects A and B eliminated 12.6 and 31.9%, respectively, of INH from the peripheral compartment.

Therefore, the overall model used to describe INH disposition is shown in Fig. 10, and the equations are given in the Appendix. The INH blood level data and the AcINH, INA, and INU urinary excretion rate data (four equations) were fitted simultaneously to the data, and the estimated

Table V. Values Calculated to Test for Metabolism of INH to AcINH from the Peripheral Compartment

Subject	k_{14} (min^{-1})		$k_{12}k_{25}k_{54}k_{46}$[c] (min^{-4})	k_{25}[d] (min^{-1})	Percent INH lost from central compartment[e]	Percent INH lost from peripheral compartment[e]
	Model assuming sole elimination from central compartment (i.e., $k_{25} = 0$)[a]	Model assuming metabolism of INH to AcINH from peripheral compartment[b]				
A	0.00250	0.00194	1.00×10^{-8}	0.000771	87.3	12.6
B	0.0221	0.0115	5.91×10^{-8}	0.00445	68.1	31.9

[a]Taken from Boxenbaum (27).
[b]Calculated from slope of plot of equation 3, and k_{46} taken from Table 3.
[c]Intercept of plot of equation 3.
[d]Calculated from intercept of plot of equation 3, k_{12} as calculated from summation expression of α and β as presented for model B in Table 1 of Rowland *et al.* (31), k_{54} and k_{46} from Table 3.
[e]Calculated from equations 17 and 18 of Rowland *et al.* (31).

parameters and statistical data are presented in Table VI. The t tests indicate that some intersubject differences between INH parameters can be detected. The INH terminal exponential constant, β, as well as E_2 (i.e., $k_{21} + k_{25}$) and k_{13} differ between the rapid and slow acetylator subjects. Interestingly, statistically significant differences in the acetylation parameters k_{14} and k_{25} could not be detected, but this results as a consequence of the extremely large % CVs for these parameters. This might at first seem anomalous, since these parameters are primarily responsible for acetylation polymorphism, but as will be discussed subsequently the computational methods employed in this investigation inevitably resulted in parameter estimates not precisely

Table VI. Parameter Estimates from INH Administration Studies (α, β, E_2, k_{14}, k_{25}, V_1, γ, δ, k_{54}, ε, ζ, k_{87}, η, θ, and $k_{11,10}$ Were Fitted by NONLIN)

Parameter	Parameter estimate (min^{-1}) Subject A	Subject B	t test[a]
α	0.195 (11.1) (0.0897–0.300)	0.259 (10.4)[b] (0.126–0.392)[c]	+
β	0.00354 (1.36) (0.00331–0.00377)	0.00982 (0.954) (0.00936–0.0103)	–
E_2	0.0819 (5.17) (0.0613–0.102)	0.0678 (4.17) (0.0538–0.0818)	–
k_{25}	0.00062 (28,518) (−0.848–0.849)	0.00454 (376) (−0.0798–0.0888)	+
k_{14}	0.00214 (10,903) (−1.13–1.14)	0.0115 (427) (−0.231–0.254)	+
k_{13}	0.00461 (8.21) (0.00277–0.00645)	0.00862 (11.0) (0.00394–0.0133)	–
k_{17}	0.000654 (8.21) (0.000393–0.000915)	0.00466 (11.0) (0.00213–0.00719)	–
k_{10}	0.00740 (N.D.)[d] (N.D.)	0.0248 (N.D.) (N.D.)	
k_{12}	0.109 (N.D.) (N.D.)	0.176 (N.D.) (N.D.)	
k_{21}	0.0813 (2.15) (0.0278–0.0898)	0.0633 (27.2) (−0.0217–0.148)	+
V_1	20,492 (8.30) (12,221–28,763)	10,652 (12.0) (4,339–16,965)	–
γ	0.0113 (11,223) (−6.16–6.18) [+][e]	0.134 (2,980) (−19.6–19.9) [+]	+
δ	0.00401 (566) (−0.106–0.114) [+]	0.00376 (19.8) [+] (0.0000833–0.00744)	+
k_{54}	0.00734 (22,548) (−8.04–8.06) [+]	0.0277 (1,584) (−2.14–2.19) [+]	+
k_{45}	0.00180 (17,870) (−1.56–1.57) [+]	0.0919 (3,600) (−16.2–16.4) [+]	+
k_{40}	0.00617 (11,953) (−3.58–3.59) [+]	0.0182 (1,473) (−1.31–1.34) [+]	+

