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PHARMACOKINETICS OF ISONIAZID IN MAN

by

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B.S., Temple University, 1965

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

PHARMACEUTICAL CHEMISTRY

in the

GRADUATE DIVISION

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of the

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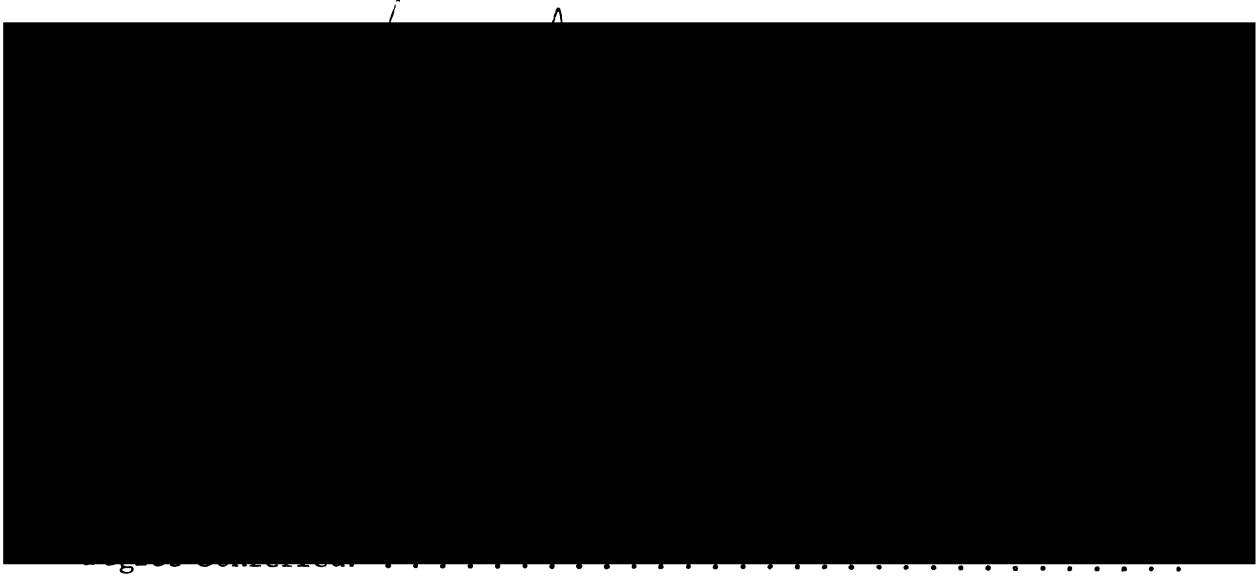

Degree Conferred: . . . . . .

ABSTRACT

Methods were developed for the determination of isoniazid and its metabolites, acetylisoniazid, isonicotinic acid, and isonicotinuric acid in biological fluids. Each of the aforementioned compounds was administered intravenously to a rapid and slow acetylator of isoniazid. Appropriate equations were fitted to the data by digital computer.

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INTRODUCTION

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

The major urinary excretion products resulting from INH administration to humans are intact INH, pyruvic acid isonicotinoylhydrazone, α-ketoglutaric acid isonicotinoylhydrazone, acetylisoniazid, isonicotinic acid, isonicotinuric acid, acetylhydrazine, and 1,2-diacetylhydrazine (4,5,6).

In humans, individuals have been classified as either rapid or slow inactivators of INH, and this capacity to inactivate (metabolize) INH is a permanent hereditary characteristic (7). Approximately half of the Caucasian and American Negro populations are rapid INH inactivators, and the other half are slow inactivators. Plasma half-lives in rapid inactivators range from 45 to 80 minutes and in slow inactivators from 140 to 200 minutes. INH inactivator status results from the capacity to acetylate INH (5). This polymorphism in the acetylation of INH by humans has also been demonstrated or suggested for other drugs. These include hydralazine (8,9), phenelzine (10), sulfamethazine (SMZ) (8,11,12), sulfamethoxypyridazine (SMP) (12), sulfamethylthiadiazole (SMTD) (13), and dapsone (14,15).

Presently, however, there is little knowledge available regarding the specific kinetics of the formation of the acetyl metabolite of INH in man. The purpose of this investigation was to study the pharmacokinetics of INH and its metabolites in man, with particular emphasis on the acetylation process. Such knowledge should be useful for a variety of reasons: (1) to aid in the formulation of better dosage regimens for the clinical use of INH in each of the inactivator groups, (2) to aid in the sophistication of the genetic hypothesis regarding acetylation polymorphism, (3) to serve as a model for comparison to other drugs which are acetylated, and (4) so that additional investigations on INH may be better formulated.

LITERATURE REVIEW

Metabolism of Isoniazid in Man

Metabolites in the urine of man following isoniazid (INH) administration are 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) (5,6,16). Ellard and Gammon (6) reported average recoveries of 95.8 and 87.0% of INH oral doses as total isonicotinoyl compounds in the 48 hour urines of rapid and slow inactivators, respectively. The metabolic scheme proposed by Ellard and Gammon (6) for the metabolism of INH is shown in Figure 1. Hydrazine 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 and co-workers (17,18) 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 hydrazine indicated that the hydrazine was subsequently converted to ammonia (19-23). Schmidt (24) reported that INH-PA and INH-KA were cleaved promptly in pH 6.0-6.5 buffer to free INH and the corresponding keto acids. Taking cognizance of the lability of the hydrazones, Schmidt suggested that their cleavage back to INH in the

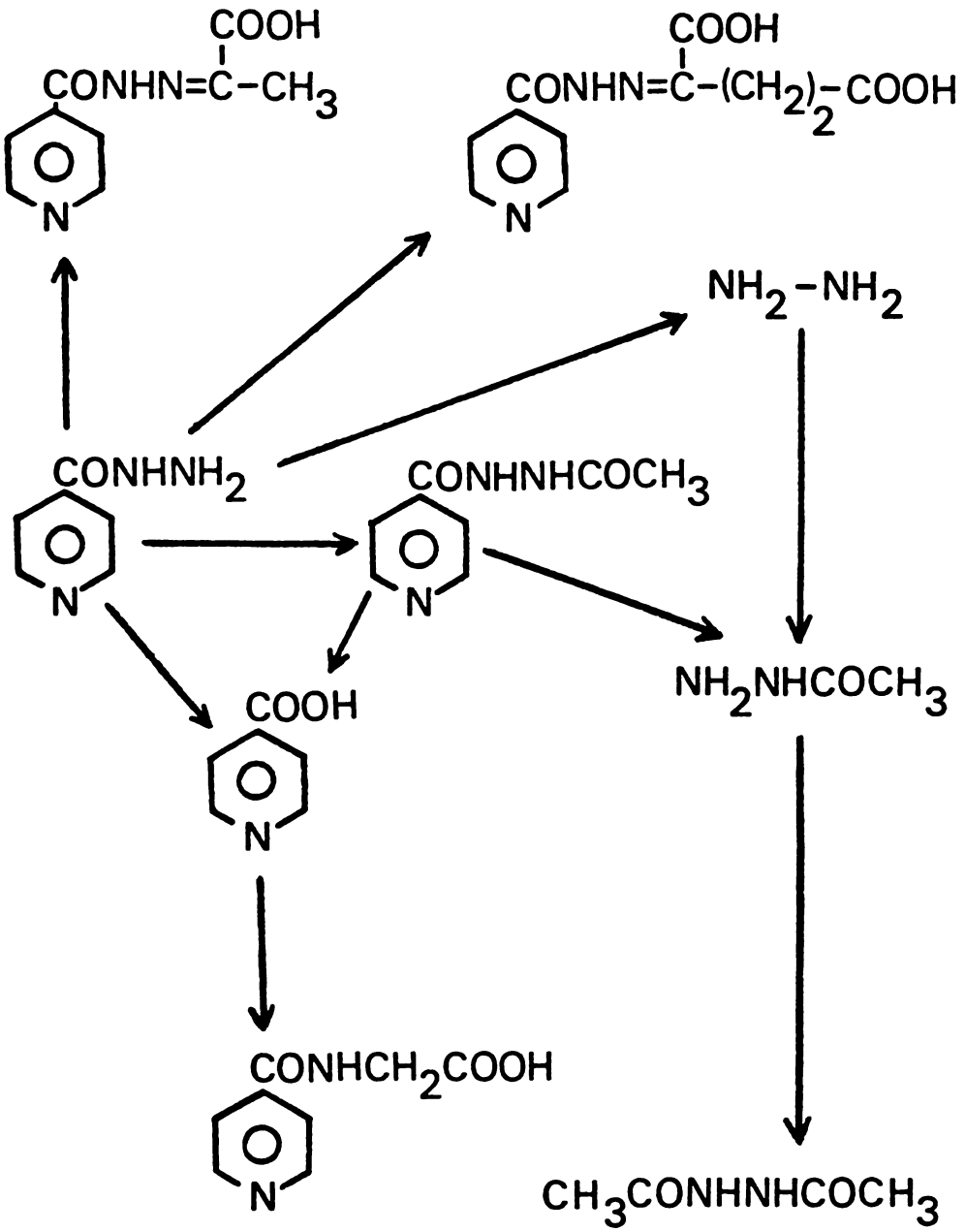

Figure 1. Scheme for the Metabolism of Isoniazid in Man

body is possible. The direct conversion of INH to INU is suggested by the experiments of Wenzel (25-27). Incubation of INH and glycine with or without human serum resulted in the production of INU. Incubation of INA with glycine under the same conditions did not produce INU.

The data from the paper of Ellard and Gammon (6) are summarized in Table 1 in order to provide some idea of the relative importance of the metabolic pathways. Assay limitations did not permit the quantitation of AcHy in these experiments.

Genetic Aspects of Isoniazid Metabolism

Introduction

In medicine, the term "idiosyncrasy" has been defined as "an abnormal susceptibility to some drug, protein, or other agent which is peculiar to the individual" (28). Another definition (29) of "idiosyncrasy" is "a genetically determined abnormal reactivity to a drug." The later definition has resulted from an increased awareness of genetic factors in relation to drug action. The merging of pharmacology with genetics has resulted in a relatively new branch of pharmacology called "pharmacogenetics." This term was first used by Vogel (30) in 1959 and was defined as "heredity variations in reactions to drugs and other exogenous compounds." Genetic influence on the acetylation of INH is an example of "genetic polymorphism"[1]. The term "genetic polymorphism" was described (31) as "a type of variation in which individuals with sharply distinct qualities co-exist as normal members of a population.

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[1]The term "polymorphism" will generally be used, and it is to be understood that this refers to "genetic polymorphism."

TABLE 1

Cumulative 48 Hour Urinary Excretion of INH and Metabolites After Oral Administration of 250 mg. INH to a Typical Rapid Inactivator (data from reference 6)

Compound(s)	% of Dose Recovered
INH + Hydrazones	16.5
AcINH	40.7
AcHy	0
DiAcHy	26.8
INA	24.5
INU	14.1
Total Hydrazino Compounds	84.0
Total Isonicotinoyl Compounds	95.8

The condition is defined as 'the occurrence together in the same habitat of two or more discontinuous forms, or 'phases,' of a species in such proportions that the rarest of them cannot be maintained merely by recurrent mutation."

Preliminary Observations[2]

In 1953, Bonicke and Reif (32) reported on the amount of free INH excreted in the 24 hour urine of human subjects given INH orally. Individuals who excreted a small percent of the dose as free INH were termed "bad" eliminators, while those who excreted a much larger percent of the dose as free INH were termed "good" eliminators. The percent of the dose excreted as free INH in the 24 hour urine was relatively constant over a period of 10 months for each individual "bad" and "good" eliminator tested, even though there were wide differences between individuals. That same year, Hughes (33) reported that AcINH was probably the major metabolite of INH in man, and that variations in the completeness of the acetylation process might well account for the relatively great differences in the toxicity of INH for different patients. Hughes et al (34) in 1954 reported on a study with tuberculosis patients receiving INH therapy in which 24 hour urine specimens were assayed for INH, AcINH, and INA-like materials. It was demonstrated that INH under went extensive metabolic transformation. Metabolism followed a relatively constant pattern in an individual patient even though patterns in different persons varied substantially. From the urinary

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[2]Much of the early work on INH utilized non-specific analytical procedures.

excretion data, individuals were divided into three groups, each having a quantitatively different metabolic pattern. The occurrence of peripheral neuritis appeared to be associated with a reduced ability to acetylate INH. In 1955, Hughes, Schmidt, and Biehl (35) reported on the metabolic patterns of INH in normal and tuberculosis subjects. In the normal subjects, essentially all the orally administered INH was excreted in the urine either as unchanged drug or metabolites. Only a relatively small fraction of the dose was eliminated as unchanged INH. However, there were wide individual variations, ranging from a minimum of 1.0% to a maximum of 17%. The elimination of AcINH and INA was inversely related to the percent free INH excreted. The amounts of INH-hydrazones and INA conjugates appeared to vary directly with the elimination of free INH. Studies on tuberculosis subjects suggested that an individual's particular pattern of metabolism was relatively constant over a period of months. In 1956, Biehl (36) again took notice of the wide individual variation in the excretion of free INH, and noted that it could not be explained. He used the term "isoniazid inactivators" for those individuals who excreted only a small percent of the administered INH intact (metabolism of INH resulted in loss of activity against the tubercle bacillus). Clinically, the frequency of emergence of INH resistance was greater in those individuals who were "isoniazid inactivators."

The first hint that the wide individual variation in INH metabolism was due to genetic factors came in 1957 from the work of Bönicke and Lisboa (37). These investigators determined the percent of an INH dose excreted intact in 24 hours in both identical and fraternal pairs

of twins. The results showed a remarkable similarity between monozygous (identical) twins and a considerable difference between dizygous (fraternal) twins. It was reported that this was due to certain hereditary properties. That same year, Mitchell and Bell (38), using a twofold serial dilution microbiological assay method for INH, classified individuals as "rapid inactivators" (0.2 μg or less INH per ml. of serum 6 hours following a standard oral dose), "slow inactivators" (0.8 μg or higher per ml.), or "intermediate inactivators" (0.4 μg per ml.). The rate of isoniazid inactivation did not appear to be influenced in adults by age, sex, extent of disease, or complicating disease processes. In 1958, Harris, Knight, and Selin (39) reported on a comparison of the concentrations of biologically active INH in the blood of individuals of Japanese and European descent. The Japanese subjects were born in the United States, and their diet and home environment was typically American. Each subject received a dose of INH orally (4 mg./kg. body weight), and blood was taken at 2, 4, and 6 hours after the test dose. Based upon the six hour serum concentration of biologically active INH, subjects were classified as "slow," "intermediate," or "rapid" inactivators of INH. Twenty-two of the 25 Japanese subjects were rapid inactivators, whereas only 12 of the 25 of those of European descent fell into this group. It was suggested that the manner in which an individual metabolizes INH is determined by genetic factors. Finally, in 1959, Knight, Selin, and Harris (40) reported on a study of the genetic factors influencing INH blood levels in humans, and for the first time, a genetic hypothesis governing INH inactivation was proposed.

Acetylation as the Genetic Determinant for Inactivator Status (*In Vivo* Studies)

In 1953, Hughes (33) reported that AcINH was probably the major metabolite of INH in man, and that variations in the completeness of the acetylation process might well account for the relatively great differences in the toxicity of INH in different patients. Investigators then began to suspect that the acetylation process may play an important role in the large inter-subject differences observed in INH disposition.

Jenne (41) administered INH intravenously to 43 human subjects, and determined serum levels of INH at 30, 90, and 150 minutes. Most of the patients studied fell into two groups, one with a mean half-life of 59 minutes (45 to 80), and the other with a mean half-life of 159 minutes (140 to 180). The former group comprised almost half of all patients tested and were considered to qualify for the term "rapid inactivation." Jenne, MacDonald, and Mendoza (42) administered INH intravenously to human subjects and followed its decline in serum. Individuals having a half-life from 40-80 minutes were considered rapid inactivators, and subjects showing half-lives greater than 80 minutes were considered slow inactivators. A comparison was then made of the pharmacokinetic parameters in the two groups. The mean apparent distribution volumes, determined by extrapolation of the semilogarithmic serum level-time curves to zero time, were almost identical in the two groups. Serum protein binding determinations demonstrated no significant binding in either group. Renal clearance, INH to creatinine renal clearance ratio, and percent tubular reabsorption were similar in the two groups. Metabolic clearance was shown to be the major component

responsible for INH serum fall-off, even in slow inactivators. It was suggested that inactivator phenotypes resulted from acetylation differences. Peters, Miller, and Brown (5) studied the metabolism of INH, AcINH, and INA by administering the respective compounds to human subjects and measuring the urinary excretion of the administered compound and its metabolites. INH-hydrazone excretion seemed to parallel approximately the INH excretion by each individual. The amounts of AcINH and INA derivatives were found to be inversely related to the INH found. Expressed as a percent of the total derivatives recovered, the amounts of INH and INH-hydrazones were smaller in rapid inactivators than in slow inactivators. However, the amounts of AcINH and INA derivatives were greater in rapid inactivators. AcINH was then administered to the subjects; INH and INH-hydrazones were not detected in urine. In these studies, both rapid and slow INH inactivators excreted essentially identical amounts of AcINH and INA derivatives. It was concluded that the capacities of rapid and slow inactivators to excrete AcINH and to form and excrete INA compounds were similar. By comparing the urinary excretion data after equimolar doses of INH and AcINH, it was believed that the data suggested a direct dependence of the formation of INA on the availability of AcINH. Consequently, the primary metabolic alteration determining inactivator status was taken to be acetylation. Additionally, no correlation between an individual's capacity to acetylate INH and conjugate INA with glycine was discerned.

Genetics of Isoniazid Inactivation (*In Vivo* Studies)

The first genetic hypothesis governing INH inactivation was proposed by Knight, Selin, and Harris (40). These investigators administered orally a test dose of 4 mg. INH/kg. body weight to Americans of northern European ancestry, and serum was obtained at 2, 4, and 6 hours. INH bioassays were performed by a twofold serial dilution technique. Histograms of the number of subjects (y-axis) *vs.* serum INH (μg/ml.) were plotted for the data at 2, 4, and 6 hours. With the 2 hour data, a single bell-shaped curve was found, whereas with the 4 and 6 hour data, bimodal curves were observed. The bimodality at the 4 and 6 hour times suggested a genetic determinant, and family studies were used to determine the pattern of inheritance. Six hour serum INH levels in 20 families, composed of 40 parents and 74 children, were measured after a dose of INH. Individuals were classified as rapid inactivators if the serum level was less than 0.4 μg/ml. and slow inactivators if the serum level was greater than 0.4 μg/ml. In one pedigree, for example, where both mother and father were slow inactivators, all offspring were slow inactivators. Where both parents were rapid inactivators, both slow and rapid inactivators were found among the children. The data did not support sex-linked inheritance, and there was no correlation between inactivator status and blood grouping. The observations in all of the family studies led to the hypothesis that slow inactivation is a simple Mendelian recessive trait, and that rapid inactivation is a dominant trait.

Evans, Manley, and McKusick (43) administered INH at a dose of 9.8-10 mg./kg. body weight orally in a single dose to a large number of

American Caucasian and Negro subjects. Blood was drawn 6 hours after administration, and INH was determined in plasma by a chemical method. The frequency distribution curve of the 6 hour plasma levels was bimodal with an antimode at 2.5 μg/ml. concentration. Persons who had INH plasma levels of less than 2.5 μg/ml. were termed rapid inactivators, and those with values above 2.5 μg/ml. were termed slow inactivators. Statistical analysis of the data indicated the following: (1) the occurrences of the two phenotypes in the American Negro population studied were not significantly different from their occurrences in the American white populations; (2) sex has no effect on the division of a population into rapid and slow inactivator phenotypes, and (3) age is without effect on the occurrence of the two phenotypes. The following hypothesis was considered: INH inactivation status is controlled by two allelic genes, and that the slow inactivator individual is an autosomal homozygous recessive. This hypothesis was tested in two ways: (1) the observed matings were compared with those expected by application of the Hardy-Weinberg law, and (2) the numbers of children of each phenotype expected were calculated and compared with those observed. Statistical analysis supported the hypothesis. Evans (44) subsequently suggested that secondary genetic influences are present which modify the expression of the major genetic polymorphism. These influences may be called gene modifiers.

Sunahara and co-workers (45-49), using a vertical diffusion culture method for determining biologically active INH concentration in plasma, determined INH plasma levels 6 hours after an INH oral dose of 4 mg./kg. body weight to human subjects (Japanese and other Asian races).

The frequency distribution curve of the plasma levels was reported to be trimodal. Individuals from 78 marriages and their children were studied, and observations on inactivation status led to the following hypothesis: (1) rapid and slow inactivators are homozygotes, and intermediate inactivators are heterozygotes, and (2) inactivation of INH is a character that is inherited without dominance. These results were essentially confirmed by Dufour, Knight, and Harris (50).

Nelson (51,52) was critical of two experimental procedures used in INH pharmacogenetic studies. He pointed out that single point measurements: (1) do not directly reflect rate of metabolism, (2) are subject to potential error in the rate of absorption among test subjects (except when drug is administered intravenously), and (3) are also subject to variation in concomitantly occurring elimination processes. Nelson also pointed out that the trimodal frequency distribution histograms for INH serum levels reported by Sunahara et al (45) and Dufour, Knight, and Harris (50) were inappropriately plotted. The abscissae of these histograms were not divided into dimensionally equal increments. Moreover, the classification of individuals as being rapid, intermediate, or slow inactivators was based on an arbitrarily selected range of serum levels. Such arbitrary manipulation of data allow correlation between experiment and theory such that any genetic hypothesis might be satisfied.

In summary, whereas rapid inactivators may be either homozygotes or heterozygotes, no unambiguous method has clearly separated the two populations.

Isoniazid Acetylation Polymorphism (*In Vitro* Studies)

Evans and White (8) obtained small pieces of healthy liver from human patients, and liver homogenates were prepared which contained an INH acetylation enzyme. The patients were subsequently administered INH and phenotyped as being either rapid or slow INH inactivators. Homogenates prepared from liver specimens of rapid inactivators had greater INH metabolism activity *in vitro* than did homogenates prepared from liver specimens from slow inactivators. The studies indicated that acetylation polymorphism lies in the activity of the acetyltransferase enzyme which transfers acetyl groups from acetyl coenzyme-A to INH. It was hypothesized that rapid inactivators may possess in the liver more of the same acetyltransferase molecules than is present in slow inactivators. Alternatively, it was thought that there may be two types of hepatic acetyltransferases with different rates of reaction.

Jenne (53) used human, post-mortem, liver specimens and reported partial purification of the enzyme believed responsible for heritable differences in INH acetylation. The enzyme was termed isoniazid transacetylase by the author. Enzyme preparations from livers of rapid INH inactivators had greater INH acetylation *in vitro* activity than enzyme preparations from livers of slow inactivators. Rapid and slow liver enzyme preparations had a number of properties in common, and it was suggested that rapid and slow INH inactivation phenotypes result from differences in concentration of this purified or a closely similar enzyme molecule.

Goedde, Schloot, and Valesky (54) studied the biochemical properties of an INH acetylating enzyme protein obtained from liver homogenates

of Macaca mulatta (rhesus monkeys). Enzyme preparations from so-called rapid and slow acetylating animals showed different maximal acetylation velocities but similar properties regarding purification values, Km values of INH, and inhibitor constants of some aromatic compounds. These results were interpreted to suggest that qualitative differences of the acetylating systems of rhesus monkeys were very small.

Weber, Cohen, and Steinberg (55) summarized their studies on partially purified N-acetyltransferase from 4 mammalian species--rabbit, human, monkey, and rat. N-acetyltransferase from all these sources catalyzed the acetylation of INH by the same mechanism. It was reported that the catalytic properties of enzyme from rapid inactivator rabbits differed from the properties of rapid inactivator human enzyme. Additionally, it was suggested that the enzyme from slow inactivator rabbits might differ from the enzyme from rapid inactivator rabbits.

Pharmacokinetic Aspects of Isoniazid

Isoniazid Binding to Plasma Proteins

Unfortunately, there is very little unambiguous information available on the protein binding of INH. This has resulted from the inherent ambiguities of the methods used, as well as from the use of non-specific analytical methods. Additionally, no attempts were made to study the stability of INH in blood, plasma, serum, or protein solutions during the course of the binding studies.

Felder (56) reported that in the electrophoresis of serum proteins INH migrated with the γ-globulin fraction. Eisfeld and Seefeldt (57) introduced INH into blood *in vivo* and *in vitro*, and the mixtures

were analyzed by paper electrophoresis. The migration rate of INH was independent of the proteins. Lass, Tepe, and Wunderlich (58) added ^{14}C-INH to human serum at concentrations of 50-1000 μg./ml., and fractionation by paper chromatography and paper electrophoresis indicated that INH was not bound to serum protein or any fraction thereof. Wenzel (59) studied the electrophoretic migration of INH in serum (25 mg. INH per ml.) and reported that INH was bound to γ-globulin. Hevér (60) reported that paper electrophoresis of mixtures of INH and serum proteins revealed that INH did not move with the proteins and was therefore not bound to them. Gomi (61) reported that INH was bound to serum protein to the extent of 15.3% as determined by electrophoresis and 28% as determined by dialysis. Sato et al (62) reported that INH showed the same electrophoretic mobility as γ-globulin, but that ultrafiltration experiments indicated that INH did not bind to serum proteins. Claunch, Castro, and Barnes (63) were unable to demonstrate any significant, consistent degree of binding of INH to serum proteins, either by electrophoretic or dialysis techniques. It was reported that there were a number of INH metabolites occurring in vivo in the rabbit which might be associated or bound with serum proteins, but that this did not appear to be so for AcINH. Okuda et al (64) studied the interaction between INH and serum protein by polarography and dialysis. Dialysis experiments indicated that in the presence of human serum containing 7.4% total protein, INH was bound to the extent of 74.1%; in the presence of 4% human albumin, INH was bound to the extent of 34.4%. After oral administration of INH to a rabbit, concentrations of protein bound and free INH in serum were determined after 1, 2, 3,

6, and 8 hours. The ratio of protein bound INH to free INH was 3.3-5.4 to 1. Jenne, MacDonald, and Mendoza (42) reported that serum protein binding of INH as determined by ultrafiltration was quantitatively negligible in both human rapid and slow inactivators. Mattila and Takki (65) estimated the binding of INH to serum protein by a Sephadex batch method and reported that INH was bound to the extent of 50% to protein of human serum.

Oral Absorption of Isoniazid in Man

Gelber, Jacobsen, and Levy (66) studied the availability of INH from commercial tablet formulations of INH. These investigators applied the Wagner-Nelson method (67) for computation of drug absorption from the tablets, and this suggested that absorption was complete by 1-1/2 hours in 3 of the 4 instances studied.

Isoniazid Distribution and Elimination in Man[3]

Peters, Miller, and Brown (5) administered INH, AcINH, and INA orally to healthy human subjects, and urine was collected for 12-24 hours. Compounds identified in the urine after an INH dose were intact INH, AcINH, INH-PA, INH-KA, INA, and INU; cumulative amounts excreted are presented in Table 2. The major compounds that contributed to the hydrazone fraction were INH-PA and INH-KA. Rapid inactivators excreted more AcINH and (INA + INU) after an INH dose than did slow inactivators. When AcINH was administered, approximately equal quantities of AcINH

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[3]Much of the data reported below are subject to errors resulting from non-specific assay methods.

TABLE 2

Mean 24 Hour Cumulative Urinary Excretion of INH and Its Metabolites by Human Subjects Receiving INH, AcINH, and INA Orally (data from reference 5)

INH 5 mg./Kg.

INH Inactivator Status	Total Derivatives (% of Dose)	% of Total Derivatives Found as:			
		INH	Total INH Hydrazones	AcINH	(INA + INU)
Rapid	87.5	3.6	4.3	43.8	48.4
Slow	76.3	12.7	20.8	34.3	32.3

INH 10 mg./Kg.

INH Inactivator Status	Total Derivatives (% of Dose)	% of Total Derivatives Found as:				
		INH	INH-PA	INH-KA	AcINH	INA + INU
Rapid	96.1	3.9	6.09	2.41	33.8	53.7
Slow	90.1	11.9	15.6	6.5	17.8	48.2

AcINH 5 mg./Kg. (INH equivalent)

INH Inactivator Status	Total Derivatives (% of Dose)	% of Total Derivatives Found as:			
		INH	INH Hydrazones	AcINH	INA + INU
Rapid	96.5	0	0	52.1	47.8
Slow	91.0	0	0	48.2	51.8

INA 5 mg./Kg. (INH equivalent)[a]

INH Inactivator Status	Total Derivatives (% of Dose)	% of Derivatives Found as:				
		INH	INH Hydrazones	AcINH	INA	INU
Rapid	94.8	0	0	0	66.6	33.4
Slow	92.5	0	0	0	63.6	36.4

[a]12 Hour cumulative urine collection.

and (INA + INU) were recovered in both inactivator groups. INH and hydrazones were not detected. These data indicated: (1) hydrazone formation involves reaction with intact INH, (2) acetylation is not reversible, (3) INA and INU recovered after INH administration are formed predominately from AcINH, and (4) inactivator status results from the capacity to acetylate INH. Administration of INA resulted in the urinary excretion of intact INA and its glycine conjugate, INU. There appeared to be no relationship between an individual's capacity to acetylate INH and glycinate INA.

Jenne (41) administered INH intravenously to tuberculosis patients (5 mg./kg. body weight) and determined INH serum levels. A linear one-compartment open model was assumed, and rapid inactivators had half-lives of 45-80 minutes (mean 59), while slow inactivators had half-lives of 140-180 minutes (mean 159). The areas under the serum concentration-time curves were approximately 2.5 times greater for the slow inactivators than the rapid inactivators. Using the same experimental methods, Jenne, MacDonald, and Mendoza (42) reported that the mean apparent distribution volumes (calculated from the extrapolated zero time concentration) for rapid and slow inactivators were almost identical with an overall figure of 61 $\pm$ 11 percent of body weight. INH renal clearances were independent of inactivation status and averaged 41 ml./minute. Assuming no binding to plasma protein, tubular reabsorption of INH was approximately 65% of the filtered load. Using a calculated mean apparent distribution volume of 43,000 ml. (70 kg. man) and a renal clearance of 41 ml./min., the INH excretion and metabolic rate constants were calculated for typical rapid and slow

inactivator subjects. The excretion rate constant was assumed equal in both subjects; a typical rapid inactivator had a metabolic rate constant approximately 3.4 times greater than the slow inactivator (10.5×10^{-3} vs. 3.1×10^{-3} min.$^{-1}$).

Andersen (68) investigated the elimination of INH after intravenous injection, and 3 rate constants were calculated assuming a linear one-compartment open model: (1) k_e, the excretion rate constant, calculated from renal clearance and distribution volume, (2) k_a, the acetylation constant, and (3) k_x, the constant representing other forms of elimination. Both k_e and k_x showed a normal distribution among 20 subjects, whereas k_a had a bimodal distribution. The rapid inactivator subjects had an average k_a of 0.01013, and the slow inactivators had an average k_a of 0.00186. Unfortunately, the results were only presented in an abstract, and the methods used to calculate the parameters as well as their units were not reported.

