THE PHARMACOKINETICS OF ETHAMBUTOL

by

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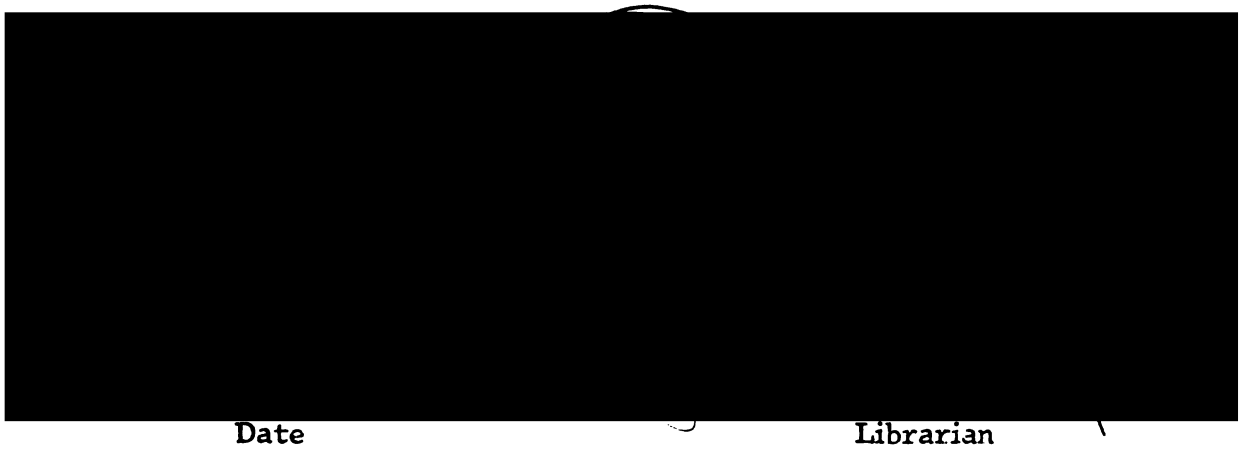

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O.K.
Leslie T. Benet

Abstract

THE PHARMACOKINETICS OF ETHAMBUTOL

Ching-san Lee

Pharmacokinetic studies of ethambutol require a sensitive and specific method of measuring the unchanged compound. We have developed a gas-liquid chromatographic electron capture detector method for ethambutol involving derivatization with trifluoroacetic anhydride and a second GLC method involving derivatization with bis(trimethylsilyl)-trifluoroacetamide and subsequent quantitation using a flame ionization detector. The former method of detection was used for plasma and dialysate assays and the latter for urine assays.

Following analytical development, I.V. and oral dosing of ethambutol in three rhesus monkeys was studied. Monkey plasma and urine data fit a two compartment body model, where the α phase had a mean half-life of 9.8 min and the half-life of the β phase ranged from 2.4 to 3.4 hr. Mean values for the central compartment and the steady-state volumes of distribution in the monkey were 0.36 and 1.58 L/kg, respectively. Fraction of the dose eliminated unchanged in the urine varied from 0.65 to 0.80. Renal clearances were 6.7 to 9.4 ml/min kg indicating an active secretion mechanism. Deconvolution of oral plasma curves indicated that the absorption in the monkey may be described by two consecutive first-order processes.

Human I.V. and oral ethambutol dosing studies were initiated in 6 normal volunteers. A four compartment body model was used for the I.V. plasma data analysis. The α, β, γ and δ phases in these subjects had a mean half-life of 8.6, 40.1, 135 and 924 min. respectively. Mean values for the central compartment and steady-state volumes of distribution were 0.42 and 4.02 L/kg, respectively. The large extravascular volume reflects the significance of the longer γ and δ phases in estimating total amounts of drug in the body. Total body and renal clearance averaged 8.5 and 6.7 ml/min kg, respectively indicating active renal secretion in man as well as in monkey.

Bioavailability was estimated from areas under the plasma curve and 72 hr urinary excretion data. Approximately 75% of the oral dose is available to the systemic

circulation. No significant bioavailability difference was found between solution and tablet dosing. The oral human data exhibited an unusual absorption pattern. Absorption rate plots seem to suggest that ethambutol absorption may be described by three first-order processes in these subjects. Confirmation of this must wait further studies. The unusual absorption of ethambutol in man and monkey might be explained by binding in the gastrointestinal tract which can become rate limiting in the absorption process.

Three patients with tuberculosis and normal kidney function were studied. Peak times, terminal half-lifes and renal clearance values were similar to those reported in our studies with normals. However, the percent of the unchanged drug excreted in the urine and peak plasma concentrations tended to be lower than that found in our previous studies with normal volunteers. We speculate that the decreased plasma levels and percent of drug excreted unchanged in tuberculosis patients are due to decreased bioavailability. A pharmacokinetic study in a patient with tuberculosis and renal insufficiency (creatinine clearance = 14 ml/min) resulted in a low urinary recovery of ethambutol (30.3% of dose) and decreased renal clearance (60 ml/min).

One anuric patient with tuberculosis undergoing hemodialysis was studied. The dialysis plasma clearance ranged from 48.4 to 61.5 ml/min and averaged 54.6 ml/min. Two dialysis blood clearances values were obtained with an average of 57.7 ml/min. The plasma volume of distribution for ethambutol in this patient averaged 62.4 L, approximating 80% of his body weight. In the patient studied, the dialysis clearance contributes 40.6% to the total body clearance. This significant contribution was also reflected by the on and off dialysis half-lives ($t_{\frac{1}{2}}$, on = 5.7 and $t_{\frac{1}{2}}$, off = 9.6 hr).

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CHAPTER 1

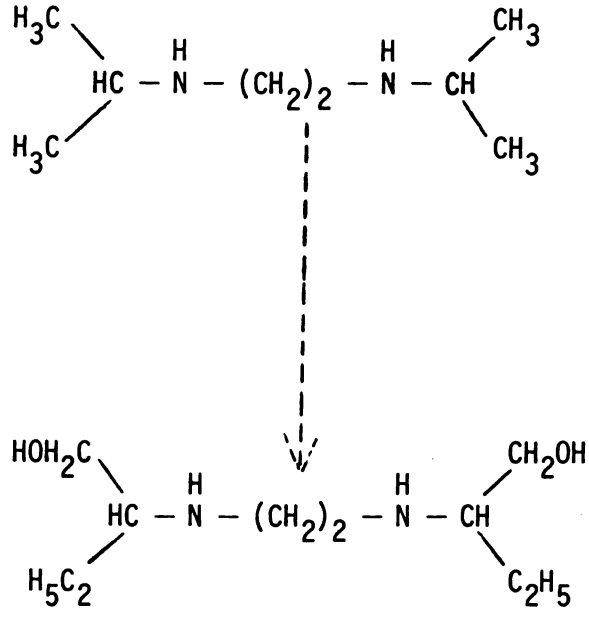
INTRODUCTION

Ethambutol (EMB) is a valuable and effective drug used in the treatment of tuberculosis. It was first considered as an oral replacement for streptomycin in 1968 (1). The chemistry of EMB has been extensively discussed and reviewed (2, 3, 4, 5) since its introduction in 1961. The pharmacological, biochemical and toxicological (6, 7, 8) properties of EMB in laboratory animals have been investigated. The basic features of the clinical pharmacology of EMB following oral ingestion and I.V. injection were determined by Place (9) in 1964. In the first portion of this introduction we shall review the chemistry, the pharmacology, biochemistry, toxicology and pharmacokinetics of EMB in various animal species as well as human subjects.

Chemistry

While screening selected compounds, Thomas and coworkers (10) found that N, N'-di-isopropylethylenediamine was effective in the treatment of experimental tuberculous infections in mice. A number of congeners of this compound were examined; the one that eventually proved to be most tuberculostatic was ethylenediimino-di-1-butanol dihydrochloride (Fig. 1-1). Ethambutol is a weak base with pK_a values of 6.6 and 9.5 (5). The summary formula for the dihydrochloride salt of EMB is $C_{10}H_{24}O_2N_2(2HCl)$, with a corresponding molecular weight of 277.5. The dihydrochloride salt of EMB is a white, scentless, bitter tasting, thermostable crystalline powder, easily soluble in water and dimethylsulfoxide; soluble in glycol; sparingly soluble in ethanol and very difficultly soluble in acetone and chloroform (11). On the contrary, the free base of EMB is very soluble in chloroform, methylenechloride, and ethylenedichloride; less soluble in benzene and carbon tetrachloride; and sparingly soluble in water (12).

N, N'-di-isopropylethylenediamine



Ethambutol

Fig. 1-1 The birth of ethambutol.

The EMB molecule is optically active. The levo isomer is tuberculostatic inactive, the meso isomer has approximately 1/8 to 1/10 of the activity of the dextro isomer and the racemate is only half as active as the dextro isomer. Therefore only the dextro isomer is used therapeutically. Ethambutol is structurally analogous to EDTA (ethylene diamine tetraacetic acid) and possesses strong chelating capacity. According to Shepherd et al. (3, 5), the presence of two basic centers is essential for the antibacterial activity. Chelation via the basic centers may produce activity directly by interferring with one of the many metal-containing enzyme systems, such as alcohol dehydrogenase or cytochrome oxidase.

Pharmacology

About 75% of strains of the human type of M. tuberculosis are sensitive to 1 mcg/ml of EMB in vitro. After infecting mice with the strain $H37R_v$, the survival rate for 50 mg/kg doses administered subcutaneously was 100% (10). For oral treatment with 50 mg/kg, the survival rate in mice was 70% (11). Substantial antituberculous activity was found for daily oral doses of 12.5 to 100 mg/kg in rhesus monkeys (7). EMB also suppresses the growth of isoniazid and streptomycin resistant tubercle bacilli. Resistance to EMB develops only very slowly in vitro.

Mycobacteria take up EMB rapidly when the drug is added to cultures that are in the exponential growth phase. EMB has no effect on the viability of nonproliferating cells, its effect is limited to cells in the multiplication phase (13). Chemical determinations show that ethambutol-inhibited cells become deficient in RNA (13). Isotope incorporation studies using ^{32}P also confirmed that the rate of RNA synthesis was decreased in the presence of EMB (13).

Optimal therapeutic serum levels in the mouse, monkey and human are

similar; 3 to 5 mcg/ml. The therapeutic ratio of EMB given orally to animals infected with M. tuberculosis is similar to that of isoniazid (14).

Biochemistry

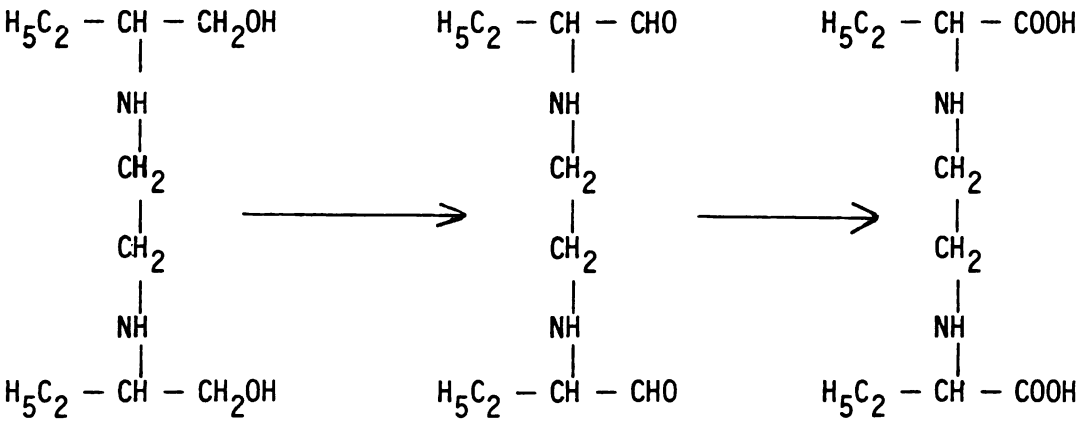
An initial metabolism of EMB results from the oxidation of the primary alcohol groups to an aldehyde. This is followed by a further oxidation to a dicarboxylic acid to produce the end metabolite. This extremely polar metabolite is excreted readily by the kidneys without further conjugation. The biotransformation of EMB is shown in Fig. 1-2.

The catalytic enzyme responsible for EMB metabolism, alcohol dehydrogenase is a zinc containing protein. It has been shown that this enzyme can utilize the primary alcohol groups of ethambutol as a substrate and convert them to aldehydes (15). Peets has reported (16) an interaction between EMB and alcohol dehydrogenase (ADH) from yeast that results in physical changes in the enzyme and an increase in the ability of the enzyme to oxidize ethanol. This stimulatory activity of EMB is restricted to a alcohol dehydrogenase from yeast. Ethambutol has no effect on crystalline ADH from horse liver or a semipurified fraction from the liver of the rat. The reasons for these differences are unknown but may be due to the considerable differences in the molecular dimensions and reaction kinetics of the enzyme from the different sources.

Several enzymes essential to the normal functions of the body contain copper or zinc or require these trace metals as cofactors. For example, cytochrome C oxidase and DOPA decarboxylase both require iron for maximal activity. Using semipurified preparations from mammalian sources, EMB was shown to have no effect on the activity of these enzymes in vitro.

Toxicology

Acute toxicity studies using the oral route of administration were



ethambutol dialdehyde diacid

Fig. 1-2 The biotransformation of ethambutol.

conducted in rats and mice. The LD_{50} was found to be 8.9 mg/kg for mouse and 6.8 mg/kg for rat (17).

In chronic toxicity studies with dogs over a period of 6-12 months all animals survived with a dosage of 400 mg/kg daily (18). Toxicity studies with monkeys at a dose of 200 mg/kg over a period of 6 months proceeded without abnormal clinical and/or pathologic-histological changes (7).

The most important side effect of EMB is optic neuritis, resulting in decrease of visual acuity and loss of ability to perceive the color green. In monkey visual impairment was demonstrated with daily doses which produced peak serum levels of approximately 130 mcg/ml (17). In human the visual toxicity is quite uncommon with an oral dose of 25 mg/kg per day but occurs more often with 50 mg/kg per day. Recovery usually occurs when EMB is withdrawn (19). According to Pernod et al. (20), tolerance was excellent and no ocular toxicity was encountered in 65 patients each receiving 60-90 infusions of 10-25 mg/kg of EMB intravenously.

Absorption

Following the oral administration of EMB approximately 80% of the drug is absorbed (20, 21, 22). Place et al. (23) gave various single oral doses of EMB (4, 8, 12.5, 25 and 50 mg/kg) to normal subjects and the resulting peak serum levels were 0.7, 1.3, 2.0, 4.2, and 8.6 mcg/ml, respectively. Peak time ranged from 2 to 4 hrs after drug ingestion demonstrating a relatively rapid absorption. As reported by Dume et al. (24), the mean peak serum level at 2 hr was 2 mcg/ml when EMB was given to 11 normal subjects at a single dose of 25 mg/kg orally. Anex et al. (33) reported peak plasma concentrations ranging from 1 to 6 mcg/ml after oral doses of 30 mg/kg. After administration of a dose of 25 mg/kg ,

Delaude (26) obtained blood levels of 4.1-6.0 mcg/ml; the average residual level after 24 hrs was 0.8 mcg/ml. According to Tacquet (27), serum levels of 2 to 4 mcg/ml were attained within 2 to 4 hrs after administration of a single oral dose of 25 mg/kg of EMB; these levels then decreased and fell to less than 10% of the maximum concentrations after 24 hrs. Pujet and Pujet (28) found maximum serum levels averaged 3.4 mcg/ml following a 20 mg/kg dose. The differences among values reported by these groups are significant. Table 1-1 exemplifies the variation found in peak plasma levels reported following oral dosing.

Distribution

Generally speaking, EMB absorption is terminated 3 to 4 hrs after ingestion. Following this, EMB becomes distributed throughout the extracellular fluids and in the tissues.

The distribution of EMB in dog was studied by Arnaud et al. (35). They reported moderate levels in the blood, very high levels in the viscera (liver, lungs, kidneys) with very low levels in cerebrospinal fluid. In one case studied in man by Pujet and Pujet (29), the intravascular and the intrapulmonary levels of EMB after intravenous infusion of 20 mg/kg over 2 hrs were:

red blood cells	5 mcg/ml
serum	9.5 mcg/ml
whole blood	6.5 mcg/ml
deep layers of healthy lung	10 mcg/ml
subpleural layers of healthy lung	8.5 mcg/ml
neoplastic lung	9 mcg/ml
tuberculous lung	8.5 mcg/ml

Their study of the distribution of EMB in blood, body fluids and lung

Table 1-1

Variation in Reported Peak Plasma Levels of

Ethambutol Following Oral Dosing

Reference	Dose (mg/kg)	Oral Dosing Peak Concentration (mcg/ml)	Peak Time (hr)	Assay Method
23	4	.7	2 - 4	microbiol.
	8	1.3		
	12.5	2.0		
	25	4.2		
	50	8.6		
27	25	2 - 4	2 - 4	microbiol.
32	25	2 - 5	3	colorimetric
24	25	2	2	microbiol.
33	25	1 - 6	2	colorimetric
26	15	2 -2.5	2 - 4	microbiol.
	20	3.1-3.5		
	25	4 - 5		
	50	8.6		
29	20	3.4	3	microbiol.

parenchyma shows that distribution in these tissues is quite adequate. The drug concentrations in the lung tissues are comparable to those in the vascular system. Delaude and Albarede (30) also found intrapulmonary levels to be at least equal to the corresponding serum concentrations.

It has been demonstrated both in vivo and in vitro that EMB has a preference for partition into red blood cells (31). The studies performed by Peets et al. (31) with EMB tagged with ^{14}C demonstrated that at the end of one hour the intraerythrocytic concentration of EMB was twice as high as the serum concentration. Between one and two hours, the plasma was cleared of ^{14}C faster than were the cells, and the enrichment factor (ratio of ^{14}C in cells to ^{14}C in plasma) increased to a value of 3. At eight hours following the dose, the erythrocytes still contained twice the levels of ^{14}C associated with EMB as did the plasma. Thus EMB quickly penetrates the red corpuscles, is stored there and is released progressively in the hours following its administration.

The protein binding of EMB has been stated to be "probably slight" by Jenne and Beggs (21). The apparent volume of distribution of EMB has been estimated to be approximately 80% of the body weight (25). Jenne and Beggs (21) cited a value of 30% body weight which probably corresponds only to the central compartment volume. These values are compared in Table 1-2.

Elimination

Pharmacokinetic parameters relating to EMB elimination were summarized in Table 1-2.

According to Peets et al. (22), from 79 to 94% of an oral dose and 82% of an I.V. injection of the drug are recovered in the urine and feces 144 hrs after administration. The fraction of an oral dose excreted

Table 1-2

Summary of Ethambutol Pharmacokinetic Parameters Reported in Man

Reference	Elimination Half-Lives in Normals (hrs)	Fraction Excreted Unchanged	Renal Failure Half-Life (hrs)	Volume of Distribution (% body weight)	Plasma Clearance (ml/min)	Renal Clearance (ml/min)	Assay
23	4	0.6-0.8 (oral)					microbiol.
24	4.2	0.46 (oral)	7.2				microbiol.
33	12-15		12-15				colorimetric
25	3.0[a] 1.2[b]	0.84 (i.v.)	7.4	80%[c]	532	446	radiochem.
21	2.5			30%[d]	100		microbiol.

[a] Estimated from Fig. 3 in reference 25.

[b] Calculated from clearance data in reference 25.

[c] Two compartment model was used for data analysis.

[d] Probably correspond only to the central compartment volume.

unchanged was 0.6-0.8 in Place's (9), 0.46 in Dume's (24), and 0.84 in Christopher's (25) work.

The plasma clearance reported by Christopher et al. (25) was 532 ml/min which corresponds to a renal clearance of about 450 ml/min. This would indicate an active secretion mechanism for this drug. However, calculated from the cited data of Jenne and Beggs (21), the plasma clearance was only 100 ml/min. Since little protein binding was demonstrated, this value would indicate an elimination mechanism of either glomerular filtration only or active secretion which is equally counteracted by reabsorption. Strauss and Erhardt (32) proposed that drug elimination occurred by only glomerular filtration based on the following two observations: (1) serum levels rose with diminished glomerular function, (2) decreases in tubular function were not associated with elevated serum level of the drug. Anex found no statistical correlation between EMB blood concentration and inulin clearance (33).

The metabolism of EMB was found not to be extensive, only 8 to 15% of a given oral or I.V. dose appears in the urine as metabolite in 24 hrs (9, 23). The metabolites, EMB aldehyde and its dicarboxylic acid derivative, are believed to be inactive microbiologically (22).

Jenne and Beggs (21) calculated a half-life of 2.5 hr from an I.V. dose of the drug. They stated that this value was "calculated from an I.V. decay curve of Place and Thomas" (9) but actually there is no I.V. data reported in that reference. Dume et al. (24) measured a half-life of 4.2 hr in 11 normal subjects after an oral dose. Christopher et al. (25) showed a half-life of 3 hr in the plasma decay curve given as Fig. 3 in their paper, yet a half-life of 1.2 hr was calculated from their clearance data. The half-life values reported by Jenne and Dume were

obtained considering the body as a single compartment, whereas a two compartment open body model was used in Christopher's work. The half-life obtained in the two pool model was designated as $t_{\frac{1}{2},\beta}$, the elimination half-life when distribution has reached equilibrium. However, the use of different model should not yield differences in half-life between the three studies since all investigators would presumably calculate the half-life from the terminal log linear slope.

Fecal excretion in a 48 hour collection after an oral ^{14}C dose was found to be 19% (22, 23). Eleven normal volunteers given 25 mg/kg of EMB orally in a single dose excreted up to 9.1% of the dose in the feces collected over 36 hours (24). The fecal excretion of the drug following oral administration actually represents the fraction of the dose unabsorbed.

By contrast, pharmacokinetic parameters relating to the I.V. administration of EMB are rather scare, due to the limited use of this route of administration. Place et al. (23) published the first data on the metabolism and excretion of EMB following I.V. injection, using ^{14}C-labelled drug. Rossi et al. (34) and Pujet and Pujet (28) have determined the blood levels and body fluid concentrations of EMB using bacteriological methods of determination when the drug was administered as an I.V. perfusion. The results from the I.V. studies correspond almost point to point with those from oral studies, differing slightly in the following respects: For the I.V. studies (a) urinary elimination is slightly more rapid, (b) there is essentially no fecal excretion, (c) greater and more prolonged intra-erythrocytic penetration is observed and (d) a more fleeting maximum blood concentration was noted. However, the peak levels following intravenous infusion were not established with precision. This is best exemplified by the variation summarized in Table 1-3. In 1968 Rossi et al. (34)

Table 1-3

Variation in Reported Peak Plasma Levels

of Ethambutol following Intravenous Dosing

Reference	Dose (mg/kg)	Intravenous Dosing Peak Concentration (mcg/ml)	Infusion Time (hr)	Assay Method
34	15-20	35-45	2	microbiol.
35	15-25	5-23	1.5	colorimetric
26	20-25	1-10	2	microbiol.
28	20	2.4-26	2	microbiol.
30	25	4.1- 6	2	microbiol.

observed peak values ranging from 35 to 45 mcg/ml following an infusion of 15-20 mg/kg of EMB over a 2 hr period. By contrast, Delaude and Albarede (30) observed peak serum concentrations of the same order as that seen after administration of the same dose of EMB by the oral route (4.1-6 mcg/ml intravenously and 4-5 mcg/ml orally at the same dose of 25 mg/kg). In Pujet and Pujet's (28) study of 22 patients infused with a 20 mg/kg dose, peak serum levels ranged from 2.4 to 26 mcg/ml (mean = 12.6 mcg/ml). These results are quite close to those obtained by Arnaud et al. (35) of 11.7 ± 4.6 mcg/ml following a standard dose of 1200 mg infused over the same time period. Based on their preliminary laboratory findings, Delaude et al. (30) indicated that EMB injectable did not possess any therapeutic properties that greatly differ from those that characterize the oral form. Majority of the I.V. studies resulted in maximum levels above 10 mcg/ml, levels at which EMB has not merely a bacteriostatic action but a bacteriocidal action for a high percentage of Mycobacterium tuberculosis strains. These results (29, 34, 35) led to the suggestion that an injectable form of EMB be used in human therapy.

Dosage in Renal Failure

The half-life in renal failure calculated from Christopher's data (25) was 9.6 hr which was more than 3 times longer than that found in normals. Dume et al. (24) found a half-life of 7.2 hr in nephrectomized patients which was 1.7 times longer than that of normals. Anex (33) reported half-lives of 3-30 hrs from a population of 50 patients with different degrees of renal function.

There has been no adquate study performed to determine the dosage requirements of EMB in patients with renal failure. Bailey (36) recommends that normal doses be used in renal failure. However, due to the large

percent of drug excreted unchanged in the urine it seems that dosage adjustments are necessary if renal function is reduced. Based on a 25 mg/kg/day dosage, Dume et al. (24) suggested that patients with severe renal failure be given 17.9 mg/kg/day. Strauss and Erhardt (32) recommended that the dose in patients with creatinine clearance below 70 ml/min be adjusted to less than 15 mg/kg. Christopher et al. (25) suggested a 5.0 mg/kg/day dose for anephrics. In general, these recommendations are very empirical and arbitrary in nature.