Table VI. Continued

k_{46}	0.00412 (11,953) (−2.39–2.40) [+]	0.0108 (143) (−0.775–0.796) [+]	+
k_{47}	0.00205 (11,953) (−1.19–1.19) [+]	0.00735 (1,473) (−0.527–0.542) [+]	+
ε	0.161 (3,139) (−24.4–24.7) [+]	0.185 (3,539) (−32.1–32.5) [+]	+
ζ	0.0159 (1,044) (−0.791–0.823) [+]	0.0124 (39.2) (−0.0116–0.0364) [+]	+
k_{87}	0.0239 (1,707) (−1.96–2.01) [+]	0.0823 (1,579) (−6.34–6.50) [+]	+
k_{78}	0.0460 (13,813) (−30.9–30.9) [+]	0.0872 (5,394) (−23.1–23.3) [+]	+
k_{70}	0.107 (6,851) (−35.5–35.8) [+]	0.0279 (1,999) (−2.73–2.78) [+]	+
k_{79}	0.0670 (6,851) (−22.3–22.4) [+]	0.0181 (1,999) (−1.77–1.80) [+]	+
$k_{7,10}$	0.0400 (6.851) (−13.3–13.4) [+]	0.00979 (1,999) (−0.957–0.976) [+]	+
η	0.147 (1,771) (−12.5–12.8) [+]	0.0636 (6,019) (−18.8–19.0) [+]	+
θ	0.00114 (1,248) (−0.0680–0.0703) [+]	0.0462 (6,745) (−15.3–15.4) [+]	+
$k_{11,10}$	0.00136 (1,338) (−0.0871–0.0898) [+]	0.0296 (1,659) (−2.40–2.45) [+]	+
$k_{10,12}$	0.123 (1,722) (−10.2–10.4) [+]	0.0993 (928) (−4.45–4.65) [+]	+
$k_{10,11}$	0.0238 (2,146) (−2.46–2.51) [+]	−0.0191 (−1,676) (−1.60–1.56) [+]	+

[a]Plus sign indicates acceptance of the null hypothesis, meaning that differences between "true" parameters from the two subjects cannot be detected; minus sign indicates rejection (5% level of significance) (26).
[b]Percent coefficient of variation (26).
[c]Approximate *S*-plane 90% confidence intervals (26).
[d]N.D., not determinable by equations cited in Boxenbaum *et al.* (26).
[e]Plus sign in square brackets next to % CV indicates acceptance of the null hypothesis, meaning that differences between "true" parameters from this study and the AcINH study cannot be detected; minus sign indicates rejection (5% level of significance) (26).

determined. This did not result as a consequence of an ill-defined or ill-conceived experiment, but rather as a consequence of fitting four equations simultaneously to four sets of data, and employing a large number of parameters. This matter has been discussed previously (26).

Nonetheless, ratios for the acetylation parameter estimates of k_{14} and k_{25} (rapid acetylator/slow acetylator) were calculated. The values were 5.37 and 7.42 for k_{14} and k_{25}, respectively. Thus there exist vast differences in both acetylation parameter estimates. Although one cannot validate statistically from the aforementioned analysis that differences in the "true" acetylation parameters exist, it is nonetheless quite clear that differences in the

Table VII. Percent Isoniazid Eliminated from Central and Peripheral Compartments by Various Pathways

	Slow acetylator (Subject A)	Rapid acetylator (Subject B)
Percentage lost by acetylation in central compartment	26.7	31.5
Percentage lost by acetylation in peripheral compartment	7.66	32.2
Percentage lost by excretion and hydrazone formation in central compartment	57.5	23.6
Percentage lost by direct conversion to INA in central compartment	8.16	12.7
Total percentage lost	100	100

parameter *estimates* have a profound effect and play a major role in the differences observed in INH terminal exponential half-lives in these two subjects.

A rather interesting finding in the INH studies is the fact that inter-subject differences for the parameters k_{13} and k_{17} could be detected. The ratios (rapid acetylator/slow acetylator) of k_{13} and k_{17} were 1.87 and 7.13, respectively. Thus the shortened terminal exponential half-life in the rapid as compared to the slow acetylator subject in these studies was also due to differences in these parameters. It is disquieting that the rapid acetylator should apparently convert INH by direct hydrolysis to INA at about seven times the rate of the slow acetylator. One would think, almost as a matter of principle, that the rates of acetylation and hydrolysis of INH would not be correlated. One wonders, therefore, if this could be a consequence of some fault in the basic assumptions employed in the INH disposition model. In this regard, additional studies are required to ascertain the significance of these initial and preliminary observations. It is at least conceivable that the processes represented by these parameters somehow contribute to the polymorphism observed in INH terminal exponential half-lives. In this regard, the fractions of INH eliminated by the various processes in the central and peripheral compartments of the two subjects are indicated in Table VII. It is clear from this table that excretion and hydrazone formation as well as direct INH conversion to INA are major elimination pathways and that major differences in the parameters representing these processes could, in a very realistic sense, contribute to the observed polymorphism. Once again, however, it must be stressed that additional studies are required before any definitive statements can be made.