The most sophisticated work on the pharmacokinetics of INH and metabolites is that of Ellard and Gammon (6). These investigators administered INH, AcINH, INA, AcHy, and DiAcHy orally to human subjects, and rate constants were determined assuming a linear one-compartment open model. These results are summarized in Table 3. Where rate constants appeared equal in the rapid and slow inactivator subjects, the values were averaged by the authors and were reported to be the same. It should be noted that the acetylation rate constant for INH was 4 times greater in the rapid inactivator. Additionally, the rate constant for acetylation of AcHy to DiAcHy was 3 times greater in the rapid INH

TABLE 3

Approximate Rate Constants (Hours^{-1}) for the Metabolism and Excretion of Isoniazid and its Metabolites in a Typical Slow and Rapid Inactivator (data from reference 6)

Compound Administered (Dose)	Elimination Route	Rate Constants	
		Slow Inactivator	Rapid Inactivator
INH (250 mg.)	Excretion + Hydrazone Formation	.085	.085
	Acetylation	.1	.4
	Hydrolysis to INA	.03	.03
AcINH (500 mg.)	Excretion	0.15	0.15
	Hydrolysis to INA	0.1	0.1
INA (25 mg.)	INA Excretion	0.75	0.75
	Glycine Conjugation	0.30	0.30
	INU Excretion	0.85	0.85
AcHy (Fumarate Salt) (190 mg.)	Excretion	.05	.05
	Acetylation to DiAcHy	0.1	0.3
DiAcHy (116 mg.)	Excretion	0.15	0.15

inactivator. These data suggested that AcHy may be polymorphically acetylated in a similar fashion to INH.

Levi, Sherlock, and Walker (69) administered INH intravenously to human subjects, and plasma half-lives were determined. Pretreatment with a variety of drugs known to induce microsomal enzymes did not shorten INH half-lives.

Influence of Prolonged Administration of Isoniazid on its Disposition in Man

Investigations of urinary excretion products (32,35) after INH administration for several months indicated that the relative amounts of INH and its metabolites did not change. Jenne (41) determined INH serum half-lives of tuberculosis patients upon initiation of INH therapy and after two months of treatment. With some exceptions, the half-lives determined after the 2 months were close to the initial half-lives in both rapid and slow inactivator groups.

Influence of Dose of Isoniazid and Metabolites on their Disposition in Man

Hughes, Schmidt, and Biehl (35) studied the urinary excretion of INH and its metabolites in tuberculosis patients. The relative amounts of INH and metabolites appearing in the urine did not change when the INH dose was raised from 5 to 20 mg./kg. body weight/day. Short (70) made a similar observation for the excretion of free INH. These results are in contrast to those of Schmidt (24). In the latter study, the percent of a dose recovered as intact INH and some metabolites after doses of 5 and 20 mg. INH/kg. body weight were reported.

Total recoveries of drug and metabolites were equally satisfactory, but there was a systematic increase in recovery of unchanged INH at the higher dose. These increases in the percent of the dose recovered as free INH at the 20 mg./kg. dosage were paralleled by decreases in the proportions of the dose excreted as AcINH and INA.

Peters (39) and Jenne (41) both reported that INH plasma or serum half-lives remained unchanged after intravenous administration of either 5 or 10 mg. INH/kg. body weight.

Peters, Miller, and Brown (5) administered INH and metabolites orally to subjects. In all but one subject, there was a decreased ratio of INU to INA in urine after equimolar doses of INH, AcINH, and INA, respectively. It was thought that this observation reflected saturability of the enzyme which converts INA to INU. Additionally, the extent of glycination decreased when the dose of INH was doubled. Ellard and Gammon (6) made a similar observation; when the oral dose of INA was raised from 25 to 250 mg., the ratio of INU to INA in the cumulative urine of a typical subject decreased from 0.76 to 0.43. These investigators also observed a marked dosage effect on the acetylation of INH; increasing the INH oral dose from 10 to 250 mg. approximately halved the ratio of cumulative urinary AcINH to the sum of INH plus INH hydrazones. Peters, Miller, and Brown (5) had previously obtained a similar dosage effect.

Influence of Tuberculosis on the Disposition of Isoniazid in Man

The influence of tuberculosis on INH disposition is important, since many of the pharmacokinetic studies utilized tuberculosis patients. Hughes, Schmidt, and Biehl (35) noted that in some tuberculous patients that the percent of the INH dose recovered in urine as AcINH was well below the percent eliminated by normal subjects. Bell and Riemensnider (71) reported the pattern of metabolic degradation of INH in a patient with tuberculosis, as measured by microbiological assay of INH in serum, is constant in spite of lessening of fever, anorexia, weight loss, and other signs of toxicity. On the basis of the percent of an INH dose recovered in the urine as intact INH and AcINH, Schmidt (24) reported that the disease status of subjects was without influence on the metabolic pattern. This independence was demonstrated in two tuberculosis patients studied first when critically ill and later after dramatic recoveries. Short (70) investigated INH serum levels as well as urinary excretion of free INH and its derivatives after administration of INH to normal subjects and patients with tuberculosis. No significant differences were observed in the behavior of normal subjects and patients in regard to the manner in which the drug was absorbed, excreted, and metabolized.

OBJECTIVES AND PLAN OF WORK

The influence of heredity on an individual's responses to a drug has become increasingly apparent to workers in the areas of pharmacology and pharmacokinetics. Pharmacogenetic differences in the metabolic conversion of INH are firmly established. Generally speaking, the world population may be broadly divided into two INH inactivation or acetylation phenotypes. It was the purpose of this investigation to study the pharmacokinetics of INH and its metabolites in one rapid and one slow acetylator subject. Emphasis was placed on the development of models and the accurate determination of the parameters. A primary goal was to determine differences in rate processes between the two subjects, particularly with respect to acetylation.

The first step was to develop methods for the determination of INH and its metabolites in biological fluids. Specificity and accuracy were the two essential criteria to be satisfied. With the large doses of compounds administered, sensitivity was no problem. In most instances, modification of previously published methods proved satisfactory. Methods were developed for the determination of INH in blood and the sum of INH plus hydrazones, AcINH, INA, and INU in urine.

Once these methods were developed, INH and its metabolites were injected intravenously into each of the two subjects. Blood and urine were collected at appropriate intervals, and the amounts of the administered compounds and its metabolites were determined. From these data, pharmacokinetic models were developed, and the constants describing these models were determined.

EXPERIMENTAL METHODS AND PROCEDURES

Materials

Syntheses

Pyruvic acid isonicotinoylhydrazone (INH-PA) was prepared by the method of Wenner (72), m.p. 209-211°C. dec. (corr.). Anal. Calc'd for $C_9H_9N_3O_3 \cdot H_2O$; C, 48.00; H, 4.92; N, 18.66. Found. C, 47.81; H, 4.92; N, 18.24.

α-Ketoglutaric acid hydrazone (INH-KA) was prepared by dissolving 14.6 Gm. α-ketoglutaric acid in a minimum quantity of water and adding this to 13.7 Gm. INH dissolved in 100 ml. water. The mixture was heated on a steam bath five minutes, cooled in a refrigerator, and filtered. The precipitate of INH-KA was stirred with 200 ml. water, filtered, and this was repeated a second time. The compound had a m.p. of 178-179°C. (corr.), and its ultraviolet spectrum was identical to that reported by Zamboni and Fachinelli (124).

Acetylisoniazid (AcINH) was prepared by the method of Fox and Gibas (74). The compound was recrystallized twice from ethyl acetate, m.p. 159-160°C. (corr.). Anal. Calc'd for $C_8H_9N_3O_2$: C, 53.63; H, 5.06. Found. C, 53.75; H, 5.09.

Isonicotinuric acid (INU) was prepared by a modification of the method used by Rohrlich (75) for the preparation of nicotinuric acid. One normal NaOH was used in place of 0.1 N NaOH, and concentrated HCl

was subsequently used to achieve neutralization. The product was recrystallized four times from 50% ethanol, m.p. 224-225°C. dec. (corr.). Anal. Calc'd for $C_8H_8N_2O_3$: C, 53.33; H, 4.48; N, 15.55. Found. C, 53.10; H, 4.24; N, 15.68.

Acetylhydrazine (AcHy) was prepared by the method of Kost and Sagitullin (76) and recrystallized four times from chloroform/ether. The product was dried over silica gel <u>in vacuo</u>, m.p. 63-64°C. (corr., sealed capillary). The infrared spectrum was identical to those appearing in the literature (77,78). The compound was extremely hydroscopic; concentrations of fresh solutions were determined by hydrolyzing to hydrazine and measuring hydrazine colorimetrically (for procedure, see section on AcINH determination).

1,2-Diacetylhydrazine (DiAcHy) was prepared by the method of Turner (79). Recrystallization once from methanol gave a product with m.p. 136-137°C. (corr.). Anal. Calc'd for $C_4H_8N_2O_2$: C, 41.37; H, 6.94; N, 24.13. Found. C, 41.22; H, 7.01; N, 24.30.

Chemicals

Isonicotinic acid hydrazide (Eastman), recrystallized thrice from 95% ethanol, m.p. 169-170°C. (corr.). Anal. Calc'd for $C_6H_7N_3O$: C, 52.54; H, 5.15. Found. C, 52.31; H, 5.10; Isonicotinic acid (Eastman), recrystallized thrice from water, m.p. 304-305°C. (corr., sublimation), Anal. Calc'd for $C_6H_5NO_2$: C, 58.53; H, 4.09; Found. C, 58.59; H, 4.22; Barbituric acid (Aldrich), recrystallized five times from water, m.p. 249°C. dec. (corr.); Chloramine-T (Eastman), $C_7H_7O_2NSClNa \cdot 3H_2O$; Potassium cyanide (Mallinckrodt), purified grade;

Vanillin (Eastman), recrystallized once from 95% ethanol, m.p. 80.5-82.5°C. (corr.); Salicylaldehyde (Eastman, from bisulfite addition compound); 2-Mercaptoethanol (Eastman); Isobutanol (Eastman), extracted with equal volumes of 0.10 N HCl, 0.10 N NaOH, and water, respectively; Sodium 1,2-naphthoquinone-4-sulfonate (Eastman), purified twice by the method of Folin (80); p-Dimethylaminobenzaldehyde (Eastman); Hydrazine dihydrochloride (Eastman); all other chemicals were reagent grade.

Apparatus

A Perkin-Elmer Model 203 fluorescence spectrophotometer equipped with a Model 150, Xenon lamp, power supply was used for all fluorescence measurements. Matched cells with a 1 cm. path length were employed with this instrument.

A Corning Model 10 pH meter was used for all pH measurements.

A Bausch and Lomb Spectronic 20 spectrophotometer using 1/2" O.D. x 4" length colorimeter tubes was used for all colorimetric determinations. When filled with a colored solution absorbing at 600 mμ, the absorbance from any two tubes differed by no more than 2.0%.

A Castle Model 999-C autoclave was used.

Melting points were determined in a Thomas-Hoover Uni-Melt, capillary tube apparatus.

Analytical Methods

Introduction

The volume of literature on the determination of INH and metabolites in biological fluids is immense. An exhaustive literature review was prepared by Boxenbaum (81). The methods presented here were developed or adapted from existing methods. Subsequent to the completion of the pharmacokinetic studies presented here, Ellard, Gammon, and Wallace (16) published a report on methods for the determination of INH and metabolites in biological fluids.

In each urine assay, standard solutions were prepared in water and carried through the experimental procedure. Water was also carried through the procedure and provided a "blank." Unless otherwise stated, for each urine assay, standards and blanks prepared in water and urine resulted in identical calibration curves. For each assay, freshly prepared standards were run concomitantly with the unknowns. Metabolite interferences are reported on a molar basis. Representative calibration curves are described in each section; in every case, the intercept of the regression lines did not differ significantly from zero at the 5% level of significance (82).

Determination of INH in Aqueous Solution

This method was modified from Peters (83) and involves an addition reaction between INH and sodium 1,2-naphthoquinone-4-sulfonate (84).

The naphthoquinone reagent consists of a freshly prepared solution of sodium 1,2-naphthoquinone-4-sulfonate (1.00 mg./ml.) in 1/15 M pH 8.00 phosphate buffer.

The INH in 1/15 M pH 8.00 phosphate buffer is diluted so as to contain approximately 5 μg INH/ml. within 1/15 M pH 8.00 phosphate buffer. One ml. naphthoquinone reagent is added to 9.00 ml. of the INH solution, and this is placed in the dark for 20 minutes. Absorbance is determined at 460 nm against a blank. Beer's law is obeyed for solutions containing up to 10 μg INH/ml. initial concentration ($A \approx 0.82$). A typical calibration curve had a correlation coefficient of 0.9999.

INH hydrazones, AcINH, INA, and INU do not interfere with the determination. The method can also be adapted for determinations at pH 7. While not well suited for the determination of INH in biological fluids (83), the method is simple and accurate for determination of INH in aqueous solutions in the absence of interfering substances.

Determination of the Sum of INH, INH-PA, and INH-KA in Urine

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. Using a concentration of INH-PA that might normally occur in the urine, approximately 30% of the INH-PA in 1/15 M pH 8.00 phosphate buffer was hydrolyzed to INH in 30 minutes at room temperature (initial concentration of INH-PA·H_2O was 11.2 mg./ml.). When a freshly collected urine (pH 5.9) was spiked with an amount of INH-PA which might normally

be present after an INH dose, 35 minutes at room temperature resulted in approximately 25% hydrolysis to INH. On the basis of these experiments, it seems likely that INH-PA in the urinary bladder would undergo hydrolysis. Hence, a measurement of intact urinary INH would not reflect the actual amount excreted from the renal tubules. It was therefore decided to measure the sum of INH and its hydrazones in urine.

The method used was modified from Peters (83) and involves the reaction between INH and vanillin to form a colored hydrazone (85). In this method, a weak acid hydrolysis is used to quantitatively split INH-PA and INH-KA to INH. The vanillin reagent consists of a 10.0% W/V solution of vanillin in 95% ethanol (may be stored at -18° for several months).

Urine is diluted so as to contain approximately 4 μg./ml. of INH plus hydrazones (expressed in terms of INH), and 5.00 ml. is mixed with 1.00 ml. 0.200 N HCl in a culture tube. The capped tube is placed in a 52°C. water bath for 60 minutes and then cooled to room temperature. One ml. each of 1.00 N HCl and vanillin reagent are added. The tube is mixed and permitted to stand at room temperature for 10 minutes; absorbance is determined at 365-370 nm against a blank. The yellow color of urine does add slightly to the absorbance, and a correction for each sample is necessary. For example, a 2 in 10 dilution would have an absorbance of approximately 0.3. For many determinations however, a common dilution was 3-5 in 100. Consequently, a second sample of diluted urine is also carried through the procedure, but 1.00 ml. 95% ethanol is used in place of the 1.00 ml. vanillin reagent. The

absorbance of this solution is measured against its blank, and this later absorbance is subtracted from the original absorbance value. The concentration of INH and hydrazones in urine is then determined by comparing this corrected absorbance to that of a calibration curve prepared using water. Beer's law is obeyed for initial concentrations of INH up to 8 μg./ml. ($A \approx 0.35$). A typical calibration curve had a correlation coefficient of 0.9996. INA, INU, and DiAcHy do not interfere with this method, whereas 1% of the AcINH present is detected as INH; presumably this results from its hydrolysis. AcHy interferes to the extent of 1.8%, while hydrazine (not a urinary metabolite) interferes to the extent of 242%. Substances were present in blank urines that did react as apparent INH, and these substances were equivalent, in a 24 hour period, to approximately 6 mg. INH.

Determination of Acetylisoniazid in Urine

A modification of the Peters' extraction method (86) is used; AcINH is extracted from diluted urine into an organic solvent mixture and subsequently re-extracted into dilute HCl. AcINH is separated from other hydrazino compounds by a modification of the ion-exchange chromatography procedure of Belles and Littleman (87).

The Ehrlich's hydrolysis reagent is prepared fresh by adding 0.60 Gm. p-dimethylaminobenzaldehyde to 20.0 ml. 10.0 N HCl. The organic extraction solvent is 3:7 isopentyl alcohol-1,2-dichloroethane, water saturated (hood required). The elution solution consists of an aqueous solution of 0.100 N HCl, 0.200 M LiCl, 0.1 M KCl, and 10.0% V/V 95% ethanol. The resin is AG 50W-X4, 100-200 mesh, hydrogen form,

analytical grade cation exchange resin (Bio-Rad Laboratories, 32nd and Griffin, Richmond, Ca., U.S.A.). Prior to use, the resin is soaked in 0.100 N HCl for at least 12 hours and then placed into columns. The columns are Bio-Rad disposable columns, glass barrel, 0.70 cm. internal diameter, cut from 30 to a length of 15.0 cm. The resin slurry is added to the column to a height of 3.0 cm., and 25 ml. 0.100 N HCl is passed through; if necessary, the resin bed height is then readjusted to a height of 3.0 cm. Immediately prior to use, the 0.100 N HCl above the resin bed is permitted to fall to the level of the bed. Columns are prepared no sooner than 3 days prior to the assays, and 0.100 N HCl is permitted to remain atop the resin bed during storage.

Urine is diluted so as to contain approximately 50 μg AcINH/ml., and 3.00 ml. is pipetted into a 90 ml. prescription bottle containing 3.2 Gm. ammonium sulfate. This is followed by 1.00 ml. 0.100 N NaOH and 40.0 ml. organic extraction solvent, and the mixture is shaken vigorously for 30 minutes. The contents are transferred to a 25 x 150 mm. culture tube and centrifuged 5 minutes at 1,750 rpm. Thirty-five ml. of the organic phase (top) is transferred to another 25 x 150 mm. culture tube containing 6.00 ml. 0.100 N HCl; this is shaken vigorously for 30 minutes and centrifuged at 1,750 rpm for 5 minutes. Four ml. of the aqueous layer (top) is gently pipetted onto the ion exchange resin and permitted to flow through. Just as the liquid runs to the level of the resin bed, 4.00 ml. 0.100 N HCl is gently pipetted onto the column, and this is also permitted to run through. This HCl wash does not elute AcINH but does remove substances that interfere with the assay. As soon as the 0.100 N HCl level runs to the level of the resin bed, a

25 ml. volumetric flask is placed under the column to collect effluent. Immediately thereafter, elution solution is run through the column until the 25 ml. flask is full. Reservoir caps for the columns, provided by the manufacturer, may be placed atop the glass barrels to provide for a greater volume and pressure head for the elution solution (these caps hold approximately 7 ml.). No attempt is made to control flow rate; however, a rate of approximately 1.5 ml./minute is achieved with a maximum pressure head. Four ml. of the 25.0 ml. effluent is added to a calibrated test tube containing 1.00 ml. Ehrlich's hydrolysis reagent. The uncapped tube is placed in a boiling water bath for 2.0 hours, cooled to room temperature, and made up to a volume of 5.0 ml. with 0.100 N HCl. Absorbance is measured at 450 nm and AcINH is determined from a calibration curve. Beer's law is obeyed up to initial AcINH concentrations of 100 μg./ml. ($A \approx 0.92$). A typical calibration curve had a correlation coefficient of 0.9995.

AcINH is quantitatively eluted from the column, and the amount recovered in the 25 ml. effluent is approximately 54% of that present in the initial 3.00 ml. diluted urine. This recovery is a consequence of non-quantitative extraction and the use of aliquots. An elution chromatogram is shown in Figure 2. The 2.0 hour hydrolysis period quantitatively converts AcINH to hydrazine.

INH, INH-PA, INH-KA, INA, INU, and DiAcHy do not interfere with this determination. Whereas INH and the hydrazones are extracted, they are not eluted from the column with this procedure. INA and INU can not interfere, since they have no hydrazine moiety. DiAcHy is extracted but elutes prior to the addition of the elution solution.

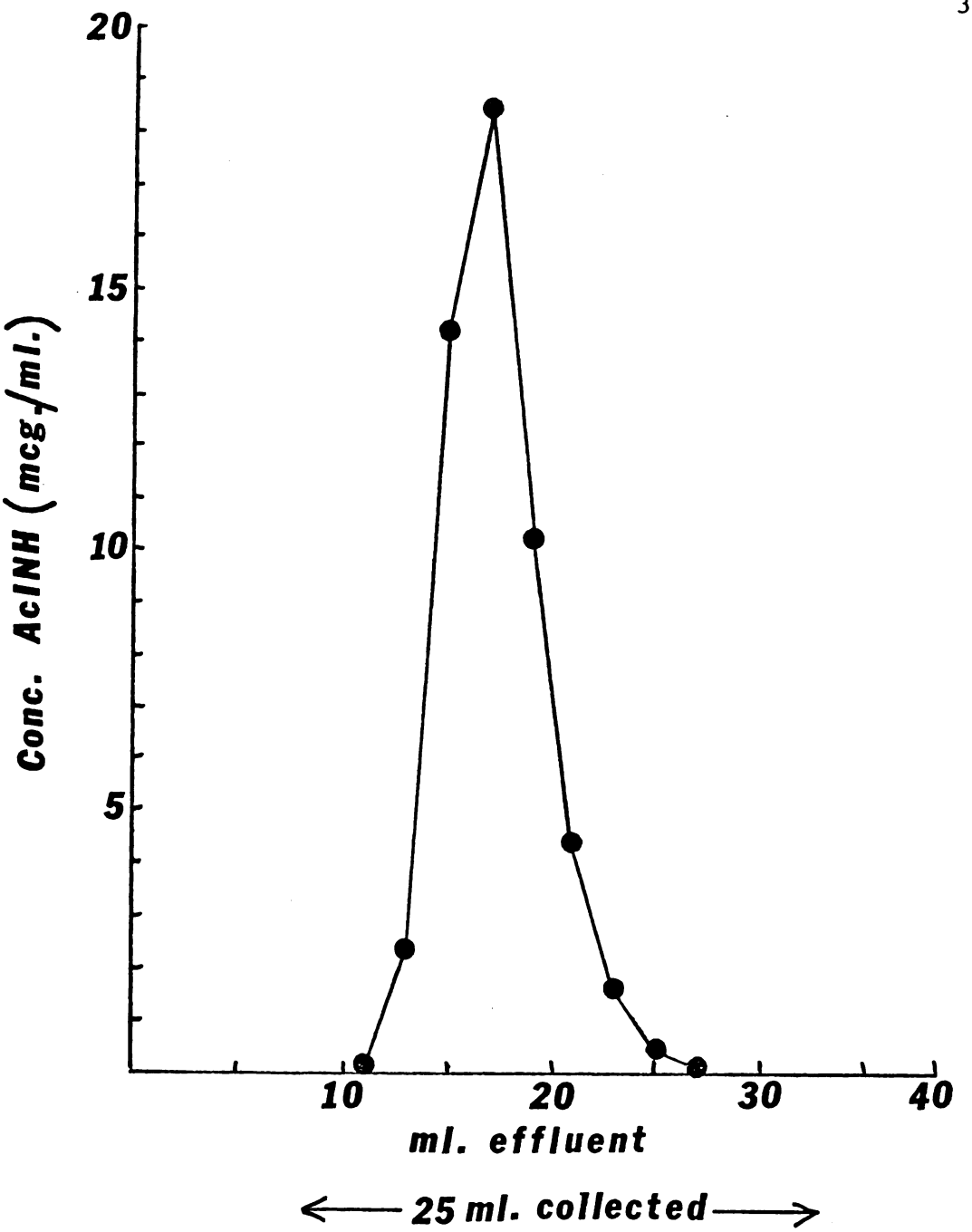

Figure 2. Elution Chromatogram of AcINH

When urines were spiked with Hy and AcHy, these compounds interfered to the extent of approximately 7.8 and 9.0%, respectively. Whereas hydrazine is not found in urine after INH administration, AcHy is definitely present (6). An estimation of the error resulting from the AcHy interference was calculated from the data of Ellard and Gammon (6). These investigators administered 190 mg. monoacetylhydrazinium fumarate (equivalent to 123 mg. AcHy) orally to a slow INH inactivator subject. The 48 hour urinary recovery for DiAcHy was 44.5% of the dose; whereas urinary AcHy could not be determined accurately, the maximum amount that could be excreted was 55.5% of the oral dose. These data may be applied to the data reported herein. After intravenous administration of INH to subject SR (see Table 8), equimolar cumulative amounts of AcINH and INA derivatives (INA and INU) were excreted in the urine. Since INA and INU do not contain a hydrazine moiety, their former hydrazine content would necessarily have to be accounted for elsewhere. For the purpose of this calculation, it was assumed that these hydrazine moieties were solely converted to AcHy and DiAcHy. If the 55.5% figure of Ellard and Gammon is used, the ratio of cumulatively excreted AcINH:AcHy is 1:0.555. Since only 9.0% of AcHy is detected as AcINH, 0.05 moles (0.555 x 0.09) AcHy would be detected as AcINH for each mole of AcINH actually excreted. This results in the cumulative AcINH determination being 105% of the true amount. Hence, the contribution of AcHy to the AcINH assay results in a maximum 5% error. In rapid acetylator subjects, more

AcHy is converted to DiAcHy, and the error would be even smaller. Conversion of the hydrazine moieties to ammonia would reduce the error to a greater extent.

Another measure of the error in the AcINH determination is provided by the cumulative urinary excretion data after intravenous administration of AcINH to subject SR. The cumulative excretion of INH and hydrazones, AcINH, INA, and INU was 670 mg. Total isonicotinoyl compounds[4] accounted for 682 mg. If there were a significant interference from AcHy in the AcINH determination, the sum of the urinary components would exceed total isonicotinoyl compounds; this did not occur.

Determination of Isonicotinic and Isonicotinuric Acids in Urine

This two component colorimetric analysis is modified from Nielsch and Giefer (88-90), and is based upon polymethine dye formation (90). Both INA and INU form colored dyes, and by measuring absorbance at two wavelengths, INA as well as INU may be simultaneously determined.

Concentrated acetate buffer is prepared according to Nielsch and Giefer (90); on dilution with 4 parts water, a solution of pH 5.0 is obtained. Barbiturate buffer is also prepared according to Nielsch and Giefer (90), and this is an approximately 2% solution of pH 5.0. On standing, this solution develops a precipitate which is removed by filtration or decantation before use.

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[4]The assay for total isonicotinoyl compounds is subsequently discussed.

Urine is diluted so that the μg concentration of (INA + 1/2 INU) is approximately 10-15 μg./ml.; this concentration may not exceed 20 μg./ml. To a 25 ml. volumetric flask under a hood, the following are added with shaking in the sequence: (1) 1.25 ml. concentrated acetate buffer; (2) 0.50 ml. 2.0% aqueous KCN-prepared fresh under a hood; (3) 5.0 ml. 1.0% chloramine-T·$3H_2O$--prepared fresh; (4) 0.50 ml. 8.6% V/V glacial acetic acid; (5) 6.50 ml. barbiturate buffer; (6) 2.50 ml. acetone; and (7) 1.00 ml. unknown solution. Using automatic pipetting devices, 15-20 samples may be processed simultaneously; each reagent is added to all flasks before proceeding to the next reagent. The volume of each flask is brought to 25.0 ml. with water, and the flasks are placed in the dark for 1.0 hour. Absorbances are determined at 590 and 625 nm. Standard solutions of INA (10, 15, and 20 μg./ml.) and INU (20, 30, and 40 μg./ml.) are carried through the procedure. INA and INU concentrations are determined by solving two simultaneous equations with two unknowns (90). Table 4 shows typical data from calibration curves. The absorbance maxima for the polymethine dyes of INA and INU are 600 and 615 nm, respectively. The wavelengths of 590 and 625 nm were selected because they provided greater accuracy than 600 and 615 nm. In order to minimize errors, it is important to make all absorbance measurements at one wavelength setting before proceeding to the second setting. Additionally, greater accuracy is achieved when the μg./ml. concentration of (INA + 1/2 INU) is 10 or above. The ratio of INA to INU in a sample should be less than 4 and greater than 0.25 or else accuracy in the determination of the lesser component is significantly reduced.

TABLE 4

Absorbances from INA and INU from Typical Calibration Curves (concentration of each component was 10 μg./25 ml. final solution)

Absorbance[a]			
INA 590 mμ	INA 625 mμ	INU 590 mμ	INU 625 mμ
0.410	.103	.120	.135
(.9999)	(.9991)	(.9997)	(.9994)

[a]Values in parenthesis indicate correlation coefficient of calibration curve.

The nominal wavelength settings on a Spectronic 20 spectrophotometer are not accurate, and therefore wavelength settings for each Spectronic 20 are selected to provide absorptivities similar to those indicated herein; these nominal settings would not be expected, however, to differ by more than about 5 mμ from those reported here.

An important presumption in the method presented here is that the absorbances from the two components be additive; this presumption was shown to be valid.

INH, AcINH, INH-PA, INH-KA, Hy, AcHy, and DiAcHy do not interfere with this determination.

Determination of Total Isonicotinoyl Compounds in Urine (Total INA)

Total INA includes INH, INH-PA, INH-KA, AcINH, INA, and INU, and is determined by a modification of the method of Peters (83). The principle of the method is to quantitatively convert all the aforementioned compounds to INA by strong acid hydrolysis. The INA is subsequently measured colorimetrically.

Urine is diluted so as to contain approximately 30-120 μg. total INA/ml. (expressed in terms of INA). Two ml. of this solution is added to a 5 ml. ampule containing 2.00 ml. 10.0 N H_2SO_4, and the ampule is sealed. The ampule is placed in an autoclave at 125-130°C. (27 psig) for 3.0 hours, and 1.00 ml. hydrolysate is neutralized with 5.00 ml. 1.00 N NaOH. Alternatively, 2.00 ml. hydrolysate may be neutralized with 5.00 ml. 2.00 N NaOH. An appropriate aliquot of the neutralized solution is subsequently used for INA determination. The

colorimetric method is the same as that described for INA and INU, except that the wavelength is 600 nm. One ml. each of INA solutions (5, 10, 15, and 20 μg./ml.) are used to prepare standards. Beer's law is obeyed up to 20 μg./25 ml. final volume ($A \approx 0.88$). A typical regression equation had a correlation coefficient of 0.99995.

With this procedure, all isonicotinoyl compounds are quantitatively converted to INA. While HCl is suitable for hydrolysis of aqueous isonicotinoyl solutions, it does not work when urine is present; in such situations, recoveries of approximately 90-95% are obtained. The reason for this anomaly is unclear.

The use of an autoclave is essential; placing these ampules in a 130°C. oven frequently resulted in violent explosions.

Determination of the Sum of INH, INH-PA, and INH-KA in Whole Blood

This assay was developed and used for a single experiment subsequently described; due to inherent technical difficulties, it is not advocated for general use.

The method is modified from Deeb and Vitagliano (85) and involves the reaction of INH with vanillin. INH hydrazones are first hydrolyzed to INH.

The vanillin reagent is a 10.0% V/V solution in 95% ethanol (may be stored several months at -18°C.).