Effect of Dialysis

Bailey (36) stated that EMB is not dialyzable and that the blood level of drug is not significantly reduced by hemodialysis. However, he presents no specific data in support of this statement. Dume et al. (24) observed a half-life of 5.1 hr on dialysis compared to an off dialysis half-life of 7.2 hr during a 30 hr peritoneal dialysis, while on an 8 hr hemodialysis, half-life was 2.1 hr. Based on these values, they suggested a 23 mg/kg dose be given immediately following an 8 hr period of hemodialysis and 17.8 mg/kg per 24 hr during a 30 hr period of peritoneal dialysis. These amounts represent the quantity of drug lost during the dialysis interval. Replacement of the drug at the doses suggested should maintain serum levels of EMB at 3-5 mcg/ml. Christopher et al. (25) measured a dialysis clearance for EMB of 37-52 ml/min. Assuming a dialysis clearance of 50 ml/min and a metabolic clearance of 90 ml/min in anephrics, they suggested that the EMB dose be increased from 1.5 mg/kg three times a day to 2.0 mg/kg tid for anephrics on hemodialysis. This increase corresponds to a decreased half-life of 34%. A summary of dosage recommendations of EMB for patients with renal insufficiency and uremic patients undergoing dialysis is given in Table 1-4.

Table 1-4

Dosage Recommendations for Patients with Renal Insufficiency and Uremic Patients undergoing Dialysis (mg/kg/day)

	Normal		Renal Failure			Dialysis	
Reference	$t_{1/2}$	Dose	Degree	$t_{1/2}$	Dose	$t_{1/2}$	Dose
36		25			25		25
24	4.2	25	anephric	7.2	17.9	2.1	23[a]
						5.1	17.8[b]
32		25	<70 ml/min		<15		
25	3.0	25	anephric		5		2.0 tid[c]

[a] hemodialysis, dose given at the end of 8 hr.

[b] peritoneal dialysis, dose given at the end of 24 hr.

[c] predialysis dose

CHAPTER 2
ANALYTICAL METHODS

INTRODUCTION

Because of greater tuberculostatic activity, lower incidence of toxic side effects, and better patient acceptance (9), ethambutol has become increasingly popular in the treatment of tuberculosis since its introduction in 1961. However, until very recently, specific and sensitive chemical assays for the drug in biological fluids have not been available.

In the following, the analytical methods for ethambutol will be reviewed in chronological order.

1961 Elemental analysis (as $C_{10}H_{24}O_2N_2 \cdot 2HCl$)

Element	% Theory	Reported (3)
C	43.3	43.5
H	9.5	9.7
N	10.1	10.4
Cl	25.6	25.6

1965 Microbiological analysis

This technique consists of measuring the diameter of the zone of inhibition of disks impregnated with the sample to be tested, or of cylinders containing the sample to be tested, on a homogeneous culture of Mycobacterium smegmatis (9), Mycobacterium aurum (28), Mycobacterium parafortuitum (28) or Mycobacterium diernhoferi (29). The lower limit of sensitivity of this method is 0.5 mcg/ml.

1967 Colorimetric analysis

This method is based on the formation of a complex of bromothymol blue and ethambutol, a complex whose yellow color may be quantitatively

measured by means of spectrophotometry. The technique was first described by Kelley and Huerga (37), modified by Strauss and Erhardt (32) and by Froseth (38). According to Strauss and Erhardt (32), the sensitivity for the determination of ethambutol in serum is 0.5 mcg/ml.

1968 Gas chromatographic - FID

Calo et al. (39) published a gas chromatographic method for the separation of three antitubercular drugs, isoniazid, iproniazid and ethambutol. The benzene solution of the trimethylsilyl ethambutol, injected along with an alcohol solution of isoniazid and an ethereal solution of iproniazid was chromatographed using a programmed temperature system. The retention time for the TMS ethambutol was 12 min. The sensitivity of detection was not reported.

1969 Reineckate analysis

The Reineckate assay procedure (40) is a gravimetric determination. Ethambutol in prepared test sample solution is precipitated by adding saturated ammonium reineckate solution. Each gram of the dried precipitate at 105^{o} is equivalent to 0.3292 g of ethambutol hydrochloride. The washed precipitate can be further dissolved in methanol and the absorbance read at 525 nm. The chelation of ethambutol with reineckate produces a stable color with low absorptivity, and small amounts can not be determined with sufficient accuracy.

1970 Gas chromatographic - FID

Richard et al. (41) reported a gas chromatographic determination of ethambutol involving derivatization with N-trimethylsilylimidazole. The method made use of a single step derivatization, isothermal temperature and a multipurpose column. The retention time for the TMS ethambutol was 4 min. No internal standard was used.

1974 GC-CIMS analysis

The method (42) involves the use of a tetradeuterated ethambutol standard and derivatization with trimethylsilylimidazole prior to injection onto a GC-CIMS. Mass fragmentography was monitored at m/e 349 and 353, the M+1 ions for the di-TMS derivatives of ethambutol and internal standard respectively, as well as at m/e 333 and 337, corresponding to the $M-CH_3$ ions for ethambutol and ethambutol-d_4. The nominal sensitivity in a 0-20 mcg/ml range is 50 ng/ml.

Except for the recently developed GC-CIMS method which involves relatively complicated procedures and requires costly equipment , other available methods may only be used to measure quantities of drug much greater than those found in biological samples. Pharmacokinetic studies of EMB require a sensitive and specific method of measuring the unchanged compound. A gas-liquid chromatographic (GLC) method has been developed for EMB involving derivatization with trifluoroacetic anhydride (TFAA) and subsequent quantitation using an electron capture detector. The electron capture method of detection is required for samples such as blood, plasma and in particular, dialysate in which low levels of EMB are anticipated. A second GLC method involving derivatization with bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and subsequent quantitation using a flame ionization detector is described. The flame ionization method is suitable for urine samples or for dosage form analysis in which high levels of EMB are anticipated. Using a dual column and dual detector gas liquid chromatograph, simultaneous micro (plasma, blood and dialysate) and macro (urine, dosage form) determination of EMB becomes possible.

The biotransformation of ethambutol has been discussed in Chapter 1. Two metabolites, 2,2'-(ethylenediimino)di-butyric aldehyde and 2,2'-

(ethylenediimino)di-butyric acid, have been identified by countercurrent distribution analysis of radioactive ethambutol in the urine of human and dog (22). The diacid metabolite has also been identified by comparative paper chromatography.

We developed an ion exchange liquid chromatographic method for the identification of the diacid metabolite. The reaction of ninhydrin with the diamino dicarboxylic acid metabolite yields a purple substance that absorbs maximally near 570 nm. An automatic amino acid analyzer (Beckman Model 116C) was used for separation and identification. The ion exchange resin used was Beckman UR-30. Using norlucine as an internal standard, it is possible to quantitate the metabolite in the urine.

Finally, the radioactivity assay of ^{14}C-EMB in monkey plasma and monkey urine samples will be discussed.

EXPERIMENTAL

Materials

EMB (Myambutol^{R}), ^{14}C-EMB (2.16 μCi/mg), dextro-2,2'-(ethylenediimino)-di-1-propanol (MEMB), and dextro-2,2'(ethylenediimino)di-1-butyric acid were graciously supplied by Lederle Laboratories, Pearl River, N.Y., Decanediol[1] (DDOL), bis(trimethylsilyl)trifluoroacetamide[2], trifluoroacetic anhydride[3], potassium hydroxide[4], and chloroform[4] were nanograde quality.

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1. Aldrich Chemical Co, Milwaukee, Wisc.
2. Regis, Chicago, Ill.
3. Pierce, Rockford, Ill.
4. Mallinckrodt, St. Louis, Mo.

Spectroquality chloroform[5] was used as a solvent in the derivatization of ethambutol by bis(trimethylsilyl)trifluoroacetamide. For the metabolite analysis, the following reagents were used: ninhydrin[3], sodium acetate buffer[3], methyl cellosolve[3], stannous chloride[3] and sodium citrate buffers (pH 4.25 and 3.25)[6]. For the radioactivity assay, Oxifluor[7] was used as the scintillation fluid.

Fig. 2-1 depicts the chemical structures of:

I. ethambutol

II. d-2,2'-(ethylenediimino)di-1-propanol, internal standard for plasma, blood or dialysate assay using electron capture detector

III. decanediol, internal standard for urine assay using flame ionization detector

IV. d-2, 2'-(ethylenediimino)di-1-butyric acid, the diacid metabolite

V. 1-norlucine, internal standard for the metabolite assay

Instrumentation

Gas liquid chromatography

Gas liquid chromatographic analyses were carried out on a dual column instrument[8] equipped with a flame ionization detector and a scandium tritide electron capture detector[9]. The columns were coiled,

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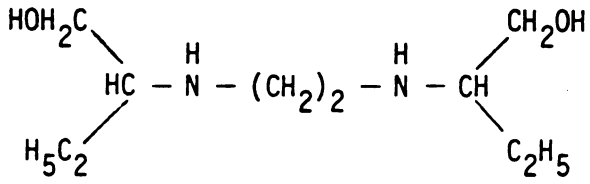
5. MCB, Norwood, Ohio
6. Beckman, Fullerton, Calif.
7. New England Nuclear, Boston, Mass.
8. Varian Aerograph, Model 2700
9. Tritium content: 1 Ci adsorbed on scandium

I



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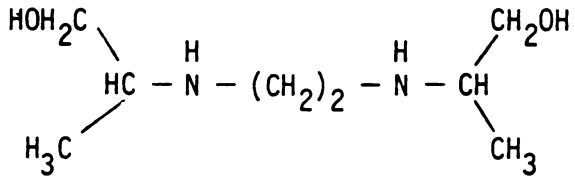

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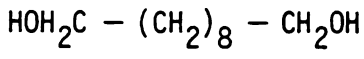

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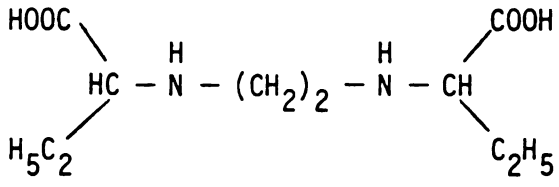

V

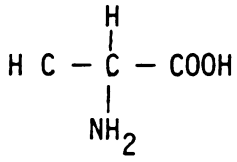

Fig. 2-1 Chemical structures of ethambutol (I), dextro 2, 2'-(ethylenediimino)di-l-propanol (II), decanediol (III), dextro 2, 2'-(ethylenediimino)di-l-butyric acid (IV) and norlucine (V).

1.8 m x 3 mm i.d., silanized glass, packed with 3% OV-17 on 100/120 mesh Chrom W A/W DMCS[10] (leading to the flame ionization detector) and with 3% OV-17 on 100/120 Gas Chrom Q[11] (leading to the electron capture detector). Nitrogen was used as the carrier gas at flow rates of 66.6 ml/min and 20 ml/min measured at the flame ionization detector base and the electron capture detector base, respectively. The air flow rate was 300 ml/min and hydrogen flow 27 ml/min. The temperature settings were: oven, 157°; injection ports, 210°; and detectors, 230°.

Mass spectrometry

Chemical ionization mass spectra were obtained using a quadrupole mass spectrometer.[12] Source pressures were maintained at 0.5 torr (isobutane). The source temperature was 150-170°. Samples (1 ± 0.5 mcg) were placed into a glass capillary by a direct-insertion probe, which was then gradually heated above 200° to effect volatilization.

Ion exchange liquid chromatography

A Beckman amino acid analyzer was used for the separation and identification of the EMB metabolite and the general amino acids contained in the urine sample. Table 2-1 lists the conditions applied during the metabolite analysis.

Liquid scintillation spectrometry

All ^{14}C-EMB samples were counted for a minimum of one thousand counts per sample in 10 ml aliquots of Oxifluor on Program #3 (high efficiency-^{14}C) of a Searle Mark III 6880 Liquid Scintillation Spectrometer.

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10. Varian Aerograph, Walnut Creek, Calif.

11. Applied Science Laboratories, State College, Pa.

12. Model 3200, Finnigan Corp., Sunnyvale, Calif.

Table 2-1

Conditions Applied for the Analysis of Ethambutol Metabolite

METHODOLOGY	**2 hour physiological fluid on the model 116C**
column size	0.9 X 69 cm
resin type	Beckman UR-30
height of resin column	56.0 cm
column flow rate	68 ml/min
column back pressure	250 psi
first buffer	3.25 ± .01 (0.2 N)
second buffer	4.25 ± .02 (0.2 N)
buffer change time	55 min
column temperature	55.5° C

Preparation of Standard Solutions

Ten milligrams of the hydrochloride salt of EMB was dissolved in distilled water to yield a stock solution of 1 mg/ml. Working standard solutions of 2, 4, 6, 10, 14 and 20 mcg/ml of EMB were prepared and used to establish a GLC calibration curve and for addition to dialysate, plasma or blood for the determination of percent recovery. A stock solution of MEMB containing 1 mg/ml of its hydrochloride salt was diluted ten fold to give the working standard solution. Working standard solutions of 100, 200, 300, 500, 700 and 1000 mcg/ml of EMB were also prepared and used to establish a GLC calibration curve and for addition to urine for the determination of percent recovery. Because of the sparing solubility of decanediol in water, one mg of decanediol was first dissolved in 250 µl of n-propanol and thendiluted with distilled water to concentration of 100 mcg/ml.

Ten µmoles of the diacid metabolite was dissolved in 1 N HCl to yield a stock solution of 5 µM/ml. Aliquots of 10, 20, 30, 50, 70 and 100 µl were pipetted from the stock solution and incorporated into blank urine when establishing a standard curve. Norlucine was prepared as a 2.5 µM/ml solution in distilled water.

The radioactive ^{14}C-EMB solution was prepared just before use. An appropriate amount of ^{14}C-EMB was weighed and dissolved in distilled water.

Determination of Extraction Recovery

Extraction recovery was determined with ^{14}C-EMB. Aqueous phases of 0.4, 1.0 and 2.0 ml were extracted with 8 ml of $CHCl_3$. The extraction recovery was calculated as: cpm counted in the organic phase after extraction/cpm counted in the aqueous phase before extraction.

Assay of Plasma Samples

Samples of 0.2 ml of plasma, to which 5 mcg of internal standard (dextro-2,2'-(ethylenediimino)di-1-propanol) had been added, were extracted with 8 ml of chloroform under alkaline conditions (3-4 drops 4 N NaOH). Two to three drops of 1 N HCl were added before chloroform aliquots were evaporated to dryness under nitrogen. Methylene chloride (0.5 ml) was added and evaporated to dryness. Residues were dissolved in 1 ml of benzene and made alkaline by addition of 3-4 drops of diluted pyridine (1:4 in benzene). Derivatization with 20 μl trifluoroacetic anhydride added to a closed tube is completed in 2 hrs at room temperature. Excess derivatization agent was washed into an aqueous phase with 3 ml of 0.01 M HCl. Appropriate aliquots of the benzene layer (2-3 μl) were injected into the GLC equipped with a scandium tritide electron capture detector.

Assay of Blood Samples

Red blood cells were hemolyzed by freezing. The same assay procedure was followed as described for the plasma assay. Smaller sample size was required because of the high partition of EMB in the red blood cell (31). Caution was taken in transferring the chloroform aliquots to avoid the messy residue produced by the hemolysis of the erythrocytes.

Assay of Dialysate Samples

For the dialysate assay the same extraction procedures were followed as previously described except that 2 ml of dialysate and 12.5 mcg of internal standard were used. The attenuator on the gas chromatograph panel was decreased by a factor of 2 when larger response signals were desired.

Assay of Urine Samples

Urine samples were assayed by either the electron capture method or the flame ionization method. Dilution of the urine sample up to 400 fold was required when the electron capture method was used and the procedures described for the plasma assay were followed. The following describes the flame ionization method for assay of EMB in urine.

Appropriate urine aliquots to which 10 mcg of decanediol had been added, were extracted with 8 ml of chloroform for 10 min under alkaline conditions. Portions of the chloroform were transferred to another tube. Two to three drops of 1 N HCl were added before the chloroform aliquots were evaporated to dryness under nitrogen. Residues were dissolved in 0.1 ml of spectroquality chloroform. Derivatization was initiated by adding 10 μl of bis(trimethylsilyl)trifluoroacetamide and was completed at room temperature in 30 min. Aliquots (2-3 μl) of the reaction mixture were injected into the gas chromatograph.

Assay of Urine Metabolite

Urine samples containing 0.05 to 0.5 μmoles of the metabolite were injected through a loop injector onto the ion exchange column, which was then gradually eluted by sodium citrate buffers (pH 3.25 for 55 minutes followed by pH 4.25 for 55 minutes). The separated test sample was mixed with ninhydrin to produce a color reaction. The solution was then passed through a colorimeter for quantitative analysis. The colorimeter readings at 570 nm and 440 nm were printed as absorbance peaks on a strip chart recorder. Norlucine was used as an internal standard.

RESULTS

Table 2-2 presents the different extraction recoveries as a result of different organic/aqueous phase ratios. With a constant organic phase ($CHCl_3$) of 8 ml, the extraction recoveries decreased from 57.9% to 16.9% when the aqueous phase was increased from 0.4 to 2 ml.

Under the chromatographic conditions described above, EMB and MEMB have retention times of 2.5 and 4 minutes, respectively (Fig. 2-2). A typical calibration curve prepared by extracting human plasma samples containing different concentrations of EMB and 5 mcg of MEMB is shown in Fig. 2-3. The electron capture response is linear between 0.1 and 2 mcg, but the calibration curve was constructed between 0.1 and 1 mcg where therapeutic concentrations can be conveniently interpolated. Table 2-3 contains peak height ratios obtained from calibration curves prepared in human plasma, human urine, monkey plasma and monkey urine. Each value represents the average of 3 samples at each concentration. The electron capture method was used.

The assay of EMB from whole blood was also attempted. Red blood cells were naturally hemolyzed by freezing. The same assay procedures were followed as described for the plasma assay. No interference from RBC constituents was found. The calibration curve is similar to that prepared from plasma indicating that EMB and the internal standard may be extracted equally well from blood and plasma.

Preliminary one week stability tests were performed by dissolving 0.1 and 0.5 mcg of EMB in 0.2 ml of plasma, which was then frozen for periods of 2, 4 and 7 days. The results (Table 2-4) show that EMB is stable in plasma on freezing for at least one week.

Table 2-2

Determination of Extraction Recovery with ^{14}C-Ethambutol

Aqueous Phase (ml)	Organic Phase[a] (ml)	Extraction Recovery (%)
.4	8	57.9
1.0	8	30.1
2.0	8	16.9

[a] chloroform

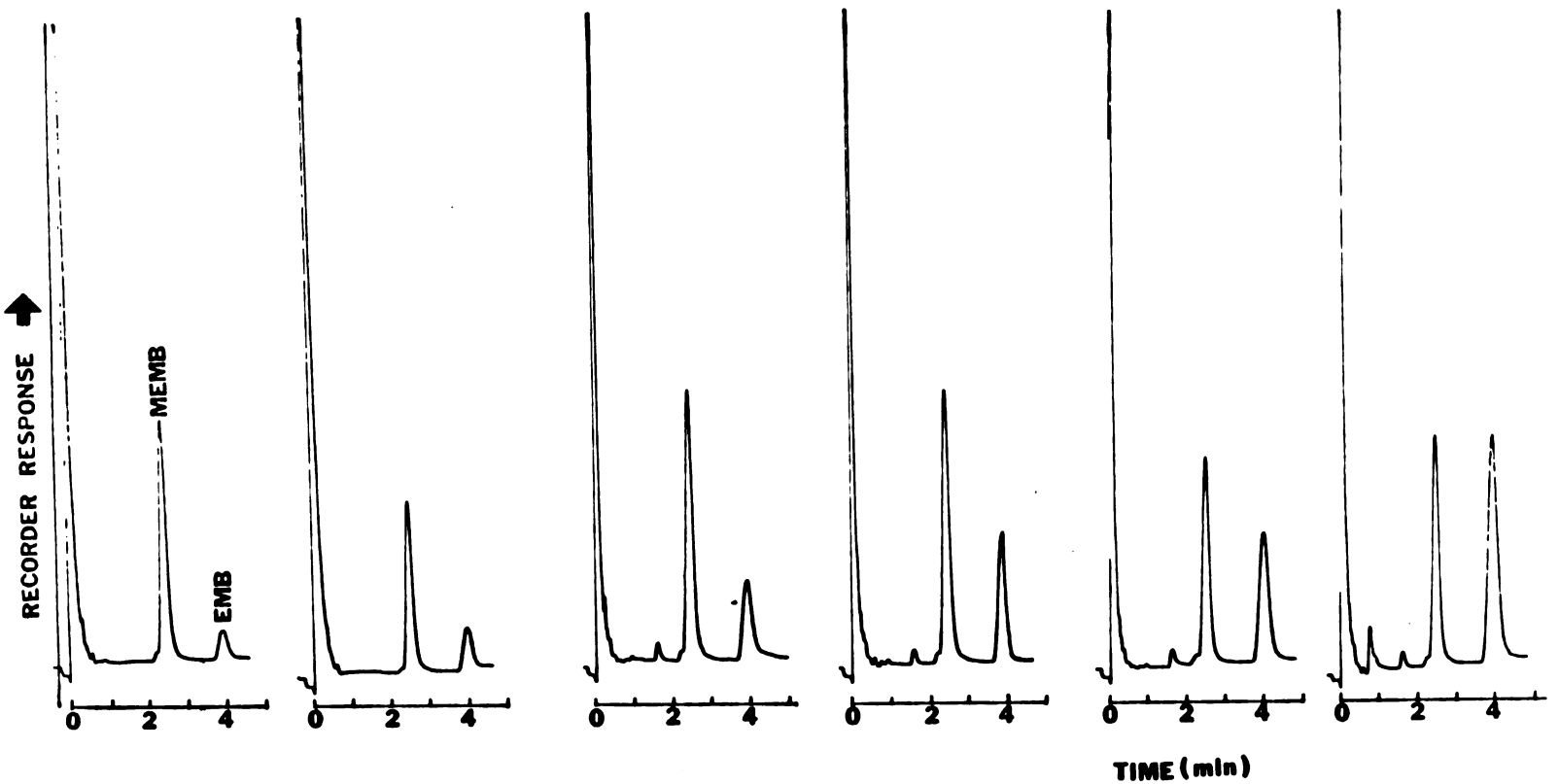

Fig. 2-2. Typical chromatogram of EMB (0.1-1.0 mcg) and MEMB (5 mcg) derivatives. Retention time 2.5 min for MEMB and 4 min for EMB.

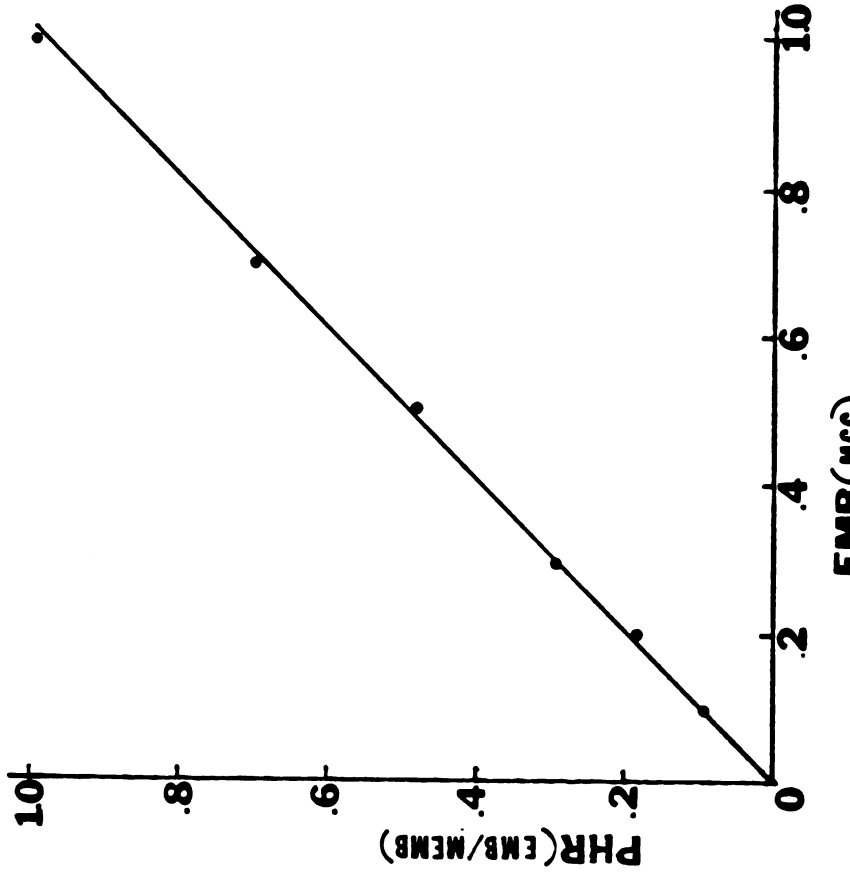

Fig. 2-3 Calibration curve relating the peak height ratios to the amount of ethambutol present in human plasma samples.

Table 2-3

Peak Height Ratios for the 4 Sets of Calibration Curves. Each Number Represents the Average of 3 Samples per Concentration. The Electron Capture Method Was Used.

EMB	Human Plasma	Human Urine	Monkey Plasma	Monkey Urine
0.1 mcg	0.10	0.10	0.11	0.09
0.2 mcg	0.21	0.18	0.21	0.19
0.3 mcg	0.30	0.30	0.28	0.28
0.5 mcg	0.51	0.47	0.47	0.48
0.7 mcg	0.67	0.67	0.69	0.70
1.0 mcg	0.97	0.89	0.90	0.89

Table 2-4

The Effect of Storage on Peak Height Ratio

Time (day) / EMB	0	2	4	7
0.1 mcg	0.104	0.106	0.111	0.107
0.5 mcg	0.495	0.506	0.511	0.508

Figure 2-4 presents gas chromatograms (electron capture detection) of (a) control dialysate, (b) EMB and MEMB added to control dialysate, and (c) dialysate collected 1 hr after initiation of hemodialysis.