Figures 11 and 12 show the theoretical blood and rate of excretion–time curves generated from the computer-estimated parameters together with the

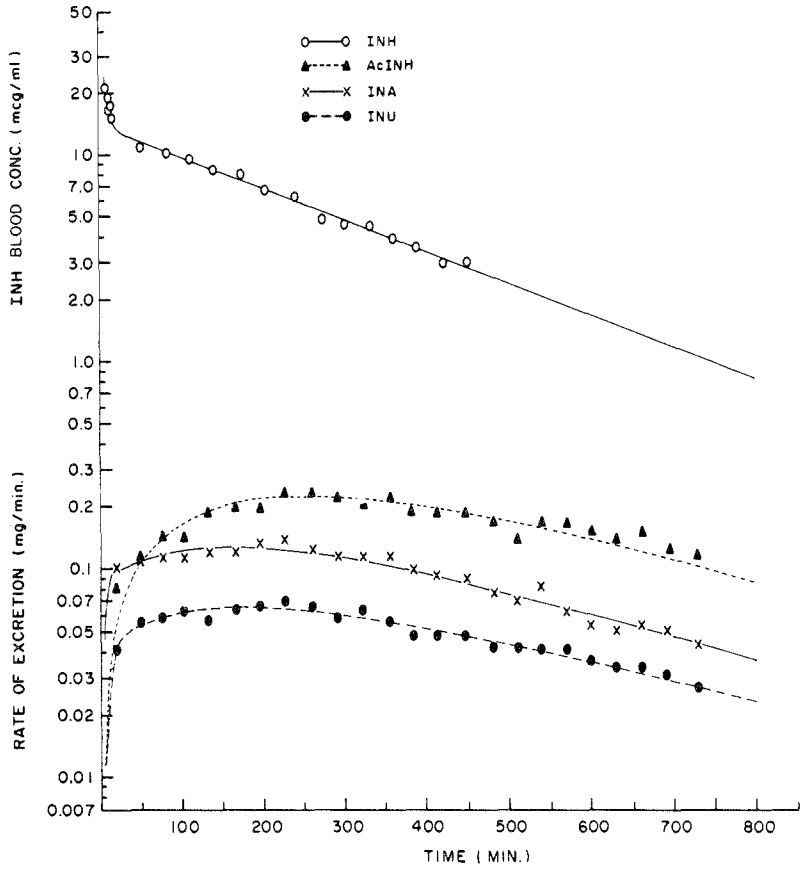


Fig. 11. INH blood levels and rates of excretion of AcINH, INA, and INU in subject A after a 5.4-min zero-order infusion of INH; curves were generated from parameter estimates.

experimental data points. For both subjects, the fit of the generated curves to the data points is good; scatter of the data points about the fitted lines generally appears random. One exception, however, is seen in Fig. 12 at the terminal segment of the INU curve. Here a "run" of data points is observed below the fitted curve. No particular significance was attributed to this finding, other than the possibility that initial parameter estimates used in the computer analysis were not sufficiently close to the true parameters, so as to assure convergence. In this regard, had the initial parameter space been sufficiently sampled, a more appropriate theoretical curve would probably have emerged.

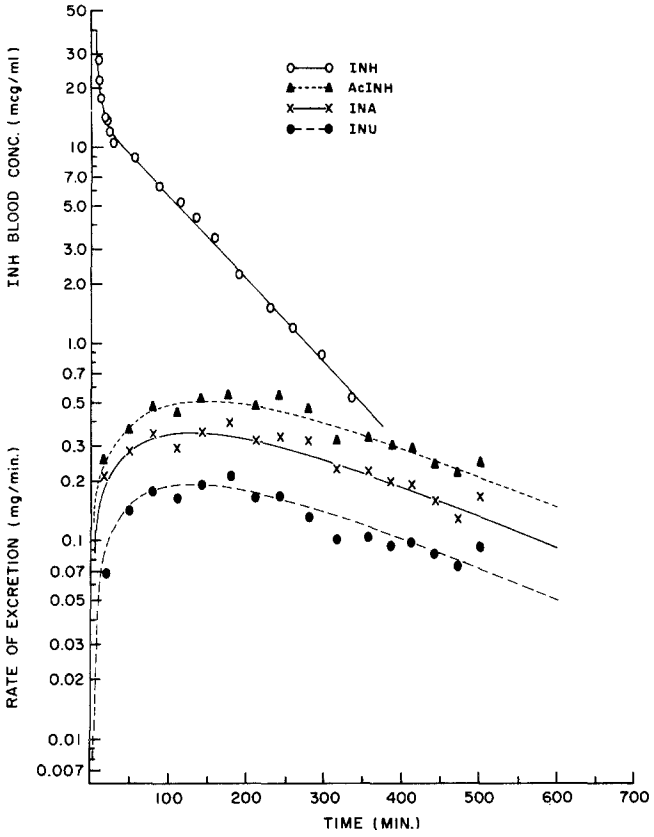


Fig. 12. INH blood levels and rates of excretion of AcINH, INA, and INU in subject B after a 5.2-min zero-order infusion of INH; curves were generated from parameter estimates.

INVESTIGATIONS DEALING WITH HYDRAZONE STABILITY AND DIRECT CONVERSION OF ISONIAZID TO ISONICOTINURIC ACID

The stability of INH hydrazones in urine was previously discussed by Boxenbaum and Riegelman (17), and it was pointed out that there probably existed some equilibrium in urine between intact INH and hydrazones. Therefore, the relative amounts of INH and hydrazones present in urine most probably do not reflect what was actually excreted by the kidneys, but rather some equilibrium condition influenced by urinary *p*H.