Freshly drawn heparinized blood (12 U.S.P. units heparin/ml.) is used. Immediately upon collection, 7.00 ml. blood is placed in a 16 x 150 mm. culture tube and 7.00 ml. 30.0% trichloracetic acid

solution is added. The tube is vigorously shaken by hand for 10 seconds, and then immediately filtered through Whatman #1 filter paper; the clear, colorless filtrate must be used 5-10 minutes after collection. Six ml. filtrate is mixed with 0.50 ml. vanillin reagent and permitted to stand at room temperature for 10 minutes. Absorbance is determined at 365-368 nm, and Beer's law is obeyed for blood levels up to 40 μg INH equivalent/ml. The lower limit of sensitivity is approximately 2.5 μg INH equivalent/ml. blood (Absorbance $\approx$ .057). Because each blood sample must be assayed immediately upon collection, this method is extremely difficult to use. For each determination, one standard and one blank need be used. Blood obtained from a blood bank interferes with the determination and is unsuitable for preparation of blank and standard solutions (contains 450 ml. human blood and 67.5 ml. anticoagulant acid citrate dextrose solution A, U.S.P.). Therefore a large volume of blood need be drawn from a subject prior to drug administration to serve as a blank and for the preparation of standards.

The trichloracetic acid precipitation effectively destroys blood cells and quantitatively converts the hydrazones to INH. INA, INU, and DiAcHy do not interfere with the determination, while AcINH and AcHy interfere to the extent of approximately 1% and 1.8%, respectively. Hydrazine (not detected as a metabolite) interferes to the extent of 230%. Freezing and thawing of the filtrates results in significant interferences from AcHy, DiAcHy, and AcINH, presumably from hydrolysis of these compounds. It is also for this reason that the filtrates should be treated 5-10 minutes after collection.

Determination of Isoniazid in Whole Blood

The method used was modified from Scott and Wright (91) and is based upon formation of the INH-salicylaldehyde hydrazone. The reduced form of the hydrazone, extracted into isobutanol, is highly fluorescent. Bisulfite is used to react with excess salicylaldehyde, thus rendering it non-fluorescent.

The salicylaldehyde-acetic acid reagent is prepared fresh according to Scott and Wright (91). The $ZnSO_4$ solution is approximately 5% $ZnSO_4 \cdot 7H_2O$ W/V, and the $Ba(OH)_2$ is approximately 0.30 N. The exact neutralization of the $ZnSO_4$ by $Ba(OH)_2$ is essential, and the procedure of Scott and Wright is used (91). The acetate buffer consists of 0.120 N NaOH and 0.385 M sodium acetate; sufficient acetic acid or NaOH is added to this buffer such that 1.30 ml. buffer, 1.50 ml. water, and 0.40 ml. salicylaldehyde-acetic acid reagent results in a solution of pH 5.60 $\pm$ 0.05. The 2-mercaptoethanol-bisulfite-buffer is prepared fresh by adding 1.92 ml. 2-mercaptoethanol (stench!) to 50.0 ml. acetate buffer containing 77.0 mg. sodium bisulfite (total volume approximately 51.92 ml.). The isobutanol, water saturated, is especially purified as indicated in the chemical section. Water must be freshly distilled so as to provide a pH about 7. All tubes are especially "acid washed" by heating approximately 5 hours at 70°C. in 1.6 N HNO_3; the tubes (teflon lined caps) are subsequently rinsed 5 times with distilled water and oven-dried.

One-half ml. heparinized blood is immediately placed in a culture tube containing 15.0 ml. distilled water; this addition should

be made within 1 minute after blood sampling. A period of 45 seconds is allowed for hemolysis to be complete, and 1.00 ml. $ZnSO_4$ solution is added. The tube is vigorously shaken by hand for 5 seconds, and 1.00 ml. $Ba(OH)_2$ solution is added. After shaking vigorously for 5 seconds, the mixture is immediately filtered through Whatman #1 filter paper into a culture tube resting in an ice-water bath. The clear, colorless filtrate is immediately frozen in dry ice-isopropanol for 2 minutes[5] and stored at -18°C. Within 24 hours from the time of freezing, the filtrate is thawed and brought to room temperature in a 50°C. water bath; 1.50 ml. filtrate is mixed with 0.40 ml. salicylaldehyde-acetic acid reagent and the capped tube is kept at room temperature 20 minutes. After that reaction period, 1.35 ml. 2-mercaptoethanol-bisulfite-buffer is added, and the capped tube is placed in a 50°C. water bath for 10 minutes. The tube is cooled to room temperature by immersing in an ice-water bath for 15 seconds, and 4.00 ml. isobutanol is added. The tube is vigorously shaken by hand for 5 seconds to extract the reduced form of the hydrazone into the isobutanol. The tube is subsequently placed in an ice-water bath 5 minutes and centrifuged 5 minutes at 2,400 rpm (without prior chilling, the heat generated by centrifugation causes turbidity). The top isobutanol layer is removed, and fluorescence determined (activation wavelength 392 nm, emission wavelength 478 nm). With the instrument used, the blank is set to 0 units and the standard of maximum concentration is set to 100 units. With a wide concentration range of unknowns, several

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[5]Thick-walled pyrex culture tubes should be used to minimize the possibility of breakage.

calibration curves of limited range need be prepared (e.g., 0.05-1 μg. INH/ml. blood and 1-30 μg. INH/ml. blood). In the examples just cited, linear curves were obtained with correlation coefficients of 0.9997 and 0.99998, respectively. The limit of sensitivity is approximately 0.01 μg INH/ml. blood; whereas the upper limit of measurability was not determined, this is at least 30 μg INH/ml. blood. The amount of salicylaldehyde would be expected to become saturated at much higher concentrations of INH. Standards are prepared by having the requisite amount of INH in the 15.0 ml. distilled water and adding 0.50 ml. blood. The blood must be obtained from the subject receiving the INH, and this should be drawn just prior to injection. In this way, standards are prepared immediately before injection.

Filtrates stored at -18°C. for 26 days lost approximately 24% of their INH content with this method, whereas filtrates stored 24 hours were unaffected. For this reason, filtrates must be assayed within 24 hours after freezing; this is the primary disadvantage of the method.

INA, INU, Hy, AcHy, DiAcHy, and AcINH do not interfere with this determination. INH-KA and INH-PA interfere to the extent of 1.1 and 7.8%, respectively.

To quantitate the interferences from hydrazones in these experiments, a study was undertaken with subject HB with urine pH maintained at 8 by sodium bicarbonate administration (for protocol, see section on subjects and test procedures). Approximately 700 mg. INH were infused at a zero-order rate over a period of 5.48 minutes. Blood was drawn at approximate 50 minutes intervals for 550 minutes;

assays were performed on each sample for INH by the fluorometric method and for (INH plus hydrazones) by the vanillin colorimetric method. The concentrations at each time point were analyzed by the t-test for paired observations (92); at a 5% level of significance, the concentrations were not identical. This indicated that hydrazones were present in blood. Assuming that INH-PA was the only hydrazone in blood, the concentration of INH and INH-PA in each sample was calculated by solving two equations with two unknowns.

$$V = 100\%\ \text{INH Concentration} + 100\%\ \text{INH-PA Concentration} \quad (1)$$

$$F = 100\%\ \text{INH Concentration} + 7.8\%\ \text{INH-PA Concentration} \quad (2)$$

where

V = concentration determined by vanillin colorimetric method, and

F = concentration determined by fluorometric method.

The maximum concentration of INH-PA in any sample was 19.9% of the concentration of INH present. The interference in the fluorometric method would be 7.8% of that value, for an approximate overall 1.6% error in the INH blood level. The average blood level of INH-PA was calculated as 4.65% of the INH blood level (average error of INH by fluorimetry, approximately 0.36%).

When urine pH is maintained at a value of 8, INH hydrazones present in renal tubules would predominantly exist as salts. Renal tubular reabsorption would probably be reduced and hydrazone blood level consequently reduced. Under this condition of urinary alkalinization, the INH fluorometric method is specific for INH.

Experimental Trial of Urine Assay Methods

The data of Peters (5) were utilized to prepare spiked urines containing INH, INH-PA, INH-KA, AcINH, INA, INU, and DiAcHy (at the time of these studies, AcHy had not been established as a urinary metabolite). DiAcHy was added in equal molar concentration to the sum of INA and INU. The urines were prepared in such a manner as to mimic what might be expected in the 24 hour urines of rapid and slow INH inactivators who were administered a 1.00 Gm. I.V. INH dose; a urine flow rate of 1.00 ml./min. was assumed. The rapid inactivator urine contained the following molar percentages: (1) INH, 3.6; (2) INH-PA, 2.9; (3) INH-KA, 1.4; (4) AcINH, 43.8; (5) INA, 27.1; (6) INU, 21.3; and (7) DiAcHy, 48.4. The slow inactivator urine contained the following molar percentages: (1) INH, 12.7; (2) INH-PA, 13.9; (3) INH-KA, 6.9; (4) AcINH, 34.3; (5) INA, 18.1; (6) INU, 14.2; and (7) DiAcHy, 32.3. Each urine assay was performed on these samples, and results are presented in Table 5. On the basis of these results, it was concluded that the assays are valid. AcHy interferes somewhat in the AcINH determination; this was previously discussed.

Additionally, it was demonstrated from urine collected after INH administration that these urine assays gave identical results when the urine was assayed shortly after collection and after 6.0 months storage at $-18^{\circ}C$.

TABLE 5

Experimental Trial of Urinary Assay Methods
(see text for explanation)

Compound(s)	Rapid Inactivator Urine % of Theoretical Amount Found	Slow Inactivator Urine % of Theoretical Amount Found
INH + Hydrazones	99.6	98.0
AcINH	96.9	97.0
INA	95.6	97.9
INU	101	95.4
Total INA	98.8	98.8

Subjects and Test Procedures

Two subjects in apparent good health were used in these experiments. Subject HB was a Caucasian male, age 29 years, weight 69.0 kg., and height 165 cm. Subject SR was a Caucasian male, age 50 years, weight 80.0 kg., and height 181 cm. Table 6 shows calculated body surface areas (93), body weights and volumes (94), and liver blood flows (95). Neither subject smoked tobacco; subject SR had not received other drugs for several months prior to and during these experiments. Subject HB was maintained euthroid (T_4 = 5.7 μg./100 ml. serum) by daily administration of 130 mg. thyroid U.S.P.; since this subject was administered thyroid to achieve a normal blood level of thyroid hormones, this was not expected to affect the pharmacokinetics. Subject HB received no other drugs prior to and during the experiments. Experiments in any one subject were conducted at least 2 weeks apart.

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.; this injection, when assayed by the naphthoquinone method, contained 99.6% of the labeled concentration of INH. INA could not be detected. All INH injections were from a single lot (control #OK 630). Injections of AcINH, INA, and INU were prepared no more than 12 hours prior to use, and were then stored in a refrigerator. Whenever possible, glassware used in the preparation of these injections was autoclaved prior to use. The requisite amount of compound was dissolved in approximately

TABLE 6

Body Parameters Calculated for the Two Subjects

Parameter	Subject	
	HB	SR
Body Surface Area, m^2	1.76	2.01
Body Weight, kg.	69.0	80.8
Age, years	29	50
Total Body Water, liters	40.4	42.5
Intracellular Water, liters	23.3	23.1
Fat Free Body Weight, kg.	55.2	58.1
Body Fat, kg.	13.8	22.7
Fat Free Solids, kg.	14.8	15.6
Extracellular Water, liters	17.1	19.4
Red Cell Volume, liters	1.98	2.09
Blood Volume, liters	5.21	5.50
Plasma Volume, liters	3.23	3.41
Liver Blood Flow, liters/min.	1.50	1.58

60 ml. Sodium Chloride Injection U.S.P. in a beaker containing a stirring bar. With continuous stirring, a dilute NaOH solution was added dropwise until the pH reached 7.4. The solution was subsequently sterilized by passing through a micropore filter into a sterilized multiple dose ampule. The sterilized filter unit used was a Millipore Swinex-25, SXGS 025 OS 0.22 μ (type GS filter, 0.22 μ mean pore size ± 0.02 μ pore size variation).

Each subject participated in a total of 4 studies and received INH, AcINH, INA, and INU intravenously. Each injection was administered intravenously at a zero-order rate through a 19 gauge needle (Abbott's Butterfly-19) located in a vein on the dorsum of the forearm. In the INH studies, blood was collected from an indwelling needle (Abbott's Butterfly-19) located in the opposite forearm. Infusions were controlled using a Harvard Model 931 infusion pump. The approximate doses were: (1) INH, 675 mg; (2) AcINH, 580 mg; (3) INA, 200 mg; and (4) INU, 120 mg. The exact dose for each study was calculated by assaying the injection solution and measuring the volume injected. The duration of infusion for INH was approximately 5.3 minutes, whereas AcINH, INA, and INU were infused for exactly 60.0 minutes. This lengthy infusion time was elected in order to minimize risks.

A preliminary study indicated that the pattern of INA excretion was affected by changes in urinary pH; consequently, urine pH was maintained at a value of 8.0 ± 0.2 in all experiments by administration of sodium bicarbonate. The regimen was modified from Kostenbauder, Portnoff, and Swintosky (96) and utilized 325 mg. Sodium Bicarbonate

Tablets U.S.P. Thirteen tablets were orally administered 2.0, 1.5, 1.0, and 0.5 hours prior to injection (52 tablets total). Urine was collected for the 30 minute time interval just prior to drug administration, and the pH was measured. A dose of sodium bicarbonate was then administered in accordance with that pH measurement. Additionally, doses of sodium bicarbonate were administered every 30 minutes for a total of approximately 12 hours. Urinary pH was determined shortly after voiding, and each 30 minute dose depended on the preceeding pH measurement (96). In most cases, after initial alkalinization was achieved, 4 tablets every 30 minutes maintained the urine pH at 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 minute intervals during and for some time after the infusion. Blood collections were later reduced to approximate 30 minute intervals. Urine was collected at approximate 30 minute intervals for about 12 hours, and then ad libitum for a total of 48 hours post-infusion.

For the AcINH, INA, and INU studies, urine was generally collected at 15 minute intervals during and for one hour post-infusion (total of 8 collections). Urine collections were subsequently made at approximate 30 minute intervals for about 11 hours post-infusion, and then ad libitum for 48 hours total.

In each study, urine pH was measured within 5-10 minutes after voiding, and all samples were frozen at -18^{o}C. until assay. INH in

blood was measured within 24 hours from time of filtrate preparation. Urine assays were performed within 2 weeks after collection.

Data Treatment

Preliminary Remarks

Table 7 shows cumulative urinary recoveries of administered compounds and metabolites after intravenous administration. The "injection amount" was calculated from assay of the concentration of the injected solution and measurement of the volume injected during the infusion period. The "sum of assays," expressed as a percent of the injection amount, is the sum of administered compound and metabolites excreted in urine samples as measured by the specific assays reported previously for the individual compounds. "Cumulative total INA" is the sum of the data obtained from the acid hydrolyzed urine samples when assayed by the total INA method; this value is also expressed as a percent of the injection amount. All compound amounts were corrected to correspond to their mole equivalents of parent compound. No less than 95.1% of the injection amount was recovered in urine. The assays of the injection solutions were subject to approximately the same relative errors as the urine assays. Since these values were very close, it was therefore assumed that differences in injection amount and "sum of assays" reflected assay error, and that 100% of the intravenous doses were excreted in the urines. Consequently, the sums of assays were used in the computer analyses and

TABLE 7

Urinary Recovery of INH and Metabolites
After Intravenous Administration
(see text for explanation)

Compound Administered	Subject: HB Injection Amount	HB Sum of Assays (% of injection amount)	HB Total INA (% of injection amount)	Subject: SR Injection Amount	SR Sum of Assays (% of injection amount)	SR Cumulative Total INA (% of injection amount)
INU	117 mg.	100	--	124 mg.	98.2	--
INA	201 mg.	99.5	--	200 mg.	98.4	--
AcINH	587 mg.	99.9	99.6	606 mg.	95.1	96.4
INH	682 mg.	99.8	97.8	675 mg.	99.3	101

taken to be the "dose." This presumes that the error in urine assays and collections were random, and no special assay contributed more than any other to the error.

Post-infusion INH blood curves could be described by bi-exponential equations. Additionally, after intravenous administration of each of the INH metabolites, post-infusion rate of excretion curves (intact compound) were also bi-exponential. Two-compartment open-system models with metabolism and excretion occurring from the central compartment were therefore constructed to describe disposition kinetics; reasons for the use of such a model were advanced by Riegelman, Loo, and Rowland (97). Figure 3 illustrates the basic model.

Table 8 shows the cumulative urinary excretion data for INH and metabolite administration following I.V. doses of INH and each of several metabolites. A cursory examination indicates the greatest differences between the data from the two subjects occur after INH administration.

Computer and Statistical Methods

Appropriate equations were fitted to the observed data using the NONLIN program[6]. This program (98) uses an adaptation of Hartley's modification (99) of the Gauss-Newton method for the fitting of non-linear regression functions by least squares.

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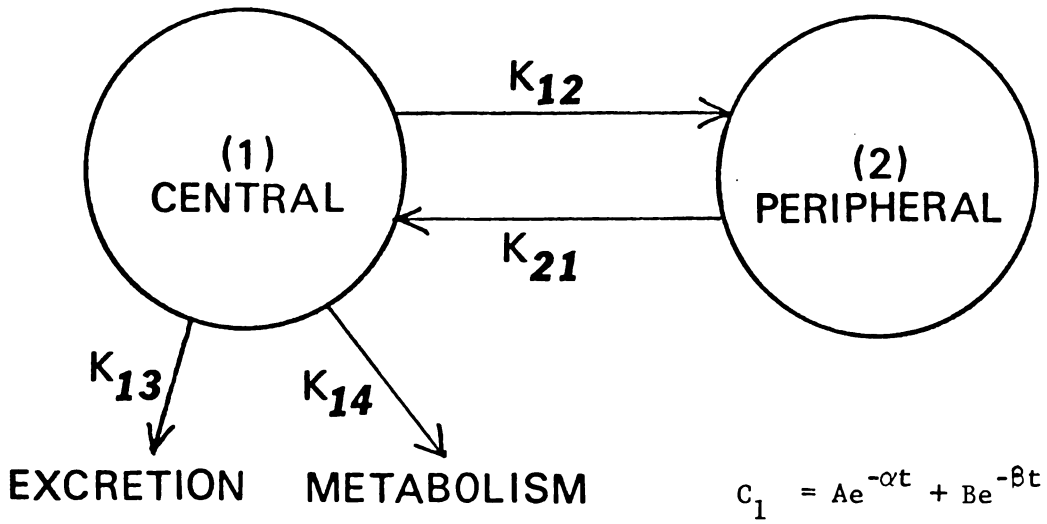
[6]This program was kindly supplied by Carl M. Metzler, The Upjohn Company, Kalamazoo, Michigan 49001. The computer used was an IBM 360/50.



$$C_1 = Ae^{-\alpha t} + Be^{-\beta t}$$

$$A = \frac{C_p^{\,o}\,(k_{21} - \alpha)}{\beta - \alpha}$$

$$B = \frac{C_p^{\,o}\,(k_{21} - \beta)}{\alpha - \beta}$$

$$C_p^{\,o} = A + B = \frac{\text{Dose}}{V_1}$$

$$b = k_{12} + k_{21} + k_{e1}$$

$$\alpha = \frac{b + \sqrt{b^2 - 4k_{e1}k_{21}}}{2}$$

$$\beta = \frac{b - \sqrt{b^2 - 4k_{e1}k_{21}}}{2}$$

$$k_{e1} = k_{13} + k_{14}$$

$$\alpha\beta = k_{21}k_{e1}$$

$$\alpha+\beta = k_{12} + k_{21} + k_{e1}$$

Figure 3. Two-Compartment Open-System Model With Metabolism and Excretion Occurring from the Central Compartment

(The dose enters the central compartment instantaneously from an intravenous injection. Arrows indicate first-order rate processes with appropriate constants. V_1 is the volume of the central compartment, and C_1 is the concentration of intact drug in the central compartment.)

TABLE 8

Cumulative Urinary Excretion of INH and Metabolites After Intravenous Administration[a]

Compound	% of Total Recovered Compounds Subject HB	 Subject SR
	INU Administration	
INU	100	100
	INA Administration	
INA	75.7	73.8
INU	24.3	26.2
	AcINH Administration	
AcINH	66.8	59.6
INA	21.1	28.6
INU	12.2	11.8
	INH Administration	
INH + Hydrazones	54.7	23.0
AcINH	25.1	38.5
INA	12.7	25.0
INU	7.57	13.5

[a]Metabolite recoveries are expressed in terms of equivalent moles of administered compound.

Least squares parameter estimates as well as their "approximate" or "asymptotic" standard deviations[7] are calculated and reported. Standard deviation (S.D.) is calculated as follows (98,100):

$$S.D. = \sqrt{S^2 \cdot C_{ii}} \tag{3}$$

where

S.D. is the standard deviation of the i^{th} estimated parameter

S^2 is the sum of the weighted squared deviations divided by the degrees of freedom, and

C_{ii} is the i^{th} diagonal element of the variance-covariance matrix of the estimates.

This S.D. approaches the true S.D. as the number of data points approaches ∞. Simulations have indicated (101) that it is not infrequent that this S.D. is 2-3 fold different from the true S.D. The estimate of the S.D. is more frequently underestimated than overestimated. The calculation of S.D. assumes and requires independence of errors at each data point (101).

Although the computer program does not make the calculation, percent coefficient of variation (%CV) may be calculated as follows (102):

$$\%CV = \frac{S.D.}{\hat{\theta}_i} \times 100 \tag{4}$$

where $\hat{\theta}_i$ is the computer estimated parameter.

---

[7]The term standard deviation or S.D. will be used throughout; it is understood that this always refers to the approximate or asymptotic standard deviation.

The computer print-out also reports the S-plane approximate 95% confidence interval[8] of the parameter estimate. In these studies the basic equation was modified, and approximate 90% confidence intervals[9] were calculated using the formula:

$$90\%\ \text{C.I.} = \hat{\theta}_i \pm \sqrt{F_{\alpha=0.1,NP,DF} \times NP \times \text{Variance}} \qquad (5)$$

where

90% C.I. is the approximate 90% confidence interval

$\hat{\theta}_i$ is the computer estimated parameter

F is derived from an F distribution table at $\alpha = 0.1$ (numerator is NP and denominator is DF)

NP is the number of parameters determined

DF is the degrees of freedom (equal to the number of data points minus NP)

Variance equals $(\text{S.D.})^2$.

If the S.D. is a large value relative to $\hat{\theta}_i$, the %CV and 90% C.I. will also be large. This means (98) that any parameter estimate in a relatively large region of the parameter space gives about equally good fit to the data. Hence the parameter may not be well determined. It should be strongly emphasized that this situation does not necessarily result from poor data; indeed, good data may result in an

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[8]This interval is approximate, since the S.D. is approximate.

[9]This 90%C.I. is a measure of the confidence in the computer determined parameter for the single study only. It is a measure of the precision of that estimate. This interval may not be used to predict future parameter estimates, even for the same subject.

excellent computer fit, but with a large S.D. An excellent discussion of this difficulty in pharmacokinetic analysis may be found in the work of Westlake (103).

A great deal of consideration was given to the form of the equations used to fit the urine data. At least 3 equations could be fit: (1) rate of excretion, (2) cumulative amount excreted, and (3) ARE or amount remaining to be excreted (i.e., amount excreted at infinity minus amount excreted at any particular time). The calculation of S.D. requires independence of errors at each data point. The very nature of the cumulative and ARE plots is such that the error associated with each point includes the errors associated with all previous data points. Only the rate of excretion equation satisfies this requirement for the S.D. calculation. Hence, while any of the 3 equations could be used, only the rate plot would result in a valid estimate of S.D.

Another disadvantage of cumulative and ARE plots is that the scatter of experimental points about the fitted line frequently shows a serious lack of randomness. Mandel (104) has presented an excellent discussion of this problem in linear regression analysis, but his arguments are also valid for non-linear situations. Mandel took the original data of Boyle from 1662 and plotted it in two different ways. The basic equation was $PV = K$, where P represents pressure, V the volume of the gas, and K is a constant. The equation[10] was rearranged to yield:

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[10]The cumulative volume measurements were made of air in a closed tube; pressure was successively increased.

$$\frac{1000}{P} = \frac{1000}{K} V \qquad (6)$$

and a plot of 1000/P vs. V was constructed. It was immediately observed that the scatter of experimental points about the fitted straight line showed a serious lack of randomness. The systematic nature of the scatter made it appear that the data, instead of conforming to a straight line, lie actually on a curve. This lack of randomness of scatter, however, was attributed to cumulative errors in the volume measurement. When the equation was modified such that changes in volume (ΔV) and changes in pressure (ΔP) were used, a randomness of scatter of experimental points about the regression line was observed.

Berman (105) has indicated that when systematic deviations exist between the calculated and observed values, the model is considered inconsistent and must be modified. While not all investigators would agree fully with Berman's analysis, randomness of scatter is generally considered to be an important criterion of goodness of fit. Hence systematic deviations arising from cumulative or ARE plots could be misinterpreted as suggesting an incorrect model, when in fact, this might only be an artifact.

The nature of scatter of experimental points about the fitted line may be investigated by the examination and analysis of residuals (106-108). An unweighted residual is defined by the following equation:

$$\begin{pmatrix}\text{Unweighted}\\ \text{Residual}\end{pmatrix} = \begin{pmatrix}\text{Observed}\\ \text{Value}\end{pmatrix} - \begin{pmatrix}\text{Calculated}\\ \text{Value}\end{pmatrix} \qquad (7)$$

A weighted residual is given by:

$$\begin{pmatrix}\text{Weighted}\\ \text{Residual}\end{pmatrix} = \begin{pmatrix}\text{Unweighted}\\ \text{Residual}\end{pmatrix} \times \left(\text{Weight}\right)^{1/2} . \qquad (8)$$

An appropriate method to examine scatter is to plot (Weighted Residual) against (Calculated Value). However, before proceeding to the examination of these plots, the subject of weighting should be discussed.

In a non-weighted least-squares analysis, $\sum_{i=1}^{N} \left(y_{i_{Obs}} - y_{i_{Calc}}\right)^2$ is minimized. Here, $y_{i_{Obs}}$ is the observed value of the i^{th} measurement, $y_{i_{Calc}}$ is the calculated or fitted value, and N is the number of data points. Daniel and Wood (109) have indicated that this method is statistically valid only when all observations in y have the same (though possibly unknown) variance. For the data presented here, this is not the case, and weighting is therefore required. When observed values in y_i do not have the same variance, each value of y_i should be weighted inversely as its variance (110). If one assumes that the variance of y_i is proportional to y_i, each y_i may be weighted as $1/y_i^2$ (111).

In each of the urine assays in these studies, urine was diluted such that each sample contained approximately the same concentration of compound to be determined. Consequently, each determination would have approximately the same percent error (this assumes no errors in urine volumes and dilutions). With the condition that each sample have the same percent error, a weighting factor of $1/y_i^2$ is appropriate (112). Thus the weight assigned each data point (w_i) may be determined by

substitution of y_i into the expression, $1/y_i^2$. A normalization of the weights may be achieved by applying the following equation (112):

$$\frac{\frac{1}{y_i^2}}{\sum_{i=1}^{N} \frac{1}{y_i^2}} \times N \qquad (9)$$

In the weighted least squares analysis, $\sum_{i=1}^{N} \left(y_{i_{Obs}} - y_{i_{Calc}}\right)^2 w_i$ is minimized. If one used the normalized weights obtained from Equation (9), comparison between non-weighted and weighted sums of squared deviations may be made (112). This is a consequence of the sums of weights being equal in both situations (in a non-weighted analysis, each weight actually has a value of one). Thus if weighting resulted in a lower sum of weighted squared deviations than the non-weighted sums of squared deviations, it could be concluded that weighting improved the fit.

In all the computer analyses reported herein, the normalized weighting factor defined by Equation (9) was used to determine weights.[11] In the case of INH blood concentrations it was also felt that all measurements had <u>approximately</u> the same percent error. This was a consequence of using several calibration curves, and setting the sample of greatest concentration from each calibration curve to a meter reading of 100. Thus each INH unknown was determined by reading

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[11] While the normalization procedure was originally adopted for the purpose of comparing weighted and non-weighted fits of data, these comparisons were never made; all data were weighted.

the meter in the vicinity of 50-100. In the concentration ranges determined, sensitivity was not a problem. Each of the calibration curves appeared to be equally well fit by a straight line. Thus while it is likely that not all INH blood levels were determined with precisely the same percent error, it was felt that this was not a poor approximation. Consequently, the normalized weighting factor previously described was used.

As was mentioned previously, plots of weighted residuals against calculated values may be used to examine the nature of scatter.[12] It should be apparent that squaring and subsequent summing of the weighted residuals gives the quantity which is minimized in a weighted least squares analysis. The urinary excretion data for INU in subject HB was fitted by NONLIN in three ways: (1) rate of excretion _vs_. time, (2) cumulative amount excreted _vs_. time, and (3) ARE _vs_. time. The model used to develop the equations is shown in Figure 8. INU was infused at a zero order rate for 60.0 minutes, and the infusion was then discontinued. Appropriate equations are given in the appendix. The primary disadvantage of rate of excretion plots is the assumption that the instantaneous rate of excretion (dAe/dt) can be approximated by $\Delta Ae/\Delta t$ (ΔAe is the amount excreted in the time interval Δt). The time corresponding to the rate of excretion, $\Delta Ae/\Delta t$, is taken to be the mid-point of the time interval. Residual plots of the computer fitted data are shown in Figures 4-6. When scatter is random, points tend to be randomly scattered above

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[12] Plots of weighted residuals _vs_. time are also useful.

and below the horizontal line. Examination of Figure 4 (rate of excretion residual plot) reveals a satisfactory randomness of scatter. On the contrary, residual plots in Figures 5 and 6 for the cumulative and ARE data, respectively, indicate systematic clustering of data (encircled by dotted lines). Comparison of the data in Figure 4 to those in Figures 5 and 6 appear to support the contention that the rate of excretion equation offers the best alternative for the fitting of urinary excretion data.