Figure 2-5 presents gas chromatograms of the following samples using the flame ionization detector: (a) control human urine, (b) control urine to which EMB and decanediol were added, and (c) human urine obtained 1 hr after dosing.

The reversed time axis in Fig. 2-5 reflects the use of a dual pen recorder where urine and dialysate samples were measured simultaneously. As indicated in Figs. 2-4a and 2-5a, no interfering peaks were found in the control dialysate or urine specimens. The trifluoroacyl derivative of EMB exhibited a retention time of 4 min, and the corresponding internal standard had a retention time of 2.5 min (Fig. 2-4). The trimethylsilyl derivative of EMB exhibited a retention time of 6 min, and the corresponding internal standard had a retention time of 4 min (Fig. 2-5).

Table 2-5 summarizes the results obtained following electron capture analysis of various amounts of EMB(10-1000 ng) added to dialysate. The mean recovery of EMB from control dialysate in the 10-1000 ng range was 99.7 ± 6.7% (n = 36).

A summary of the results obtained following analysis of various amounts of EMB in control human urine is presented in Table 2-6. In the 10-100 mcg range, the mean recovery of EMB from control urine was 99.4 ± 5.5% (n = 42).

Figure 2-6 shows the liquid chromatogram of 0.1 ml of human urine spiked with the diacid metabolite and norlucine as the internal standard. The upper peak depicts the absorbance measured at 570 nm and the lower peak the absorbance measured at 440 nm. The diacid metabolite exhibited

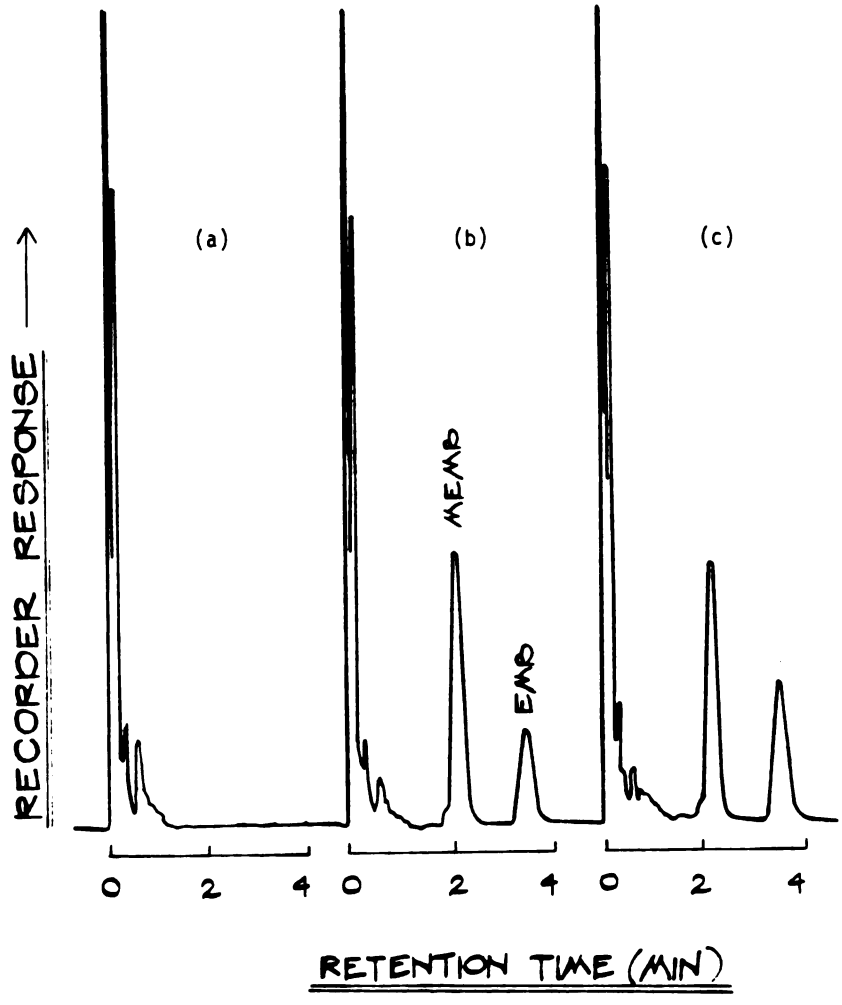

Fig. 2-4 Gas chromatograms of: (a) control dialysate, (b) EMB (0.3 mcg) and MEMB (12.5 mcg) added to control dialysate, and (c) dialysate collected 1 hr after initiation of hemodialysis.

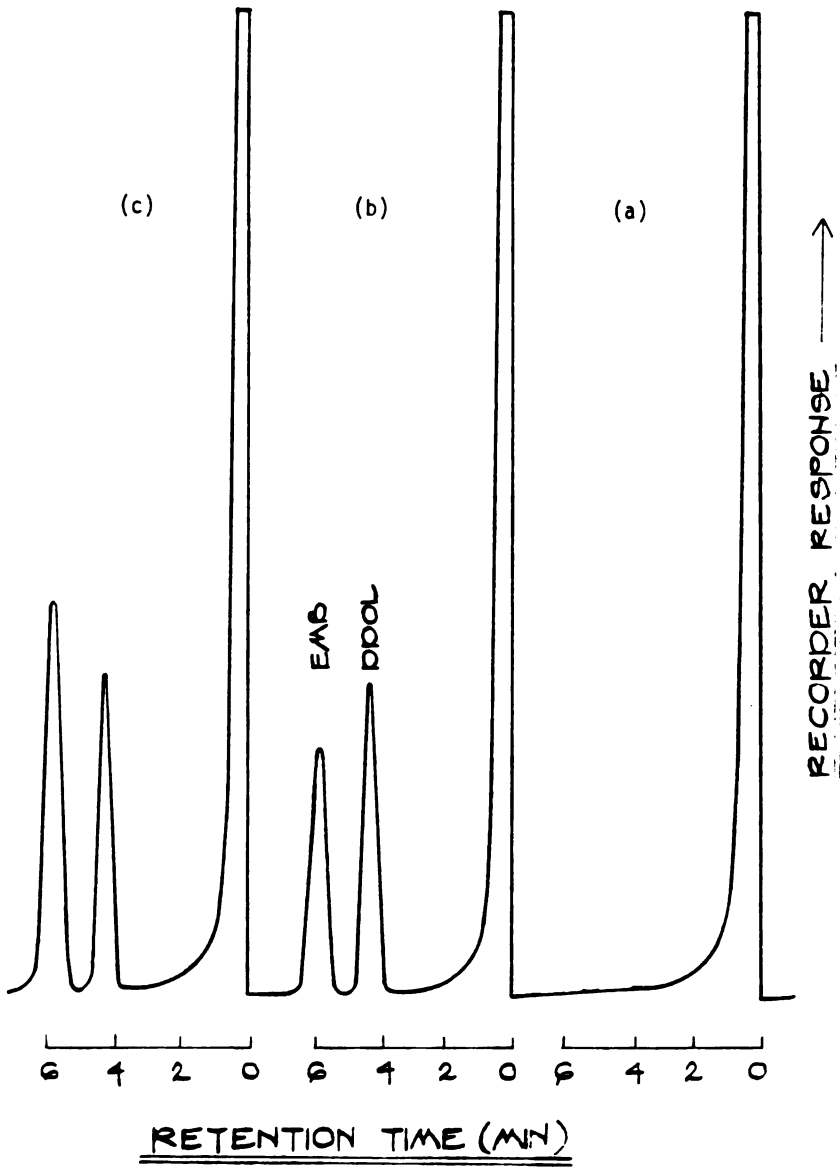

Fig. 2-5 Gas chromatograms of: (a) control human urine, (b) control to which EMB (50 mcg) and DDOL (10 mcg) were added, and (c) human urine obtained 1 hr after dosing.

Table 2-5

Recovery of Ethambutol (I) from Dialysate

Using Electron Capture Detection Method

I Added (ng)	n	I Found (ng)	Recovery[a] (%)
100	6	102.2 ± 12.3	102.2 ± 12.3
200	6	200.3 ± 18.9	100.2 ± 9.5
300	6	297.1 ± 16.5	99.0 ± 5.5
500	6	491.6 ± 22.9	98.3 ± 4.6
700	6	692.8 ± 33.0	99.0 ± 4.7
1000	6	991.6 ± 37.6	99.2 ± 3.8
100-1000	36	---	99.7 ± 6.7

[a] Expressed as Mean ± SD

Table 2-6

Recovery of Ethambutol (I) from Urine Using

Flame Ionization Detection Method

I Added (mcg)	n	I Found[a] (mcg)	Recovery[a] (%)
10	7	9.9 ± 0.79	99.0 ± 7.9
20	7	19.7 ± 1.50	98.5 ± 7.5
30	7	29.8 ± 1.64	99.3 ± 5.5
50	7	49.8 ± 2.16	99.7 ± 4.3
70	7	69.9 ± 2.83	99.9 ± 4.0
100	7	100.3 ± 3.60	100.3 ± 3.6
10-100	42	---	99.4 ± 5.5

[a] Expressed as Mean ± SD

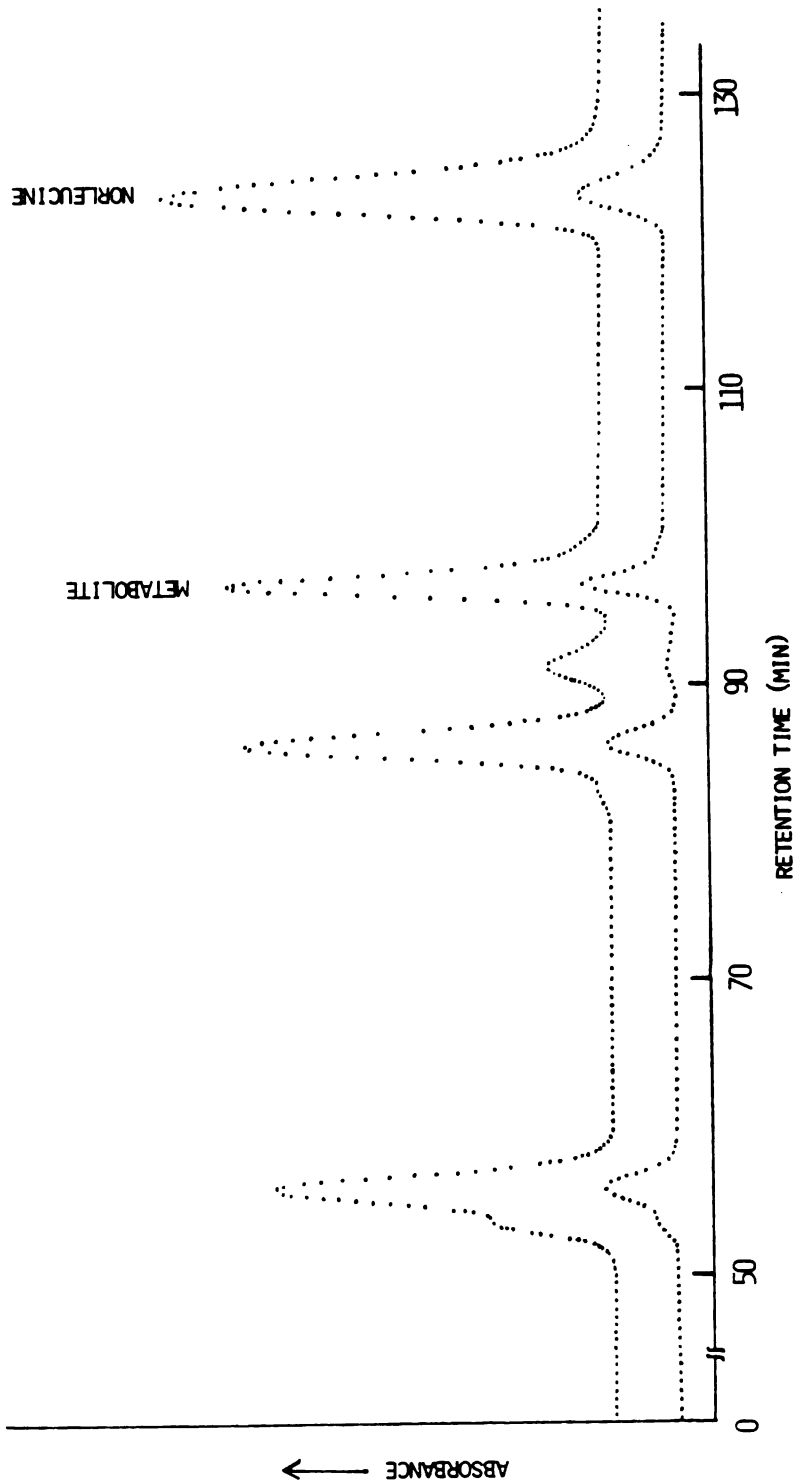

Fig. 2-6 Chromatogram of 0.1 ml of human urine spiked with 0.25 μM of ethambutol metabolite and 0.15 μM of internal standard (norleucine).

a retention time of 95 min and the corresponding internal standard had a retention time of 125 min. A calibration curve prepared by injecting human urine samples containing different concentrations of the diacid metabolite and 0.25 μmoles of norlucine is shown in Fig. 2-7. The absorbance is linearly correlated with the metabolite content ranging from 0.05 to 0.35 μmoles in the urine.

DISCUSSION

Extraction recovery is an important factor in determining assay reproducibility. Because of the presence of the relatively polar hydroxyl and amine groups in the EMB molecule, the water solubility of the EMB free base is considerable. As indicated in Table 2-2, extraction recoveries vary noticeably with the organic/aqueous phase ratios. Therefore, it is important to keep a constant organic/aqueous phase ratio in a whole set of extractions to assure a constant extraction recovery. This can be achieved by adding sufficient water to the aqueous samples to match in total volume the largest sample while keeping a constant volume of the organic phase.

The electron capture detector and the flame ionization detector have different detecting sensitivities for EMB. As indicated in Tables 2-5 and 2-6, the lower detection limit is 0.1 mcg using the electron capture detector and 1.0 mcg using the flame ionization detector. Comparing the electron capture response and the flame ionization response, an approximate hundred fold difference in detecting sensitivity is evident. The electron capture method of detection is required for samples such as blood, plasma and in particular, dialysate in which low levels of EMB are anticipated.

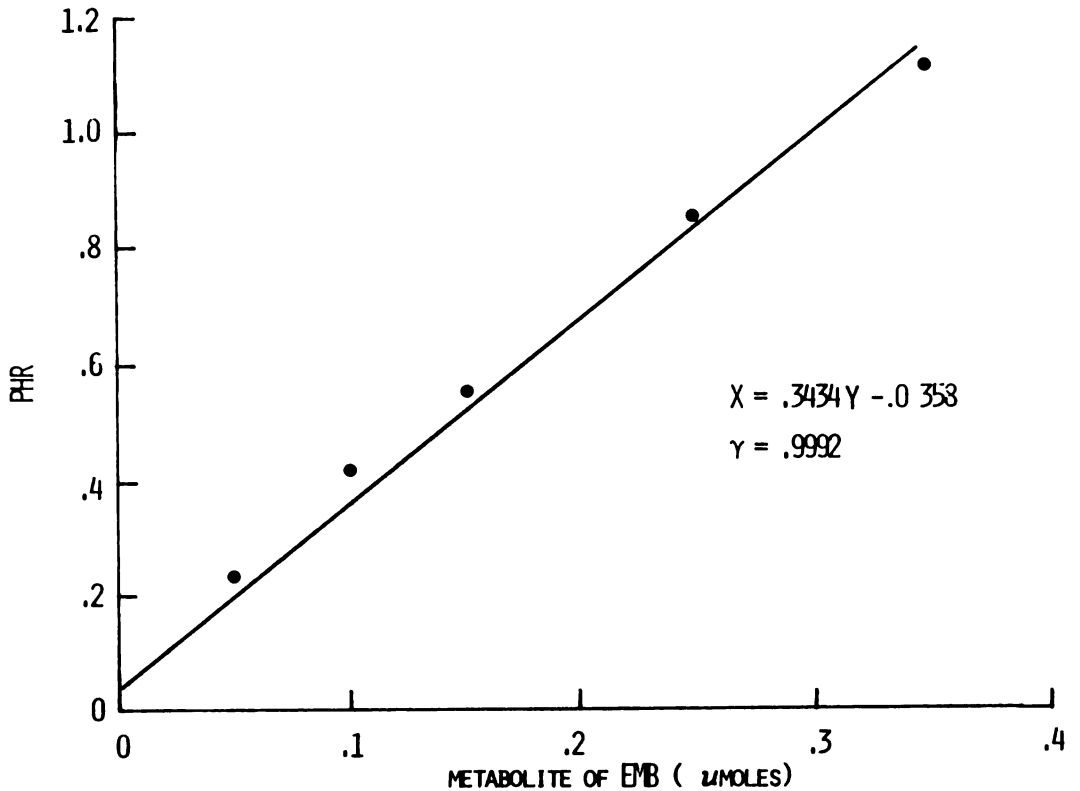

Fig. 2-7 Calibration curve relating the peak height ratio (PHR) to the amount of metabolite present in 0.1 ml of human urine sample.

Drug removal by dialysis is oftentimes difficult to determine due to the large dialysate volume collected during the dialytic process (20-30 liters per hr interval). Concentration procedures (e.g., rotary evaporation or extraction of the large dialysate volume with an organic solvent and subsequent evaporation of this solvent) are almost always required to bring the dialysate samples within the assay detection limits. With the highly sensitive electron capture GLC method described here, only 2 ml of dialysate is needed for extraction.

The flame ionization method is suitable for urine samples or for dosage form analysis in which high levels of EMB are anticipated. The flame ionization method avoids the tedious and possibly erroneous dilution of the samples when the GLC electron capture detector assay is used to measure the high drug concentrations found in urine.

Decanediol was chosen as the internal standard for the flame ionization urine assay because it is commercially available at low cost. Since high concentrations of drug are measured in this assay relatively large amounts of internal standard are required. Decanediol is not a good internal standard in the plasma or dialysate assay when trifluoroacetic anhydride is used as the derivatizing agent due to peak overlap.

Several column packings were tested and a 3% OV-25 on Chrom Q was found useful. However, since OV-17 is a universal liquid phase which has multiple utility, our assays were carried out exclusively on OV-17.

Heptafluorobutyric anhydride was also found useful as a derivatizing agent for EMB, but minor peaks eluted with longer retention times tended to slow down the assay process. Trifluoroacetic anhydride does not give these minor peaks and should be considered as a first choice.

Richard et al. (41) pointed out that only trimethylsilyl imidazole derivatized EMB immediately and completely and other trimethylsilylating agents required mild heating at 50° to complete the derivatization. We found that bis(trimethylsilyl)trifluoroacetamide with 1 percent of trimethylchlorosilane satisfactorily derivatized EMB at room temperature and less solvent interference was obtained than when trimethylsilyl imidazole was used. The use of an internal standard in the methods described here presents an improvement over the other published chromatographic techniques (39, 41).

The isobutane chemical ionization mass spectra of EMB, the trimethylsilyl derivative of EMB and the trifluoroacyl derivative of EMB can be seen in Figs. 2-8, 2-9 and 2-10, respectively. The major molecular ion at m/e 349 in Fig. 2-9 corresponds to the ditrimethylsilylated derivative of EMB. No peaks were found beyond m/e 349 probably indicating trimethysilylation occurred at the 2 hydroxyl groups but not at the 2 secondary amines in EMB. The major molecular ions at m/e 589 and 493 in Fig. 2-10 correspond to the tetra- and tri-trifluoroacylated derivatives of EMB, respectively, with relative abundance of approximately 100:15. However, the tri-trifluoroacyl derivative of EMB was not detected by the electron capture detector due to the relatively less abundance of fluorine in addition to the relatively less intensity of the peak compared to the tetra derivative. The peak at m/e 475 (M^+) respresents a break down product of the protonated molecular ion (MH^+) at m/e 589.

The trifluoroacetic anhydride derivatives of EMB and MEMB are stable in benzene solution for several weeks. Nevertheless, the peak height ratio may change if the tubes are not tightly capped and vaporization occurs.

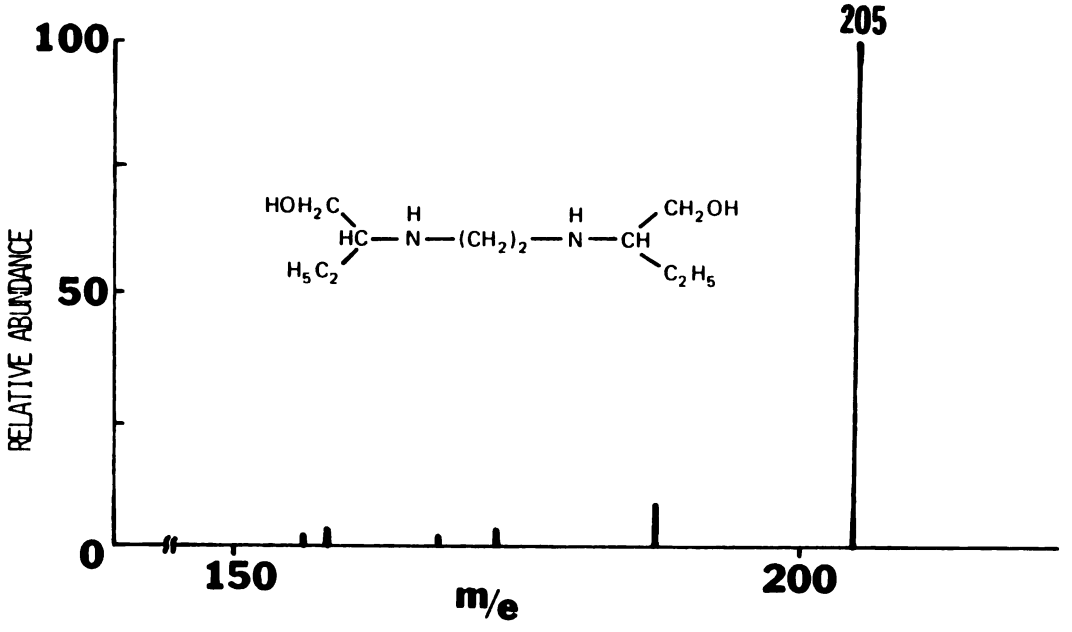

Fig. 2-8 Chemical ionization mass spectrum of ethambutol using isobutane as the reagent gas.

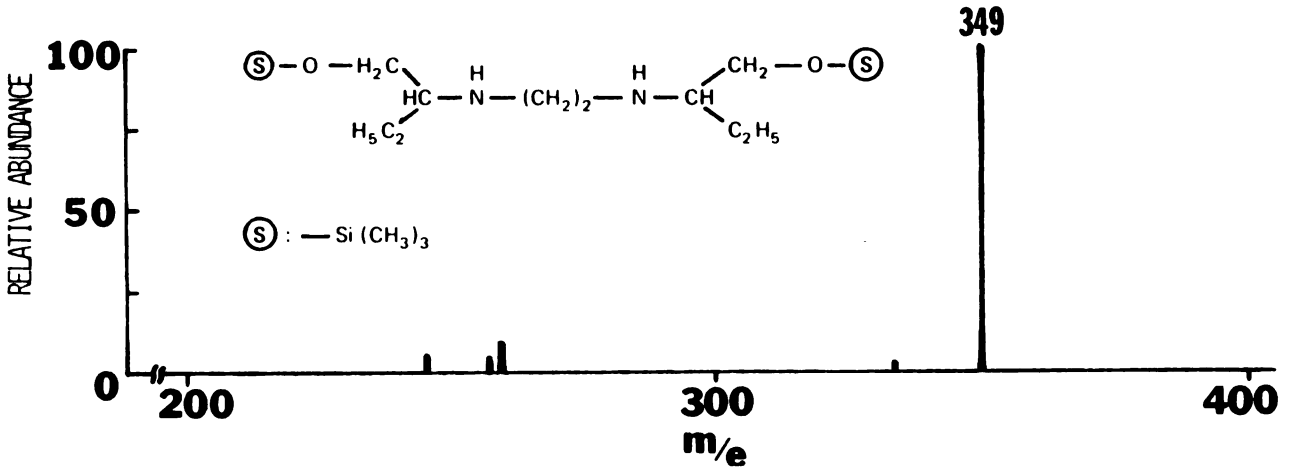

Fig. 2-9 Chemical ionization mass spectrum of trimethylsilylated ethambutol using isobutane as the reagent gas.