An additional consideration relevant to the pharmacokinetics of isoniazid was initially raised by Wenzel (12–14). It was reported that incubation

of INH with glycine with or without the presence of human serum resulted in the direct formation of INU. These experiments were duplicated in our laboratory. Incubation of either INH or INA, separately, did not result in the formation of INU. Therefore, there appears to be no evidence for the direct formation of INU from INH *in vivo*.

THE TWO-COMPARTMENT OPEN MODEL WITH ELIMINATION FROM BOTH THE CENTRAL AND PERIPHERAL COMPARTMENTS

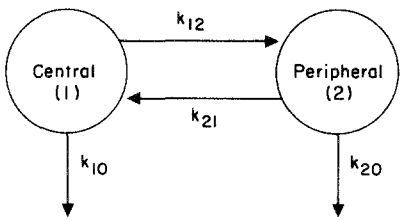
The model illustrated in Fig. 13 was used to describe INH disposition and has been discussed by various investigators (31,38,39).

The volume of distribution at steady-state ($V_{d_{ss}}$) is given by (39)

$$V_{d_{ss}} = \left(\frac{k_{12} + E_2}{E_2}\right) V_p \tag{4}$$

where C_1^0 is the concentration in compartment 1 at zero time, V_p = dose/C_1^0, and $E_2 = k_{21} + k_{20}$. An additional volume of distribution, V_{d_β}, is given by (40)

$$V_{d_\beta} = \frac{\text{volume of central compartment } (V_1)}{\text{fraction of drug in central compartment at pseudo distribution equilibrium, i.e., terminal exponential phase}} \tag{5}$$



$$E2 = k_{21} + k_{20}$$

$$\alpha + \beta = k_{12} + k_{10} + E2$$

$$\alpha\beta = ((k_{12} + k_{10})\,E2) - k_{12}\,k_{21}$$

Fig. 13. Two-compartment open model with elimination from both the central and peripheral compartments (arrows represent first-order processes).

When elimination occurs from both the central and peripheral compartments, it may readily be demonstrated that V_{d_β} is given by

$$V_{d_\beta} = V_1 \Big/ \left(\frac{E_2 - \beta}{E_2 - \beta + k_{12}} \right) \tag{6}$$

where the denominator is as described in equation 5.

It may also be readily demonstrated that the area under the central compartment concentration–time curve $0 \rightarrow \infty$ (AUC) is given by

$$\text{AUC} = \frac{\text{dose}}{(k_{12} + k_{10})V_1 - (k_{12}k_{21}V_1/E_2)} \tag{7}$$

Describing V_2 in relation to V_1 as suggested by Rowland *et al.* (31), and substituting into equation 7, one gets

$$V_2 = k_{12}V_1/E_2 \tag{8}$$

$$\text{AUC} = \frac{\text{dose}}{(k_{12} + k_{10})V_1 - k_{21}V_2} \tag{9}$$

An interesting observation comes from these relationships. Let us assume that a drug is administered intravenously, and blood is assayed for intact drug as a function of time. A two-compartment open model with elimination occurring *solely* from the central compartment is constructed. Let us further assume, however, that this is an incorrect model, because elimination also occurs from the peripheral compartment. The $V_{d_{ss}}$ will not be affected (see equation 4), since E_2 is mistakenly taken to be equal to k_{21}. However, V_{d_β} will be *incorrectly* determined. The degree to which this parameter is incorrectly calculated will depend on the relative magnitude of the various rate constants of the models. In this regard, the mistaken value of V_{d_β} will no longer be the proportionality constant between the concentration of drug in the central compartment at the terminal exponential phase and the total amount of drug in the body.

ISONIAZID PHARMACOKINETICS IN PERSPECTIVE: GENERAL OVERVIEW OF DATA

At this point, it is advisable to examine the data in order to determine the correctness of the models. If the models used were correct, parameter estimates would not be expected to change to any appreciable extent when

different compounds were administered, i.e., when the system was perturbed. One approach would be to utilize the appropriate *t* test (26) and determine if indeed the parameters did change. Considering the observed progressive increases in SDs, such analyses would probably result in questionable conclusions. A more simple and illustrative test of the models was made as follows. The disposition parameters for each compound, determined after administration of the respective compounds, were used to generate blood and urine curves for each of the subjects following administration of each of the compounds; the appropriate models illustrated in Figs. 5, 7, and 10 were used. Following INA administration, Fig. 14 indicates that the INU parameters reasonably well predict the INU rate of excretion curve in subject A; the same was true for subject B. Figure 15 indicates that following AcINH administration a systematic error in both the INA and INU curves is evident with subject A; this did not occur with subject B. It is believed that this inconsistency in subject A may result from the fact that INA conjugation