Another important consideration in the fitting of data is the form in which the equations are fitted. For the model shown in Figure 3, four parameters are needed to fully describe the system. These parameters could be α, β, k_{21}, and V_1 or, alternatively, k_{12}, k_{21}, k_{e1}, and V_1. The constants k_{13} and k_{14} may be determined from knowledge of the cumulative amounts of metabolite(s) formed and intact compound excreted at infinite time. When there are no blood data, but urinary excretion data for intact compound are available, V_1 may <u>not</u> be determined; in such a situation, only 3 parameters need be determined. For the studies reported herein, equations were derived and are included in the appendix. These contain hybrid constants (indicated by Greek letters), volume of the central compartment (when appropriate), and the constants describing transfer of the compounds from the peripheral to central compartments. The best values of the parameters were estimated by the computer program. Other rate constants (e.g., k_{e1}, k_{13}, k_{14}, and k_{12} of Figure 3) were then determined by standard methods (97,113). When α, β, k_{21}, and V_1 were estimated

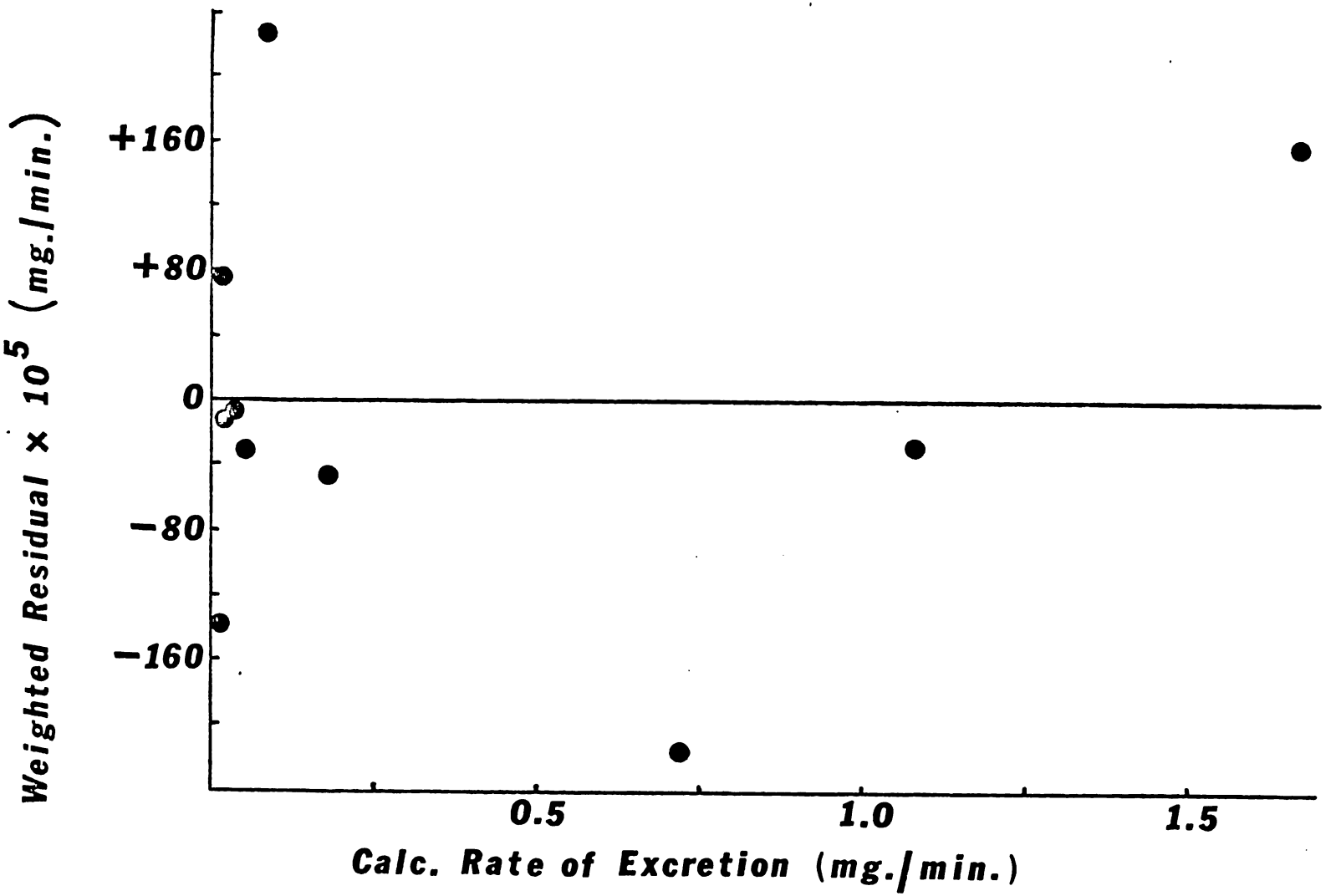

Figure 4. Weighted Residual Plot of Rate of Excretion Data from INU Administration Study in Subject HB (see text for explanation).

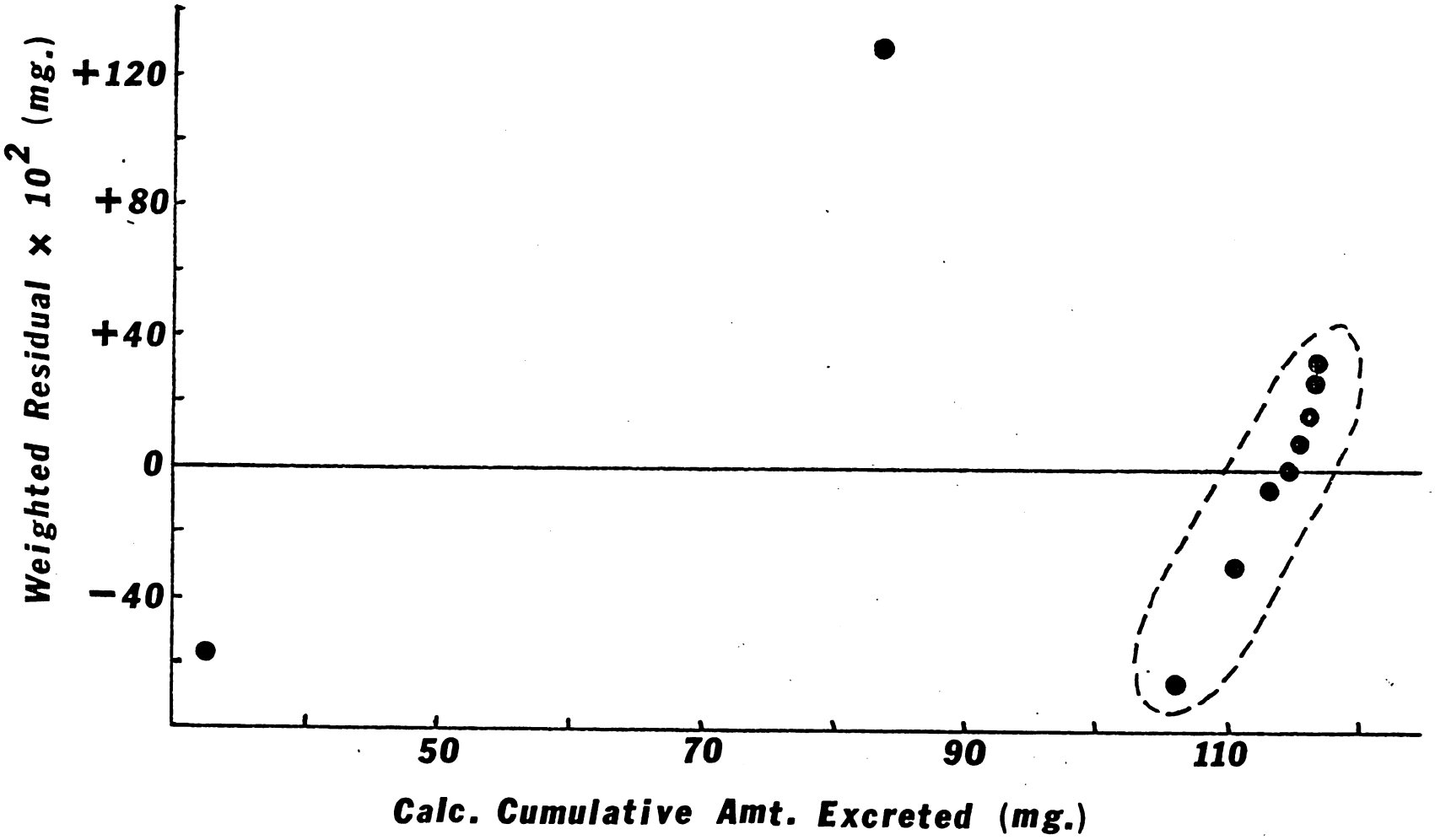

Figure 5. Weighted Residual Plot of Cumulative Excretion Data from INU Administration Study in Subject HB (see text for explanation)

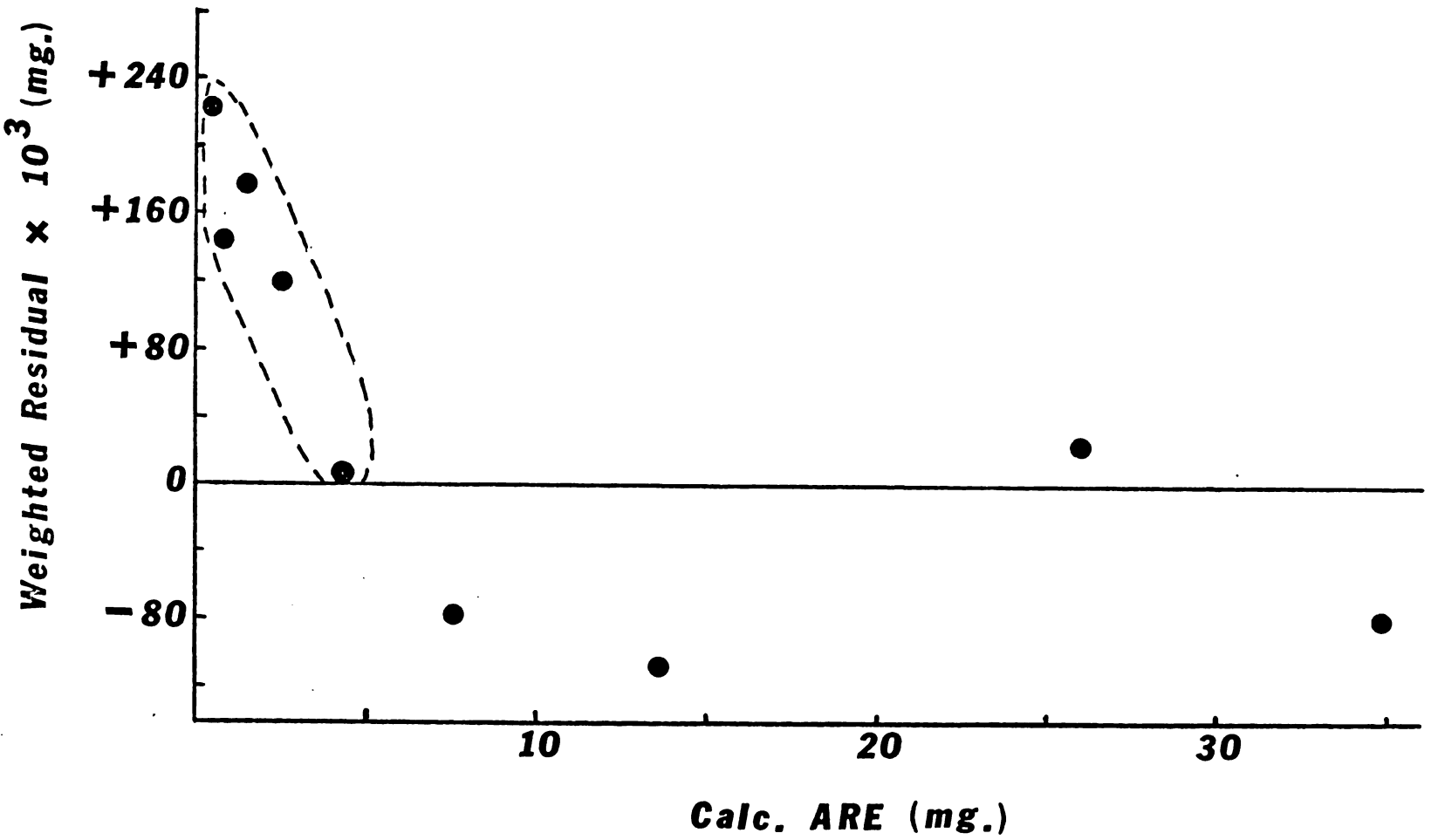

Figure 6. Weighted Residual Plot of ARE Data from INU Administration Study in Subject HB (see text for explanation)

by the computer program, S.D.'s of these estimates were calculated and printed out. To determine the S.D.'s of k_{e1}, k_{13}, k_{14}, and k_{12}, the following equation was used (101):

$$(S.D._F)^2 = (F_a S.D._a)^2 + (F_b S.D._b)^2 + (F_c S.D._c)^2 + \ldots$$
$$+ 2(F_a F_b COV_{a,b} + F_a F_c COV_{a,c} + F_b F_c COV_{b,c} \ldots) \qquad (10)$$

where $F_a = \frac{\partial F}{\partial a}$; $F_b = \frac{\partial F}{\partial b}$; $F_c = \frac{\partial F}{\partial c}$; and

F is the defining function,

$S.D._i$ is the standard deviation of the i^{th} estimated parameter,

a,b,c, ... are computer estimated parameters and are functions of F,

$COV_{i,j}$ is the covariance of the i^{th} and j^{th} parameters,

F_a, F_b, F_c, ... are evaluated at the computer estimated values for a, b, c, ...

For example, the standard deviation of k_{e1} (see Figure 3) would be calculated as follows:

$$k_{e1} = \frac{\alpha\beta}{k_{21}} \qquad (11)$$

$$F_\alpha = \frac{\beta}{k_{21}} \quad F_\beta = \frac{\alpha}{k_{21}} \quad F_{k_{21}} = \frac{-\alpha\beta}{k_{21}^2} \qquad (12a,b,c)$$

$$\left(S.D._{k_{e1}}\right)^2 = \left(\frac{\beta}{k_{21}}\right)^2\left(S.D._\alpha\right)^2 + \left(\frac{\alpha}{k_{21}}\right)^2\left(S.D._\beta\right)^2 + \left(\frac{-\alpha\beta}{k_{21}^2}\right)^2\left(S.D._{k_{21}}\right)^2$$
$$+ \left(2\right)\left(\frac{\beta}{k_{21}}\right)\left(\frac{\alpha}{k_{21}}\right)\left(COV_{\alpha,\beta}\right) + \left(2\right)\left(\frac{\beta}{k_{21}}\right)\left(\frac{-\alpha\beta}{k_{21}^2}\right)\left(COV_{\alpha,k_{21}}\right) +$$
$$2\left(\frac{\alpha}{k_{21}}\right)\left(\frac{-\alpha\beta}{k_{21}^2}\right)\left(COV_{\beta,k_{21}}\right) . \qquad (13)$$

The variance of k_{el} or $(S.D._{k_{el}})^2$ would then be evaluated by substituting the computer estimates of α, β, k_{21} and the appropriate S.D.'s and covariances into the equation. The covariances are calculated by the computer and are printed out.[13]

Initial estimates of the parameters are required by the program. Graphical estimates of the parameters for the administered compound were determined by analysis of the post-infusion blood or post-infusion rate of excretion curves (114). Computer estimates of INU parameters were used as initial estimates to describe INU disposition in the INA computer analysis. Computer estimates of INU and INA parameters from the INA analysis were subsequently used as initial estimates to describe disposition of these compounds in the AcINH analysis. Likewise, the INU, INA, and AcINH computer parameter estimates from the AcINH analysis were used as initial estimates in the INH analyses. When multiple curves were being fit, and the initial estimates were poor, some computer estimated parameters were poorly determined (i.e., certain curves were poorly fit). This most frequently occurred in rate of excretion plots of terminal compounds in a catenary chain with more than 2 compounds being involved. More realistic initial estimates subsequently were used, and the data were then better fitted.

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[13]The "Variance-covariance matrix of estimates" on the print out are actually the variances and covariances divided by S^2. These values therefore need be multiplied by S^2 to obtain the variances and covariances.

The t statistic, used to compare parameter estimates from different studies, was calculated from the formula (101):

$$t = \left| \frac{\hat{\theta}_{ij} - \hat{\theta}_{ik}}{[(S.D._{ij})^2 + (S.D._{ik})^2]^{1/2}} \right| \qquad (14)$$

where

$\hat{\theta}_{ij}$ is the computer estimated i^{th} parameter in study j

$\hat{\theta}_{ik}$ is the computer estimated i^{th} parameter in study k

$S.D._{ij}$ is the standard deviation of the i^{th} computer estimated parameter from study j

$S.D._{ik}$ is the standard deviation of the i^{th} computer estimated parameter from study k.

A null hypothesis was established: $\theta_{ij} = \theta_{ik}$ (θ_{ij} and θ_{ik} are the true parameter values for the respective studies). The t statistic, derived from Equation (14), was then compared to a value of t determined from a statistics table (2 tail, P = 0.05, DF = (# data points j^{th} study) + (# data points k^{th} study) - NP_j - NP_k). The later terms are the total number of parameters determined in the respective studies. When the calculated value of t was less than the value of t for conditions above, the null hypothesis was accepted. The application of the t test assumes that true values for the i^{th} parameter are normally distributed. One may not place absolute faith in the t test, because the S.D.'s are approximate.

Another statistic calculated in some studies was the F ratio. This was used to compare weighted sums of squared deviations obtained

when different equations were used to fit the same data. This statistic was calculated from the formula (101):

$$\frac{WSS_j - WSS_k}{WSS_k} \times \left(\frac{DF_k}{DF_j - DF_k}\right) = F \qquad (15)$$

where

WSS_j was the weighted sums of squared deviations obtained from the j^{th} set of parameters

WSS_k was the weighted sums of squared deviations obtained from the k^{th} set of parameters

DF is equal to the number of data points used to fit the curve(s) minus the number of parameters fit ($DF_j > DF_k$).

The calculated F was compared to the F statistic derived from a table (numerator has ($DF_j - DF_k$) degrees of freedom and denominator has DF_k degrees of freedom). When the calculated F ratio was less than that determined from the table, it was concluded that the weighted sums of squared deviations were not significantly different.

RESULTS AND DISCUSSION

Preliminary Remarks

Before analyzing the data, it is appropriate to refer to Figure 1 in which a scheme for the metabolism of INH in man is presented. During the studies, it became clearly apparent from the data that INH, AcINH, INA, and INU disposition each would require multicompartmental analysis. A two compartment body model such as illustrated in Figure 3 was proposed for each of the aforementioned compounds. A system of greek letters was used to discriminate between the exponential hybrid rate constants for each compound, and appropriate "microscopic" constants were utilized to describe transfer between compartments. Figure 7 illustrates the total model.

INU Administration Studies

Following intravenous infusion of INU in the two test subjects, only intact compound was detected in urine. Post-infusion rate of excretion-time curves appeared to be biexponential, and the model shown in Figure 8 was used to describe INU disposition. A single equation describing the rate of urinary excretion of INU both during and after the zero-order infusion was derived and is presented in the Appendix. The equation was fitted to the data by computer, and the estimated parameters are presented in Table 9. The %CV's of the estimated

Hybrid Rate Constants

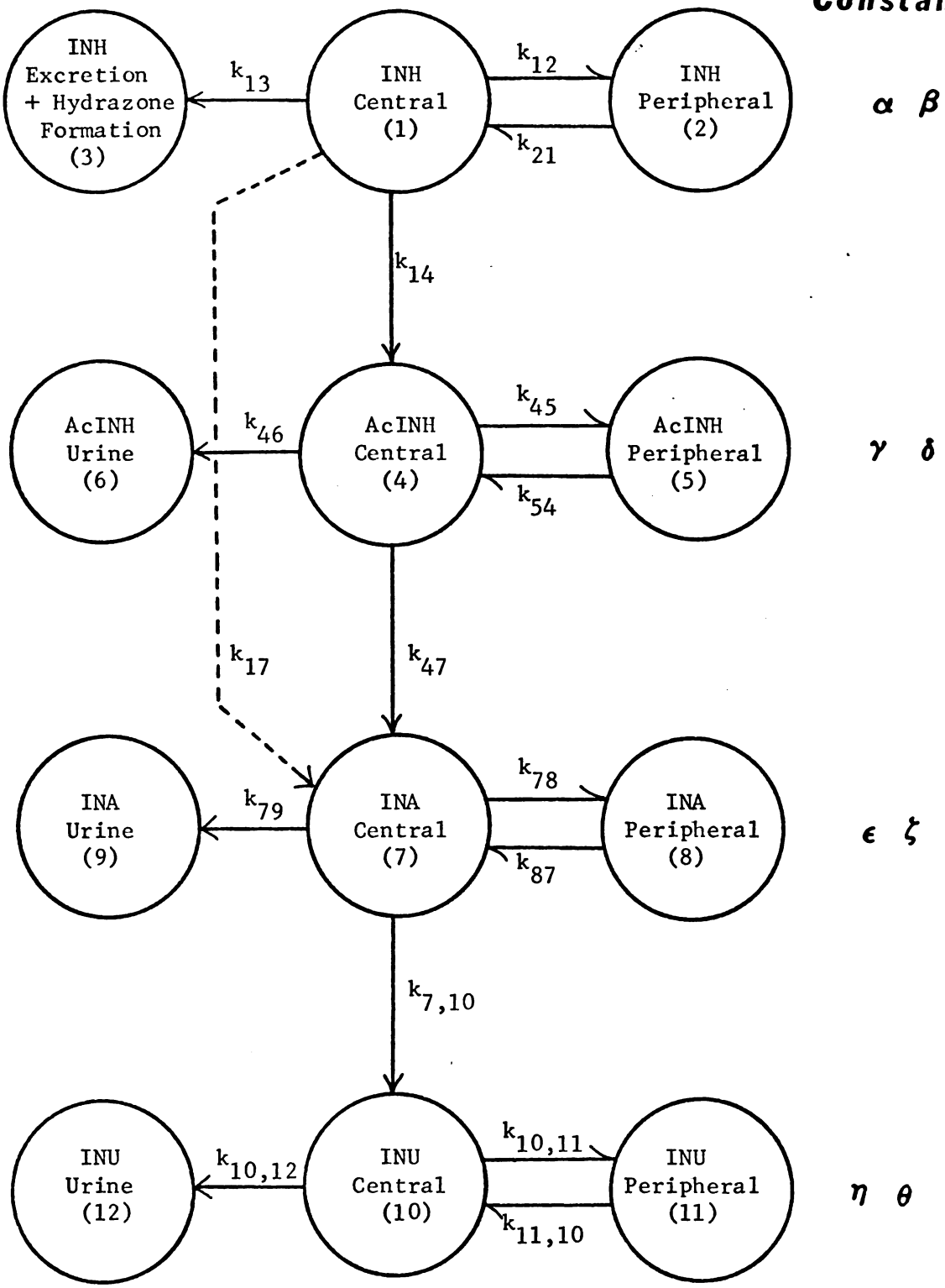
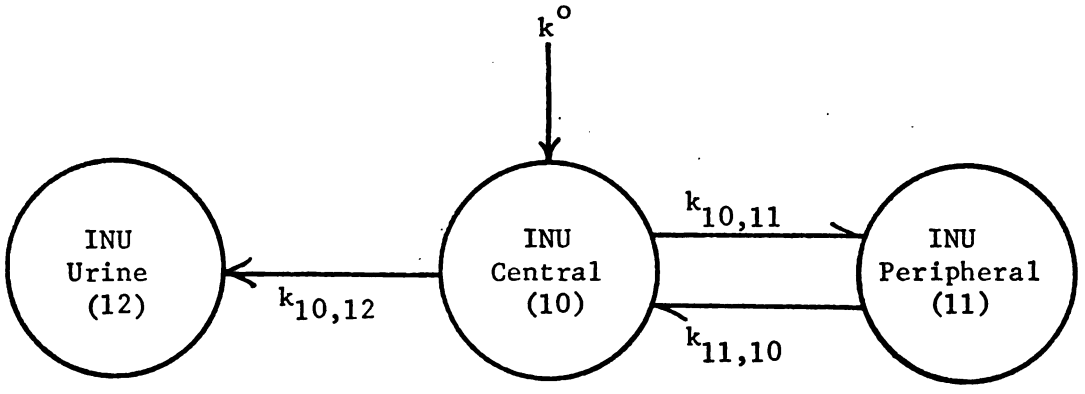


Figure 7. Model Used to Describe Disposition of INH and Its Metabolites. (Arrows represent first-order rate processes; appropriate hybrid rate constants are shown beside the corresponding compounds. The existence of the pathway described by k_{17} is discussed in the text.)



k^o = zero-order infusion rate

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

Figure 8. Model Used to Describe INU Disposition Including Zero-Order Input. (With the exception of the zero-order input, arrows represent first-order processes.)

TABLE 9

Parameter Estimates from INU Administration Studies

Parameter	Parameter Estimate ($min.^{-1}$) Subject HB	Subject SR	t test[a]
η	.0675 (2.42)[b] (.0625-.0725)[c]	.0473 (6.81) (.0380-.0566)	-
θ	.0114 (1.73) (.0108-.0120)	.0129 (6.55) (.0105-.0153)	+
$k_{11,10}$	.0133 (1.65) (.0126-.0140)	.0158 (6.01) (.0130-.0186)	-
$k_{10,11}$	.00787 (4.04) (.00690-.00884)	.00579 (17.3) (.00289-.00869)	+
$k_{10,12}$	.0577 (2.44) (.0534-.0620)	.0386 (6.30) (.0315-.0457)	-

[a]Positive sign indicates acceptance of the null hypothesis that the "true" parameters from the two subjects are equal; negative sign indicates rejection (5% level of significance).

[b]Percent coefficient of variation.

[c]Approximate 90% confidence interval.

parameters are relatively small (maximum of 17.3); consequently, the approximate 90% confidence intervals are relatively narrow. The t test indicates that not all parameters are the same for the two subjects.

Theoretical rate of excretion-time curves were generated using the estimated values of the parameters, and these are shown in Figures 9 and 10 with the experimental data points. An examination of the scatter of the data points about the fitted lines indicates satisfactory randomness of scatter.

Ellard and Gammon (6) 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 (115)), and indicates active tubular secretion of INU by the renal tubules. Since INU renal clearance is equal to $k_{10,12}V_{10}$, V_{10} may be estimated.[14] This value was calculated to be approximately 10 liters, which is almost twice the estimated blood volumes.

Ellard and Gammon (6) 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 θ reported herein (0.0114 $min.^{-1}$ and 0.0129 $min.^{-1}$ in subjects HB and SR, respectively).

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[14] V_{10} is the volume of compartment 10.

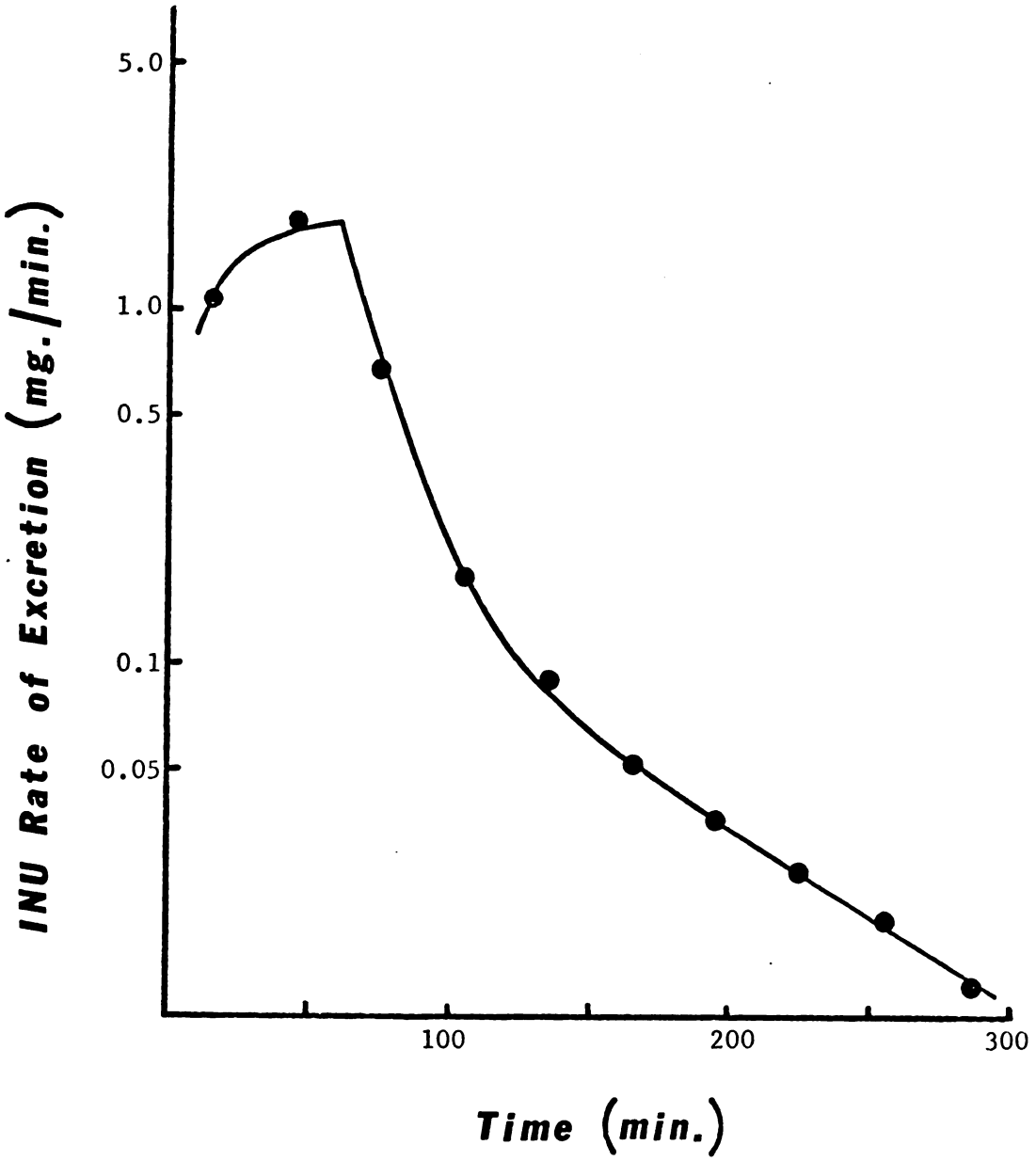


Figure 9. Rate of Excretion of INU in Subject HB. (INU was infused over a period of 60.0 minutes.)

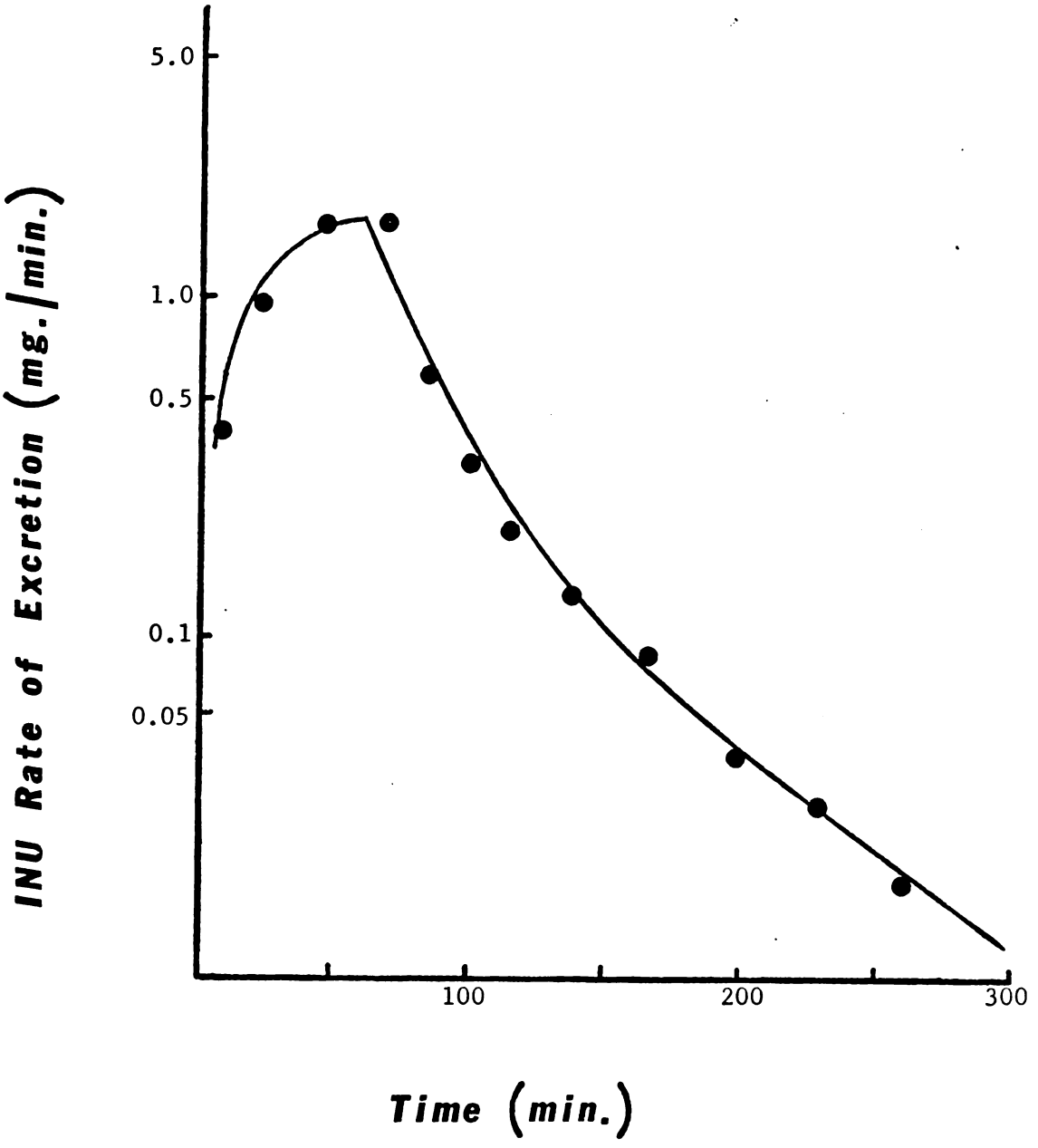


Figure 10. Rate of Excretion of INU in Subject SR. (INU was infused over a period of 60.0 minutes.)