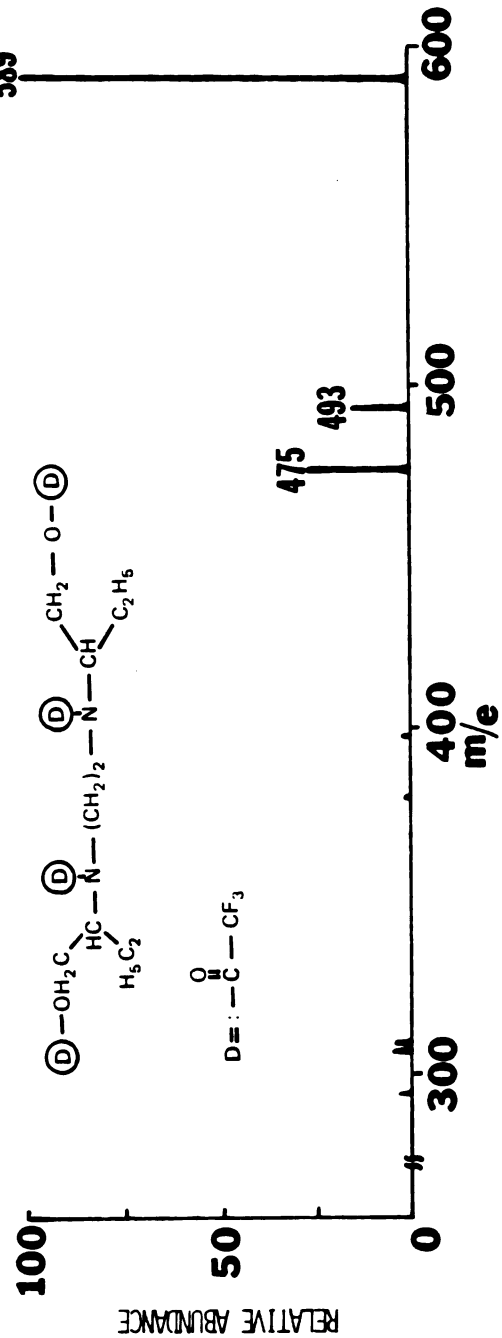

Fig. 2-10 Chemical ionization mass spectrum of trifluoroacylated ethambutol using isobutane as the reagent gas.

Due to the low boiling point of bis(trimethylsilyl)trifluoroacetamide and the minute volume of chloroform used in dissolving the trimethylsilyl derivatives, vaporization of the TMS reaction mixture occurs easily. Although residues can be redissolved if repeated injection is required, the results may turn out to be unsatisfactory. To prevent the reaction mixture from vaporizing, immediate injection or use of a nipple tube for storage is suggested.

Arguments have been raised regarding the advantage of using peak area over peak height (43, 44). A theoretical discussion on this was presented by Janik (45). For comparison purposes, the peak heights were measured using an A-25 Varian recorder and the peak areas integrated using a 3370B Hewlett Packard integrator. Figure 2-11 contrasts the calibration curves prepared from peak height ratio and peak area (plasma electron capture assay done on Varian 1400, oven: 168°, other conditions the same). Linear regression shows that both curves are equally good with $\gamma^2 = 0.96$ for the former and $\gamma^2 = 0.95$ for the latter. There is no essential difference between choosing peak height or peak area as a measure of the detector response.

About 20% of ethambutol is metabolized to the carboxylic acid derivative and excreted in the urine (22). This highly polar compound is very poorly extracted into the organic phase in the procedure described here and thus does not interfere with the assay of unchanged drug.

Although retention times are unusually long for the diacid metabolite and norlucine in the liquid chromatographic process, assays can be done on an automatic amino acid analyzer, thus reducing the concern for man power and time consumption. In addition, no pretreatment of the urine samples is required.

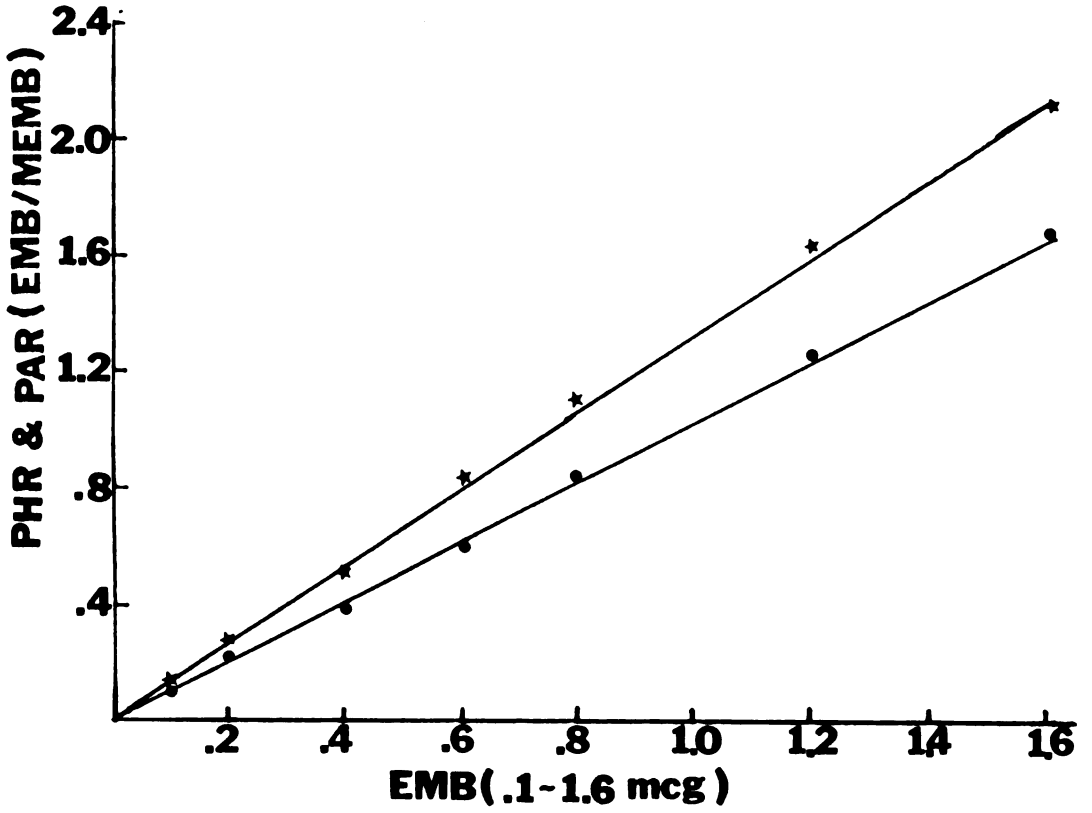

Fig. 2-11 Calibration curves contrasting the use of peak height ratio and peak area ratio as a measure of detector response. ● PHR; ★ PAR

The calibration curve depicted in Fig. 2-7 is applicable only when the urine sample injected is less than 0.1 ml. However, larger volumes are often required for assaying the metabolite content in urine collected in the early and late phases of a kinetic study. When an undiluted urine sample of greater than 0.1 ml was injected, a natural amino acid in the urine, valine, overlapped the metabolite peak and thus interfered with the assay. The overlapped peaks were resolved by replacing the ion exchange resin UR-30 with AA-20 and using a high pressure automatic amino acid analyzer. The resolution is shown in Fig.2-12.

Pharmacokinetic studies in 2 rhesus monkeys were done using ^{14}C-EMB. Plasma and urine samples were assayed for radioactivity. Plasma concentrations were expressed as cpm/ml and excretion rates as cpm/min. For 0.2 ml plasma or urine samples, the absolute counting efficiency was calculated to be 76.4%. The counting efficiency of a scintillation spectrometer is defined as cpm/dpm. As indicated by Gustafson (46), the ratios of efficiencies are essentially independent of the volume of plasma or urine added in the range of 0 to 1.0 ml. Since efficiency remains constant, a pharmacokinetic analysis based on cpm will be equally valid as the one based on dpm. Therefore, cpm data was used without quench correction to dpm in the monkey data analysis.

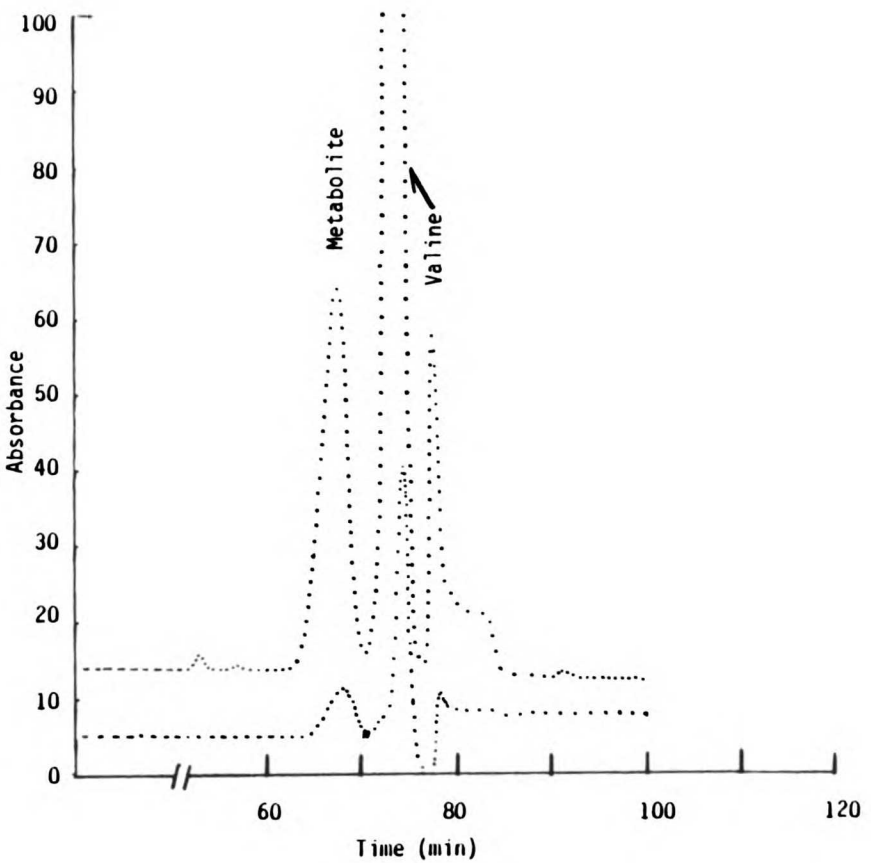

Fig. 2-12 Resolution of ethambutol metabolite and valine. The third peak resulted from the buffer change.

CHAPTER 3

ANIMAL STUDIES

THE ANIMAL MODEL

Restrained male rhesus monkeys (Macaca mulatta) weighing from 4 to 10 kg with permanent catheters implanted in the iliac vein and artery were used as described by Nayak and Benet (47). One of the monkeys was also implanted with a stomach cannula for oral drug administration. The rhesus monkey was chosen as an animal model for preliminary study since this species resembles the human with respect to kidney function, body fluid compartment, gastrointestinal absorption and metabolism. Rhesus monkeys were used for intravenous studies with EMB before our INDA (Investigational New Drug Application) was approved by the FDA (Food and Drug Administration). Although toxicity studies of EMB in rhesus monkeys were reported by Schmit in 1966 (7), a literature review reveals that no kinetic studies were performed on this animal species.

EXPERIMENTAL

Unanesthetized restrained monkeys were fasted overnight before each study. The monkey was allowed water ad lib during the study.

For the intravenous studies, the drug was dissolved in double distilled water and filtered through a millipore filter (Millex TM, Sterile 0.22 μM) into a sterile syringe before administration to the monkey. One ml of the drug solution was infused over a 3 minute period followed by a saline wash of 2 ml. Oral drug doses were given through the stomach cannula.

Three rhesus monkeys, designated A, B and C, were studied. Monkey A was studied once with 100 mg of unlabelled EMB given intravenously. Monkey

B was studied three times: the first time 100 mg of unlabelled EMB was given intravenously, the second time 100 mg of unlabelled EMB was given orally and the third time 0.81 mg of ^{14}C-EMB diluted with 100 mg of unlabelled EMB was orally administered. The three studies were separated by several weeks. Monkey C was simultaneously dosed with 0.9381 mg of ^{14}C-EMB intravenously and 100 mg of unlabelled EMB orally. The radiolabelled EMB used in these studies has a specific activity of 2.16 μCi/mg and was kindly supplied by Lederle Laboratories, Pearl River, N.Y.

During the intravenous studies with unlabelled EMB in monkeys A and B, 1 to 1½ ml blood samples were collected at the following times: 0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 210, 270, 330, 390, 450, 570, 690 and 1440 minutes after the fast I.V. infusion. In the oral study in monkey B with unlabelled EMB, 1 to 1½ ml blood samples was collected at: 0, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, 360, 420, 480, 600, 720 and 1440 minutes after oral administration. Urine collections were made following natural voiding in these studies for up to 72 hrs. In the intravenous and oral studies in monkeys B and C with ^{14}C-EMB, no blood samples were taken. Urine samples were collected again following natural voiding but close attention was paid to the voiding time in the first 12 hrs. Total urine collections were made up to 72 hrs.

The collected blood samples were placed immediately into heparinized tubes and centrifuged for 5 minutes to separate the plasma aliquots. The plasma and the urine samples were frozen until they were assayed for EMB content. The analytical procedures for labelled and unlabelled drug in the plasma and urine samples were described in Chapter 2.

RESULTS AND DISCUSSION

Following intravenous administration, EMB plasma concentration and urinary excretion rate rapidly dropped in all monkeys (Fig. 3-1). Extrapolated plasma levels at time zero were 55.1 and 48.3 mcg/ml for monkeys A and B, respectively. In the 12-hr period immediately following termination of the fast infusion, EMB levels exhibited biexponential decay. The plasma data from monkeys A and B, and urine data from monkey C following I.V. administration were fitted with the NONLIN program (48) to a two compartment open body model. The pharmacokinetic parameters obtained are presented in Table 3-1. The α phase in these monkeys had a mean half-life of 9.75 min while the half-life of the β phase ranged from 2.36 to 3.40 hr (mean 2.75 hr). Since ^{14}C-EMB was administered to monkey C, urinary analysis inevitably included EMB metabolite as well as the unchanged compound. The kinetic parameters thus obtained from urine data failed to represent the exclusive kinetic characteristics of the parent drug. Therefore the following values are discussed only with reference to the studies in monkeys A and B. The mean values for the volume of the central pool and the steady-state volume of distribution for this drug were 0.36 and 1.58 L/kg, respectively. The fraction of the dose administered that was eliminated by the urinary route varied from 0.65 to 0.80 (mean 0.72). Renal clearance was calculated by multiplying the fraction excreted unchanged by the total body clearance; the latter value was determined by dividing the dose administered by the total area under the plasma concentration-time curve (AUC). The value of AUC was calculated by:

$$AUC = \int_0^t C_p dt + \frac{C_{P,t}}{\gamma}$$

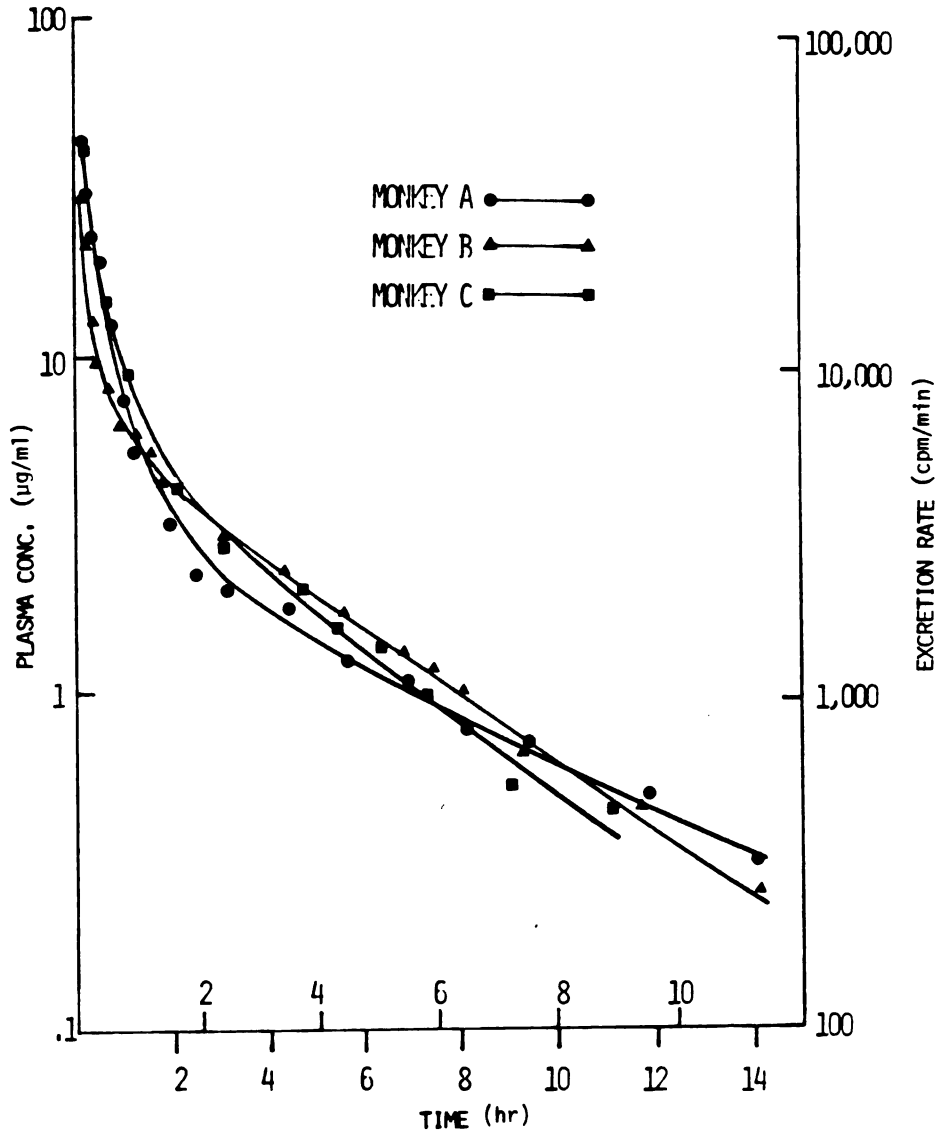

Fig 3-1 Ethambutol plasma concentration vs. time plot (●——● and ▲——▲) and urinary excretion rate vs. time plot (■——■) in monkeys A, B and C. The upper time scale is for excretion rate on right axis and lower time scale for plasma concentration on left axis.

Table 3-1

Comparison of the Pharmacokinetic Constants of the Two-compartment Body Model in Three Rhesus Monkeys following I.V. Bolus Doses of Ethambutol*

Monkey	Weight (kg)	α min^{-1}	β min^{-1}	K_{12} min^{-1}	K_{21} min^{-1}	K_{10} min^{-1}	V L/kg	V_{dss} L/kg	Cl_R ml/min/kg	%E.U.
A	8.5	.1245	.0049	.0777	.0201	.0317	.2137	1.0275	6.70	79.7
B	4.0	.0512	.0034	.0214	.0068	.0265	.5170	2.1345	9.78	64.6
C	10.0	.0375	.0043	.0138	.0082	.0197	--	--	--	--
Mean	7.5	.0711	.0042	.0376	.0117	.0259	.3653	1.5810	8.24	72.20
SD	3.1	.0460	.0008	.0349	.0007	.0060	.2144	.7827	2.17	10.67

* For monkeys A and B, 100 mg dose was given, plasma data were fitted to a NONLIN program; monkey C was given .9381 mg of ^{14}C labelled ethambutol, urine data were fitted.

where t is the time of the last recorded plasma concentration ($C_{P,t}$) and γ is the terminal elimination rate constant estimated from the 12 and 24 hr plasma concentrations. In calculating clearance, the 24 hr plasma concentration was used as the value for $C_{P,t}$. The area up to time t was estimated using the trapezoidal method. In monkeys A and B, renal clearances were 6.7 and 9.4 ml/min/kg, respectively. These clearance values are 2-3 times the normal GFR of the rhesus monkey (3.08 ± 0.939 ml/min/kg (49)) and hence indicate an active secretion mechanism.

Table 3-2 compares the terminal half-lives following oral and intravenous administration of EMB in the three rhesus monkeys. In monkey B the intravenous and oral studies were carried out on separate occasions. We observed a significant difference in terminal half-life, 3.40 vs. 4.31 hrs following intravenous and oral dosing. In order to find out whether such a difference was real or simply an artifact, we carried out another experiment in monkey C with simultaneous dosing of ^{14}C-EMB intravenously and unlabelled EMB orally. We again observed a difference in the terminal half-life (2.68 vs. 3.38 hrs). Note that the half-life derived after intravenous administration in this case should already be an overestimate of the true half-life due to the inability of the assay method to differentiate metabolite from parent drug. Following oral and intravenous routes of EMB administration in human subjects, we also observed such a difference in terminal half-lives. These results will be presented in Chapter 4. A second study was carried out in monkey B where the ^{14}C-EMB was isotopically diluted and given orally. Both the labelled and unlabelled drug were assayed. A slight difference in terminal half-lives was observed, 4.3 hr measuring ^{14}C-EMB and 4.0 hr measuring unlabelled EMB. This indicates a possible overestimate of terminal half-life when radiolabelled EMB is

Table 3-2

Terminal Half-lives of Ethambutol in Rhesus Monkeys following Oral and Intravenous Administration

Monkey	Terminal half-life (hr)	
	I.V.	Oral
A	2.36	--
B[1]	3.40	--
B[2]	--	4.31
B[3]	--	4.32
	--	4.02
C[4]	2.68	3.38

[1] First study in monkey B with 100 mg of unlabelled EMB given intravenously.

[2] Second study in monkey B with 100 mg of unlabelled EMB given orally.

[3] Third study in monkey B with .81 mg of ^{14}C-EMB diluted with 100 mg of unlabelled EMB given orally. The half-life was 4.32 hr measuring ^{14}C-EMB and 4.02 hr measuring unlabelled EMB.

[4] Simultaneous dosing of ^{14}C-EMB intravenously and unlabelled EMB orally. The half-life was 2.68 hr measuring ^{14}C-EMB and 3.38 hr measuring unlabelled EMB.

administered. From the percent recovery of the labelled and unlabelled drug, we estimate that approximately 14% of the absorbed dose was metabolized. In studies in monkeys A and B, the time intervals between urination were not accurately known so that kinetic analysis of urine samples was not justified.

Figure 3-2 depicts the excretion rate plot upon simultaneous dosing of ^{14}C-EMB intravenously and unlabelled EMB orally in monkey C. A difference in terminal half-life was found as noted in Table 3-2. Figure 3-3 shows the excretion rate plot for a second study in monkey B with isotopically diluted EMB. A slight difference in terminal half-life was observed probably as a result of an overestimate of the true half-life in the radiolabelled drug study. Figures 3-2 and 3-3 graphically depict the results presented in Table 3-2.

Attempts were made to fit the monkey oral data to a two compartment model with first order absorption. The resulting fit was quite poor. Therefore, an alternative approach was taken to solve this problem.

There are three methods which may be used to identify the absorption profile. Namely, the Wagner-Nelson method (50), the Loo-Riegelman method (51), and the Benet-Chiang deconvolution method (52). The Wagner-Nelson method is used to identify the absorption profile for a drug following one compartment body model kinetics whereas the Loo-Riegelman method is used for a drug following a two compartment body model. Neither of these methods makes any assumptions concerning the linearity or continuity of the absorption process. The Benet-Chiang deconvolution method makes no assumptions concerning the number of compartments in the model but does assume linearity and continuity for both the absorption and elimination processes. The Loo-Riegelman method was used since the intravenous data

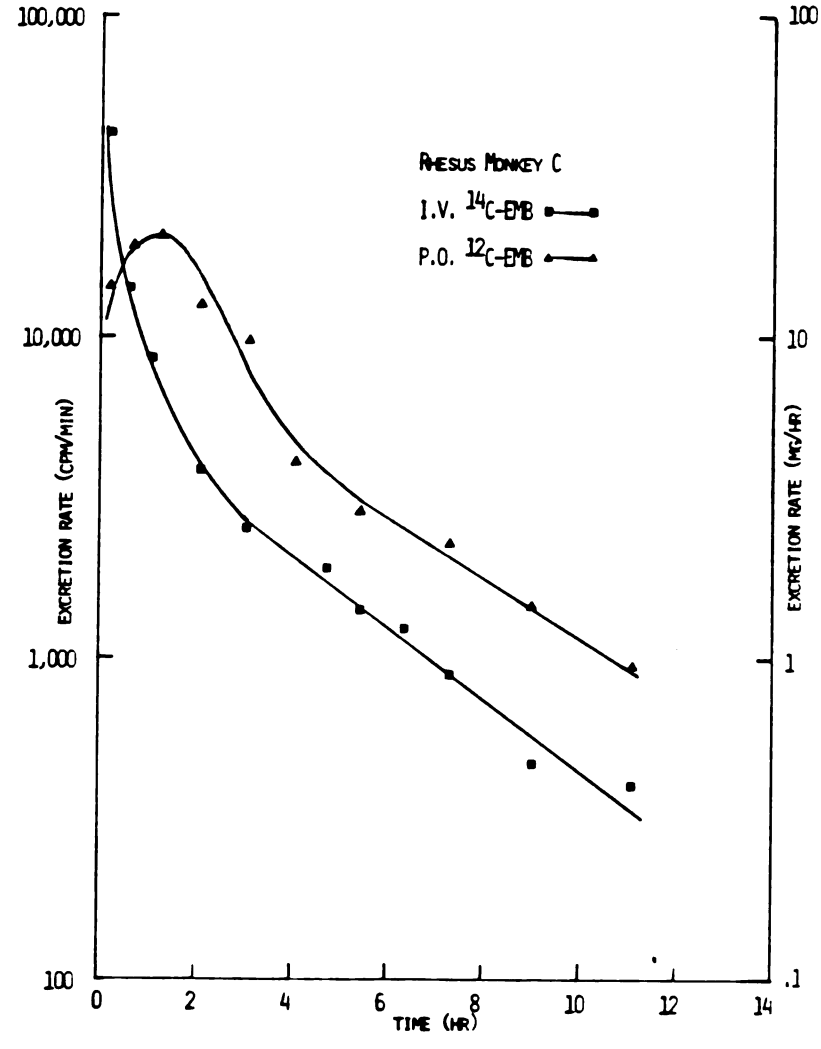

Fig. 3-2 Excretion rate plots following simultaneous dosing of ^{14}C-EMB intravenously and unlabelled EMB orally. Both axes represent excretion rate with the left one expressed in cpm/min and the right one in mg/hr.