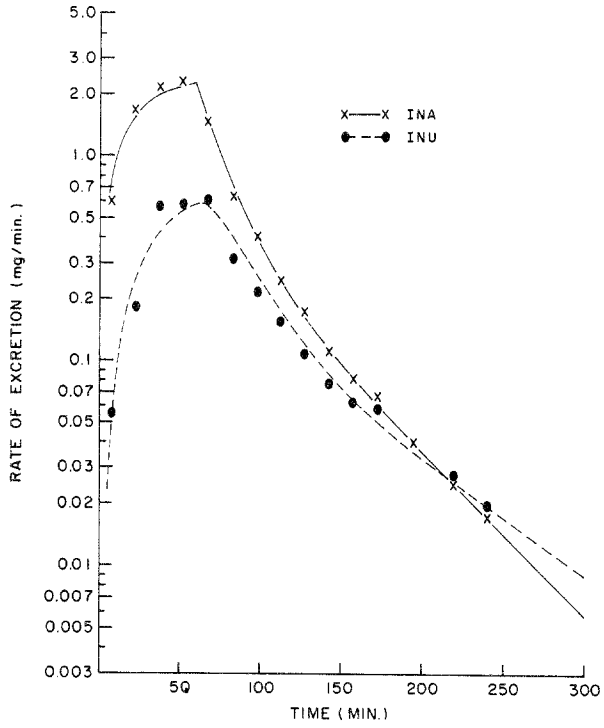


Fig. 14. Comparison of expected curves to data obtained following INA administration to subject A (see text for explanation).

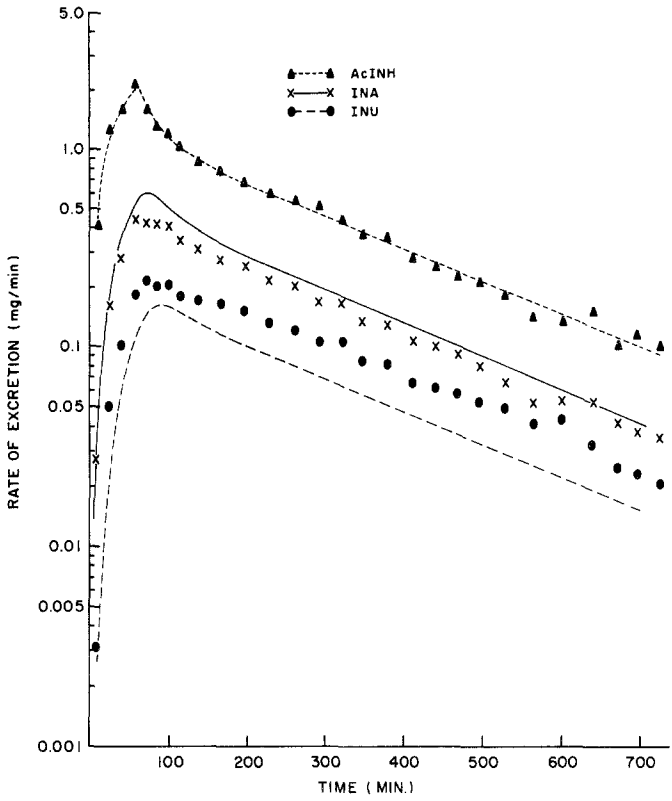


Fig. 15. Comparison of expected curves to data obtained following AcINH administration to subject A (see text for explanation).

with glycine is saturable (18), and that the linear two-compartment open model describing INA disposition is inadequate. A similar error pattern is observed in Figs. 16 and 17 following administration of INH to both subjects. In this regard, the metabolism of INA to INU might better have been described by applying a nonlinear model utilizing the Michaelis–Menten equation. Consideration of such a model at this time, however, is beyond the goals of this investigation.

Additionally, Fig. 17 indicates a systematic error of another type in the INA rate of excretion curve. The initial predicted INA rate of excretion pattern is considerably overestimated. This could conceivably result in an overestimation in the parameter k_{17}, describing direct conversion of INH to INA. In this regard, the value of this parameter in subject B was 7.1 times greater than in subject A.