INA Administration Studies

The two test subjects were administered approximately 200 mg. INA by a one-hour intravenous infusion. Subsequently, INA and INU appeared in urine; subjects HB and SR excreted 24.3 and 26.2% of the dose, respectively, as INU. Peters, Miller, and Brown (5) administered INA orally to 7 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 percent of the dose recovered as INU (range of 19.3 to 48.3%). Ellard and Gammon (6) 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 30.0% of the recovered dose was excreted as INU after the higher dose. Both Peters, Miller, and Brown (5) and Ellard and Gammon (6) found no correlation between an individual's acetylation phenotype and his capacity to conjugate INA with glycine.

The data reported herein as well as that of Peters, Miller, and Brown (5) and Ellard and Gammon (6) are summarized in Table 10. A decreasing percent conversion of INA to INU with increasing INA dose would be expected if the enzyme responsible for glycine conjugation were becoming saturated or a rate limitation was imposed by co-factor or co-substrate availability; such a pattern is not observed.

Prior to the studies with controlled urinary pH reported herein, a preliminary study was undertaken. A 1.00 Gm. dose of INH was administered orally to subject HB on an empty stomach; no attempt

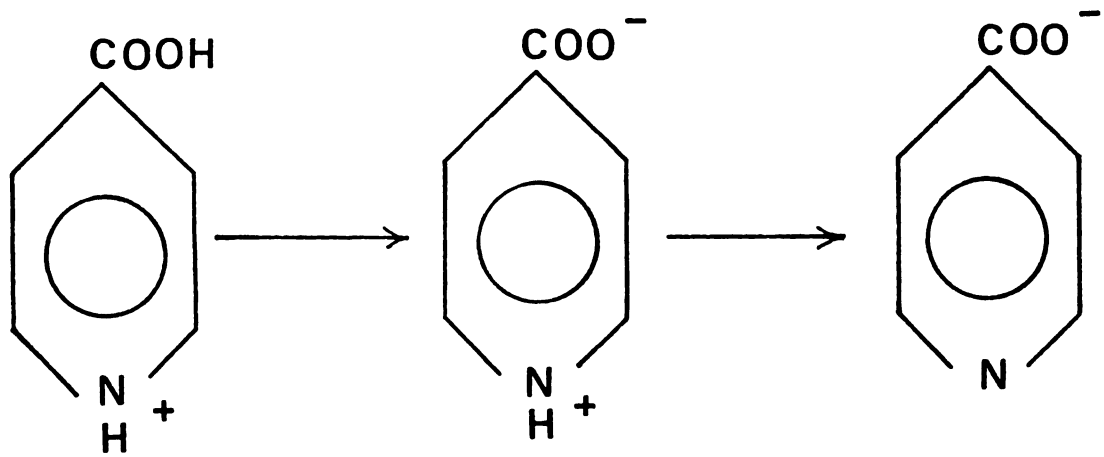
TABLE 10

Average Percent of INA Doses Recovered in Urine as the Glycine Conjugate (INU)

Approximate Dose	Route of Administration	Reference	# Subjects	INU as a % of Total Recovered Compounds[a]	Urine pH Control
25 mg	Oral	6	2	43.6	No
200 mg	I.V.	Herein	2	25.2	Yes
250 mg	Oral	6	1	30.0	No
315 mg	Oral	5	7	34.9	No

[a]Average for the number of subjects noted in Column 4.

was made to control urine volume or pH. Figure 11 shows the data[15] from this study. The rate of excretion of INA was unusually low between 60 and 230 minutes, and this appeared to be related to urine pH. As the urine pH dropped to a value of approximately 5, the rate of excretion dropped. The molecular dissociation characteristics of INA[16] are shown below:



At pH 5, the zwitterionic species of INA would be more prevalent than at higher pH's. Quite possibly this neutral species was

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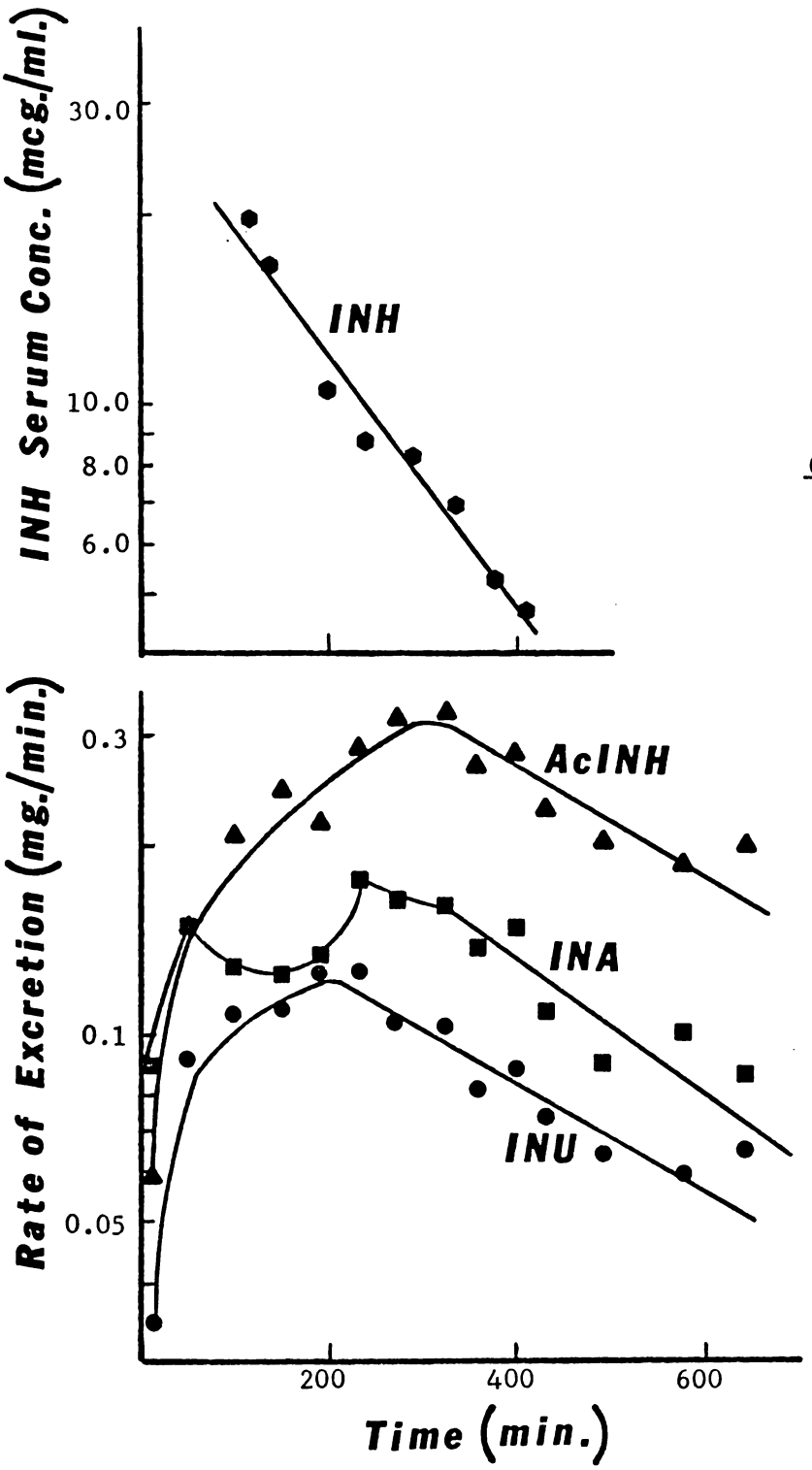
[15]INH was assayed in serum by the method of Scott and Wright (91); urine assays were those reported herein.

[16]The pKa's of INA are well established; the dissociation of the carboxylic hydrogen prior to the pyridinium hydrogen is assumed.



Time (min.)	Urine Flow Rate (ml./min.)	Urine pH
13.5	.811	5.10
49.0	.700	7.00
99.5	.524	5.20
149.0	.495	5.05
191.5	.484	5.07
229.5	1.02	6.41
273.0	1.06	7.40
321.5	1.14	7.19
357.5	.966	6.99
394.0	1.70	6.74
428.5	3.02	6.13
490.5	1.67	5.69
574.0	.941	6.30
643.5	.803	4.80

Figure 11. Serum Levels of INH and Rates of Excretion of AcINH, INA, and INU After Oral Administration of 1.00 Gm INH on an Empty Stomach. (Curves are empirically drawn; urine flow rates and pH values are given.)

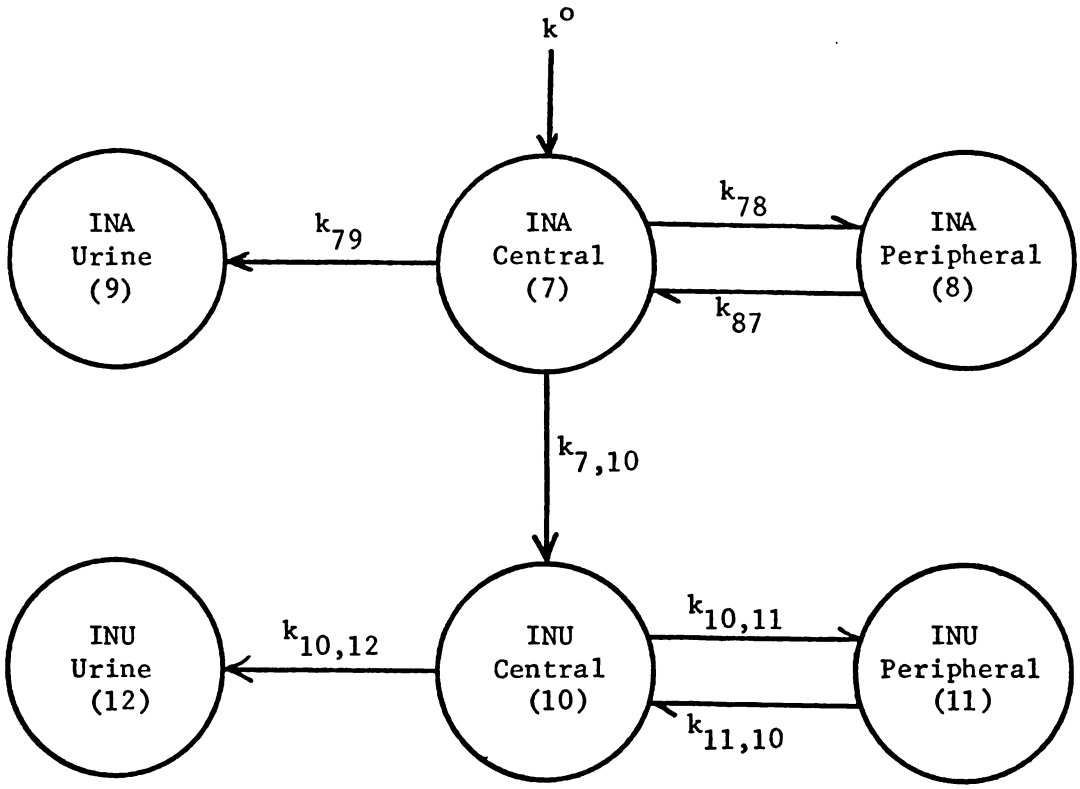
being reabsorbed from the renal tubules to a greater extent than the negatively charged carboxylate species. Hence, one could rationalize the depressed rate of excretion as being caused by an increased rate of renal tubular reabsorption. A decision was therefore made to conduct the urinary excretion studies under conditions in which the urine was alkalinized by administration of sodium bicarbonate in appropriate dosage. Loading doses of bicarbonate were given and maintenance doses were adjusted during the studies by monitoring the pH of each urine collection (see experimental section for details).

It should be noted that an alternative explanation for the results shown in Figure 11 might be that the excretion was limited by urine flow rate. Urine flow rates, however, did not seem to affect INA excretion when the compound was administered with sodium bicarbonate.

On the basis of the preceding arguments, it seems clear that future studies to determine the quantitative properties of the INA conjugating system should be undertaken with conditions of controlled urine pH.

In the present analysis, a linear pharmacokinetic model was employed (i.e., all processes were treated as being first-order). Following intravenous infusion of INA, post-infusion INA rate of excretion-time curves appeared to be biexponential for each subject. As is customary (97), a two-compartment body model for INA disposition was formulated; metabolism and excretion were assumed to occur from the central compartment. 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 Figure 12, and the equations for the rates of excretion of INA and INU are in the Appendix. The two equations were fitted simultaneously to the data by computer, and the estimated parameters and statistical data are presented in Table 11. Before discussing these data, a number of important points need be mentioned. The equations fitted to the data contain the term f_7 (see Figure 12); the value of this term was determined from urinary excretion data $\left(f_7 = \frac{A_9^{\infty}}{(A_9 + A_{12})^{\infty}}\right)$ 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 6 to 7. For a given subject, statistical analysis by the t and F tests indicated that the values of f_7 and the weighted sums of 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 S.D.'s. When parameters 1 through 6 were defined as ϵ, ζ, k_{87}, η, θ, and $k_{11,10}$, respectively, different S.D.'s of all parameters were calculated than when parameters 1 through 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 S.D.'s (116).



k^o = Zero-order infusion rate

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

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

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

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

Figure 12. Model Used to Describe INA Disposition Including Zero-Order Input. (With the exception of the zero-order input, arrows represent first-order processes.)

TABLE 11

Parameter Estimates from INA Administration Studies

Parameter	Parameter Estimate ($min.^{-1}$) Subject HB	Subject SR	t test[a]
ϵ	.0621 (9.03)[b] (.0425-.0817)[c]	.0416 (8.82) (.0286-.0546)	-
ζ	.0184 (8.09) (.0128-.0240)	.0118 (18.7) (.00401-.0196)	-
k_{87}	.0228 (12.7) (.0127-.0329)	.0136 (22.8) (.00262-.0246)	-
k_{78}	.00769 (24.1) (.00118-.01420)	.00380 (29.0) (-.000095-.00770)	+
k_{70}	.0499 (5.58) (.0401-.0597)	.0359 (5.49) (.0289-.0429)	-
k_{79}	.0378 (5.58) (.0304-.0452)	.0265 (5.49) (.0213-.0317)	-
$k_{7,10}$	.0121 (5.58) (.00973-.0145)	.00941 (5.49) (.00758-.0112)	-
η	.0991 (12.5) [-][d] (.0557-.1425)	.102 (23.1) [-] (.0184-.186)	+
θ	.0113 (19.5) [+] (.00360-.0190)	.0157 (14.9) [+] (.00738-.0240)	+
$k_{11,10}$	.0138 (21.3) [+] (.00348-.02412)	.0204 (20.9) [+] (.00528-.0355)	+
$k_{10,11}$	.0158 (21.6) [-] (.00383-.0278)	.0187 (45.0) [+] (-.0112-.0486)	+
$k_{10,12}$	.0807 (11.0) [-] (.0498-.112)	.0786 (19.3) [-] (.0248-.132)	+

[a]Positive sign indicates acceptance of the null hypothesis that the "true" parameters from the two subjects are equal; negative sign indicates rejection (5% level of significance).

[b]Percent coefficient of variation.

[c]Approximate 90% confidence intervals.

[d]Positive sign in square bracket next to % CV indicates acceptance of the null hypothesis that the "true" parameter from this study is equal to the "true" parameter from the INU studies; negative sign indicates rejection (5% level of significance).

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 S.D.'s, this order was adopted in these and subsequent studies. Lastly, the influence of fitting two or more curves on the S.D.'s of the parameter estimates needs be mentioned. For the data reported here, INA and INU curves were fit simultaneously. One could, however, just fit the INA curve with 3 parameters; when this approach was taken with the data from subject HB, slightly different estimates of the 3 parameters were computed, but the %CV's were substantially lower (approximately 1.7 to 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 11, the t test indicates that the "true" values of all but 1 of the INA disposition parameters are different for the two subjects; the "true" values of all the INU disposition parameters appear equal between subjects.[17] Comparing the INU disposition parameters in these studies to those determined after INU administration reveals that some of the parameter values are found to be equal by the t test whereas others are not. A trend begins here

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[17] In this and subsequent t tests, small values for t result as a consequence of large values for the S.D.'s; 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 S.D.'s.

which continues in subsequent studies. The %CV's 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 (98). The large %CV's 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.

Figures 13 and 14 show the theoretical rate of excretion-time curves generated from the computer estimated parameters together with the experimental data points. For both subjects, 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 (6) reported the renal clearance of INA in one subject to be 453 ml./min.; because renal clearance is equal to $k_{79}V_7$, V_7 may be estimated.[18] The calculated value is approximately 14 liters, and this is almost 3 times the estimated blood volumes. As with INU, INA is actively secreted by the renal tubules.

Ellard and Gammon (6) 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 HB and SR, respectively). The metabolism rate constant reported was 0.0050 $min.^{-1}$, and this also is in good agreement

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[18] V_7 is the volume of compartment 7.

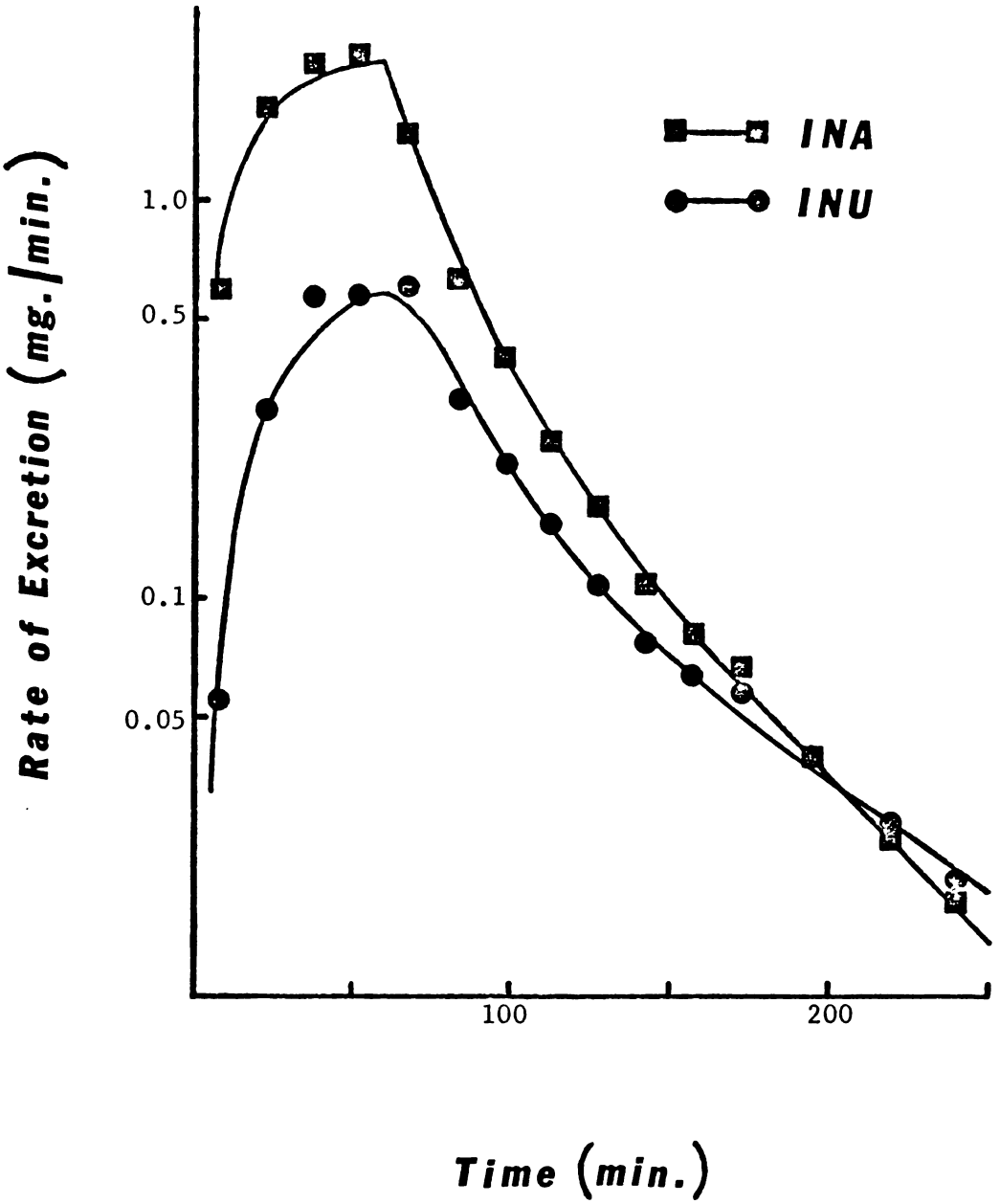


Figure 13. Rate of Excretion of INA and INU in Subject HB After INA Administration. (INA was infused over a period of 60.0 minutes.)

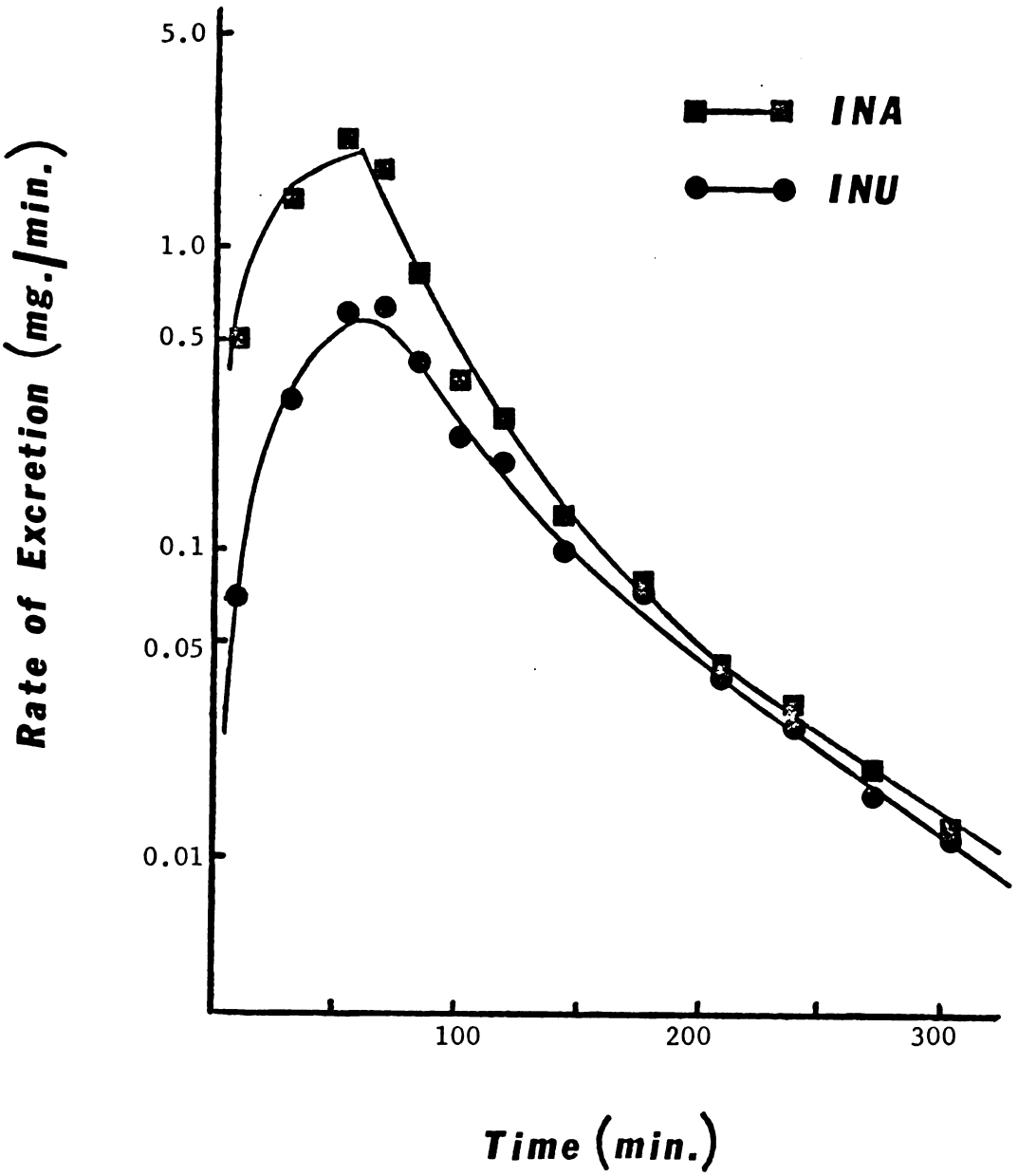


Figure 14. Rate of Excretion of INA and INU in Subject SR After INA Administration. (INA was infused over a period of 60.0 minutes.)

with the values calculated here (0.00447 $min.^{-1}$ and 0.00309 $min.^{-1}$ for subjects HB and SR, respectively).

AcINH Administration Studies

Following intravenous infusion of AcINH in the two test subjects, AcINH, INA, and INU were detected in urine. Table 12 shows the percentages of the dose excreted as the various compounds; the data of Peters, Miller, and Brown (5) and Ellard and Gammon (6) are also included for comparison. There is good agreement among all 3 sets of data.

Following intravenous infusion of AcINH in these studies, post-infusion AcINH rate of excretion-time curves appeared to be bi-exponential. A two-compartment body model for AcINH was formulated with metabolism and excretion occurring from the central compartment. Two compartment body models were also used to describe both INA and INU disposition; metabolism and excretion of these metabolites were also assumed to occur from their central compartments. The overall model used to describe AcINH disposition is shown in Figure 15, and the equations for the rates of excretion of AcINH, INA, and INU are in the Appendix.

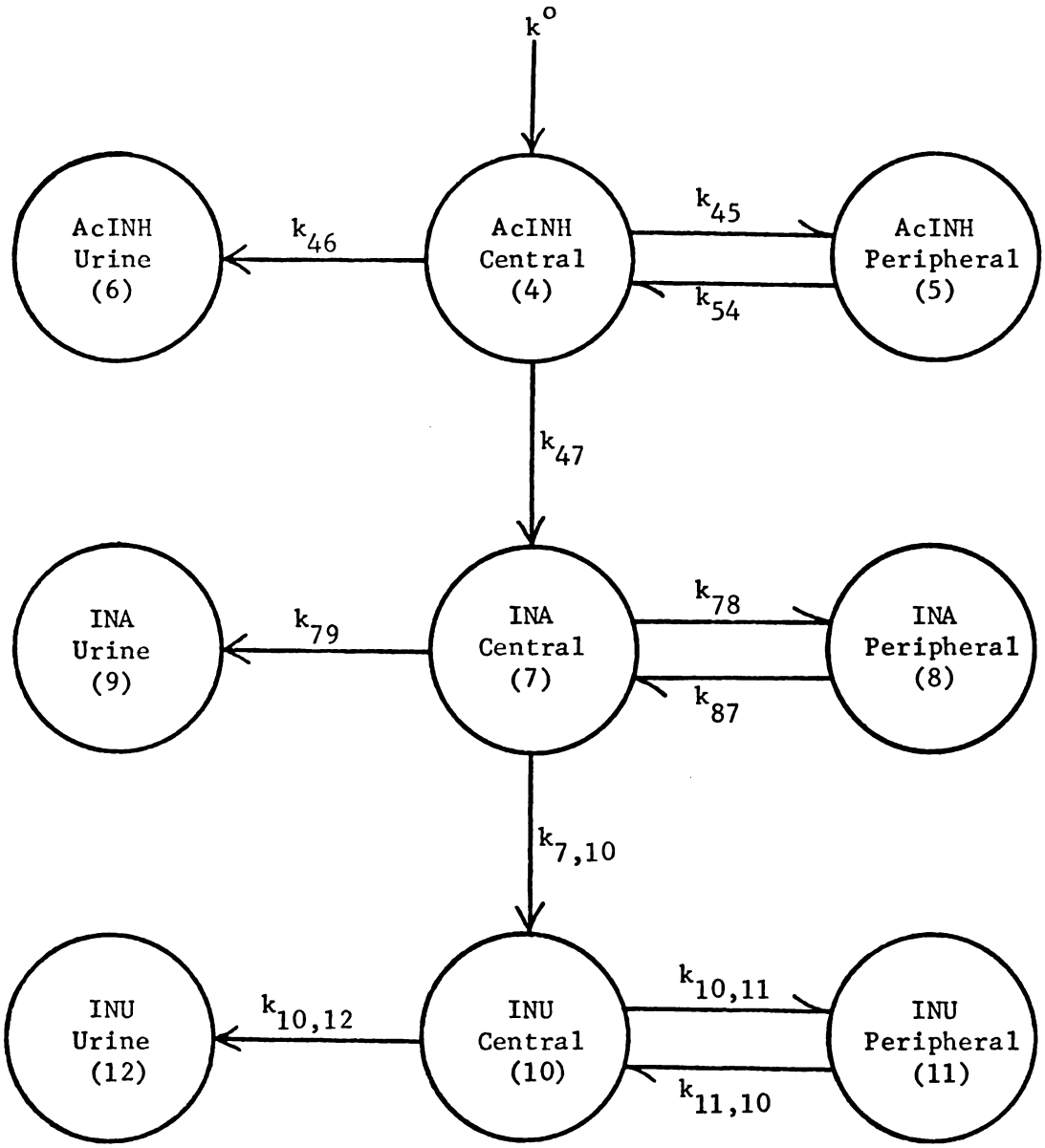
All three equations were fitted to the data simultaneously, and the estimated parameters and statistical data are presented in Table 13. The values of f_4 and f_7 (see Figure 15) were determined by standard methods (113, 117) and were held constant in the computer analyses. The t test indicates that most parameters from the two subjects are equal. Acceptance of the null hypotheses in many of these tests

TABLE 12

Average Percent of AcINH Doses Recovered in Urine Intact and as Metabolites

Approximate Dose	Route of Administration	Reference	# Subjects	Urine pH Control	% of Total Recovered Compounds as[a]		
					AcINH	INA	INU
458 mg	Oral	5	7	No	49.9	32.5	17.6
500 mg	Oral	6	2	No	60.3	25.2	14.5
580 mg	I.V.	Herein	2	Yes	63.2	24.8	12.0

[a]Average for the number of subjects noted in Column 4.



k^o = Zero-order infusion rate

$\gamma\delta = k_{40}k_{54}$ $\quad \varepsilon\zeta = k_{70}k_{87}$ $\quad \eta\theta = k_{10,0}k_{11,10}$

$k_{40} = k_{46} + k_{47}$

$k_{70} = k_{79} + k_{7,10}$ $\quad k_{10,0} = k_{10,12}$

$f_4 = \frac{k_{46}}{k_{40}}$ $\quad f_7 = \frac{k_{79}}{k_{70}}$

Figure 15. Model Used to Describe AcINH Disposition Including Zero-Order Input. (With the exception of the zero-order input, arrows represent first-order processes.)