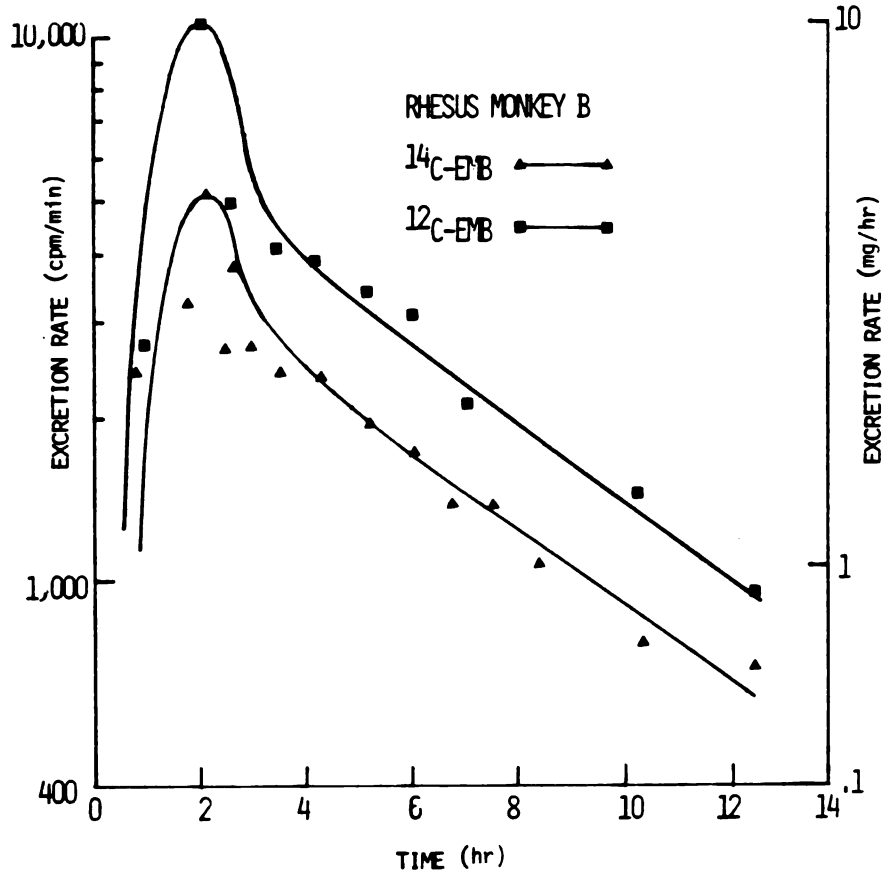

Fig. 3-3 Semilogarithmic excretion rate plots for 100.81 mg oral dose of ethambutol (0.81 mg ^{14}C-EMB and 100 mg unlabelled EMB) to rhesus monkey B.

was consistent with a two-compartment body model, and the absorption process did not appear to follow simple first order kinetics.

Figure 3-4 shows the oral and I.V. curves following administration of the same dose (100 mg) of EMB to monkey B. The I.V. data was then treated according to the Loo-Riegelman method (51). Upon plotting percent of the drug remaining to be absorbed vs. time and the absorption rate vs. time (Figure 3-5), we observed a biexponential absorption process. This was indicated by the curvature in the two log linear plots. Using the method of residuals, we estimated these two input rate constants and used them as initial guesses to fit a four exponential function shown in the following equation as its Laplace Transform:

$$C_P = \frac{FD\, k_{1i} k_{2i} (s + k_{21})}{V_1 (s + k_{1i})(s + k_{2i})(s + \alpha)(s + \beta)}$$

where k_{1i} is the first input rate constant and k_{2i} the second input rate constant. Other parameters are conventionally defined (53).

The computer fit with the NONLIN program resulted in 2 input rate constants (k_{1i}, k_{2i}) which have correspondingly similar half-lives of approximately 30 min. The same order of magnitude of the two rate constants indicates no predominance of one kinetic process over the other. It was necessary to incorporate a lag time into the integrated equation in order to obtain a good fit of the monkey oral data. This lag time indicated a delay in absorption after oral dosing and was found to be 6.7 min for this monkey.

The implications of this unusual absorption profile will be further discussed in Chapter 4.

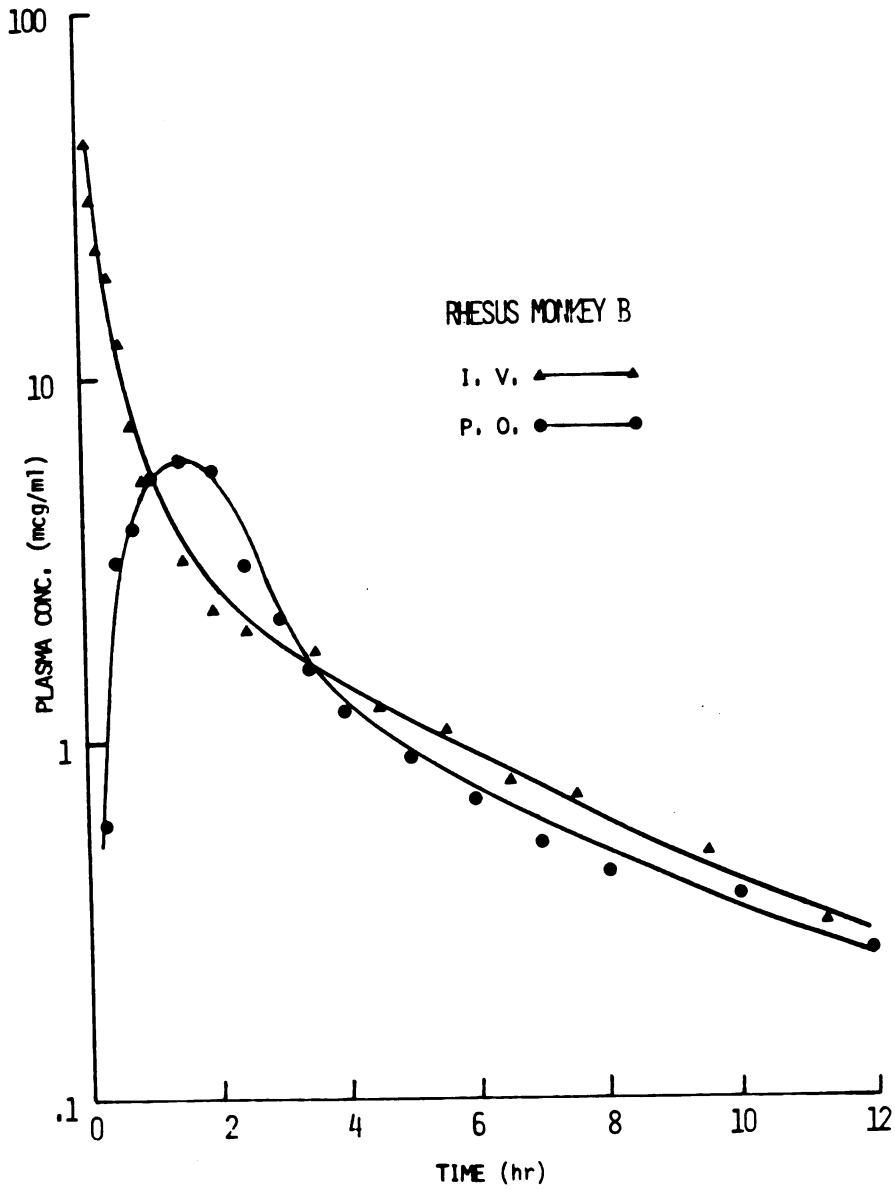

Fig. 3-4 Plasma concentration decay curves following administration of identical doses (100 mg) of ethambutol orally (●——●) and intravenously (▲——▲).

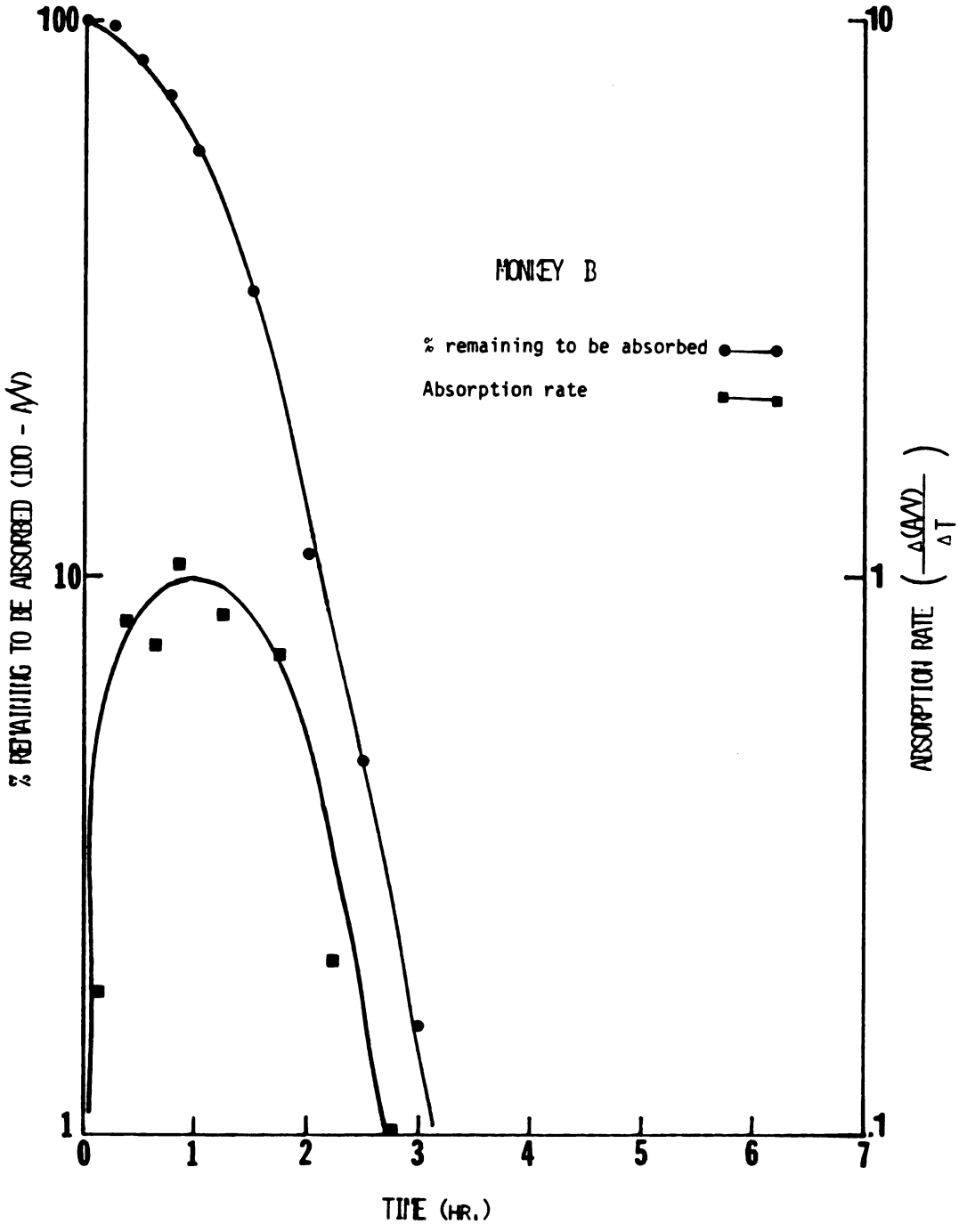

Fig. 3-5 Loo-Riegelman treatment of absorption profile for ethambutol on monkey B after 100 mg of oral dosing. Left axis: percent remaining to be absorbed; right axis: absorption rate.

CHAPTER 4

HUMAN STUDIES IN NORMAL VOLUNTEERS

METHODS

Subject Selection

The subjects studied were selected from the students, employees and staff of U. C. Medical Center, as well as the investigators of this study.

All subjects studied received a routine medical history, physical examination and a panel of laboratory tests (e.g., SMA-12, creatinine clearance, WBC and differential, hematocrit and hemoglobin). Specific data on each subject are given in Table 4-1. All subjects had normal SMA-12, renal and hepatic function.

Informed consent was obtained from each subject prior to participation.

Oral Study Design

Six healthy adult volunteers completed the study within a period of 5 weeks. Each subject received oral doses of approximately 15 mg/kg EMB. The sequence of oral solution and tablet administration was randomized and separated by at least 7 days.

For oral solution administration, drug powder was dissolved in 40 ml of distilled water and the solution was swallowed. An additional 40 ml of water was taken for rinsing the mouth and the rinse was swallowed. For tablet administration, a dose closest to 15 mg/kg where only unbroken commercial tablets (Myambutol^{R}, Lederle) was administered.

Blood samples were collected over 12 hours, at the following times: 0, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, 360, 420, 480, 600,

Table 4-1

Subject Characteristics

Subject	Age (Yrs)	Sex	Dose (mg)	Weight (Kg)	Serum Creatinine* (mg%)	Creatinine Clearance* (ml/min/Kg)
AL	30	F	800	52	0.85	1.56
SL	28	M	800	48	0.85	2.08
JG	28	M	1100	71	1.10	1.94
CB	31	M	1100	71	1.10	1.70
BS	29	F	800	56	0.85	1.59
CL	31	M	800	55	1.30	1.53

*Average of two determinations.

and 720 min. One additional sample was collected at 24 hrs. Urine was collected for 72 hrs at 0, 1, 2, 3, 4, 5, 6, 8, 12, 24, 48 and 72 hrs. The frequent blood sampling during the initial phase of the oral study was chosen to facilitate the observation of drug absorption characteristics. Urine collections were synchronized with blood sampling to allow calculation of renal clearance by excretion rate and midpoint plasma concentration.

Intravenous Study Design

The same 6 subjects participated in the I.V. study 6 months after completion of the oral studies. The intravenous study was completed within a period of 4 weeks. Each subject received the same dose as he did in the oral studies. Administration of the same dose in the oral and intravenous studies allowed absolute bioavailability to be calculated without correcting for dose.

For the I.V. administration, the infusion was carried out over a one hour period using a Harvard infusion pump (Model #975 Harvard Apparatus, Millis, MA) with the rate calibrated to 8 or 12 ml/hr.

Three ml blood samples were collected at the following times: 0, 5, 10, 15, 20, 30, 45, 60, 65, 75, 90, 120, 150, 180, 210, 240, 270, 300, 360, 420, 480, 600, 720 and 1440 mins after the start of infusion. Total urine collections were made at: 0, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 hrs.

Blood was sampled frequently during the first hour and soon after termination of infusion to facilitate characterization of the volume of distribution and the fast disposition parameters. Blood sampling schedules were identical for both oral and intravenous studies 300 min after dosing. Elimination essentially characterizes the post infusion curve from 300 min

on.

Blood samples and urine collection were arranged similar to that described for oral studies to allow convenient calculation of renal clearance.

Subject Set Up

Subjects fasted overnight and for up to 4 hrs after an early morning administration of the drug. Water was allowed ad libidum. Subjects were encouraged to drink water in the first 4 hrs to facilitate the urine collections. To minimize the discomfort of multiple venipunctures for blood sampling, a scalp vein needle with a heparin lock was left in place in a superficial arm vein for the initial 12 hr sampling period.

Plasma Protein Binding

Pooled plasma protein solutions containing 0.5, 1 and 2 mcg/ml of EMB were dialyzed against freshly prepared Krebs-Henseleit buffer (pH = 7.4) for 4 hrs. Both plasma and dialysate were removed and assayed for EMB. Protein binding was also determined for the same solutions using the micro-ultrafiltration technique of Shah et al. (54).

RBC Partitioning

One ml of heparinized whole blood was incubated with EMB (concentrations 0.5, 1 and 2 mcg/ml) at 37^{o} for 30 mins. After incubation, the plasma was separated by centrifuging for 2 mins using a Beckman microfuge and assayed for EMB.

Assay Procedure

Ethambutol concentrations in plasma, blood and urine samples were measured as previously described in Chapter 2.

I.V. Plasma Data Treatment

Model dependent kinetic analysis

Preliminary graphical analysis indicated 4 disposition phases following drug infusion and therefore a 4 compartment body model was used. The terminal elimination rate constant was estimated from the 24, 48 and 72 urine samples and was designated as δ with a corresponding extrapolated intercept D. The other 3 phases resolved from step by step peeling were designated as α, β, and γ with corresponding extrapolated intercepts A, B and C. The total area under the plasma concentration-time curve (AUC) was calculated according to

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta} + \frac{C}{\gamma} + \frac{D}{\delta} \qquad \text{(Eq. 2-1)}$$

Each term on the right hand side of Eq. 2-1 may be expressed as a percent of the total area under the curve related to that particular disposition phase. The volume of distribution in the central compartment was calculated as

$$V_1 = \frac{\text{Dose}}{A + B + C + D} \qquad \text{(Eq. 2-2)}$$

and the steady state volume of distribution as (55).

$$V_{dss} = \frac{\text{Dose} \left(\frac{A}{\alpha^2} + \frac{B}{\beta^2} + \frac{C}{\gamma^2} + \frac{D}{\delta^2} \right)}{(AUC)^2} \qquad \text{(Eq. 2-3)}$$

The total body clearance was calculated as

$$Cl_T = \frac{\text{Dose}}{AUC} \qquad \text{(Eq. 2-4)}$$

and renal clearance as

$$Cl_R = fe \cdot Cl_T \qquad \text{(Eq. 2-5)}$$

where fe is the fraction of drug excreted unchanged.

Model independent calculation of Cl_T and Cl_R

Total body clearance was also determined independent of the fit of the plasma data where the value of AUC substituted into Eq. 2-4 was calculated according to

$$AUC = \int_0^t C_P dt + \frac{C_{P,t}}{\gamma'} \qquad \text{(Eq. 2-6)}$$

where t is the time of the last recorded plasma concentration ($C_{P,t}$) and γ' is the terminal elimination rate constant estimated from 12 and 24 hr plasma samples. The area up to t was estimated using the trapezoidal method. Renal clearance was also calculated by the excretion rate method

$$Cl_R = \frac{\Delta Ae}{\Delta t} / C_{P,mid} \qquad \text{(Eq. 2-7)}$$

where the numerator is the urinary excretion rate over a given time interval and the denominator the midpoint plasma concentration during that interval.

RESULTS

Following intravenous infusion, EMB plasma concentrations rapidly rose in all subjects until cessation of administration. Semilogarithmic plots of plasma concentration versus time and urinary excretion rate

versus time over a 12 hr period are shown for subject CL in Fig. 4-1. In the 11-hr period immediately following termination of infusion, EMB concentrations and excretion rates showed multiexponential decay. The pharmacokinetic constants for the 4-compartment body model in 6 subjects following I.V. administration of EMB are presented in Table 4-2. The α, β, γ and δ phase in these subjects had a mean half-life of 8.6, 40.1, 135 and 924 min, respectively. The corresponding areas under the 4 phases are 21.5, 17.8, 31.3 and 29.3%, respectively. The mean values for the volume of the central pool and the steady-state volume of distribution for EMB were 0.42 and 4.02 L/kg, respectively. The total body clearance and renal clearance, derived from a 4-compartment analysis were 8.52 and 6.75 ml/min/kg, respectively. These values are contrasted with those obtained from model independent calculations as given in Table 4-3. Clearance values are fairly consistent with one another when the model dependent and independent methods are compared.

Semilogarithmic plots of the plasma concentration versus time over a 12 hr period following solution and tablet dosing are shown for subject JG in Fig. 4-2. A distinct distribution phase lasting approximately 5 hrs was followed by a longer terminal phase. Analysis of the terminal log linear phase by least squares regression for plasma samples up to 12 hrs post dosing gave the apparent half-lives as reported in Table 4-4. However, plasma samples at 24 hrs and analysis of urine samples from 24 to 72 hrs indicated the presence of an even longer half-life. Interestingly, all average half-lives calculated were longer following dosing with the aqueous solution. Although the difference is small for the values in Table 4-4, it was shown to be significantly different ($p<0.005$) by a paired t-test. Half-lives calculated from 12 and 24 hrs plasma samples only, yielded an average half-life of 11.2 $\pm$ 3.6 hrs for the

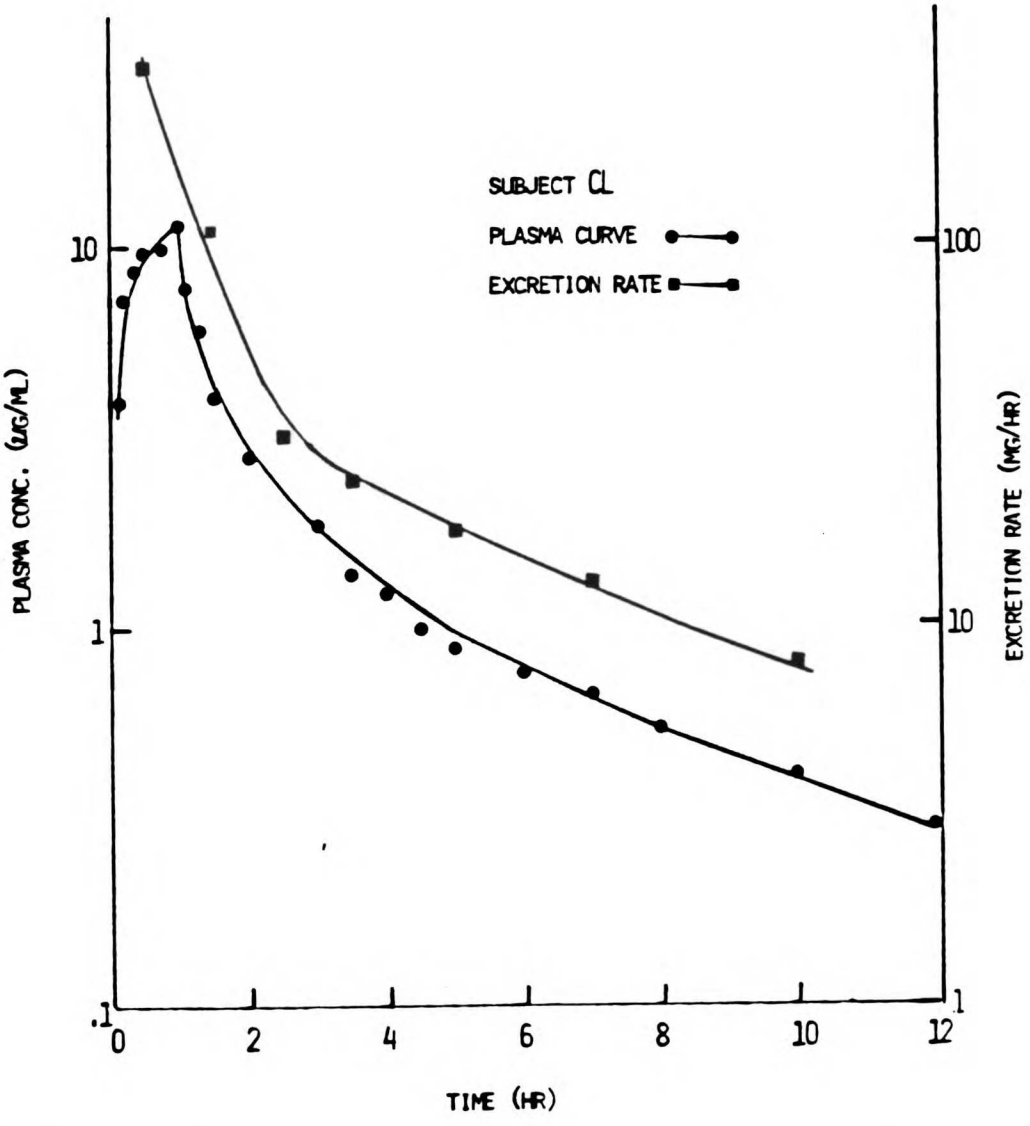

Fig. 4-1 Ethambutol plasma concentration vs. excretion rate plot, single intravenous dose 800 mg infused over 1 hr period.

Table 4-2

Four Compartmental Analysis on Ethambutol in Man following Intravenous Infusion of 15 mg/kg Dose[a]

Subject	$\alpha \cdot 10^{-2}$ min^{-1}	$t_{\frac{1}{2},\alpha}$ min	AUC_α %	$\beta \cdot 10^{-2}$ min^{-1}	$t_{\frac{1}{2},\beta}$ min	AUC_β %	$\gamma \cdot 10^{-3}$ min^{-1}	$t_{\frac{1}{2},\gamma}$ min	AUC_γ %	$\delta \cdot 10^{-4}$ min^{-1}	$t_{\frac{1}{2},\delta}$ hr	AUC_δ %	V_1 L/kg	V_{dss} L/kg	Cl_p[b] ml/min·kg	Cl_R[c] ml/min·kg
AL	9.90	7	39.1	1.65	42	18.4	5.21	133	18.3	8.86	13.0	24.2	0.21	3.34	8.96	6.69
SL	5.33	13	15.3	1.19	58	24.2	7.70	90	30.7	7.59	15.2	29.8	0.73	4.57	10.01	8.16
JG	5.33	13	19.3	1.73	40	13.5	6.60	105	31.5	8.21	14.0	35.7	0.53	3.91	7.92	6.65
CB	8.66	8	12.1	1.73	40	17.0	4.62	150	38.5	7.13	16.2	32.4	0.50	4.25	7.73	6.00
BS	13.86	5	21.0	2.88	24	18.0	3.64	190	38.0	6.51	17.7	23.0	0.21	3.54	7.76	6.17
CL	8.66	8	22.0	1.87	37	16.0	5.00	142	31.0	6.95	16.6	31.0	0.37	4.52	8.74	6.83
Mean	8.62	9.0	21.5	1.84	40.1	17.8	5.46	135.0	31.3	7.54	15.4	29.3	0.42	4.02	8.52	6.75
SD	3.18	3.3	9.4	0.56	10.9	3.6	1.45	35.3	7.3	0.87	1.7	4.9	0.20	0.51	0.90	0.76

[a] Disposition parameters were obtained by the method of residuals as described in the text.