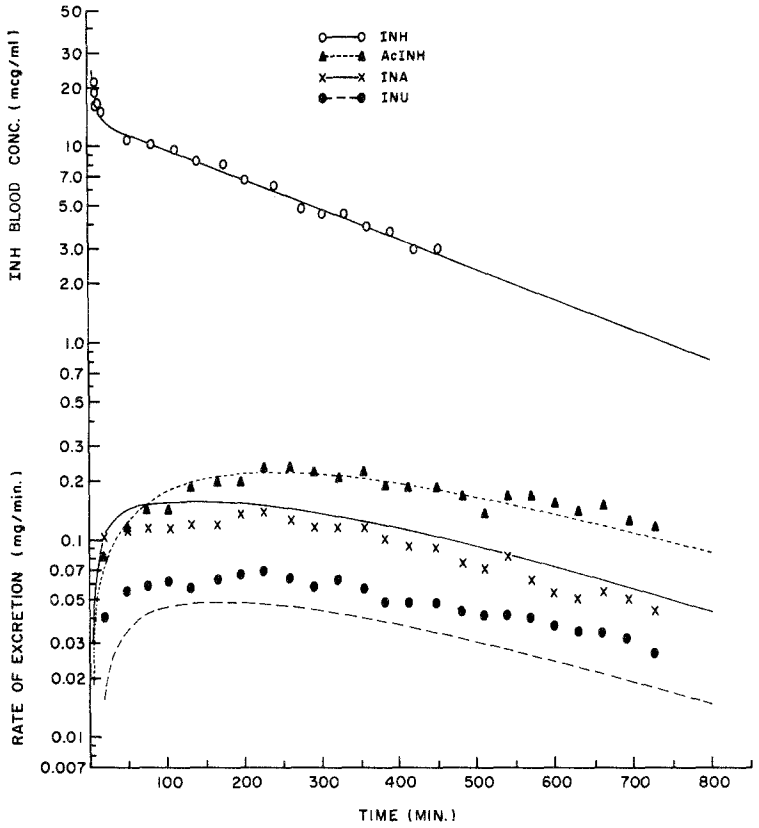


Fig. 16. Comparison of expected curves to data obtained following INH administration to subject A (see text for explanation).

Therefore, there may exist at least two inconsistencies in the present scheme describing INH disposition. An alternative explanation, however, is that it is unrealistic to expect parameters to remain reasonably constant, even within the same subject. Wagner (41) has addressed himself to this very point, and has stated that "there is evidence that with many drugs intra-subject variation in elimination rate constants and half-lives of drugs is appreciable and cannot be ignored." Interestingly, on the three occasions that subject A received 1000-, 682-, and 700-mg INH doses, terminal exponential half-life estimates were 148, 196, and 240 min, respectively.

One important aspect of these present studies is that the mathematical functions generated from the models were adequate to fit the observed data.

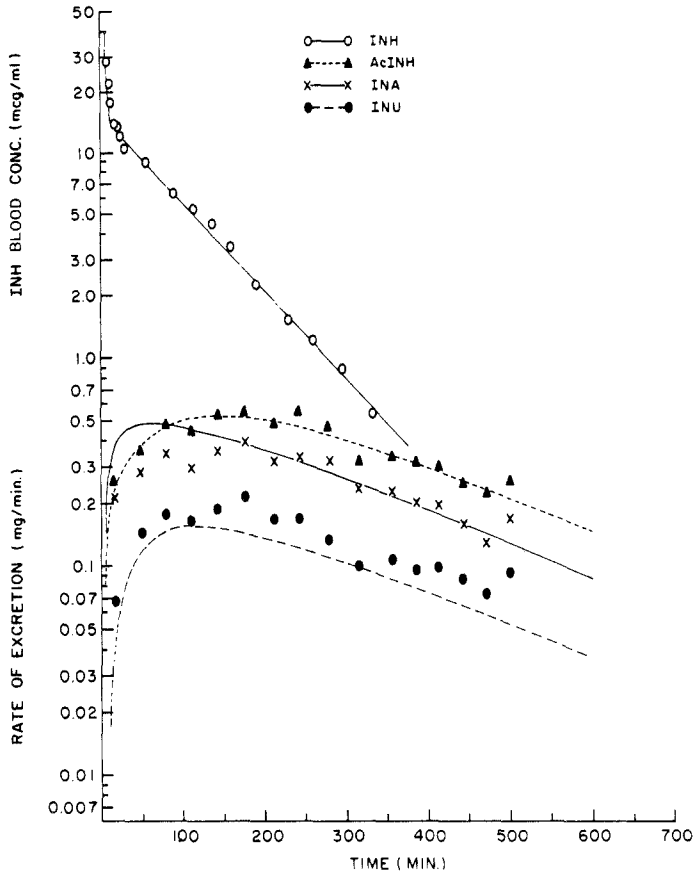


Fig. 17. Comparison of expected curves to data obtained following INH administration to subject B (see text for explanation).

This was to be expected in view of the fact that a large number of parameters were employed. Had the equations and least-squares procedures not fit the data, it would have been bewildering.

From a clinical pharmacokinetic standpoint, the sophistication achieved in the present analysis is far beyond that required. In this regard, terminal exponential half-lives probably are important parameters. Therefore, Table VIII summarizes these half-lives of elimination for the two subjects in a concise form. Values reported were those obtained following administration of the respective compounds.

Table VIII. Elimination Half-Lives of Isoniazid and Metabolites Obtained Following Administration of the Respective Compounds

Compound	Elimination half-life (min)	
	Subject A (slow acetylator)	Subject B (rapid acetylator)
INH	196	70.7
AcINH	178	178
INA	37.7	58.7
INU	60.8	53.7

APPENDIX: EQUATIONS FITTED TO DATA

A_{ij} represents the amount of compound in compartment ij.