TABLE 13

Parameter Estimates from AcINH Administration Studies

Parameter	Parameter Estimate ($min.^{-1}$) Subject HB	Subject SR	t test[a]
γ	.0456 (9.76)[b] (.0281-.0631)[c]	.0324 (11.9) (.0172-.0476)	-
δ	.00389 (1.83) (.00361-.00417)	.00389 (5.19) (.00309-.00469)	+
k_{54}	.0171 (8.15) (.0116-.0226)	.0122 (12.3) (.00629-.0181)	-
k_{45}	.0220 (13.2) (.0106-.0334)	.0137 (16.3) (.00481-.0226)	-
k_{40}	.0104 (4.55) (.00855-.0123)	.0104 (5.54) (.00812-.0127)	+
k_{46}	.00692 (4.55) (.00568-.00816)	.00617 (5.54) (.00481-.00753)	+
k_{47}	.00345 (4.55) (.00283-.00407)	.00418 (5.54) (.00326-.00510)	-
ϵ	.0934 (48.8) (-.0855-.272) [+][d]	.0643 (34.1) (-.0226-.151) [+]	+
ζ	.0141 (51.7) (-.0145-.0427) [+]	.00738 (86.0) (-.0178-.0326) [+]	+
k_{87}	.0216 (79.7) (-.0459-.0891) [+]	.00943 (99.9) (-.0280-.0468) [+]	+
k_{78}	.0251 (98.5) (-.0718-.122) [+]	.0120 (87.7) (-.0297-.0537) [+]	+
k_{70}	.0608 (22.8) (.00627-.115) [+]	.0503 (21.6) (.00704-.0936) [+]	+
k_{79}	.0385 (22.8) (.00402-.0730) [+]	.0357 (21.6) (.00510-.0663) [+]	+
$k_{7,10}$	.0223 (22.8) (.00233-.0423) [-]	.0147 (21.6) (.00216-.0272) [+]	+

TABLE 13 (Continued)

Parameter	Parameter Estimate ($min.^{-1}$) Subject HB	Subject SR	t test[a]
η	.111 (85.0) (-.259-.481) [+]	.614 (264) (-6.07-7.35) [+]	+
θ	.00720 (96.4) (-.0200-.0344) [+]	.0184 (97.5) (-.0526-.0894) [+]	+
$k_{11,10}$	.00872 (103) (-.0265-.0439) [+]	.0485 (132) (-.205-.302) [+]	+
$k_{10,11}$	.0178 (201) (-.123-.158) [+]	.368 (292) (-3.88-4.61) [+]	+
$k_{10,0}$	.0915 (74.4) (-.176-.359) [+]	.243 (275) (-2.41-2.89) [+]	+

[a]Positive sign indicates acceptance of the null hypothesis that the "true" parameters from the two subjects are equal; negative sign indicates rejection (5% level of significance).

[b]Percent coefficient of variation.

[c]Approximate 90% confidence intervals.

[d]Positive sign in square bracket next to % CV indicates acceptance of the null hypothesis that the "true" parameter from this study is equal to the "true" parameter from the INA studies; negative sign indicates rejection (5% level of significance).

resulted as a consequence of large values for the S.D.'s. With S.D. 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 S.D.'s are not a consequence of poor fit of the equations to the data or inadequate numbers of data points. On the contrary, the equations fitted the data extremely well, and the numbers of data points are substantial (e.g., see Figure 16). As was pointed out by Metzler (98) and Westlake (103), the large S.D.'s 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 most true values of these parameters were equal (again, acceptance of null hypotheses in these tests resulted as a consequence of large S.D.'s).

Figures 16 and 17 show the theoretical rate of excretion-time curves 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 is excellent; scatter of the data points about the fitted lines appears random.

Ellard and Gammon (6) reported the renal clearance of AcINH in one subject to be 111 ml./min.; since renal clearance is equal to $k_{46}V_4$, V_4 may be estimated.[19] The calculated value is approximately 17 liters, and this is greater than 3 times the estimated blood volumes.

[19] V_4 is the volume of compartment 4.

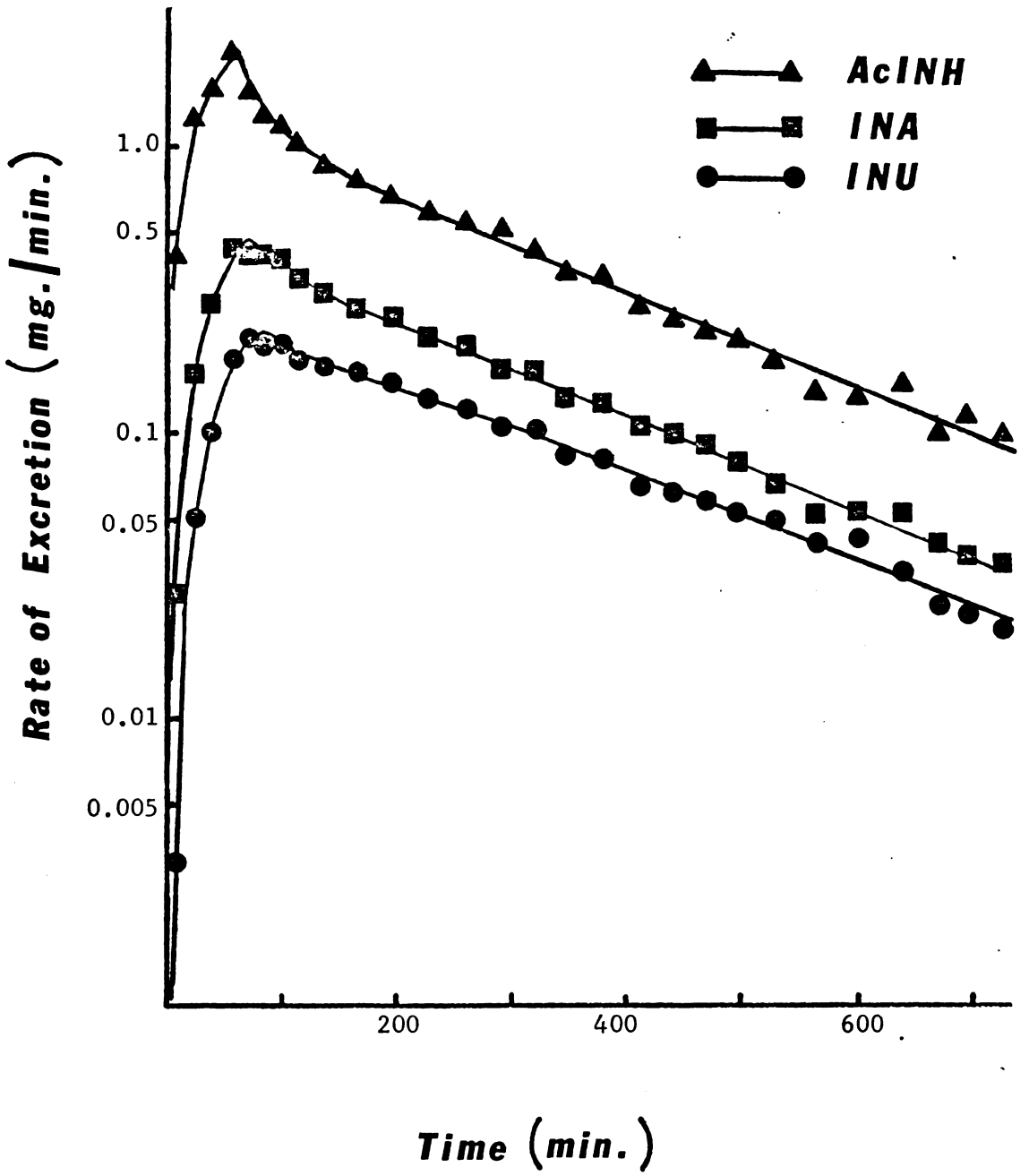


Figure 16. Rate of Excretion of AcINH, INA, and INU in Subject HB After AcINH Administration. (AcINH was infused over a period of 60.0 minutes.)

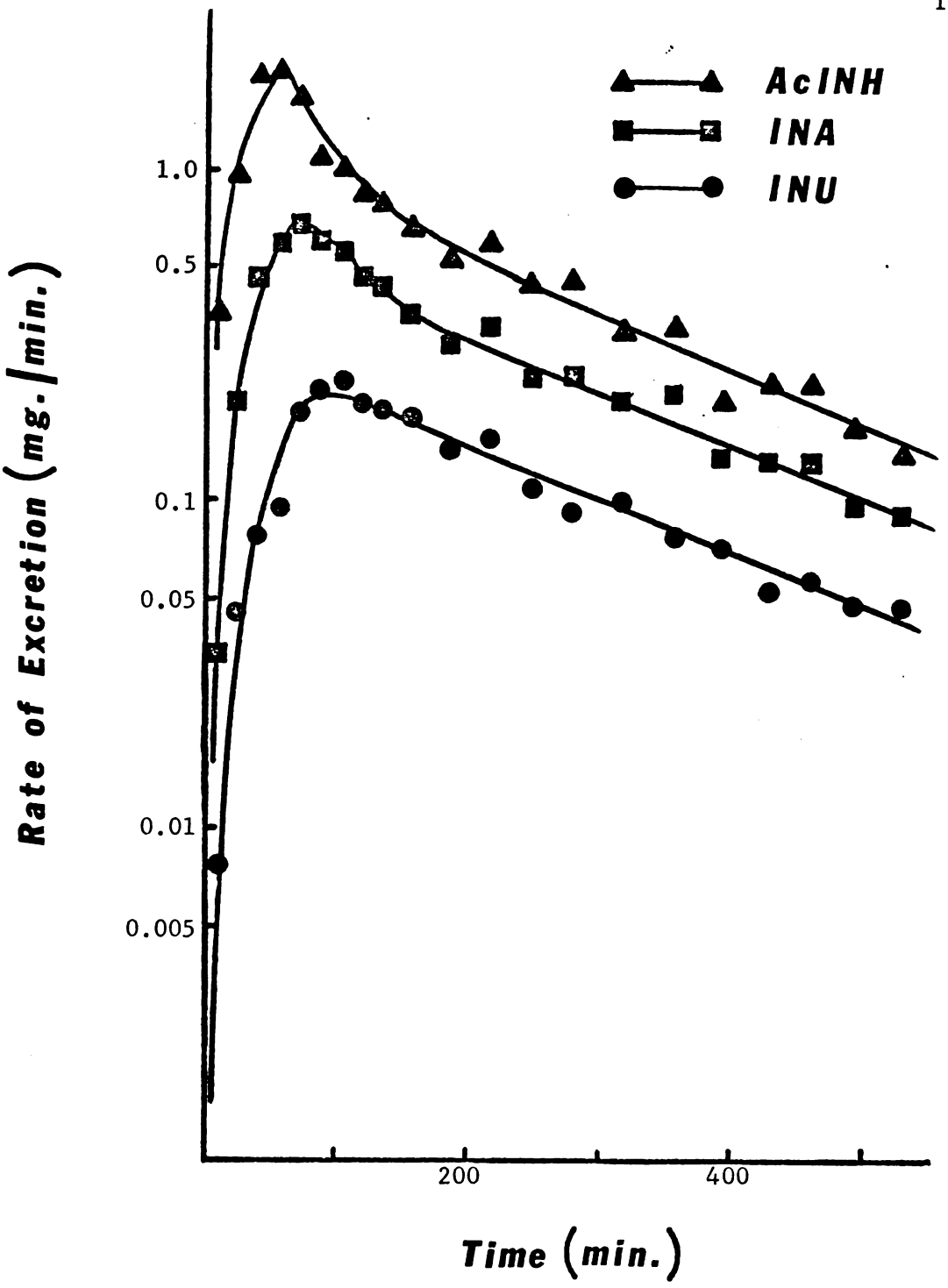


Figure 17. Rate of Excretion of AcINH, INA, and INU in Subject SR After AcINH Administration. (AcINH was infused over a period of 60.0 minutes.)

Ellard and Gammon (6) 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 HB and SR, 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 HB and SR, respectively).

INH Administration Studies

The virtual impossibility for separation of INH from its hydrazones was discussed in the analytical section; the assay utilized in the urine determinations 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. Table 14 shows the percentages of the doses excreted as the various compounds; the data of Peters, Miller, and Brown (5) and Ellard and Gammon (6) are also included for comparison. For both the rapid and slow acetylator subjects used in the present studies, excretion of INH plus hydrazones was greater and excretion of AcINH, INA, and INU was less than that reported by the other two groups. There is no apparent explanation for these differences, although possibly alkalinization of the urine in the present studies may have been responsible for the change in excretion.

Ellard and Gammon (6) reported one-compartment elimination constants for typical rapid and slow acetylators of INH (0.0086 $min.^{-1}$

TABLE 14

Average Percent of INH Doses Recovered in Urine as Various Compounds

Approximate Dose	Route of Administration	Reference	# Subjects	Urine pH Control	% of Total Recovered Compounds as[a]			
					INH + Hydrazones	AcINH	INA	INU
			Slow Acetylators					
350 mg	Oral	5	4	No	33.5	34.3	20.5	11.8
700 mg	Oral	5	2	No	30.0	22.0	28.3	19.7
250 mg	Oral	6	1	No	39.1	32.8	16.9	11.3
600 mg	Oral	6	6	No	32.5	29.5	22.1	16.2
681 mg	I.V.	Herein	1	Yes	54.7	25.1	12.7	7.57
			Rapid Acetylators					
350 mg	Oral	5	3	No	7.9	43.8	32.2	16.2
700 mg	Oral	5	5	No	14.9	34.1	33.2	17.7
250 mg	Oral	6	1	No	17.2	42.5	25.6	14.7
600 mg	Oral	6	7	No	7.94	47.8	29.8	14.5
670 mg	I.V.	Herein	1	Yes	23.0	38.5	25.0	13.5

[a]Average for the number of subjects noted in Column 4.

and 0.0036 min.$^{-1}$, respectively). These are similar to those for subjects SR and HB (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.

In subject HB, the zero-order intravenous infusion of INH lasted 5.40 minutes; at 6.15 minutes and 7.85 minutes, INH blood levels were 16.8 μg./ml. and 21.1 μg./ml., respectively. In subject SR, the infusion lasted 5.20 minutes; at 5.45 minutes and 7.70 minutes, INH blood levels were 17.1 μg./ml. and 28.0 μg./ml., respectively. Thus there were short delays (at least 0.75 minutes) 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.

An 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* (118), 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. Examination of the distribution constants for AcINH, INA, and INU determined following their intravenous administration indicates that the aforementioned assumption is reasonable. However, this assumption does not appear reasonable when INH is administered intravenously. Digital computer analysis of the post-infusion INH blood level data from subject HB (first data point used was at 7.85 minutes) resulted in an estimated value of 0.164 min.$^{-1}$ ($t_{1/2}$ = 4.2 minutes) for the first-order rate

constant describing transfer of the drug from the central to peripheral compartment. Because it took more than 0.75 minutes for the INH to distribute uniformly within the central compartment, mixing of the drug within that compartment was not rapid compared to efflux of the drug from that compartment. Nonetheless, the assumption of instantaneous mixing within the central compartment is inherent in the mathematical analyses. Consequently, inaccuracies in the estimates of α and k_{21} for INH are probable; these inaccuracies would not be reflected in the S.D.'s, because these reflect only precision. For both subjects, post-infusion INH blood level-time curves appeared to be bi-exponential. Consequently, a two-compartment body model for INH was formulated with metabolism and excretion occurring from the central compartment. Initially, only the INH blood data were utilized in the computer analyses, and the appropriate equations (see Appendix) were fitted to the data. The model, blood data, and computer generated curves are shown in Figure 18. The terminal, linear logarithmic phases of the curves had half-lives of 70.7 minutes and 196 minutes for the rapid and slow acetylator subjects, respectively. Table 15 shows the estimated parameters, some statistical data, and calculated volumes of distribution. The elimination constant, k_{10}, is 4.46 times greater in the rapid acetylator than the slow acetylator. The volumes of the central compartment are quite different in the two subjects, and there is no apparent explanation for this.

Jenne, McDonald, and Mendoza (42) reported that Vd_{extrap} was equal to total body water, and Ellard and Gammon (6) have consequently divided renal clearance by total body water to calculate one-compartment

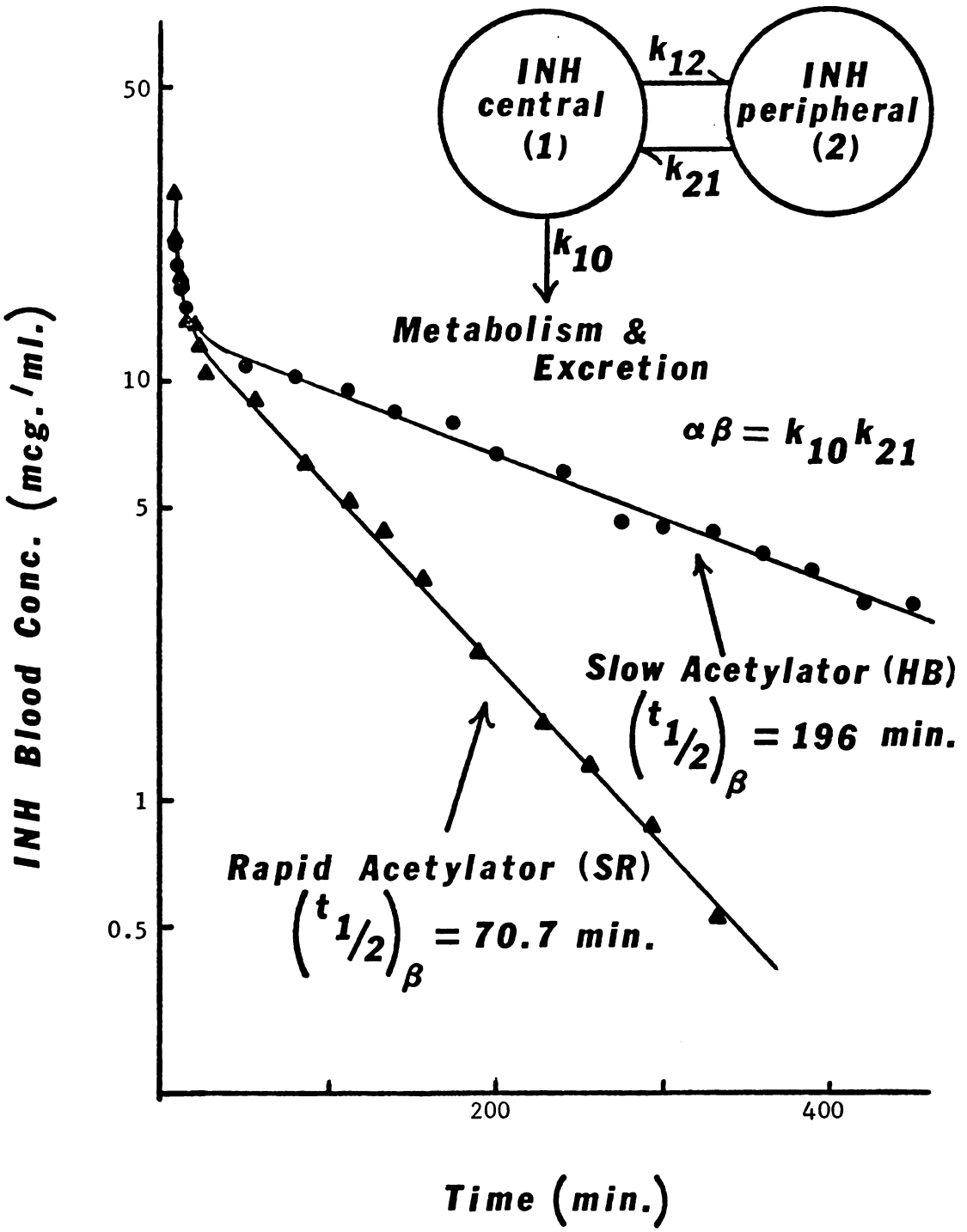


Figure 18. Pharmacokinetic Model, Blood Data, and Fitted Curves for INH Disposition. (Arrows in model represent first-order processes.)

TABLE 15

Pharmacokinetic Parameters Determined by Computer Analysis of INH Blood Data

Parameter	Subject HB	Subject SR
	Parameter Estimate ($min.^{-1}$)	
α	.195 (16.9)[a]	.260 (18.6)
β	.00354 (2.21)	.00981 (1.88)
k_{21}	.0819 (1.47)	.0679 (3.61)
k_{12}	.108	.164
k_{10}	.00843	.0376
	Parameter Estimate (ml.)[b]	
V_1	20,492 (16.1)[a]	10,652 (20.0)
Vd_{ss}[c]	47,514	36,380
Vd_{area}[d]	48,738	40,740
Vd_{extrap}[e]	50,069	45,877

[a]Percent coefficient of variation.

[b]See Reference (119) for equations used to calculate volumes.

[c]Volume of distribution at steady-state.

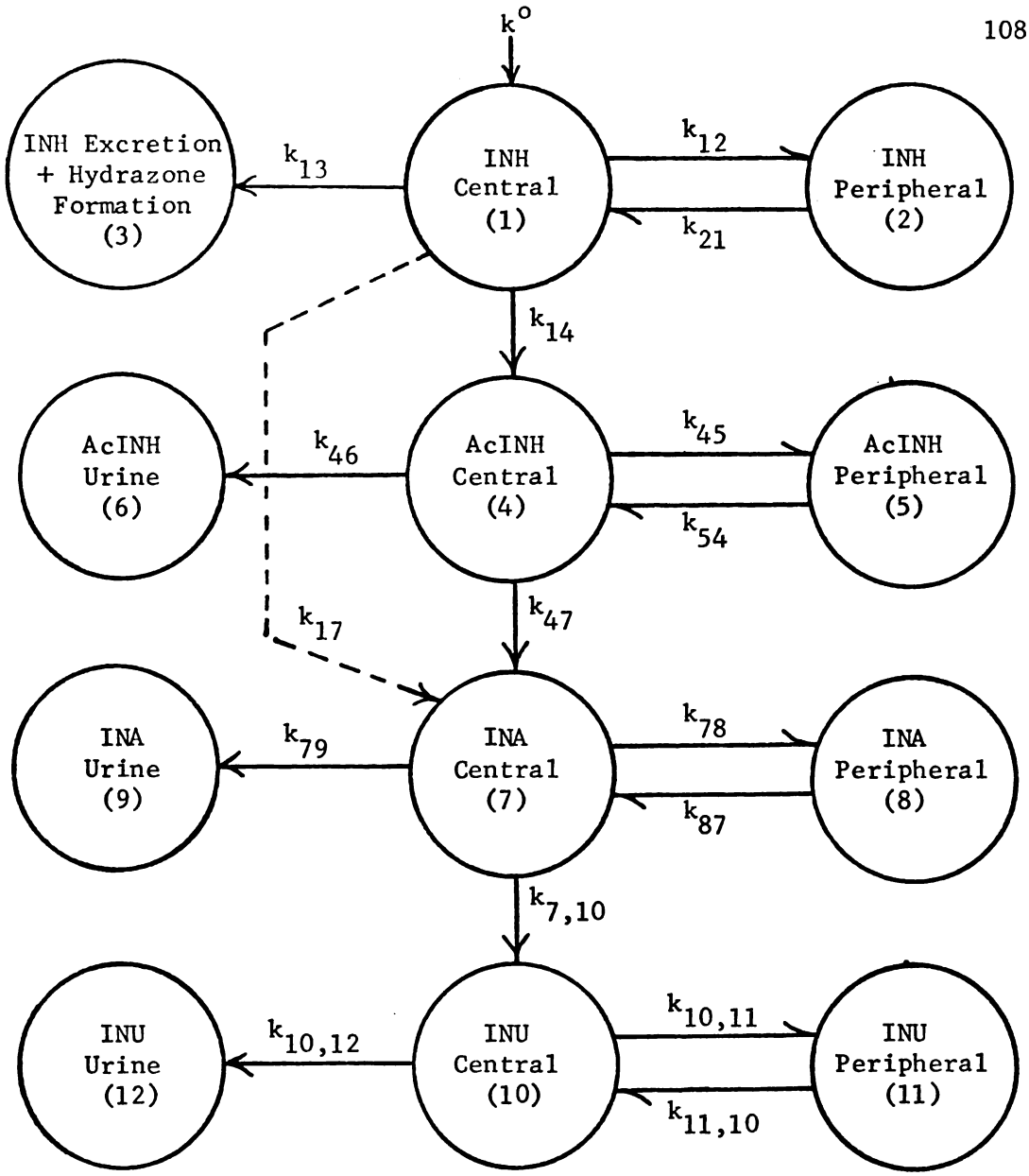
[d]Volume of distribution by area method.

[e]Volume of distribution by extrapolation method.

excretion rate constants. When the Vd_{extrap} values of Table 15 are compared to estimated total body waters of Table 6, the Vd_{extrap} values overestimate total body water by 23.9 percent and 7.95 percent in subjects HB and SR, respectively.

An attempt was next made to fit appropriate equations simultaneously to INH blood data and the AcINH and INA urine data. It was felt that fitting the appropriate equation to INU urine data would result in parameter estimates with extremely large S.D.'s. Thus, one could not place much confidence in the estimates. Therefore, INU data were not used in the computer analysis.

Initially, the model shown in Figure 19 was used and direct conversion of INH to INA was presumed to occur (equations are in the Appendix). The values of f_7 were determined from urinary excretion data. However, values for f_{14} and f_{47} could not be explicitly determined, and these were estimated as parameters by the computer program. Certain parameters describing INH disposition (α, β, k_{21} and V_1) were held constant at the values previously determined. When this was not done and they were permitted to iterate, extremely poor fits for INH blood curves associated with non-randomness of scatter sometimes resulted. The estimated parameters and statistical data are presented in Table 16. Theoretical curves were generated using the estimated values of the parameters, and these are shown in Figures 20 and 21 with the experimental data points; scatter of data points about the fitted curves appears random. Comparison of the S.D.'s of INH parameters in Tables 15 and 16 reveals that expanding the model to INH metabolites and fitting three curves simultaneously results in much greater S.D.'s.



k^o = Zero-order infusion rate

$\alpha\beta = k_{21}k_{10}$ $\gamma\delta = k_{54}k_{40}$ $\varepsilon\zeta = k_{87}k_{70}$ $\eta\theta = k_{11,10}k_{10,12}$

$k_{10} = k_{13} + k_{14} + k_{17}$ $k_{40} = k_{46} + k_{47}$ $k_{70} = k_{79} + k_{7,10}$

$$f_{13} = \frac{k_{13}}{k_{10}} \quad f_{14} = \frac{k_{14}}{k_{10}} \quad f_{17} = \frac{k_{17}}{k_{10}}$$

$$f_{47} = \frac{k_{47}}{k_{40}} \quad f_4 = f_{46} = \frac{k_{46}}{k_{40}} \quad f_7 = f_{79} = \frac{k_{79}}{k_{70}}$$

Figure 19. Model Used to Describe INH Disposition Including Zero-Order Input. (With the exception of the zero-order input, arrows represent first-order processes. The existence of the pathway described by k_{17} is discussed in the text.)

TABLE 16

Parameter Estimates from INH Administration Studies, Assuming Direct Conversion of INH to INA

Parameter	Parameter Estimate ($min.^{-1}$) Subject SR	Subject HB	t test[a]
α	.260 (804)[b] (-9.26-9.78)[c]	.195 (127) (-.915-1.31)	+
β	.00981 (62.0) (-.0179-.0375)	.00354 (40.7) (-.00291-.00999)	+
k_{21}	.0679 (540) (-1.60-1.74)	.0819 (143) (-.442-.606)	+
V_1	10,652.4 (-) --	20,492 (-) --	-
f_{14}	.589 (731) (-.607-.651)	.296 (27.8) (-.0724-.664)	+
f_{47} ($\equiv f_4$)	.355 (1331) (-21.2-21.9)	.139 (188) (-1.03-1.31)	+
k_{12}	.164 (997) (-7.26-7.59)	.108 (133) (-.532-.748)	+
k_{10}	.0376 (321) (-.514-.589)	.00843 (38.5) (-.00607-.0229)	+
k_{13}	.00864 (321) (-.118-.135)	.00461 (38.5) (-.00331-.0125)	+
k_{14}	.0221 (626) (-.607-.651)	.00250 (48.5) (-.00292-.00792)	+
k_{17}	.00681 (2565) (-.791-.804)	.00133 (63.5) (-.00245-.00511)	+
γ	.0367 (563) (-.906-.980) [+][d]	.0980 (113) (-.394-.590) [+]	+
δ	.00347 (19.3) (.000417-.00652) [+]	.00294 (37.7) (-.00203-.00791) [+]	+
k_{54}	.0261 (368) (-.412-.464) [+]	.0279 (32.3) (-.0123-.0681) [+]	+

TABLE 16 (Continued)

Parameter	Parameter Estimate ($min.^{-1}$) Subject SR	Subject HB	t test[a]
k_{45}	.00919 (1186) (-.487-.506) [+]	.0627 (160) (-.385-.510) [+]	+
k_{40}	.00488 (248) (-.0503-.0600) [+]	.0103 (89.0) (-.0308-.0514) [+]	+
k_{46}	.00315 (967) (-.135-.142) [+]	.00887 (117) (-.0377-.0554) [+]	+
k_{47}	.00173 (1104) (-.0853-.0888) [+]	.00143 (117) (-.00605-.00891 [+]	+
ϵ	.0148 (463) (-.297-.327) [+]	.139 (106) (-.524-.802) [+]	+
ζ	.00662 (1505) (-.447-.460) [+]	.00611 (120) (-.0266-.0388) [+]	+
k_{87}	.0103 (1139) (-.523-.543) [+]	.0180 (87.5) (-.0527-.0887) [+]	+
k_{78}	.00161 (3635) (-.265-.268) [+]	.0799 (150) (-.453-.613) [+]	+
k_{70}	.00951 (2171) (-.929-.948) [+]	.0472 (71.0) (-.103-.197) [+]	+
k_{79}	.00617 (2171) (-.604-.617) [+]	.0296 (71.0) (-.0644-.124) [+]	+
$k_{7,10}$	.00334 (2171) (-.327-.337) [+]	.0176 (.0125) (-.0384-.0736) [+]	+

[a]Positive sign indicates acceptance of the null hypothesis that the "true" parameters from the two subjects are equal; negative sign indicates rejection (5% level of significance).