[b] Calculated using AUC determined by Eq. 2-1

[c] Calculated using Eq. 2-5

Table 4-3

Comparison of the Model Independent and Model Dependent

Plasma and Renal Clearance Values (ml/min·kg)

Subject	Model Independent			Model Dependent	
	Cl_P[a]	Cl_R[b]	Cl_R[c]	Cl_P	Cl_R
AL	9.12	6.81	6.86	8.96	6.69
SL	10.54	8.59	8.45	10.01	8.16
JG	9.22	7.75	7.13	7.92	6.65
CB	8.25	6.40	5.93	7.73	6.00
BS	7.56	6.01	6.02	7.76	6.17
CL	8.94	6.99	6.50	8.74	6.83
Mean	8.93	7.09	6.81	8.52	6.75
SD	1.01	0.94	0.92	0.90	0.76

[a] calculated by Eq. 2-4

[b] calculated by Eq. 2-5

[c] calculated by Eq. 2-7

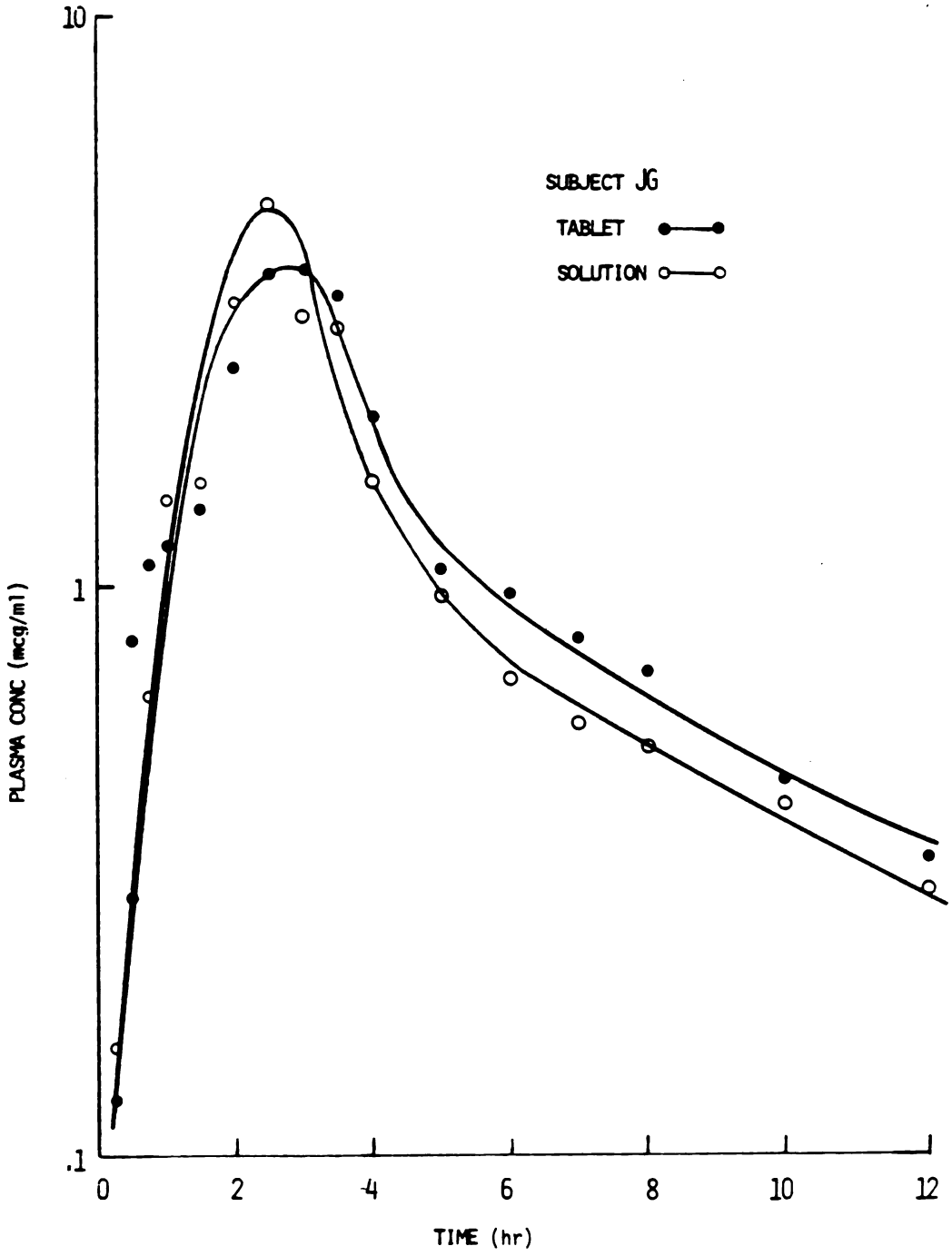

Fig. 4-2 Semilogarithmic plasma concentration-time curves for 1100 mg oral doses of ethambutol as a solution and as tablets to subject JG.

Table 4-4

Apparent Terminal Half-lives of Ethambutol after Single Oral and Intravenous Dosing

Subject	Terminal half-life (hr)		
	Injection	Solution	Tablet
AL	4.39	4.94*	3.95
SL	4.87	4.64	3.37
JG	3.57	4.48	4.02
CB	3.80	5.49	4.70
BS	4.29	4.33	3.69
CL	4.39	4.82	4.68
AVE ± SD	4.21 ± .46	4.78 ± .41	4.06 ± .53

*Sampling time up to 9 hours only, others up to 12 hours.

Tablet 4-5

Half-lives Calculated from 12
and 24 hr Plasma Samples Only

Subject	Injection	Solution	Tablet
AL	9.50	9.03	10.7
SL	12.97	7.99	--
JG	17.44	12.62	6.4
CB	10.18	7.22	7.73
BS	6.86	14.26	11.61
CL	10.45	14.64	9.50
Mean	11.23	11.35	9.19
SD	3.62	3.51	2.13

injection, 11.4 ± 3.5 hrs for the solution, and 9.2 ± 2.1 hrs for tablets. (See Table 4-5.) Half-lives calculated from 24 to 72 hr urinary samples were found to be 15.4 ± 1.7 hrs for the injection, 14.1 ± 2.3 hrs for the solution, and 13.8 ± 3.5 hrs for tablets. (See Table 4-6.)

Peak concentrations of drug in plasma following intravenous and oral administration of 15 mg/kg of EMB in 6 subjects and the times at which the peak occurred after oral dosing are summarized in Table 4-7. Following I.V. administration, peak concentrations were reached at the end of infusion. For the oral administrations, peak time ranged from 1.5 to 4 hrs. Comparing oral solution and oral tablet, peak concentrations occurred earlier with the aqueous solution of EMB.

Except in subjects CB and CL, peak plasma concentrations were lower with the commercial tablet preparation than the solution. To compute the absolute extent of availability of the solution and the tablet, areas under the curve were estimated using the trapezoidal method and Eq. 2-6; see Table 4-8. Area under the I.V. curve was the largest of the three, yet area under the solution curve and that under the tablet curve were not statistically different ($0.8 < p < 0.9$; paired t-test).

The cumulative urinary excretion of EMB as a percent of dose for the three studies are presented in Table 4-9. Approximately 75-84% of the intravenous doses and 54 to 67% of the oral doses was excreted unchanged in the urine over a 72 hr period. The percent recovery from solution and tablet preparations was similar, mean values of 63.4 and 61.1%, respectively. Absolute bioavailabilities of the solution and the tablet as estimated from area under the plasma curve and from cumulative urinary excretion, are presented in Table 4-10.

The renal clearance values of EMB in 6 subjects following intravenous and oral administrations are presented in Table 4-11. The renal

Table 4-6

Half-lives Calculated from 24, 48, and 72 hr Urinary Samples

Subject	Injection	Solution	Tablet	Subject Mean ± SD
AL	13.04	--	16.44	14.7 ± 2.4
SL	15.22	12.16	12.52	13.3 ± 1.7
JG	14.06	12.33	9.23	11.9 ± 2.4
CB	16.20	17.65	19.26	17.7 ± 1.5
BS	17.75	13.28	13.69	14.9 ± 2.5
CL	16.61	15.15	11.97	14.6 ± 2.4
Mean	15.48	14.11	13.85	
SD	1.73	2.31	3.54	

Table 4-7

Peak Times and Peak Concentrations following the Oral and the Intravenous Administration of 15 mg/kg of Ethambutol in 6 Subjects

Subject	One hr I.V. Infusion	Solution		Tablet	
	c_p(mcg/ml)	t_p(hr)	c_p(mcg/ml)	t_p(hr)	c_p(mcg/ml)
AL	15.40	2.0	4.05	2.5	3.87
SL	12.44	1.5	6.00	3.0	3.25
JG	12.68	2.5	4.67	3.0	3.58
CB	12.82	1.5	4.23	2.5	5.62
BS	13.30	2.5	4.44	4.0	3.60
CL	11.59	1.5	3.35	2.0	4.16
AVE ± SD	13.04 ± 1.28	1.91 ± .49	4.45 ± .88	2.83 ± .68	4.01 ± .84

Table 4-8

Area under the Curve following Administration of

Injection, Solution and Tablets of Ethambutol

		Area under the curve[a] (mcg·min/ml)		
Suject	Dose	Injection	Solution	Tablet
AL	800	1685	1277	1287
SL	800	1579	1148	1103
JG	1100	1670	1146	1058
CB	1100	1878	1224	1313
BS	800	1888	1641	1484
CL	800	1624	1236	1206
AVE ± SD		1720 ± 131	1278 ± 184	1241 ± 155

[a] calculated using Eq. 2-6

Table 4-9

Cumulative Excretion of Ethambutol as Percent of the Oral and the Intravenous Doses

Subject	Urine collection period (hr)	Injection	Solution	Tablet
AL	0-24	68.40	57.26	51.99
	0-48	72.89	66.66	60.08
	0-72	74.63	--	64.70
SL	0-24	78.02	54.00	53.17
	0-48	80.32	60.09	57.75
	0-72	81.48	62.17	59.40
JG	0-24	78.00	53.21	48.01
	0-48	82.19	59.26	53.38
	0-72	84.04	61.38	54.44
CB	0-24	69.80	49.24	53.78
	0-48	74.80	56.54	57.87
	0-72	77.59	61.20	60.85
BS	0-24	72.94	58.36	59.28
	0-48	76.93	64.36	62.60
	0-72	79.50	66.76	64.00
CL	0-24	72.75	54.66	53.67
	0-48	76.16	61.95	60.69
	0-72	78.14	65.60	63.02
AVE ± SD	0-72	79.23 ± 3.26	63.42 ± 2.57	61.07 ± 3.80

Table 4-10

Absolute Bioavailability of the Two Preparations, Solution and Tablet Calculated by Area under the Curve and Cumulative Urinary Excretion

Subject	Area under the curve		Cumulative urinary excretion	
	Solution	Tablet	Solution	Tablet
AL	72.16	69.44	--	86.69
SL	70.49	66.58	76.30	72.90
JG	60.44	63.51	73.03	64.78
CB	66.72	68.47	78.87	78.42
BS	82.62	77.28	83.97	80.50
CL	68.53	73.78	83.95	80.65
AVE ± SD	70.16 ± 7.32	69.84 ± 4.97	79.22 ± 4.79	77.32 ± 7.57

Table 4-11

The Average Renal Clearance of Ethambutol following Oral and Intravenous Doses

Subject	Injection	Solution	Tablet	Subject Mean ± SD
AL	386.8	345.1	412.1	381.3 ± 33.8
SL	437.8	356.0	386.7	393.5 ± 41.3
JG	593.2	347.9	496.0	479.0 ±123.5
CB	495.4	491.1	420.1	468.8 ± 42.3
BS	413.1	359.2	466.6	412.9 ± 53.7
CL	436.8	452.0	413.4	434.0 ± 19.4
AVE ± SD	460.5 ± 74.2	391.9 ± 63.1	432.4 ± 40.5	

clearance for each subject was determined using Eq. 2-7 where the urinary excretion rate over a given time interval is divided by the midpoint plasma concentration. Measurements for each individual were made up to 10 hrs following tablet, solution and intravenous dosing and then averaged.

The excretion rate versus time plot together with the plasma concentration time curve for subject SL following oral solution administration are shown in Fig. 4-3. For both curves the peak time and slope of the log linear phase are similar. Fig. 4-4 depicts a plot of excretion rate versus midpoint plasma concentration following solution dosing to subject SL. The slope of this plot is the renal clearance.

The plasma concentration time curve for subject JG following oral solution and oral tablet administrations was previously shown in Fig. 4-2. Attempts were made to fit the human oral data to a two compartment model with 2 consecutive first order absorption processes. Despite the success in fitting monkey oral data using exactly the same approach (Chapter 3), the resulting fit for the human oral data was quite poor. The absorption profile was then determined using the Loo-Riegelman method (51). The absorption rates versus time plot for each subject following oral solution and oral tablet administration are given in Fig. 4-5 through 4-10.

The partition of EMB between erythrocytes and plasma for 3 subjects is shown in Table 4-12. The concentration ratio of EMB in red blood cells to plasma ranged from 1.1 to 1.6. This is consistent with the data of Peets et al. (22).

The protein binding of EMB was determined both by equilibrium dialysis and ultrafiltration over the plasma concentration range of

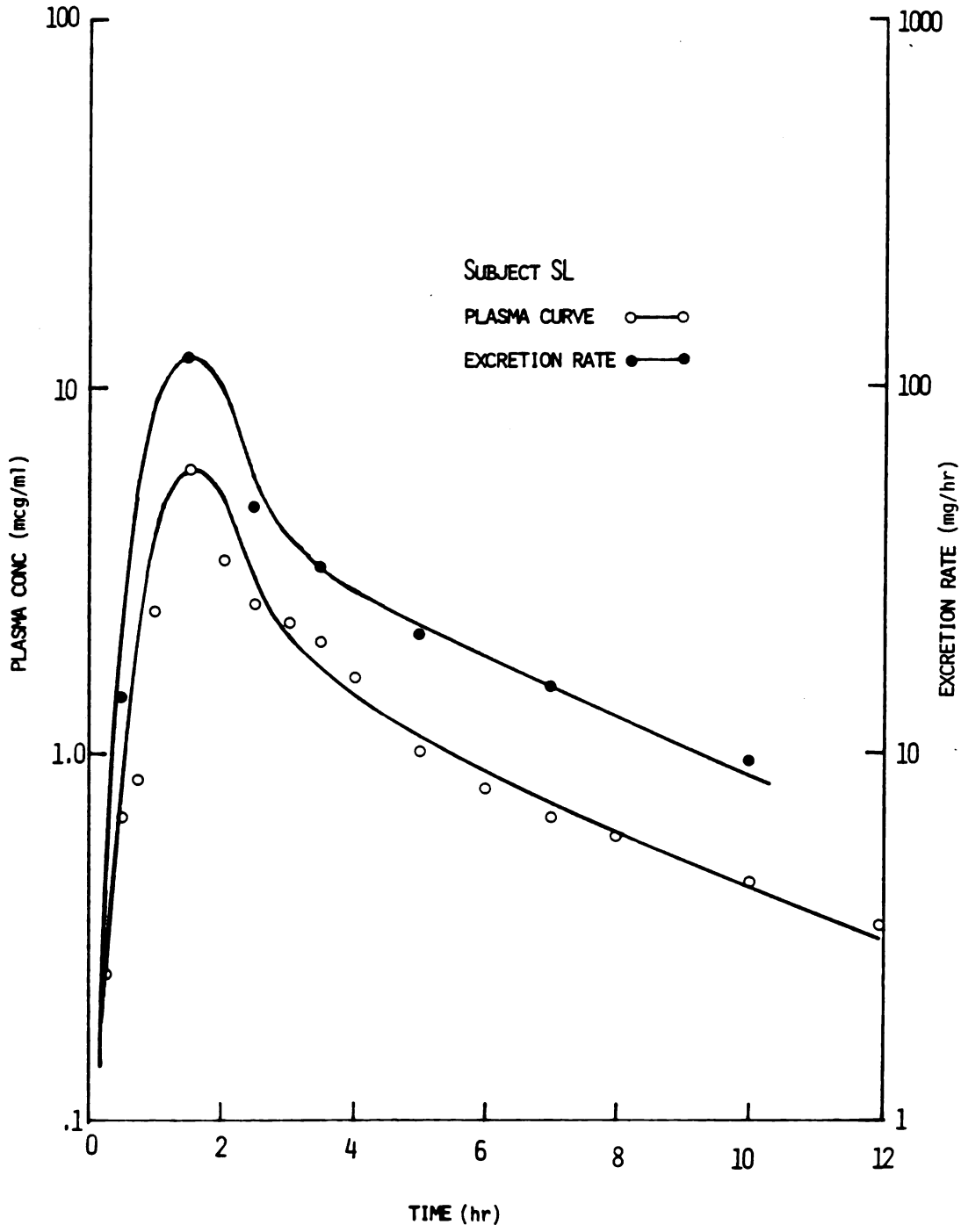

Fig. 4-3 Semilogarithmic plasma concentration and excretion rate plots for 800 mg oral dose of ethambutol as a solution to Subject SL.

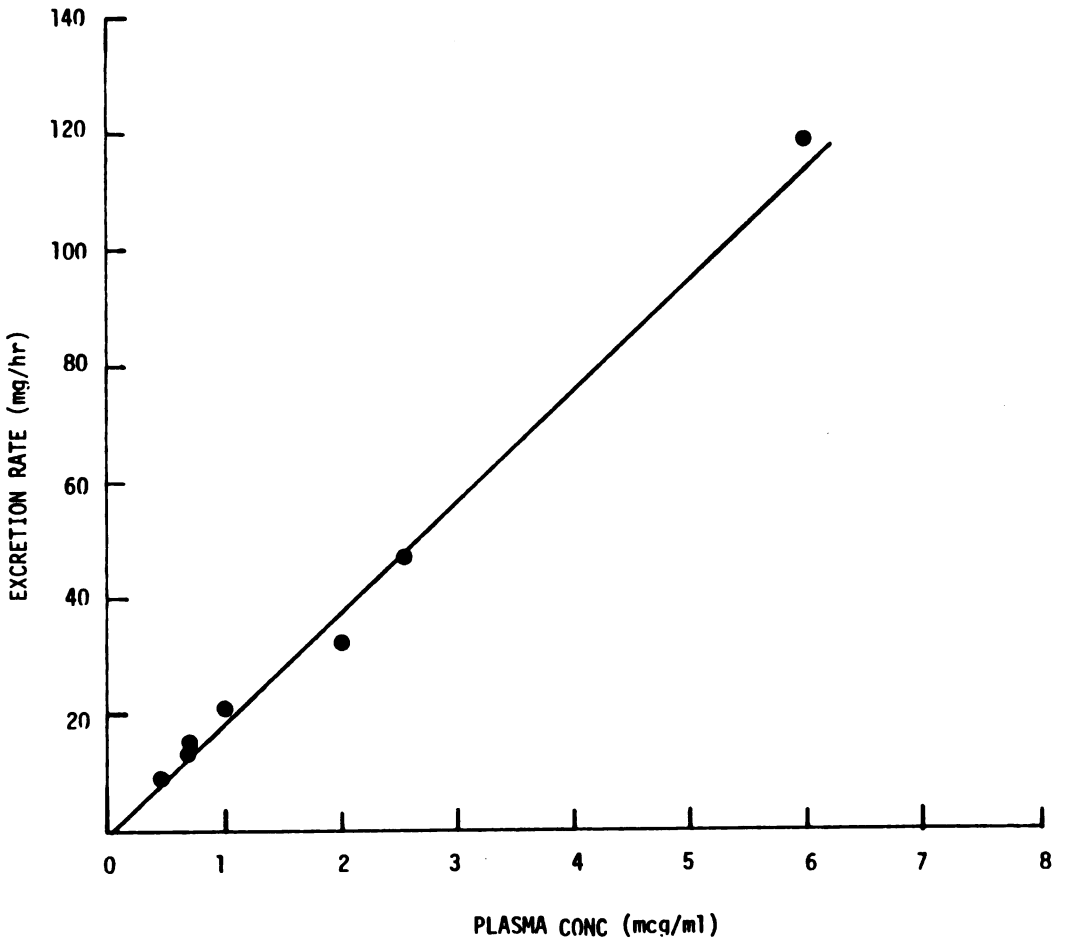

Fig. 4-4 Urinary excretion rate vs. midpoint plasma concentration up to 10 hrs following an 800 mg oral solution dose of ethambutol to subject SL. Renal clearance, i.e., the slope of the line in this plot, was 356 ml/min.

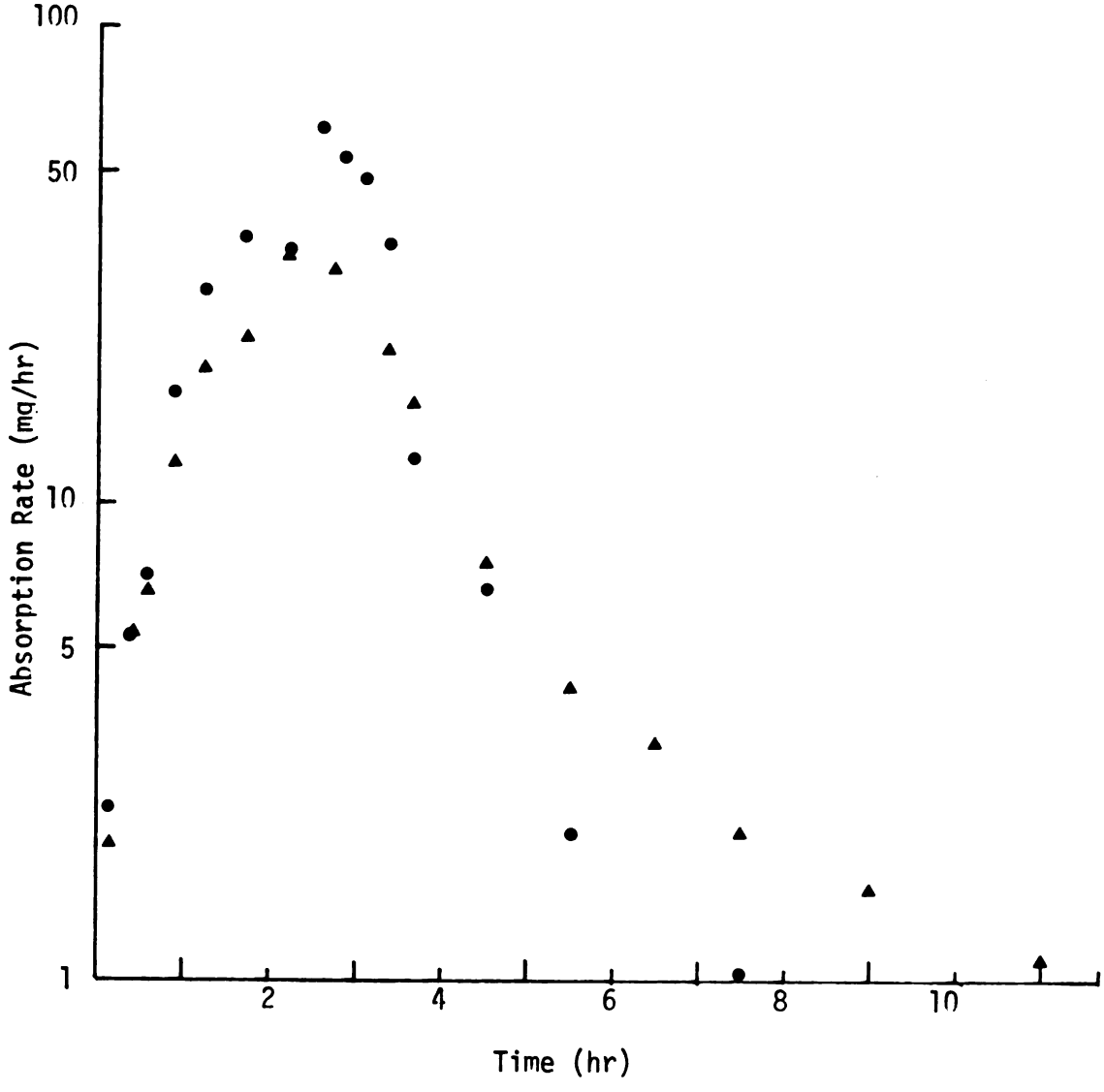

Fig. 4-5 Absorption rate vs. time plot in subject AL following oral administrations of ethambutol solution (●) and tablets (▲).