The appropriate equation describing the INU model shown in Fig. 3 is

$$\frac{dA_{12}}{dt} = \frac{\theta k^0(k_{11,10} - \eta)(1 - e^{\eta b})\, e^{-\eta t}}{k_{11,10}(\eta - \theta)} + \frac{\eta k^0(k_{11,10} - \theta)(1 - e^{\theta b})\, e^{-\theta t}}{k_{11,10}(\theta - \eta)} \tag{A1}$$

The appropriate equations describing the INA model shown in Fig. 5 are

$$f_7 = k_{79}/(k_{79} + k_{7,10}) \tag{A2}$$

$$\frac{dA_9}{dt} = \frac{f_7 \varepsilon \zeta k^0}{k_{87}}\left[\frac{(k_{87} - \varepsilon)(1 - e^{\varepsilon b})\, e^{-\varepsilon t}}{\varepsilon(\varepsilon - \zeta)} + \frac{(k_{87} - \zeta)(1 - e^{\zeta b})\, e^{-\zeta t}}{\zeta(\zeta - \varepsilon)}\right] \tag{A3}$$

$$\begin{aligned}\frac{dA_{12}}{dt} = \frac{(1 - f_7)\varepsilon\zeta\eta\theta k^0}{k_{87}k_{11,10}}\Bigg[&\frac{(k_{87} - \varepsilon)(k_{11,10} - \varepsilon)(1 - e^{\varepsilon b})\, e^{-\varepsilon t}}{\varepsilon(\varepsilon - \zeta)(\varepsilon - \eta)(\varepsilon - \theta)} \\ &+ \frac{(k_{87} - \zeta)(k_{11,10} - \zeta)(1 - e^{\zeta b})\, e^{-\zeta t}}{\zeta(\zeta - \varepsilon)(\zeta - \eta)(\zeta - \theta)} \\ &+ \frac{(k_{87} - \eta)(k_{11,10} - \eta)(1 - e^{\eta b})\, e^{-\eta t}}{\eta(\eta - \varepsilon)(\eta - \zeta)(\eta - \theta)} \\ &+ \frac{(k_{87} - \theta)(k_{11,10} - \theta)(1 - e^{\theta b})\, e^{-\theta t}}{\theta(\theta - \varepsilon)(\theta - \zeta)(\theta - \eta)}\Bigg]\end{aligned} \tag{A4}$$

The appropriate equations describing the AcINH model shown in Fig. 7 are

$$f_4 = k_{46}/(k_{46} + k_{47}) \tag{A5}$$

$$f_7 = k_{79}/(k_{79} + k_{7,10}) \tag{A6}$$

$$\frac{dA_6}{dt} = \frac{f_4\gamma\delta k^0}{k_{54}}\left[\frac{(k_{54}-\gamma)(1-e^{\gamma b})\,e^{-\gamma t}}{\gamma(\gamma-\delta)} + \frac{(k_{54}-\delta)(1-e^{\delta b})\,e^{-\delta t}}{\delta(\delta-\gamma)}\right] \tag{A7}$$

$$\frac{dA_9}{dt} = \frac{(1-f_4)f_7\gamma\delta\varepsilon\zeta k^0}{k_{54}k_{87}}\left[\frac{(k_{54}-\gamma)(k_{87}-\gamma)(1-e^{\gamma b})\,e^{-\gamma t}}{\gamma(\gamma-\delta)(\gamma-\varepsilon)(\gamma-\zeta)}\right.$$
$$+\frac{(k_{54}-\delta)(k_{87}-\delta)(1-e^{\delta b})\,e^{-\delta t}}{\delta(\delta-\gamma)(\delta-\varepsilon)(\delta-\zeta)} + \frac{(k_{54}-\varepsilon)(k_{87}-\varepsilon)(1-e^{\varepsilon b})\,e^{-\varepsilon t}}{\varepsilon(\varepsilon-\gamma)(\varepsilon-\delta)(\varepsilon-\zeta)}$$
$$\left.+\frac{(k_{54}-\zeta)(k_{87}-\zeta)(1-e^{\zeta b})\,e^{-\zeta t}}{\zeta(\zeta-\gamma)(\zeta-\delta)(\zeta-\varepsilon)}\right] \tag{A8}$$