[b]Percent coefficient of variation.

[c]Approximate 90% confidence intervals.

[d]Positive sign in square bracket next to % CV indicates acceptance of the null hypothesis that the "true" parameter from this study is equal to the "true" parameter from the AcINH studies; negative sign indicates rejection (5% level of significance).

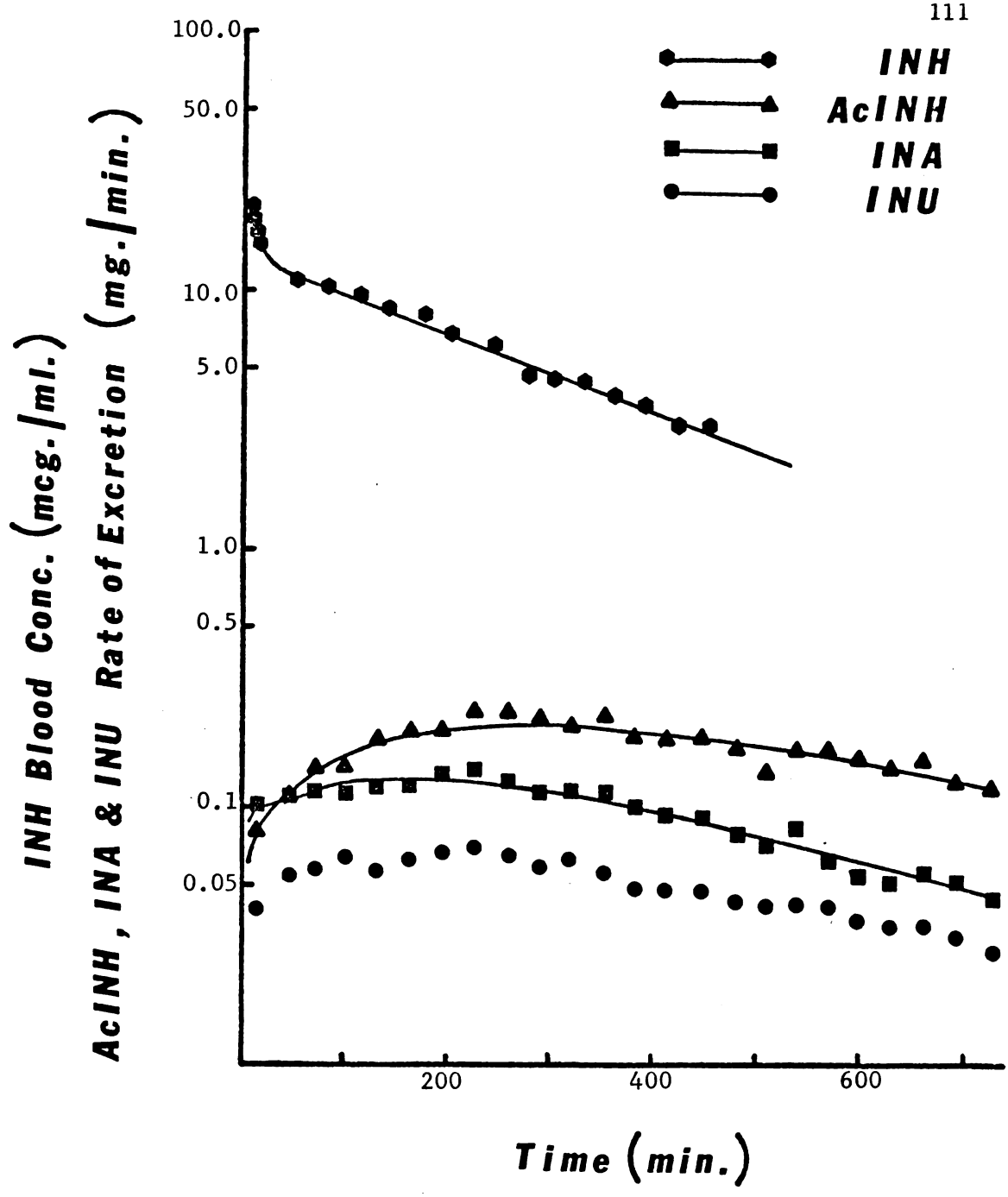


Figure 20. INH Blood Levels and Rates of Excretion of AcINH, INA, and INU in Subject HB After a 5.40 Minute Infusion of INH. (INH was presumed to be metabolized directly to INA.)

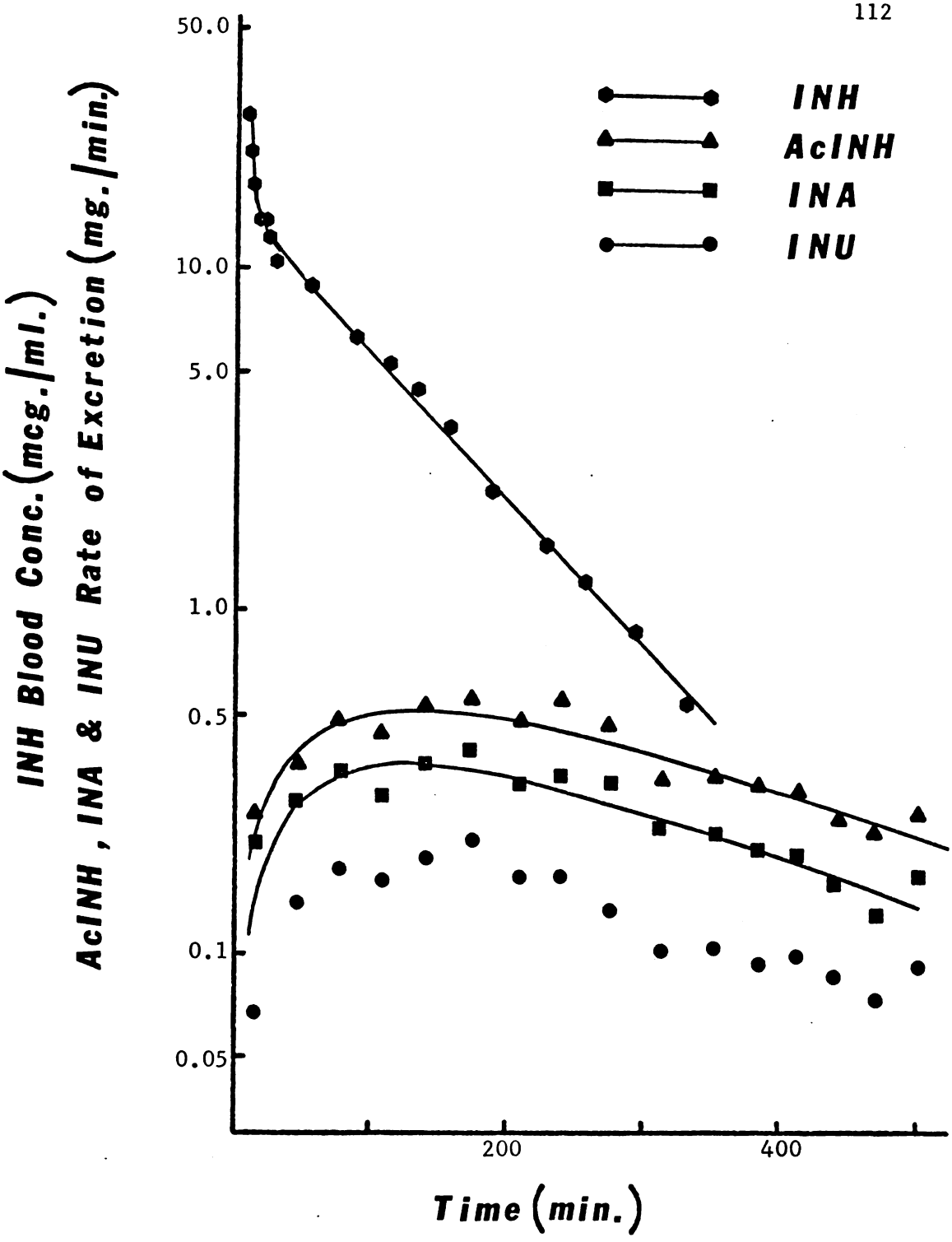


Figure 21. INH Blood Levels and Rates of Excretion of AcINH, INA, and INU in Subject SR After a 5.20 Minute Infusion of INH. (INH was presumed to be metabolized directly to INA.)

This suggests that the INH blood data reflect changes in these parameters more sensitively than the AcINH and INA urine curves. The t tests indicate that when the estimated parameters from subject HB are compared to those of subject SR the S.D.'s are sufficiently large so as to conclude that all parameters are equal. Quite obviously this is a false conclusion, particularly for the acetylation rate constants. Nonetheless, the t tests indicate that significant differences of parameters between subjects are impossible to demonstrate. The reason for this inability to demonstrate differences resides in the extremely large S.D.'s of the parameters. Comparison of the AcINH and INA disposition parameters from the INH studies to those of the AcINH studies reveals an inability to demonstrate differences.

The model shown in Figure 19 was varied inasmuch as direct conversion of INH to INA was presumed not to occur (i.e., $k_{17} = 0$); appropriate equations are in the Appendix. Under this assumption, values for f_{14} and f_{47} could be determined from urinary excretion data. The appropriate equations were fit simultaneously to the INH blood data and the AcINH and INA urine data (α, β, k_{21} and V_1 were again held constant). The estimated parameters and statistical data are presented in Table 17. Theoretical curves were generated using the estimated values of the parameters, and these are shown in Figures 22 and 23 with the experimental data points; scatter of data points about the fitted curves appears random. The t tests indicate no differences in parameters between subjects; this again results from extremely large S.D.'s. Comparison of the AcINH and INA disposition parameters from the INH

TABLE 17

Parameter Estimates from INH Administration Studies, Assuming No Direct Conversion of INH to INA

Parameter	Parameter Estimate ($min.^{-1}$) Subject SR	Subject HB	t test[a]
α	.260 (564)[b] (-5.92-6.44)[c]	.195 (73.2) (-.396-.786)	+
β	.00981 (191) (-.0689-.0885)	.00354 (19.7) (.000662-.00642)	+
k_{21}	.0679 (307) (-.807-.943)	.0819 (65.5) (-.140-.304)	+
V_1	10,652.4 (-) --	20,492 (-) --	+
k_{12}	.164 (711) (-4.76-5.09)	.108 (93.3) (-.310-.526)	+
k_{10}	.0376 (326) (-.480-.555)	.00843 (39.0) (-.00517-.0220)	+
k_{13}	.00864 (326) (-.110-.127)	.00461 (39.0) (-.00283-.0121)	+
k_{14}	.0290 (326) (-.369-.427)	.00382 (39.0) (-.00234-.00998)	+
γ	.0215 (531) (-.458-.501) [+][d]	.104 (76.7) (-.225-.433) [+]	+
δ	.00323 (15.8) (.00109-.00537) [+]	.00299 (14.1) (.00124-.00474) [-]	+
k_{54}	.0158 (601) (-.383-.415) [+]	.0281 (28.1) (-.00457-.0608) [+]	+
k_{45}	.00453 (593) (-.109-.118) [+]	.0678 (101) (-.217-.352) [+]	+
k_{40}	.00440 (140) (-.0216-.0304) [+]	.0111 (61.6) (-.0172-.0394) [+]	+
k_{46}	.00220 (140) (-.0108-.0152) [+]	.00614 (61.6) (-.00949-.0218) [+]	+

TABLE 17 (Continued)

Parameter	Parameter Estimate ($min.^{-1}$) Subject SR	Subject HB	t test[a]
k_{47}	.00220 (140) (-.0108-.0152) [+]	.00496 (61.6) (-.00769-.0176) [+]	+
ϵ	.792 (408) (-12.8-14.4) [+]	.327 (213) (-2.55-3.21) [+]	+
ζ	.000540 (924) (-.0205-.0215) [+]	.0111 (34.7) (-.00478-.0270) [+]	+
k_{87}	.000530 (923) (-.0200-.0211) [+]	.00485 (20.6) (.000715-.00899) [+]	+
k_{78}	-.0150 (-444) (-.295-.265) [+]	-.415 (-193) (-3.73-2.90) [+]	+
k_{70}	.807 (407) (-13.0-14.6) [+]	.748 (200) (-5.41-6.91) [+]	+
k_{79}	.524 (407) (-8.44-9.49) [+]	.468 (200) (-3.39-4.33) [+]	+
$k_{7,10}$	.283 (407) (-4.56-5.12) [+]	.280 (200) (-2.02-2.58) [+]	+

[a]Positive sign indicates acceptance of the null hypothesis that the "true" parameters from the two subjects are equal; negative sign indicates rejection (5% level of significance).

[b]Percent coefficient of variation.

[c]Approximate 90% confidence intervals.

[d]Positive sign in square bracket next to % CV indicates acceptance of the null hypothesis that the "true" parameter from this study is equal to the "true" parameter from the AcINH studies; negative sign indicates rejection (5% level of significance).

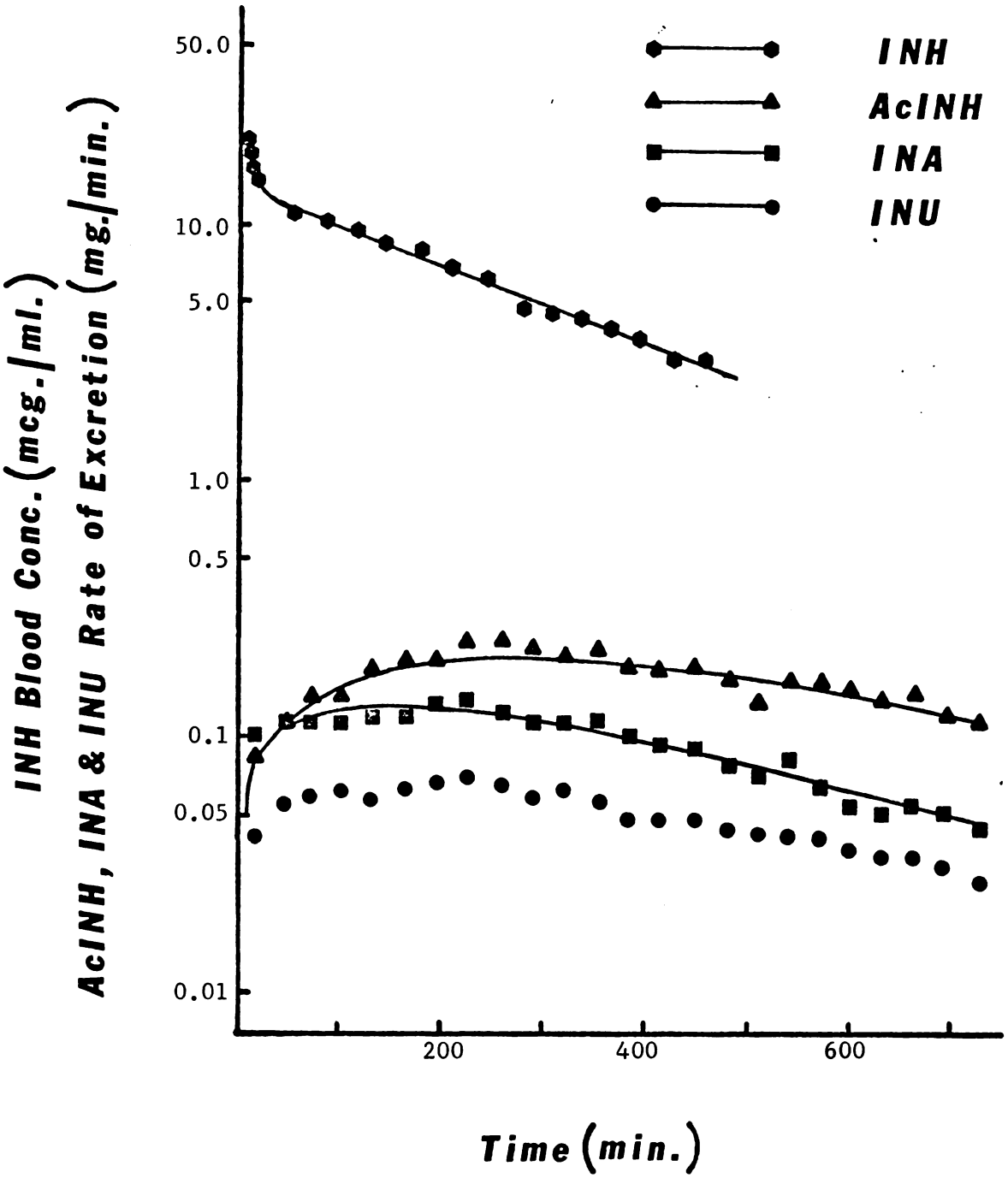


Figure 22. INH Blood Levels and Rates of Excretion of AcINH, INA, and INU in Subject HB After a 5.40 Minute Infusion of INH. (INH was presumed not to be directly metabolized to INA.)

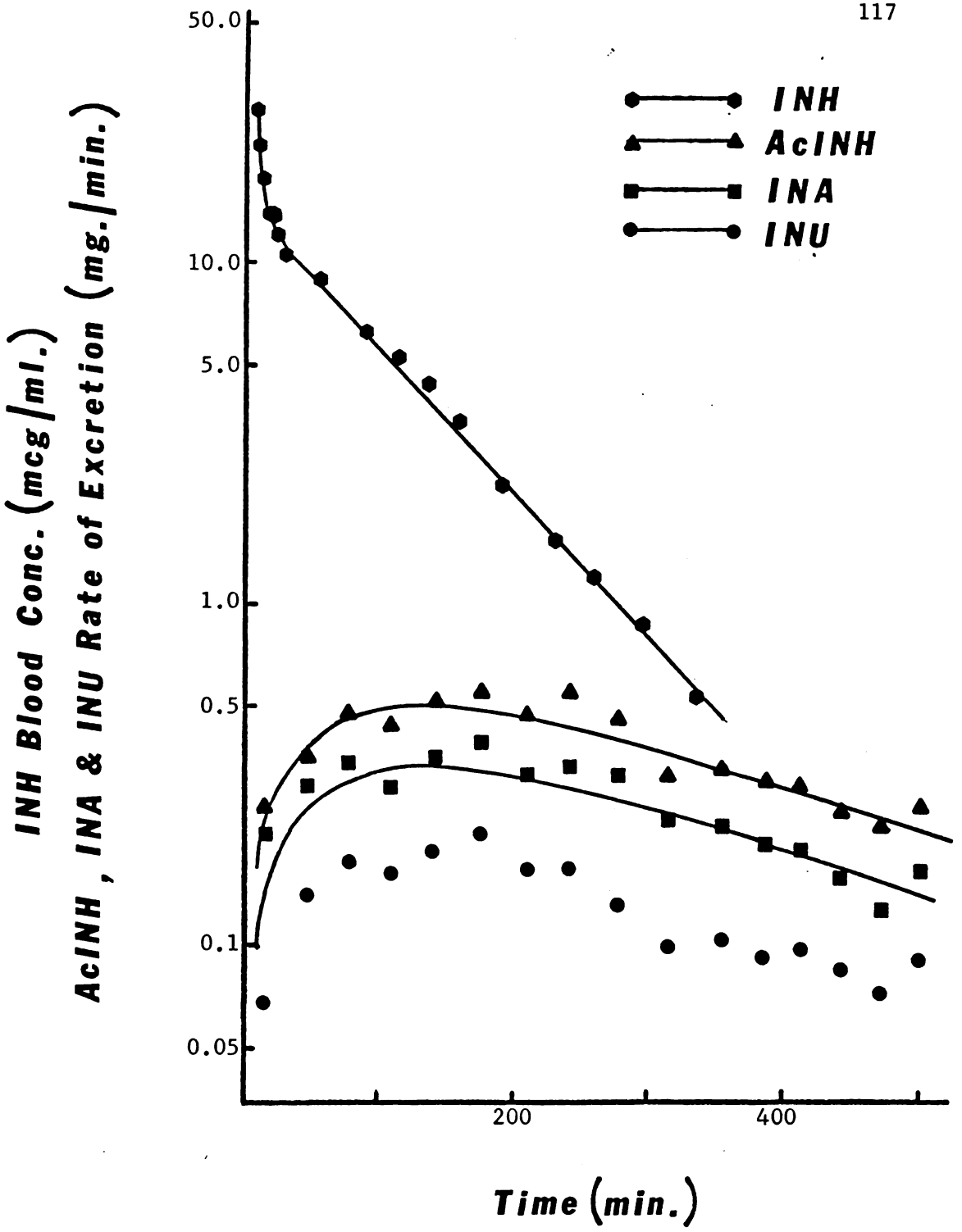


Figure 23. INH Blood Levels and Rates of Excretion of AcINH, INA, and INU in Subject SR After a 5.20 Minute Infusion of INH. (INH was presumed not to be directly metabolized to INA.)

studies to those of the AcINH studies reveals an inability to demonstrate differences (an exception is δ in subject HB).

The F test, for a given subject, was used to compare the combined weighted sums of squared differences between fits to models with and without the direct conversion of INH to INA. For both subjects, there were no differences. Hence, either model could be used to derive equations which give satisfactory fits. Additionally, scatter of data points about the fitted curves appears good for both models. It is difficult, if not impossible, to quantitate any direct conversion of INH to INA by this method, because equations derived from models which do and do not include direct hydrolysis of INH to INA may be fit equally well.

General Overview of Data

At this point, it is advisable to examine the INH data as well as the data from previously discussed studies in order to determine the correctness of the models. Table 18 shows the fractions of administered compounds excreted and converted to metabolites. If the models used are correct, the fractions excreted and metabolized would not change with the compound administered; however, the fractions metabolized and excreted appeared to change systematically. Tables 19 and 20 show parameter estimates, S.D.'s, and %CV's following administration of the various compounds. On examining the hybrid or microscopic constants right to left, in many cases the parameter estimates become larger; also, the S.D.'s and %CV's generally become much larger. This is

TABLE 18

Fraction of Administered Compounds Excreted
in Urine and Converted to Metabolites

	Drug Administered		
	INH	AcINH	INA
Fraction of AcINH metabolized to INA	.355 (SR)[a]		
	.139 (HB)	.404 (SR)	--
$\left[\frac{INA + INU}{AcINH + INA + INU}\right]_{urine}^{\infty}$	.500 (SR)[b]	.332 (HB)	
	.447 (HB)		
Fraction of AcINH excreted	.645 (SR)[c]		
	.861 (HB)	.596 (SR)	--
$\left[\frac{AcINH}{AcINH + INA + INU}\right]_{urine}^{\infty}$	.500 (SR)[b]	.668 (HB)	
	.553 (HB)		
Fraction of INA metabolized to INU	.351 (SR)	.291 (SR)	.262 (SR)
$\left[\frac{INU}{INA + INU}\right]_{urine}^{\infty}$	.374 (HB)	.367 (HB)	.243 (HB)
Fraction of INA excreted	.649 (SR)	.709 (SR)	.738 (SR)
$\left[\frac{INA}{INA + INU}\right]_{urine}^{\infty}$	.626 (HB)	.633 (HB)	.757 (HB)

[a]These fractions assume direct conversion of INH to INA and were determined by the computer (see Table 16).

[b]These calculations assume no direct conversion of INH to INA.

[c]These fractions assume direct conversion of INH to INA.

TABLE 19

Parameter Estimates ($min.^{-1}$), S.D.'s ($min.^{-1}$), and % CV's Following Administration of INH and Metabolites to Subject HB

Parameter	Compound Administered				
	INH[a]	INH[b]	AcINH	INA	INU
α	.195[c] (.143[d], 73.2[e])	.195 (.248, 127)			
β	.00354 (.000696, 19.7)	.00354 (.00144, 40.7)			
k_{21}	.0819 (.0537, 65.5)	.0819 (.117, 143)			
k_{12}	.108 (.101, 93.3)	.108 (.143, 133)			
k_{10}	.00843 (.00329, 39.0)	.00843 (.00324, 38.5)			
k_{13}	.00461 (.00180, 39.0)	.00461 (.00177, 38.5)			
k_{14}	.00382 (.00149, 39.0)	.00250 (.00121, 48.5)			
k_{17}	--	.00133 (.000844, 63.5)			
f_{14}	--	.296 (.0823, 27.8)			
f_{47} ($\equiv f_4$)	--	.139 (.261, 188)			
γ	.104 (.0796, 76.7)	.0980 (.110, 113)	.0456 (.00445, 9.76)	--	--
δ	.00299 (.000422, 14.1)	.00294 (.00111, 37.7)	.00389 (.0000713, 1.83)	--	--
k_{54}	.0281 (.00790, 28.1)	.0279 (.00899, 32.3)	.0171 (.00139, 8.15)	--	--
k_{45}	.0678 (.0688, 101)	.0627 (.100, 160)	.0220 (.00291, 13.2)	--	--
k_{40}	.0111 (.00684, 61.6)	.0103 (.00917, 89.0)	.0104 (.000472, 4.55)	--	--
k_{46}	.00614 (.00378, 61.6)	.00887 (.0104, 117)	.00692 (.000315, 4.55)	--	--
k_{47}	.00496 (.00306, 61.6)	.00143 (.00167, 117)	.00345 (.000157, 4.55)	--	--

TABLE 19 (Continued)

Parameter	Compound Administered				
	INH[a]	INH[b]	AcINH	INA	INU
ϵ	.327 (.696, 213)	.139 (.148, 106)	.0934 (.0456, 48.8)	.0621 (.00561, 9.03)	--
ζ	.0111 (.00384, 34.7)	.00611 (.00731, 120)	.0141 (.00728, 51.7)	.0184 (.00160, 8.69)	--
k_{87}	.00485 (.00100, 20.6)	.0180 (.0158, 87.5)	.0216 (.0172, 79.7)	.0228 (.00290, 12.7)	--
k_{78}	-.415 (.801, -193)	.0799 (.119, 150)	.0251 (.0247, 98.5)	.00769 (.00186, 24.1)	--
k_{70}	.748 (1.49, 200)	.0472 (.0335, 71.0)	.0608 (.0139, 22.8)	.0499 (.00279, 5.58)	--
k_{79}	.468 (.933, 200)	.0296 (.0210, 71.0)	.0385 (.00879, 22.8)	.0378 (.00211, 5.58)	--
$k_{7,10}$	.280 (.557, 200)	.0176 (.0125, 71.0)	.0223 (.00509, 22.8)	.0121 (.000677, 5.58)	--
η	--	--	.111 (.0942, 85.0)	.0991 (.0124, 12.5)	.0675 (.00164, 2.42)
θ	--	--	.00720 (.00694, 96.4)	.0113 (.00220, 19.5)	.0114 (.000197, 1.73)
$k_{11,10}$	--	--	.00872 (.00898, 103)	.0138 (.00295, 21.3)	.0133 (.000219, 1.65)
$k_{10,11}$	--	--	.0178 (.0358, 201)	.0158 (.00342, 21.6)	.00787 (.000318, 4.04)
$k_{10,0}$	--	--	.0915 (.0681, 74.4)	.0807 (.00884, 11.0)	.0577 (.00141, 2.44)

[a]No direct hydrolysis of INH to INA assumed.

[b]Direct hydrolysis of INH to INA assumed.

[c]Parameter estimate.

[d]S.D.

[e]% CV.

TABLE 20

Parameter Estimates ($min.^{-1}$), S.D.'s ($min.^{-1}$), and % CV's Following Administration of INH and Metabolites to Subject SR

Parameter	Compound Administered				
	INH[a]	INH[b]	AcINH	INA	INU
α	.260[c] (1.47[d], 564[e])	.260 (2.09, 804)			
β	.00981 (.0187, 191)	.00981 (.00608, 62.0)			
k_{21}	.0679 (.208, 307)	.0679 (.367, 540)			
k_{12}	.164 (1.17, 711)	.164 (1.63, 997)			
k_{10}	.0376 (.123, 326)	.0376 (.121, 321)			
k_{13}	.00864 (.0282, 326)	.00864 (.0278, 321)			
k_{14}	.0290 (.0945, 326)	.0221 (.138, 626)			
k_{17}	--	.00681 (.175, 2565)			
f_{14}	--	.589 (4.31, 731)			
$f_{47} (\equiv f_4)$	--	.355 (4.73, 1331)			
γ	.0215 (.114, 531)	.0367 (.207, 563)	.0324 (.00384, 11.9)	--	--
δ	.00323 (.000509, 15.8)	.00347 (.000670, 19.3)	.00389 (.000202, 5.19)	--	--
k_{54}	.0158 (.0949, 601)	.0261 (.0962, 368)	.0122 (.00149, 12.3)	--	--
k_{45}	.00453 (.0269, 593)	.00919 (.109, 1186)	.0137 (.00224, 16.3)	--	--
k_{40}	.00440 (.00618, 140)	.00488 (.0121, 248)	.0104 (.000574, 5.54)	--	--
k_{46}	.00220 (.00309, 140)	.00315 (.0305, 967)	.00617 (.000342, 5.54)	--	--
k_{47}	.00220 (.00309, 140)	.00173 (.0191, 1104)	.00418 (.000232, 5.54)	--	--

TABLE 20 (Continued)

Parameter	Compound Administered				
	INH[a]	INH[b]	AcINH	INA	INU
ϵ	.792 (3.23, 408)	.0148 (.0685, 463)	.0643 (.0219, 34.1)	.0416 (.00367, 8.82)	--
ζ	.000540 (.00499, 924)	.00662 (.0996, 1505)	.00738 (.00635, 86.0)	.0118 (.00220, 18.7)	--
k_{87}	.000530 (.00489, 923)	.0103 (.117, 1139)	.00943 (.00942, 99.9)	.0136 (.00310, 22.8)	--
k_{78}	-.0150 (.0666, -444)	.00161 (.0585, 3635)	.0120 (.0105, 87.7)	.00380 (.00110, 29.0)	--
k_{70}	.807 (3.28, 407)	.00951 (.206, 2171)	.0503 (.0109, 21.6)	.0359 (.00197, 5.49)	--
k_{79}	.524 (2.13, 407)	.00617 (.134, 2171)	.0357 (.00771, 21.6)	.0265 (.00146, 5.49)	--
$k_{7,10}$	.283 (1.15, 407)	.00334 (.0725, 2171)	.0147 (.00316, 21.6)	.00941 (.000517, 5.49)	--
η	--	--	.641 (1.69, 264)	.102 (.0236, 23.1)	.0473 (.00322, 6.81)
θ	--	--	.0184 (.0179, 97.5)	.0157 (.00235, 14.9)	.0129 (.000843, 6.55)
$k_{11,10}$	--	--	.0485 (.0638, 132)	.0204 (.00427, 20.9)	.0158 (.000948, 6.01)
$k_{10,11}$	--	--	.368 (1.07, 292)	.0187 (.00843, 45.0)	.005789 (.00100, 17.3)
$k_{10,0}$	--	--	.243 (.668, 275)	.0786 (.0152, 19.3)	.0386 (.00243, 6.30)

[a]No direct hydrolysis of INH to INA assumed.