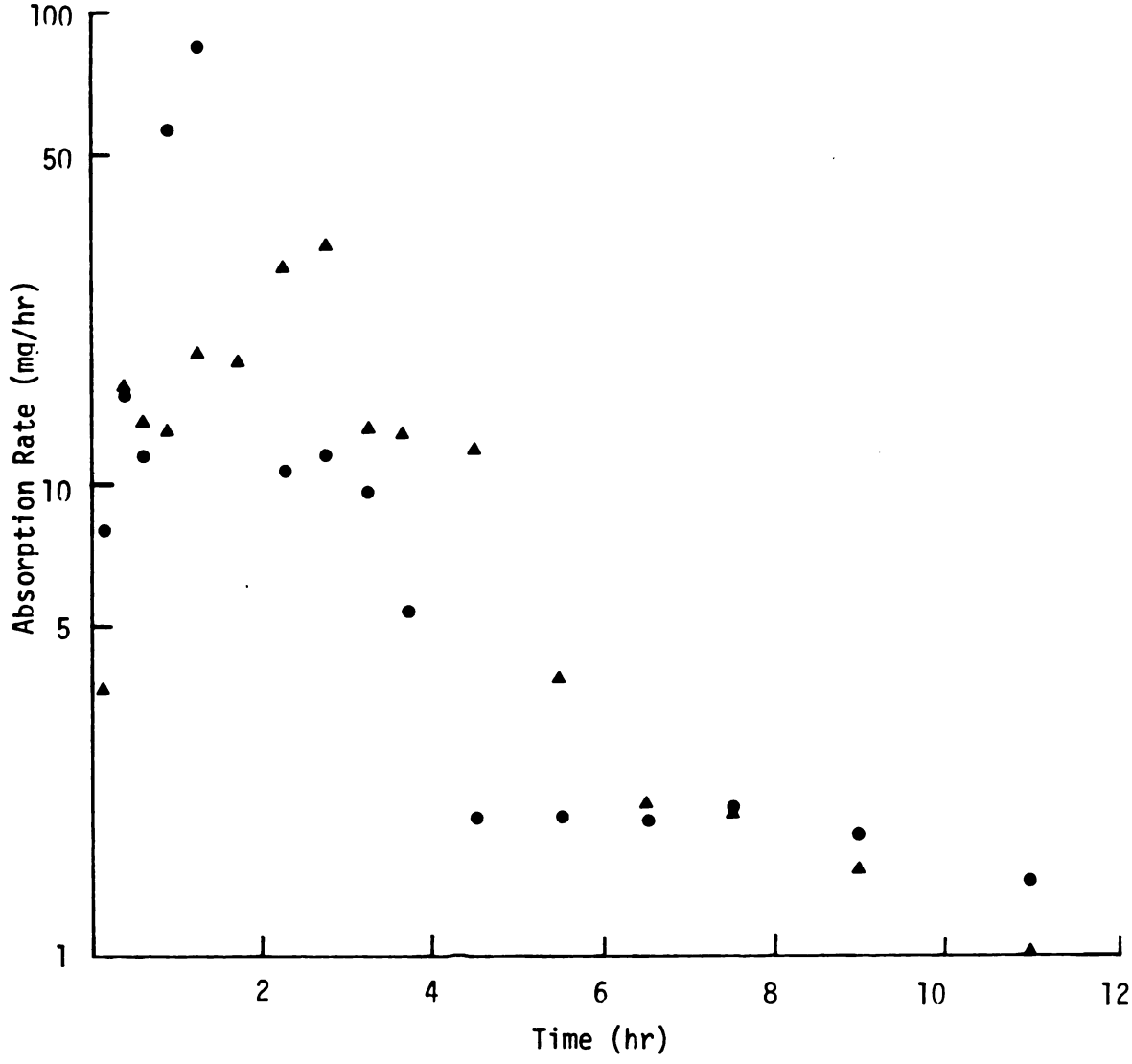

Fig. 4-6 Absorption rate vs. time plot in subject SL following oral administrations of ethambutol solution (●) and tablets (▲).

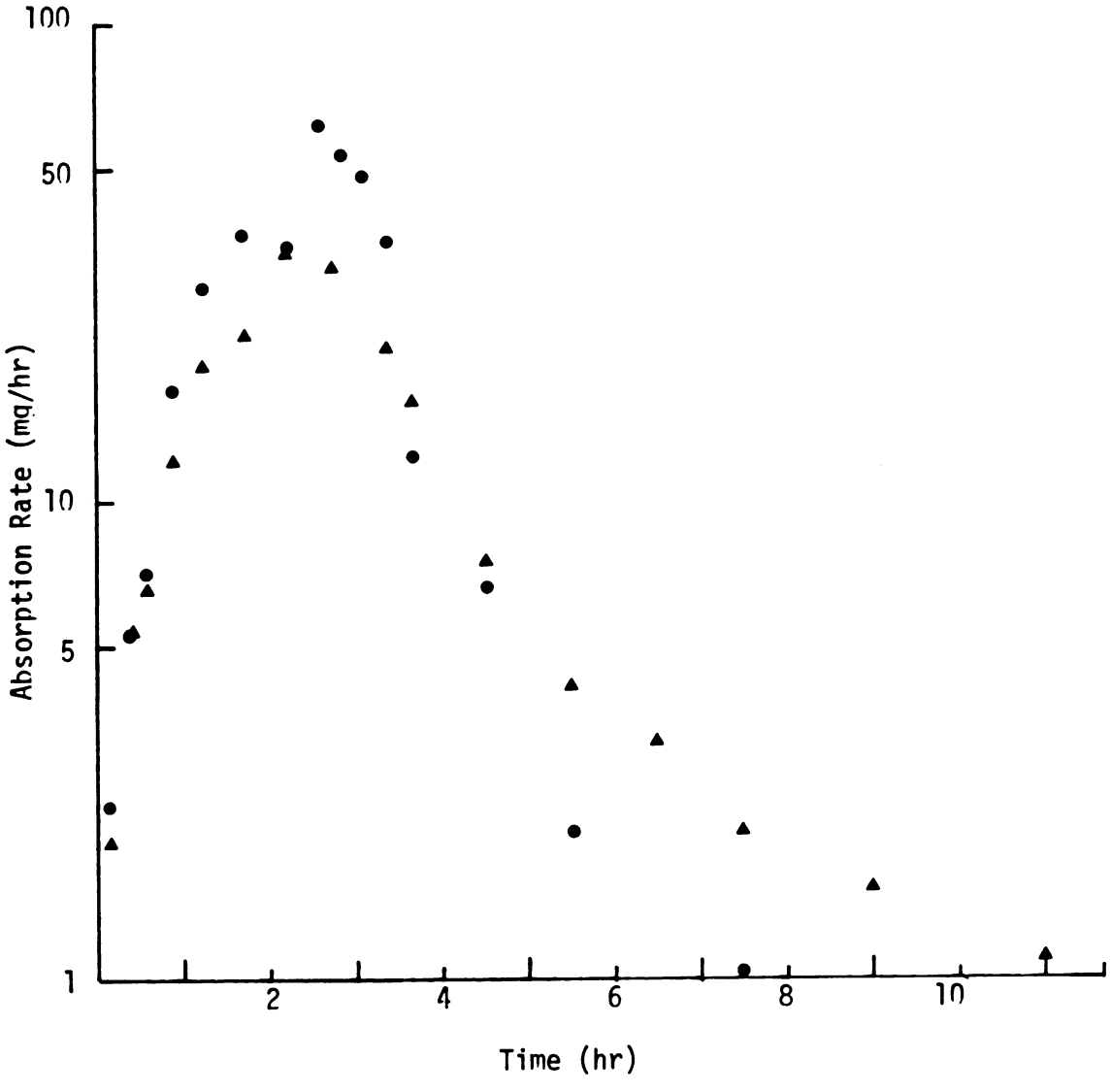

Fig. 4-5 Absorption rate vs. time plot in subject AL following oral administrations of ethambutol solution (●) and tablets (▲).

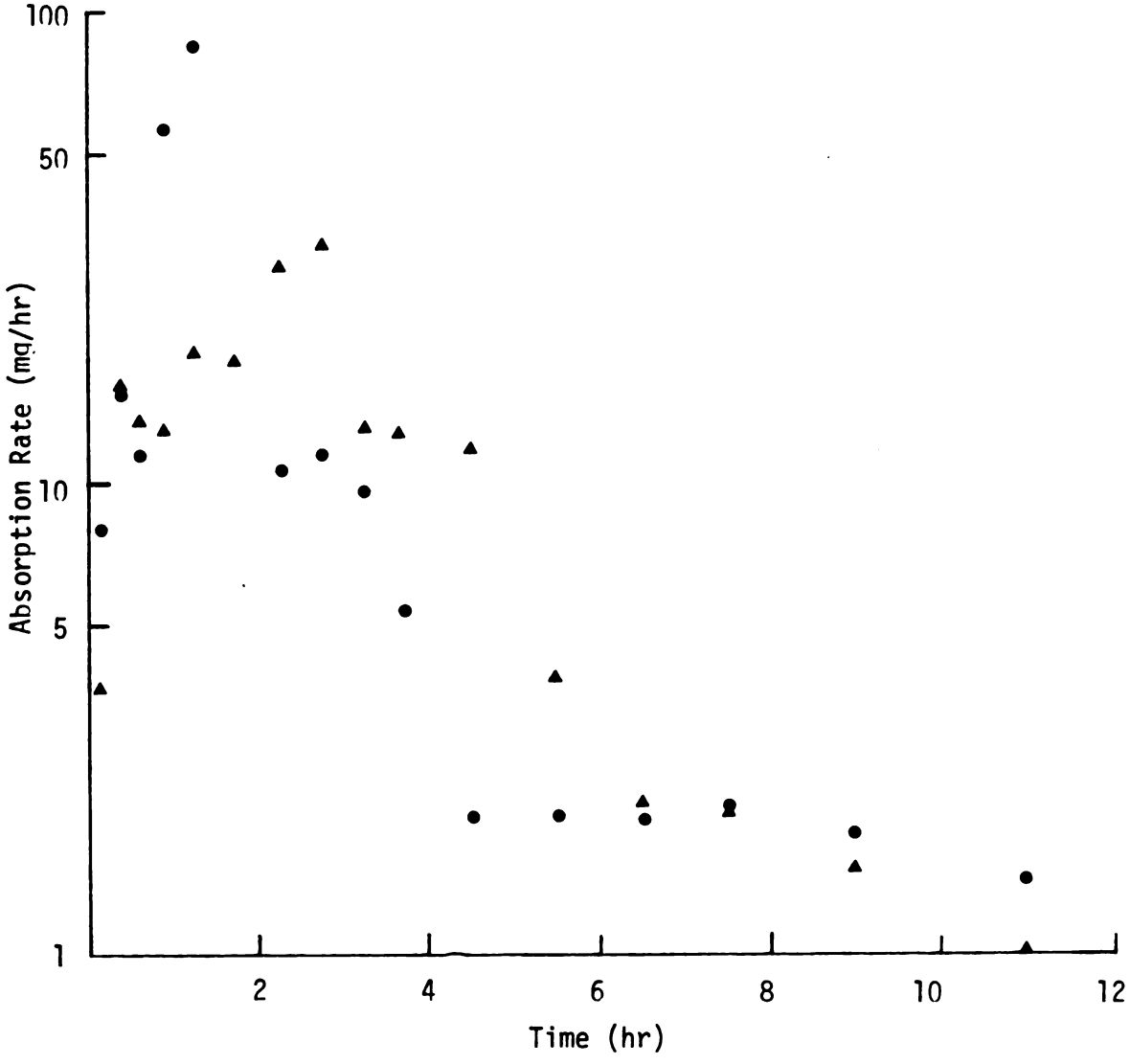

Fig. 4-6 Absorption rate vs. time plot in subject SL following oral administrations of ethambutol solution (●) and tablets (▲).

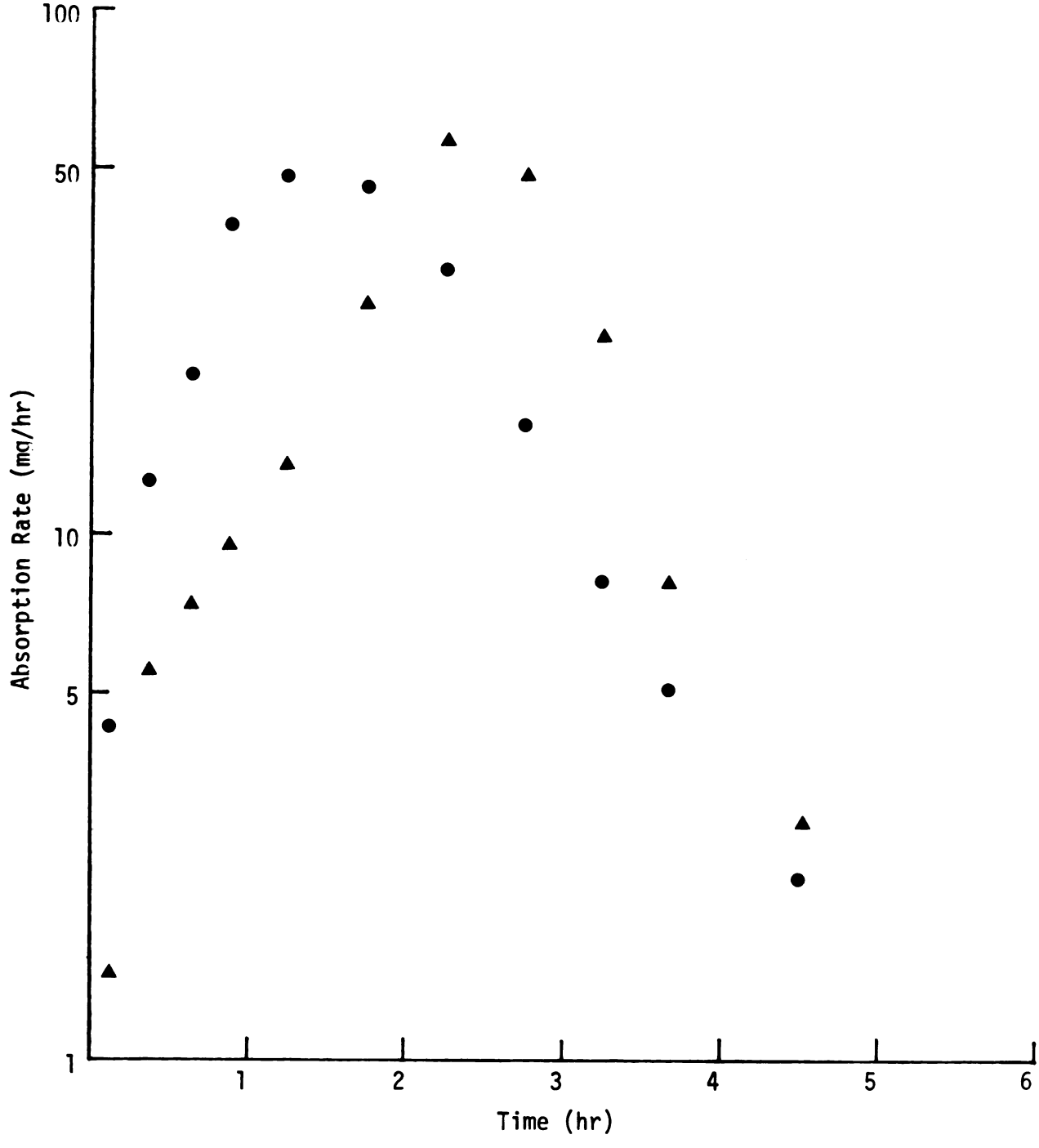

Fig. 4-7 Absorption rate vs. time plot in subject CB following oral administrations of ethambutol solution (●) and tablets (▲).

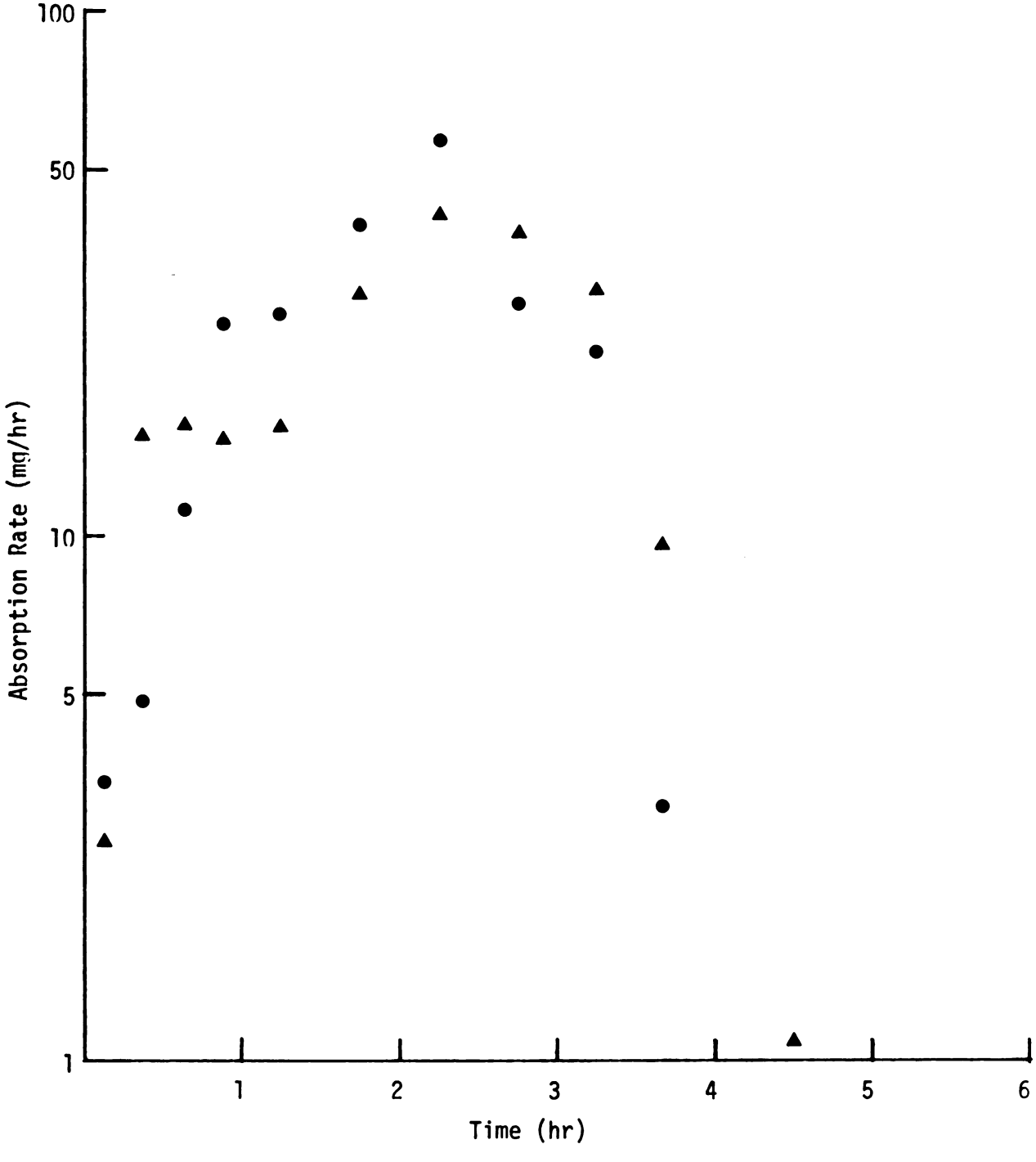

Fig. 4-8 Absorption rate vs. time plot in subject JG following oral administrations of ethambutol solution (●) and tablets (▲).

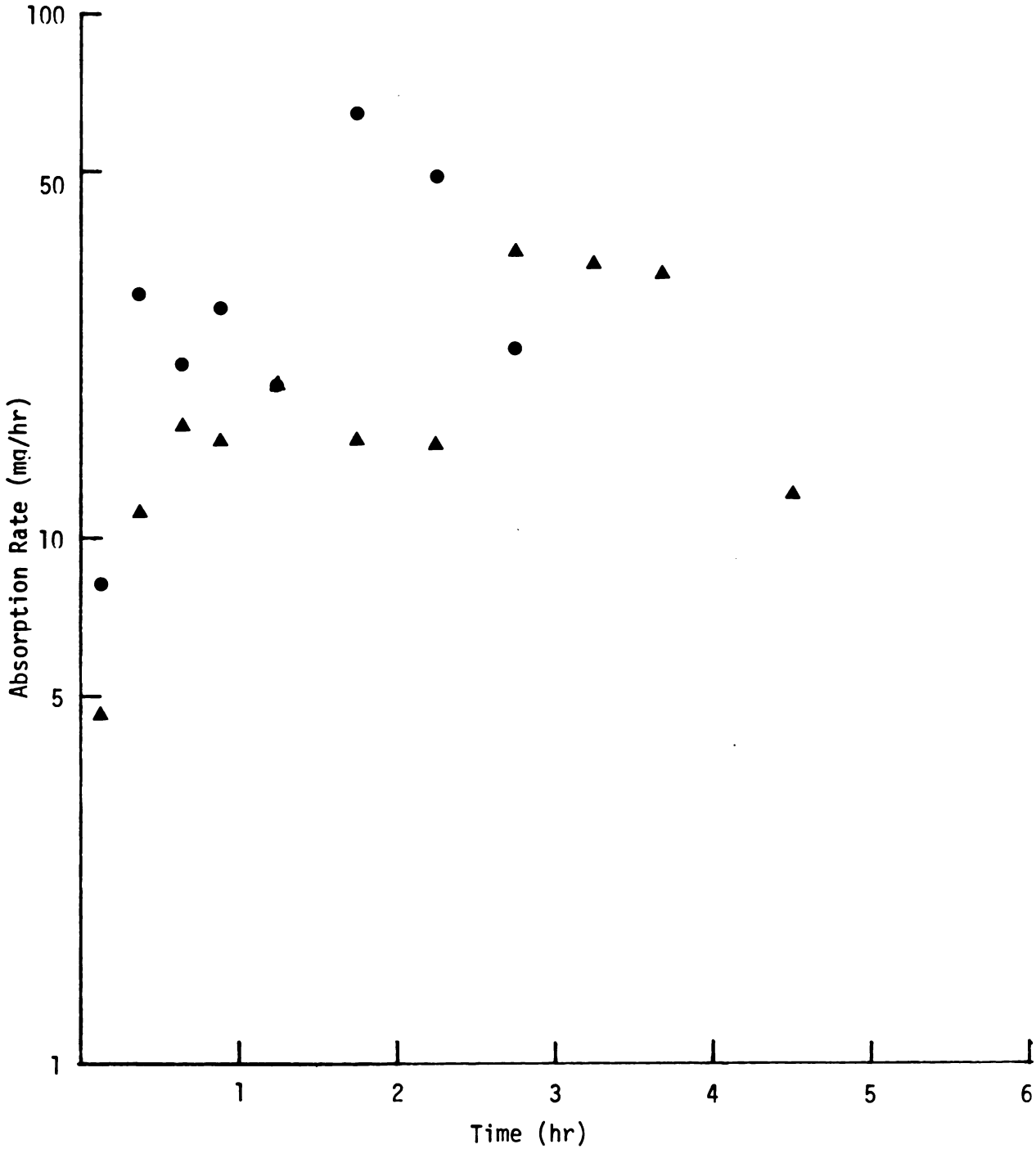

Fig. 4-9 Absorption rate vs. time plot in subject BS following oral administrations of ethambutol solution (●) and tablets (▲).

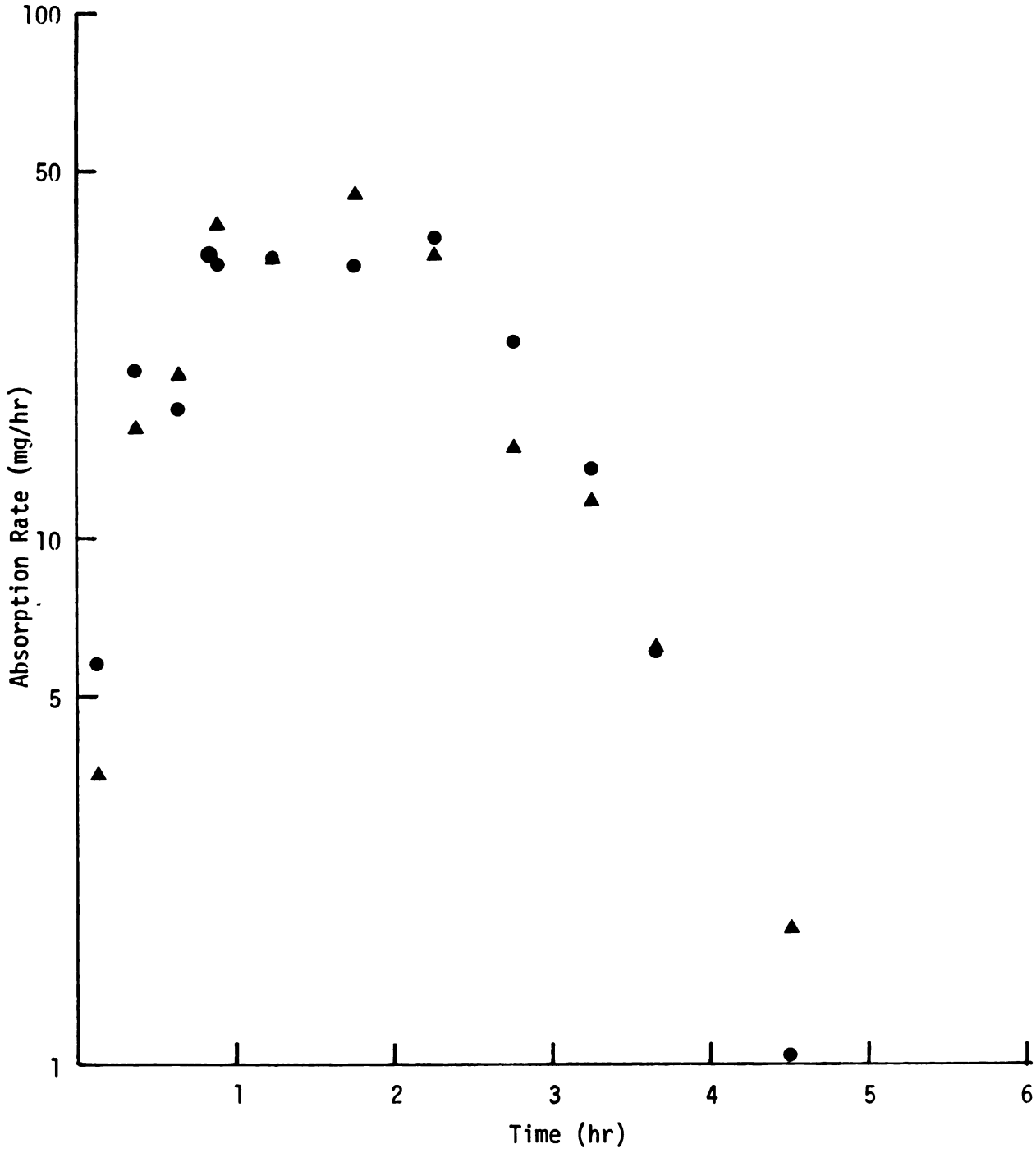

Fig. 4-10 Absorption rate vs. time plot in subject CL following oral administrations of ethambutol solution (●) and tablets (▲).