$$\frac{dA_{12}}{dt} = \frac{(1-f_4)(1-f_7)\gamma\delta\varepsilon\zeta\eta\theta k^0}{k_{54}k_{87}k_{11,10}}$$
$$\times\left[\frac{(k_{54}-\gamma)(k_{87}-\gamma)(k_{11,10}-\gamma)(1-e^{\gamma b})\,e^{-\gamma t}}{\gamma(\gamma-\delta)(\gamma-\varepsilon)(\gamma-\zeta)(\gamma-\eta)(\gamma-\theta)}\right.$$
$$+\frac{(k_{54}-\delta)(k_{87}-\delta)(k_{11,10}-\delta)(1-e^{\delta b})\,e^{-\delta t}}{\delta(\delta-\gamma)(\delta-\varepsilon)(\delta-\zeta)(\delta-\eta)(\delta-\theta)}$$
$$+\frac{(k_{54}-\varepsilon)(k_{87}-\varepsilon)(k_{11,10}-\varepsilon)(1-e^{\varepsilon b})\,e^{-\varepsilon t}}{\varepsilon(\varepsilon-\gamma)(\varepsilon-\delta)(\varepsilon-\zeta)(\varepsilon-\eta)(\varepsilon-\theta)}$$
$$+\frac{(k_{54}-\zeta)(k_{87}-\zeta)(k_{11,10}-\zeta)(1-e^{\zeta b})\,e^{-\zeta t}}{\zeta(\zeta-\gamma)(\zeta-\delta)(\zeta-\varepsilon)(\zeta-\eta)(\zeta-\theta)}$$
$$+\frac{(k_{54}-\eta)(k_{87}-\eta)(k_{11,10}-\eta)(1-e^{\eta b})\,e^{-\eta t}}{\eta(\eta-\gamma)(\eta-\delta)(\eta-\varepsilon)(\eta-\zeta)(\eta-\theta)}$$
$$\left.+\frac{(k_{54}-\theta)(k_{87}-\theta)(k_{11,10}-\theta)(1-e^{\theta b})\,e^{-\theta t}}{\theta(\theta-\gamma)(\theta-\delta)(\theta-\varepsilon)(\theta-\zeta)(\theta-\eta)}\right] \tag{A9}$$

The appropriate equations describing the INH model shown in Fig. 10 are

$$C_1 = \frac{k^0}{V_1}\left[\frac{(1-e^{\alpha b})(E_2-\alpha)\,e^{-\alpha t}}{-\alpha(\beta-\alpha)} + \frac{(1-e^{\beta b})(E_2-\beta)\,e^{-\beta t}}{-\beta(\alpha-\beta)}\right] \tag{A10}$$

$$\frac{dA_6}{dt} = \left\{k^0k_{14}k_{46}\sum_{i=\alpha}^{\delta}\left[\frac{(1-e^{ib})(E_2-i)(E_5-i)\,e^{-it}}{-i(j-i)(k-i)(l-i)}\right]\right\}$$
$$+\left\{k^0k_{12}k_{25}k_{54}k_{46}\sum_{i=\alpha}^{\delta}\left[\frac{(1-e^{ib})\,e^{-it}}{-i(j-i)(k-i)(l-i)}\right]\right\} \tag{A11}$$

where i equals either α, β, γ, or δ, while j, k, and l are Greek letter macroconstants not equal to i.

$$\frac{dA_9}{dt} = \left\{ k^0 k_{14} k_{47} k_{79} \sum_{i=\alpha}^{\zeta} \left[\frac{(1 - e^{ib})(E_2 - i)(E_5 - i)(E_8 - i)\, e^{-it}}{-i(j - i)(k - i)(l - i)(m - i)(n - i)} \right] \right\}$$
$$+ \left\{ k^0 k_{12} k_{25} k_{54} k_{47} k_{79} \sum_{i=\alpha}^{\zeta} \left[\frac{(1 - e^{ib})(E_8 - i)\, e^{-it}}{-i(j - i)(k - i)(l - i)(m - i)(n - i)} \right] \right\}$$
$$+ \left\{ k^0 k_{17} k_{79} \sum_{\substack{i=\alpha \\ i,j,k,l \neq \gamma,\delta}}^{\zeta} \left[\frac{(1 - e^{ib})(E_2 - i)(E_8 - i)\, e^{-it}}{-i(j - i)(k - i)(l - i)} \right] \right\} \tag{A12}$$

where i equals either α, β, γ, δ, ε, or ζ (except where γ and δ are prohibited), while j, k, l, m, and n are Greek letter macroconstants not equal to i.

$$\frac{dA_{12}}{dt} = \left\{ k^0 k_{14} k_{47} k_{7,10} k_{10,12} \right.$$
$$\left. \times \sum_{i=\alpha}^{\theta} \left[\frac{(1 - e^{ib})(E_2 - i)(E_5 - i)(E_8 - i)(E_{11} - i)\, e^{-it}}{-i(j - i)(k - i)(l - i)(m - i)(n - i)(o - i)(p - i)} \right] \right\}$$
$$+ \left\{ k^0 k_{12} k_{25} k_{54} k_{47} k_{7,10} k_{10,12} \right.$$
$$\left. \times \sum_{i=\alpha}^{\theta} \left[\frac{(1 - e^{ib})(E_8 - i)(E_{11} - i)\, e^{-it}}{-i(j - i)(k - i)(l - i)(m - i)(n - i)(o - i)(p - i)} \right] \right\}$$
$$+ \left\{ k^0 k_{17} k_{7,10} k_{10,12} \right.$$
$$\left. \times \sum_{\substack{i=\alpha \\ i,j,k,l,m,n \neq \gamma,\delta}}^{\theta} \left[\frac{(1 - e^{ib})(E_2 - i)(E_8 - i)(E_{11} - i)\, e^{-it}}{-i(j - i)(k - i)(l - i)(m - i)(n - i)} \right] \right\} \tag{A13}$$

where i equals either α, β, γ, δ, ε, ζ, η, or θ (except where γ and δ are prohibited), while j, k, l, m, n, o, and p are Greek letter macroconstants not equal to i.

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