[b]Direct hydrolysis of INH to INA assumed.

[c]Parameter estimate

[d]S.D.

[e]% CV.

particularly evident in the INA and INU data. If the models used were correct, parameter estimates would be expected not to change when different compounds were administered. One could utilize the t test to determine if indeed parameters changed. Considering the progressive increases in S.D.'s, such analyses would probably once again result in questionable conclusions that the null hypotheses were accepted.

A more simple and more illustrative test of the models was made as follows. The disposition parameters for each compound, determined after administration of each individual compound, were used to generate blood and urine curves for each of the subjects[20]; the model in Figure 19 was used. The generated curves are shown in Figures 24 and 25 together with the data obtained from administration of INH to each of the subjects. It is apparent that the curves do not fit the data well, particularly during the first 100 minutes.

Based upon the preceding observation, a few comments may be made about the models used in these analyses. First, the mathematical functions generated from the models, together with the parameter estimates, are adequate to predict the observed data. This has been demonstrated. Secondly, the INH and possibly several of the other models may be considered "nonunique" or "ill-conditioned" (105). In other words, the amount of information contained in the data is inadequate to define the models chosen. Thus more than one model can fit the data equally well, and it is impossible on the basis of the least square fits alone to

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[20]The rate constant k_{17} was calculated by assuming that the relative amounts of AcINH metabolized and excreted remained unchanged with either AcINH or INH administration.

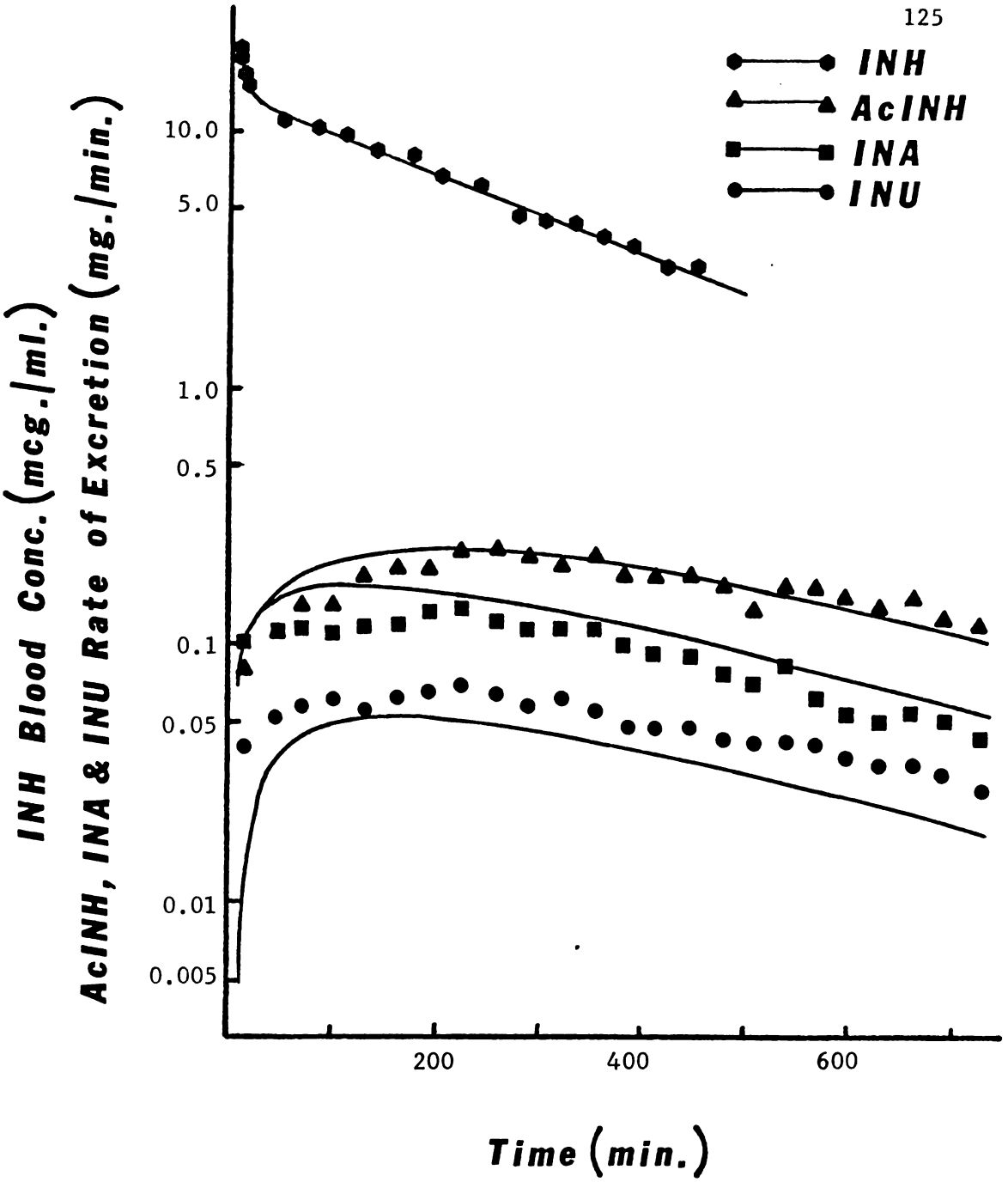

Figure 24. Comparison of Generated Curves to Data Obtained Following INH Administration to Subject HB (see text for explanation)

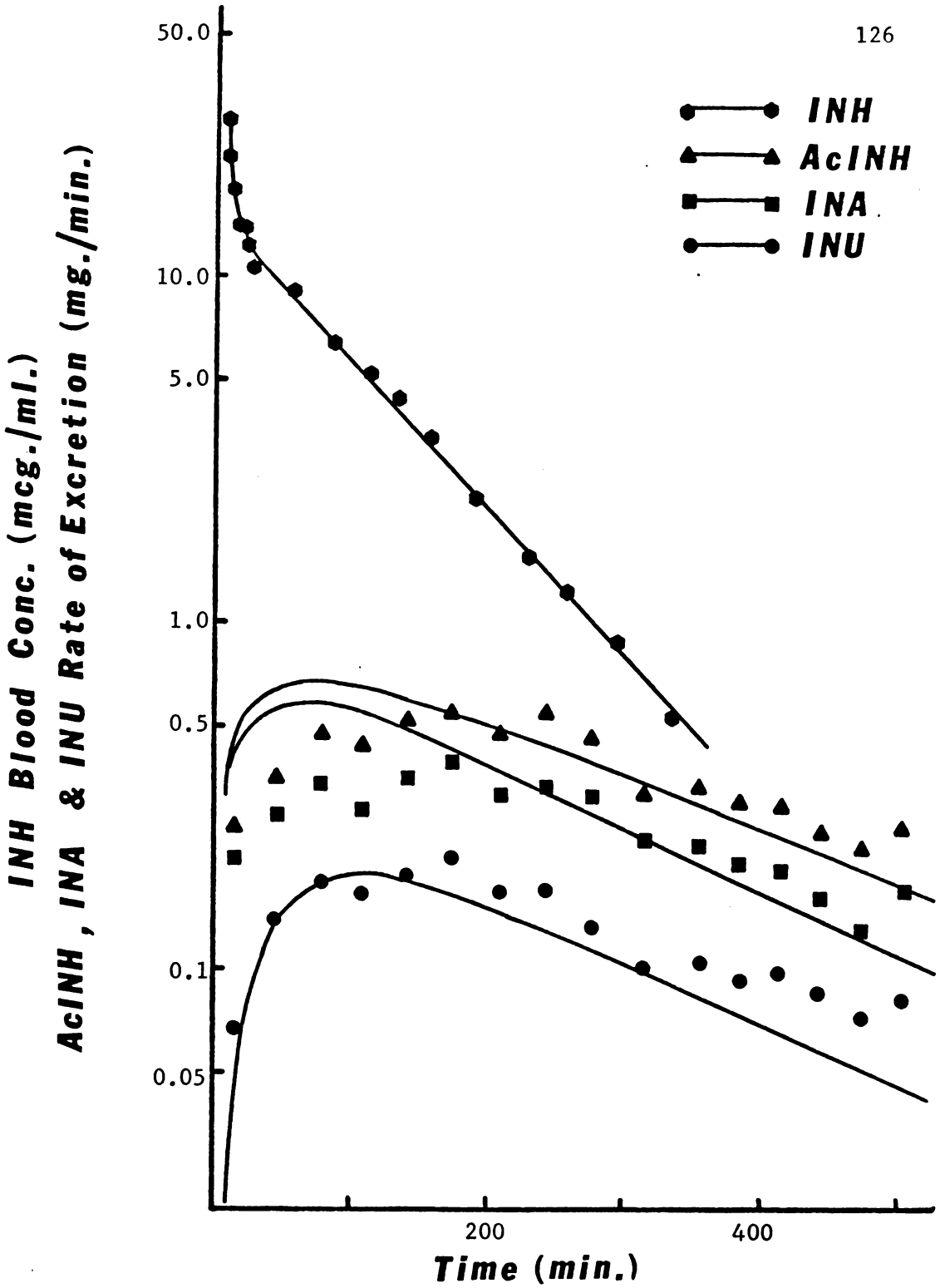

Figure 25. Comparison of Generated Curves to Data Obtained Following INH Administration to Subject SR (see text for explanation)

choose between them. Such was the situation in trying to differentiate between INH models with and without k_{17}.

In order to improve upon the models presented here, a number of alternatives may be considered. In all the models used, it has been assumed that the liver and kidney are in the central compartments and that metabolism and excretion occur from those compartments. A more acceptable alternative may be to place the liver in the peripheral compartment; metabolism could then be considered to occur in that compartment.

Another approach might be to investigate whether or not one or more of the metabolic steps are capacity rate limited. This would mean that one would have to apply Michaelis-Menten kinetics; this would inherently add more parameters to a model. Once again this could complicate the model to the extent that it is beyond the ability of the data to determine precisely the parameter values. It would probably be advisable to study the drug at several dose levels to test for dose dependent metabolism before this approach is taken.

Another approach would be to add more compartments to the models. This would complicate the models by adding more parameters and quite possibly could not be justified on the basis of bi-exponential post-infusion curves.

Another possibility is that INH is directly converted to INU, and in vitro experiments (as in References 25-27) could be undertaken to investigate this possibility.

The possibility of kidney metabolism could also be investigated; kidney metabolism would require alterations of the models (120, 121, 122).

Two alternative models[21] are shown in Figure 26a and b. Figure 26a is logical from a biochemical standpoint, because it is likely that compounds undergo metabolism in the liver, and the metabolites are returned to the blood stream after the completion of the metabolic sequence. However, this scheme lumps all the body tissues (except liver) into a single compartment, and in effect indicates that the connection from one compound to the other is entirely through the liver. One could also lump other body tissues into the liver compartment, and the same mathematical model would result.

Figure 26b subdivides the body into liver, central, and peripheral compartments, requiring the fitting of two additional microscopic constants. This model would result in tri-exponential blood curves for the administered compound provided certain microscopic rate constants were of such relative magnitude as not to reduce the model to two compartments.[22] It is likely that urinary excretion data are not adequate to justify use of Figure 26b as the preferred model. Both Figure 26a and b have an interesting feature in common, namely, they explain why a

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[21]The direct conversion of INH to INA is not considered, but this may be added to the models.

[22]An excellent discussion as to how a three-compartment model can be reduced to an apparent two-compartment model is given by Sharney, Wasserman, and Gevirtz (123).

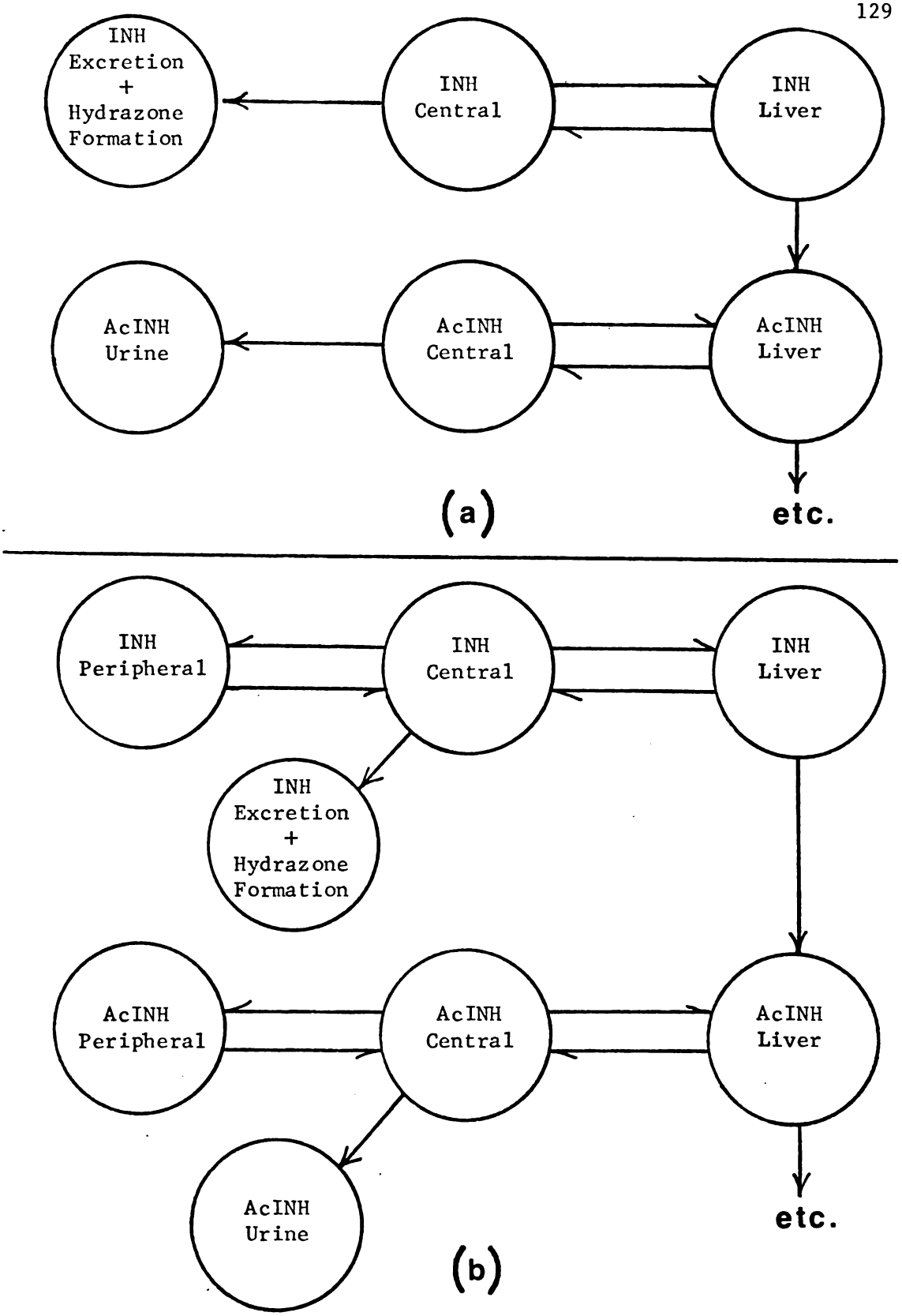

Figure 26. Alternative Models to Describe INH Disposition; Arrows Indicate First-Order Processes

different percent metabolite excretion is observed when a compound is intravenously infused as compared to when it is formed in the body (e.g., see Table 17). A further computer analysis of these data is definitely warranted to see if the scheme represented in Figure 26 is more appropriate than the present one, represented in Figure 7. Future experimental studies and computer analyses of these data are planned.

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APPENDIX

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

The Appropriate equations describing the INU model shown in Figure 8 are as follows:

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

$$A_{12} = k^{o}b + \frac{\theta k^{o}(k_{11,10} - \eta)(1 - e^{\eta b})e^{-\eta t}}{k_{11,10}\eta(\theta - \eta)} + \frac{\eta k^{o}(k_{11,10} - \theta)(1 - e^{\theta b})e^{-\theta t}}{k_{11,10}\theta(\eta - \theta)}$$

The appropriate equations describing the INA model shown in Figure 12 are as follows:

$$f_7 = \frac{k_{79}}{k_{79} + k_{7,10}}$$

$$\frac{dA_9}{dt} = \frac{f_7 \epsilon \zeta k^o}{k_{87}} \left[\frac{(k_{87} - \epsilon)(1 - e^{\epsilon b})e^{-\epsilon t}}{\epsilon(\epsilon - \zeta)} + \frac{(k_{87} - \zeta)(1 - e^{\zeta b})e^{-\zeta t}}{\zeta(\zeta - \epsilon)} \right]$$

$$\frac{dA_{12}}{dt} = \frac{(1 - f_7)\epsilon\zeta\eta\theta k^o}{k_{87}k_{11,10}} \left[\frac{(k_{87} - \epsilon)(k_{11,10} - \epsilon)(1 - e^{\epsilon b})e^{-\epsilon t}}{\epsilon(\epsilon - \zeta)(\epsilon - \eta)(\epsilon - \theta)} + \right.$$

$$\frac{(k_{87} - \zeta)(k_{11,10} - \zeta)(1 - e^{\zeta b})e^{-\zeta t}}{\zeta(\zeta - \epsilon)(\zeta - \eta)(\zeta - \theta)} +$$

$$\frac{(k_{87} - \eta)(k_{11,10} - \eta)(1 - e^{\eta b})e^{-\eta t}}{\eta(\eta - \epsilon)(\eta - \zeta)(\eta - \theta)} +$$

$$\left. \frac{(k_{87} - \theta)(k_{11,10} - \theta)(1 - e^{\theta b})e^{-\theta t}}{\theta(\theta - \epsilon)(\theta - \zeta)(\theta - \eta)} \right]$$

The appropriate equations describing the AcINH model shown in Figure 15 are as follows:

$$f_4 = \frac{k_{46}}{k_{46} + k_{47}} \qquad f_7 = \frac{k_{79}}{k_{79} + k_{7,10}}$$

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

$$\frac{dA_9}{dt} = \frac{(1 - f_4)\ f_7\gamma\delta\epsilon\zeta k^o}{k_{54}k_{87}}\left[\frac{(k_{54} - \gamma)(k_{87} - \gamma)(1 - e^{\gamma b})e^{-\gamma t}}{\gamma(\gamma - \delta)(\gamma - \epsilon)(\gamma - \zeta)} + \right.$$

$$\frac{(k_{54} - \delta)(k_{87} - \delta)(1 - e^{\delta b})e^{-\delta t}}{\delta(\delta - \gamma)(\delta - \epsilon)(\delta - \zeta)} + \frac{(k_{54} - \epsilon)(k_{87} - \epsilon)(1 - e^{\epsilon b})e^{-\epsilon t}}{\epsilon(\epsilon - \gamma)(\epsilon - \delta)(\epsilon - \zeta)}$$

$$\left. + \frac{(k_{54} - \zeta)(k_{87} - \zeta)(1 - e^{\zeta b})e^{-\zeta t}}{\zeta(\zeta - \gamma)(\zeta - \delta)(\zeta - \epsilon)}\right]$$

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

$$\frac{(k_{54} - \delta)(k_{87} - \delta)(k_{11,10} - \delta)(1 - e^{\delta b})e^{-\delta t}}{\delta(\delta - \gamma)(\delta - \epsilon)(\delta - \zeta)(\delta - \eta)(\delta - \theta)} +$$

$$\frac{(k_{54} - \epsilon)(k_{87} - \epsilon)(k_{11,10} - \epsilon)(1 - e^{\epsilon b})e^{-\epsilon t}}{\epsilon(\epsilon - \gamma)(\epsilon - \delta)(\epsilon - \zeta)(\epsilon - \eta)(\epsilon - \theta)} +$$

$$\frac{(k_{54} - \zeta)(k_{87} - \zeta)(k_{11,10} - \zeta)(1 - e^{\zeta b})e^{-\zeta t}}{\zeta(\zeta - \gamma)(\zeta - \delta)(\zeta - \epsilon)(\zeta - \eta)(\zeta - \theta)} +$$

$$\frac{(k_{54} - \eta)(k_{87} - \eta)(k_{11,10} - \eta)(1 - e^{\eta b})e^{-\eta t}}{\eta(\eta - \gamma)(\eta - \delta)(\eta - \epsilon)(\eta - \zeta)(\eta - \theta)} +$$

$$\left.\frac{(k_{54} - \theta)(k_{87} - \theta)(k_{11,10} - \theta)(1 - e^{\theta b})e^{-\theta t}}{\theta(\theta - \gamma)(\theta - \delta)(\theta - \epsilon)(\theta - \zeta)(\theta - \eta)}\right]$$

The appropriate equations describing the INH model shown in Figure 19 are indicated below. If INH is presumed not to be metabolized directly to INA, k_{17} and f_{17} are equal to zero.

$$f_{14} = \frac{k_{14}}{k_{13} + k_{14} + k_{17}} \qquad f_{17} = \frac{k_{17}}{k_{13} + k_{14} + k_{17}}$$

$$1 - f_{47} = f_4 = f_{46} = \frac{k_{46}}{k_{46} + k_{47}} \qquad f_{79} = \frac{k_{79}}{k_{79} + k_{7,10}} = f_7$$

$$C_1 = \frac{k^o}{V_1}\left[\frac{(k_{21} - \alpha)(1 - e^{\alpha b})e^{-\alpha t}}{\alpha(\alpha - \beta)} + \frac{(k_{21} - \beta)(1 - e^{\beta b})e^{-\beta t}}{\beta(\beta - \alpha)}\right.$$

$$\frac{dA_6}{dt} = \frac{f_{14}f_{46}\,\alpha\beta\gamma\delta k^o}{k_{21}k_{54}}\left[\frac{(k_{21} - \alpha)(k_{54} - \alpha)(1 - e^{\alpha b})e^{-\alpha t}}{\alpha(\alpha - \beta)(\alpha - \gamma)(\alpha - \delta)}\right.$$

$$\frac{(k_{21} - \beta)(k_{54} - \beta)(1 - e^{\beta b})e^{-\beta t}}{\beta(\beta - \alpha)(\beta - \gamma)(\beta - \delta)} +$$

$$\frac{(k_{21} - \gamma)(k_{54} - \gamma)(1 - e^{\gamma b})e^{-\gamma t}}{\gamma(\gamma - \alpha)(\gamma - \beta)(\gamma - \delta)} +$$

$$\left.\left.\frac{(k_{21} - \delta)(k_{54} - \delta)(1 - e^{\delta b})e^{-\delta t}}{\delta(\delta - \alpha)(\delta - \beta)(\delta - \gamma)}\right]\right]$$

$$\frac{dA_9}{dt} = \frac{f_{14}(1 - f_{46}) f_{79}^{\alpha\beta\gamma\delta\epsilon\zeta} k^o}{k_{21} k_{54} k_{87}} \left[\frac{(k_{21} - \alpha)(k_{54} - \alpha)(k_{87} - \alpha)(1 - e^{\alpha b}) e^{-\alpha t}}{\alpha(\alpha - \beta)(\alpha - \gamma)(\alpha - \delta)(\alpha - \epsilon)(\alpha - \zeta)} + \right.$$

$$\frac{(k_{21} - \beta)(k_{54} - \beta)(k_{87} - \beta)(1 - e^{\beta b}) e^{-\beta t}}{\beta(\beta - \alpha)(\beta - \gamma)(\beta - \delta)(\beta - \epsilon)(\beta - \zeta)} +$$

$$\frac{(k_{21} - \gamma)(k_{54} - \gamma)(k_{87} - \gamma)(1 - e^{\gamma b}) e^{-\gamma t}}{\gamma(\gamma - \alpha)(\gamma - \beta)(\gamma - \delta)(\gamma - \epsilon)(\gamma - \zeta)} +$$

$$\frac{(k_{21} - \delta)(k_{54} - \delta)(k_{87} - \delta)(1 - e^{\delta b}) e^{-\delta t}}{\delta(\delta - \alpha)(\delta - \beta)(\delta - \gamma)(\delta - \epsilon)(\delta - \zeta)} +$$

$$\frac{(k_{21} - \epsilon)(k_{54} - \epsilon)(k_{87} - \epsilon)(1 - e^{\epsilon b}) e^{-\epsilon t}}{\epsilon(\epsilon - \alpha)(\epsilon - \beta)(\epsilon - \gamma)(\epsilon - \delta)(\epsilon - \zeta)} +$$

$$\left. \frac{(k_{21} - \zeta)(k_{54} - \zeta)(k_{87} - \zeta)(1 - e^{\zeta b}) e^{-\zeta t}}{\zeta(\zeta - \alpha)(\zeta - \beta)(\zeta - \gamma)(\zeta - \delta)(\zeta - \epsilon)} \right] +$$

$$\frac{f_{17} f_{79}^{\alpha\beta\epsilon\zeta} k^o}{k_{21} k_{87}} \left[\frac{(k_{21} - \alpha)(k_{87} - \alpha)(1 - e^{\alpha b}) e^{-\alpha t}}{\alpha(\alpha - \beta)(\alpha - \epsilon)(\alpha - \zeta)} + \right.$$

$$\frac{(k_{21} - \beta)(k_{87} - \beta)(1 - e^{\beta b}) e^{-\beta t}}{\beta(\beta - \alpha)(\beta - \epsilon)(\beta - \zeta)} +$$

$$\frac{(k_{21} - \epsilon)(k_{87} - \epsilon)(1 - e^{\epsilon b}) e^{-\epsilon t}}{\epsilon(\epsilon - \alpha)(\epsilon - \beta)(\epsilon - \zeta)} +$$

$$\left. \frac{(k_{21} - \zeta)(k_{87} - \zeta)(1 - e^{\zeta b}) e^{-\zeta t}}{\zeta(\zeta - \alpha)(\zeta - \beta)(\zeta - \epsilon)} \right]$$

$$\frac{dA_{12}}{dt} = \frac{f_{14}(1 - f_{46})(1 - f_{79})\alpha\beta\gamma\delta\epsilon\zeta\eta\theta k^{o}}{k_{21}k_{54}k_{87}k_{11,10}} \quad X$$

$$\left[\frac{(k_{21} - \alpha)(k_{54} - \alpha)(k_{87} - \alpha)(k_{11,10} - \alpha)(1 - e^{\alpha b})e^{-\alpha t}}{\alpha(\alpha - \beta)(\alpha - \gamma)(\alpha - \delta)(\alpha - \epsilon)(\alpha - \zeta)(\alpha - \eta)(\alpha - \theta)} + \right.$$

$$\frac{(k_{21} - \beta)(k_{54} - \beta)(k_{87} - \beta)(k_{11,10} - \beta)(1 - e^{\beta b})e^{-\beta t}}{\beta(\beta - \alpha)(\beta - \gamma)(\beta - \delta)(\beta - \epsilon)(\beta - \zeta)(\beta - \eta)(\beta - \theta)} +$$

$$\frac{(k_{21} - \gamma)(k_{54} - \gamma)(k_{87} - \gamma)(k_{11,10} - \gamma)(1 - e^{\gamma b})e^{-\gamma t}}{\gamma(\gamma - \alpha)(\gamma - \beta)(\gamma - \delta)(\gamma - \epsilon)(\gamma - \zeta)(\gamma - \eta)(\gamma - \theta)} +$$

$$\frac{(k_{21} - \delta)(k_{54} - \delta)(k_{87} - \delta)(k_{11,10} - \delta)(1 - e^{\delta b})e^{-\delta t}}{\delta(\delta - \alpha)(\delta - \beta)(\delta - \gamma)(\delta - \epsilon)(\delta - \zeta)(\delta - \eta)(\delta - \theta)} +$$

$$\frac{(k_{21} - \epsilon)(k_{54} - \epsilon)(k_{87} - \epsilon)(k_{11,10} - \epsilon)(1 - e^{\epsilon b})e^{-\epsilon t}}{\epsilon(\epsilon - \alpha)(\epsilon - \beta)(\epsilon - \gamma)(\epsilon - \delta)(\epsilon - \zeta)(\epsilon - \eta)(\epsilon - \theta)} +$$

$$\frac{(k_{21} - \zeta)(k_{54} - \zeta)(k_{87} - \zeta)(k_{11,10} - \zeta)(1 - e^{\zeta b})e^{-\zeta t}}{\zeta(\zeta - \alpha)(\zeta - \beta)(\zeta - \gamma)(\zeta - \delta)(\zeta - \epsilon)(\zeta - \eta)(\zeta - \theta)} +$$

$$\frac{(k_{21} - \eta)(k_{54} - \eta)(k_{87} - \eta)(k_{11,10} - \eta)(1 - e^{\eta b})e^{-\eta t}}{\eta(\eta - \alpha)(\eta - \beta)(\eta - \gamma)(\eta - \delta)(\eta - \epsilon)(\eta - \zeta)(\eta - \theta)} +$$

$$\left. \frac{(k_{21} - \theta)(k_{54} - \theta)(k_{87} - \theta)(k_{11,10} - \theta)(1 - e^{\theta b})e^{-\theta t}}{\theta(\theta - \alpha)(\theta - \beta)(\theta - \gamma)(\theta - \delta)(\theta - \epsilon)(\theta - \zeta)(\theta - \eta)} \right]$$

$$+ \frac{f_{17}(1 - f_{79})\ \alpha\beta\epsilon\zeta\eta\theta k^{o}}{k_{21}k_{87}k_{11,10}} \times$$

$$\left[\frac{(k_{21} - \alpha)(k_{87} - \alpha)(k_{11,10} - \alpha)(1 - e^{\alpha b})e^{-\alpha t}}{\alpha(\alpha - \beta)(\alpha - \epsilon)(\alpha - \zeta)(\alpha - \eta)(\alpha - \theta)} + \right.$$

$$\frac{(k_{21} - \beta)(k_{87} - \beta)(k_{11,10} - \beta)(1 - e^{\beta b})e^{-\beta t}}{\beta(\beta - \alpha)(\beta - \epsilon)(\beta - \zeta)(\beta - \eta)(\beta - \theta)} +$$

$$\frac{(k_{21} - \epsilon)(k_{87} - \epsilon)(k_{11,10} - \epsilon)(1 - e^{\epsilon b})e^{-\epsilon t}}{\epsilon(\epsilon - \alpha)(\epsilon - \beta)(\epsilon - \zeta)(\epsilon - \eta)(\epsilon - \theta)} +$$

$$\frac{(k_{21} - \zeta)(k_{87} - \zeta)(k_{11,10} - \zeta)(1 - e^{\zeta b})e^{-\zeta t}}{\zeta(\zeta - \alpha)(\zeta - \beta)(\zeta - \epsilon)(\zeta - \eta)(\zeta - \theta)} +$$

$$\frac{(k_{21} - \eta)(k_{87} - \eta)(k_{11,10} - \eta)(1 - e^{\eta b})e^{-\eta t}}{\eta(\eta - \alpha)(\eta - \beta)(\eta - \epsilon)(\eta - \zeta)(\eta - \theta)} +$$

$$\left. \frac{(k_{21} - \theta)(k_{87} - \theta)(k_{11,10} - \theta)(1 - e^{\theta b})e^{-\theta t}}{\theta(\theta - \alpha)(\theta - \beta)(\theta - \epsilon)(\theta - \zeta)(\theta - \eta)} \right]$$

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