Table 4-12

Erythrocyte Partition of Ethambutol[a]

Whole blood conc (mcg/ml)	Pooled whole blood HCT=42.0	AL HCT=41.8	SL HCT=41.2	JG HCT=44.8
.5	1.3	1.5	1.1	1.4
1.0	1.2	1.1	1.5	--
2.0	1.2	1.6	1.4	1.2

[a] Reported as concentration in red blood cells divided by plasma concentration. HCT = hematocrit as a percent.

0.5 to 2.0 mcg/ml. The results of the protein binding studies in pooled human plasma and in three subjects are shown in Table 4-13. Approximately 20 to 30% of EMB is bound to plasma proteins.

DISCUSSION

In previous reports peak plasma EMB concentrations varied significantly between different studies at the same dose (22, 24, 33). Our results indicate only minor variation among individuals. For the I.V. injection, the peak concentrations ranged from 11.59 - 15.40 mcg/ml (13.04 ± 1.28), for the oral solution 3.35 - 6.0 mcg/ml (4.45 ± 0.88) and for tablets 3.25 - 5.62 mcg/ml (4.01 ± 0.84). The peak values from our intravenous study are close to those reported by Arnaud et al. (35) and Pujet and Pujet (28, 29) but remarkably different from those reported by Rossi et al. (34) and Delaude et al. (30). The peak values from our oral study tend to be higher than those reported by others (23, 24). The time at which the peak occurred has been reported to range from 2 to 4 hrs after tablet ingestion (23, 24, 33). In our study, similar peak times were found after oral administration of the commercial tablet (2.83 ± 0.68 hrs). Peak levels occurred earlier with the aqueous solution of EMB (1.91 ± 0.49 hrs).

In contrast to the reported large variation (40 - 80%) of the percent of the oral dose excreted unchanged in the urine (23, 24, 25), we found approximately 54 to 67% of the dose was excreted unchanged after a single oral dose. Literature values of renal clearance range from 100 to 447 ml/min (21, 25). The renal clearance found in this study in man (428.2 ± 59.3 ml/min) indicates active renal secretion. In addition, our study of 2 rhesus monkeys after intravenous bolus dosing yields renal

Table 4-13

Plasma Protein Binding of Ethambutol as Percent Unbound

Subject	Conc. (mcg/ml)	Equilibrium dialysis	Ultrafiltration
Pooled plasma	.5	74.6	72.4
	1.0	77.0	73.3
	2.0	78.0	81.0
AL	1.0	--	76.0
SL	1.0	--	70.0
CB	1.0	--	70.8

clearance values 2-3 times the normal glomerular filtration rate (Chapter 3). Because of the high renal clearance of EMB and the large amount excreted unchanged in the urine, changes in renal function would predictably alter the kinetics of the drug. Since it appears that EMB is actively secreted, there is also a potential for importantly affecting the renal clearance of EMB by co-administration of drugs that might compete for secretory pathways. At present, this potential is speculative.

The sharp fall in plasma EMB concentrations and urinary excretion rates following cessation of drug administration and the short $t_{\frac{1}{2},\alpha}$, $t_{\frac{1}{2},\beta}$ indicate that drug distribution in the body is quite rapid. Using a two compartment body model, the apparent volume of distribution for EMB has been estimated to be approximately 80% of the body weight (25). Jenne and Beggs (21) cited a value of 30% body weight which probably corresponds only to the central compartment volume. The mean values of V_1 and V_{dss} found in our studies are 0.42 and 4.02 L/kg, respectively, indicating that EMB distribution in the body is predominently extravascular. The large extravascular volume reflects the significance of the longer γ and δ phases in estimating total amounts of drug in the body. In order to compare the volume of distribution steady state with the value of Christopher et al. (25), i.e., 80% of body weight, the plasma data up to 12 hrs was fit to a two compartment body model. For this case a value of 1.65 L/kg was determined. This value is significantly greater than that reported by Christopher and coworkers who used a nonspecific radiolabel assay. Part of this difference is obviously a function of the longer $t_{\frac{1}{2}}$ observed by us over this time interval (4.2 hrs from Table 4-4 vs. apparent 3 hr value of Christopher et al.).

Half-life values have varied from 1.2 to 4.2 hrs in different studies (24, 25). Our data over the first 12 hrs post dosing favor longer half-life values (see Table 4-4) than those previously reported. Previous studies may have underestimated the half-life of EMB by calculating $t_{\frac{1}{2}}$ from the distribution phase rather than the terminal phases since detection of the latter phase requires prolonged plasma sampling and a sensitive, specific assay. Twenty-four hr plasma samples and 24 to 72 hr urinary samples indicate that EMB possesses an even longer terminal half-life of about 12 hrs. Although this longer half-life is not apparent before 12 hrs, the fractional area calculated under the plasma concentration-time curve after 12 hrs ranged from 15 to 28% with an average of 21.3 $\pm$ 4.8%. This relatively large area under the curve for the 12 hr half-life indicates that there should be approximately a 20% increase in steady-state levels upon multiple daily dosing (56) as compared to levels predicted using the shorter half-lives. However, for therapeutic purposes this projected increase would not be significant. Therefore, it appears reasonable to state that the 4-5 hr half-life for EMB reported in Table 4-4 is a good estimate for patients with normal renal function. The increased apparent half-lives reported in Table 4-4 for solution dosing versus tablet dosing may be explained in the following manner: Since EMB is more rapidly absorbed from the solution than from the tablets, the 4-12 hr phase following solution dosing is further into the terminal elimination phase than would be the case for the tablets. Thus, the influence of the longer 12 hr terminal phase may become apparent at an earlier time following solution dosing.

Due to the existence of a longer terminal phase beginning 12 hrs post drug administration, we found that approximately 0.1 mcg/ml plasma

concentrations of EMB can be clearly detected up to 24 hrs following either an intravenous or an oral dose. Previous studies (9) using a microbiological assay found no detectable drug concentration at this time after even higher doses (25 mg/kg).

In the present study we administered EMB solution as a reference standard and found from area under the curve and 72 hr urinary excretion data that EMB as an oral tablet was as available as its aqueous solution (97.4% plasma area and 96.3% urinary excretion measurements; see Tables 4-9 and 4-14). The lack of an important difference in bioavailability of EMB between solution and tablet implies that the formulation of EMB tested optimizes oral absorption.

As can be seen from Table 4-10, there is approximately a 10% difference in absolute bioavailability estimated from area under the curve and from cumulative urinary excretion. We believe that bioavailability estimated from urinary excretion is more reliable than that estimated from area under the plasma curve since urine collections were made over a 3 day period while blood sampling lasted for 24 hrs only and area thereafter was extrapolated from a constant determined from the 12 and 24 hr points only. As previously indicated, EMB possesses an even longer terminal half-life of about 12 hrs which becomes apparent 12 hrs post dosing. When the area under this third phase was taken into account the absolute bioavailability estimated from area under the curve was raised approximately 2% and 4%, respectively, for tablet and solution (compare Tables 4-10 and 4-15). From this study we can conclude that approximately 75% of the oral dose is available to the systemic circulation. Peets et al. (22) report EMB bioavailability as 80%. This was calculated by subtracting the fraction excreted in the feces from the

Table 4-14

Area under Curves and Relative Ratio Calculated following Administration of Ethambutol as a Solution and as Tablets

Subject	Area under the curve (mcg min/ml)		Ratio of areas (tablet/solution)
	Solution	Tablet	
AL	1277	1287	1.008
SL	1148	1103	0.961
JG	1146	1058	0.923
CB	1224	1313	1.073
BS	1641	1484	0.904
CL	1236	1206	0.976
Mean ± SD	1279 ±185	1242 ±155	0.974 ±0.061

Table 4-15

Absolute Bioavailabilities of Solution and Tablet Estimated by Area under the Curve, Taking into Account the Area under the Third Phase

Subject	Solution	Tablet
AL	75.78	76.38
SL	72.70	69.85
JG	68.62	63.35
CB	65.17	69.91
BS	86.91	78.60
CL	76.11	74.26
AVE ± SD	74.21 ± 7.52	72.06 ± 5.51

total dose. The results here confirm the earlier calculation of Peets et al.

The plasma profiles (Fig. 4-2) and even the absorption rate plots (Fig. 4-7. Fig. 4-8 and Fig. 4-10 for JG, CB and CL, respectively) found after oral dosing in human were similar to those seen in monkeys (Fig. 3-4 and Fig. 3-5). However, oral human data exhibited an unusual absorption pattern which could not be analyzed assuming two first-order processes. Absorption rate plots for subjects AL (Fig. 4-5) and SL (Fig. 4-6) seem to suggest that EMB absorption may be described by three first-order processes in these subjects. Bungay et al. (57) have demonstrated that phenol red appearance in the gall bladder can be simulated using 2 stirred tanks connected in series to represent the bile duct. When the 2 tanks were lumped into one, they failed to predict the bile concentration precisely. Compared to bile secretion, gastrointestinal absorption is a far more complicated process. Therefore, it is not impossible that three first-order processes are governing the G. I. absorption. Confirmation of this must wait further treatment of the data.

Absorption rate plots for subject BS indicates erratic absorption although her plasma concentration curve did not seem to give such an indication.

The unusual absorption of EMB might be explained by binding in the gastrointestinal tract which can become rate limiting in the absorption process. This hypothesis is based on the speculation that EMB, an EDTA analog might be expected to bind or chelate with a number of molecules in the G. I. fluids/or tissues. In vitro EMB chelates have been shown to exist (5).

The total body clearance and renal clearance obtained from both model independent and model dependent treatments are compared in Table 4-3. The closeness of these corresponding clearance values suggests that a four compartment model is a reasonable description of the disposition kinetics of EMB.

CHAPTER 5

HUMAN STUDIES IN TUBERCULOSIS PATIENTS AND UREMIC PATIENT UNDERGOING HEMODIALYSIS

METHODS

Patient Selection

The subjects studied were selected from patients diagnosed as having tuberculosis and receiving EMB as a part of their routine drug treatment. All subjects studied received a routine medical history, physical examination and a panel of laboratory tests (e.g., SMA-12, creatinine clearance, WBC and differential, hematocrit and hemoglobin). Three classes of patients were studied:

Class I - Three patients with tuberculosis and normal kidney function (creatinine clearance > 90 ml/min).

Class II - One patient with tuberculosis and renal insufficiency (creatinine clearance = 14 ml/min).

Class III - One anuric patient with tuberculosis undergoing hemodialysis.

Study Design

Subjects for Class I was selected at random from the Tuberculosis Ward at San Diego University Hospital. Subject characteristics are given in Table 5-1. The serum creatinine and creatinine clearance values indicate normal renal function for these subjects. Blood samples were collected over 12 hrs, at the following times: 0, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, 360, 420, 480, 600 and 720 minutes after dosing. One additional sample was collected at 24 hrs. Urine samples were collected for 72 hrs at 0, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 hrs. Two of the three subjects received an oral dose of 15 mg/kg of EMB in

Table 5-1

Subject Characteristics

Subject	Class	Sex	Age (yr)	Weight (kg)	Dose (mg)	Serum Creatinine (mg%)	Creatinine Clearance (ml/min)
CE	I	M	57	63	1000	.8	--
PG	I	M		60	900	.7	132
MJ	I	M	25	64	1500	.7	148*
WR	II	M	54	54.4	300		14
TM	III	M	45	81.3	400		

* average of three determinations

the commercially available tablet form. The other received 25 mg/kg of EMB. All doses were given following an overnight fast starting at 10 P.M. the night before the study day. No breakfast was given and lunch was given at least 4 hrs after the dose. Water was allowed ad libidum. To minimize the discomfort of multiple venipunctures from blood sampling a scalp vein needle with a heparin lock was left in place in a superficial arm vein for the initial 12 hr sampling period.

The Class II subject was an out patient of the Parnassus Height Medical Clinics. The patient was screened initially for elevated serum creatinine. Creatinine clearance was determined from samples collected during the study. The patient was taking 300 mg of EMB daily. Blood samples were collected over 12 hrs, at the following times: 0, 10, 30, 60, 90, 120, 150, 180, 240, 480 and 720 minutes. Urine samples were collected for 48 hrs at 0, 2, 4, 8, 12, 24, 30, 36 and 48 hrs.

The Class III subject was a patient from the Franklin Hospital Dialysis Unit, San Francisco. The patient had tuberculous pericarditis and meningitis tuberculosis and was being hemodialyzed for uremia. Blood samples were drawn before and while the patient was on hemodialysis. Hemodialysis commenced 10 hrs after the oral dose of EMB thus eliminating any concern for the absorption and distribution phases. Blood samples were drawn from the arterial line to the dialyzer during dialysis at 0, 30, 90, 150, 210 and 240 minutes. Venous line samples were drawn at 30 and 150 minutes. Total dialysate was collected at hourly intervals, the volume measured and a sample saved for analysis. The outgoing dialysate samples at 30 and 150 minutes were also collected. The patient was also studied on a non-dialysis day with blood samples collected only at 0, 30, 60, 120, 180 and 240 minutes. The dialyzer used in this

study was Cordis-Dow hollow fiber kidney. Figure 5-1 shows the clinical set up for the dialysis system used in this study as adapted from Sargent (58).

Treatment of Dialysis Data

Calculation of dialysis clearance

Dialysis clearance is defined as the hypothetical volume of body fluid that must be cleared by the dialyzer to give the amount of drug excreted per unit time. Following the treatment of Gotch (59), the solute mass balance across the dialyzer can be described as follows:

Drug in arterial blood + Drug in incoming dialysate stream
=Drug in venous blood + Drug in outgoing dialysate stream

or

$$Q_{Bi}C_{Bi} + Q_{Di}C_{Di} = Q_{Bo}C_{Bo} + Q_{Do}C_{Do}$$

where Q_B - blood flow in (i) and out (o) of the dialyzer

C_B - blood concentration

C_D - dialysate fluid concentration

Rearranging the above equation:

$$Q_{Bi}C_{Bi} - Q_{Bo}C_{Bo} = Q_{Do}C_{Do} - Q_{Di}C_{Di}$$

dividing by C_{Bi}

$$Cl_{db} = \frac{Q_{Bi}C_{Bi} - Q_{Bo}C_{Bo}}{C_{Bi}} \qquad \text{(Eq. 5-1)}$$

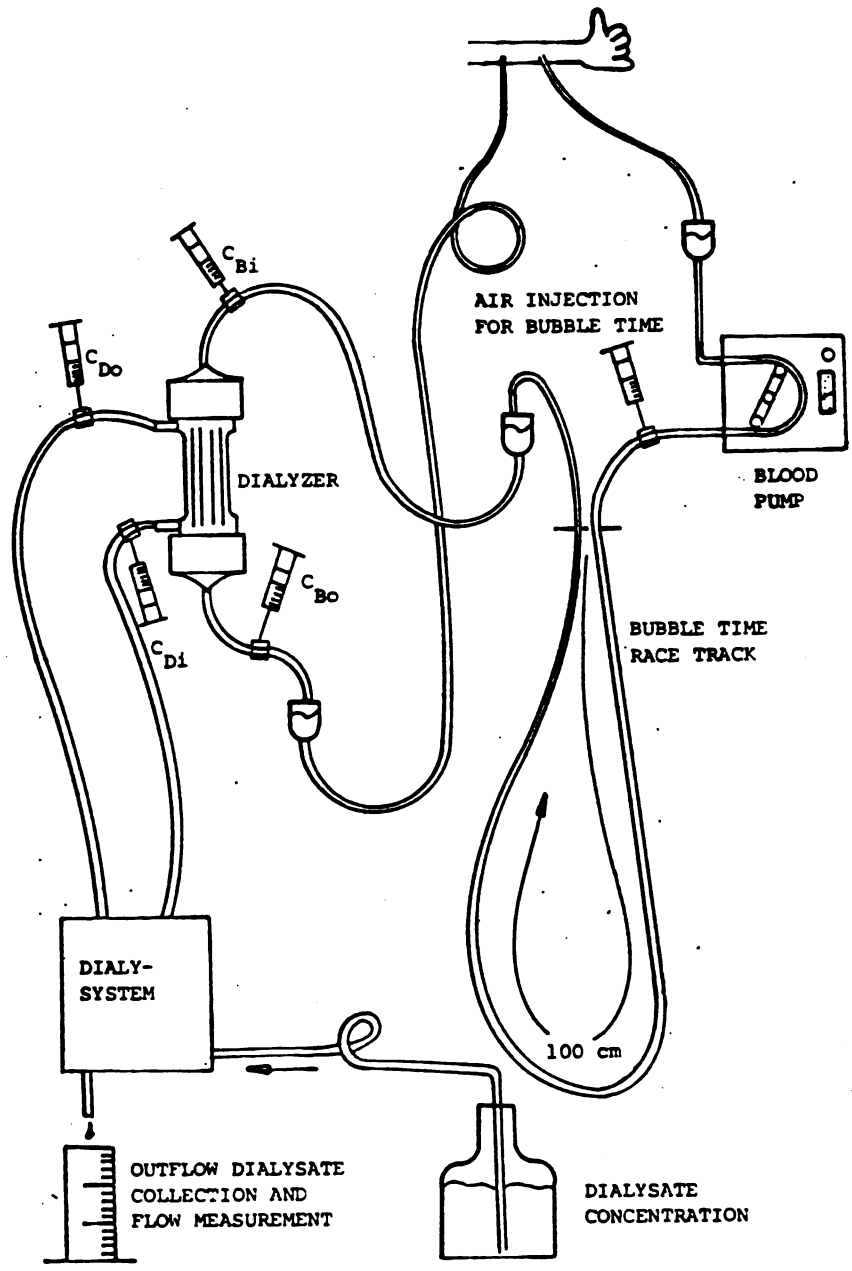

Fig. 5-1 Clinical set up for a hemodialysis system. Franklin Hospital Dialysis Unit, San Francisco.

$$Cl_{db} = \frac{Q_{Do}C_{Do} - Q_{Di}C_{Di}}{C_{Bi}} \qquad \text{(Eq. 5-2)}$$

where Cl_{db} is the hemodialyzer clearance measured with blood as the body fluid of reference.

If ultrafiltration, Q_F, is a measurable quantity,

$$Q_{Bo} = Q_{Bi} - Q_F \qquad \text{and} \qquad Q_{Do} = Q_{Di} + Q_F$$

and the clearance equations must be modified to take this into account. Therefore

$$Cl_{db} = \frac{Q_{Bi}C_{Bi} - (Q_{Bi} - Q_F)C_{Bo}}{C_{Bi}} \qquad \text{(Eq. 5-3)}$$

and

$$Cl_{db} = \frac{Q_{Do}C_{Do} - (Q_{Do} - Q_F)C_{Di}}{C_{Bi}} \qquad \text{(Eq. 5-4)}$$

However, if $Q_F = 0$ then Eqs 5-3 and 5-4 reduce to

$$Cl_{db} = Q_B \frac{C_{Bi} - C_{Bo}}{C_{Bi}} \qquad \text{(Eq. 5-5)}$$

$$Cl_{db} = Q_D \frac{C_{Do} - C_{Di}}{C_{Bi}} \qquad \text{(Eq. 5-6)}$$

Now, if concentration of drug in the incoming dialysate fluid is negligible ($C_{Di} = 0$), then

$$Cl_{db} = Q_D \frac{C_{Do}}{C_{Bi}} \qquad \text{(Eq. 5-7)}$$

In using Eq. 5-5, arterial and venous blood concentrations (C_{Bi} and C_{Bo}, respectively) are measured simultaneously. In using Eq. 5-7, arterial blood and outgoing dialysate concentrations are measured simultaneously. Since $Q_D C_{Do}$ is amount removed per unit time or the removal rate

$$Cl_{db} = \frac{\text{Drug Removal Rate}}{C_{Bi}}$$

where C_{Bi} represents the average arterial blood concentration during the time interval which can be determined by

$$C_{Bi} = \frac{\int_{t_1}^{t_2} C_{Bi} dt}{(t_2 - t_1)}$$

where the integration term represents the area under the blood concentration curve between blood sampling times t_1 and t_2. The area is usually estimated by trapezoidal method. In practice, C_{Bi} is approximated by $C_{Bi,mid}$, drug concentration in the arterial blood at the midpoint of dialysate collection interval. Thus,

$$Cl_{db} = \frac{\text{Drug Removal Rate}}{C_{Bi,mid}} \qquad \text{(Eq. 5-8)}$$

The drug removal rate can be determined hourly or over the whole dialysis period, with the arterial blood sampling arranged accordingly to obtain $C_{Bi,mid}$.

Many times, drug plasma concentrations but not drug blood concentrations are measured. If the ratio of the concentration of drug in the red blood cells to the concentration of drug in the plasma (C_{RBC}/C_P) remains constant on both arterial and venous side of dialyzer, then Eq. 5-3

becomes

$$Cl_{db} = \frac{Q_{Bi}C_{Pi} - (Q_{Bi} - Q_F)C_{Po}}{C_{Pi}} \qquad \text{(Eq. 5-9)}$$

where C_{Pi} and C_{Po} are the arterial and venous plasma concentrations. Equation 5-7 becomes

$$Cl_{dp} = \frac{Q_D \ C_{Do}}{C_{Pi}} \qquad \text{(Eq. 5-10)}$$

where Cl_{dp} is the hemodialyzer clearance measured with plasma as the body fluid of reference and Eq. 5-8 becomes

$$Cl_{dp} = \frac{\text{Drug Removal Rate}}{C_{Pi,mid}} \qquad \text{(Eq. 5-11)}$$

However, others(60) report dialysis plasma clearance as

$$Cl_{dp} = Q_P \frac{C_{Pi} - C_{Po}}{C_{Pi}} = (1 - H)Q_B \frac{C_{Pi} - C_{Po}}{C_{Pi}} \qquad \text{(Eq. 5-12)}$$

where H is the hematocrit.

It is important to realize that Eq. 5-12 is not equivalent to Eq. 5-10. Calculation using Eq. 5-12 always results in an underestimate of the true dialysis clearance (see Appendix 5-1). Therefore, Eq. 5-10 and 5-11 but not Eq. 5-12 should be used in such calculations.

Calculation of volume of distribution

The apparent volume of distribution can be determined by Eqs. 5-13 and 5-14:

$$V_{dp} = \frac{Cl_{dp}}{.693\left(\frac{1}{t_{\frac{1}{2},on}} - \frac{1}{t_{\frac{1}{2},off}}\right)} \qquad \text{(Eq. 5-13)}$$

$$V_{dp} = \frac{\text{Total Drug Removal}}{(C^{o}_{Pi} - C^{t}_{Pi}) \times \left(1 - \frac{t_{\frac{1}{2},on}}{t_{\frac{1}{2},off}}\right)} \qquad \text{(Eq. 5-14)}$$

where V_{dp} = volume of distribution defined in terms of measurements in plasma

$t_{\frac{1}{2},on}$ = half-life on dialysis

$t_{\frac{1}{2},off}$ = half-life off dialysis

C^{o}_{Pi} = initial plasma concentration of drug entering the dialyzer

C^{t}_{Pi} = plasma concentration of drug entering the dialyzer at time t

Total Drug Removal = summation of drug removal from time 0 to time t

(See Appendices 5-2 and 5-3 for formula derivations.)

The assumption involved is that the "metabolic clearance" of the drug remains constant on and off dialysis. As defined in Appendix 5-1, residual renal clearance is included in "metabolic clearance."

If dialysis clearance is expressed in blood and/or drug concentration is measured in blood, a similar volume term V_{db} may be calculated accordingly.

Blood Flow Measurement

Dialysis clearance has a fixed dependence on blood flow rate (61), particularly for a middle size molecule (M. W. < 500) such as EMB. Although the average blood flow rate readily achieved with present day

blood access is approximately 200 ml/min, in order to control dialysis clearance over a broad range and tailor performance to individual patient needs, it is often necessary to control blood flow rate over a range of 100 to 300 ml/min. The most widely used technique to measure blood flow is the bubble time method. A small air bubble of approximately 0.5 ml is injected into the blood tubing and the velocity is determined by measuring the time required for the air bubble to traverse a "racetrack" (Fig. 5-1) of known length. Blood flow was maintained at 200 ml/min during the course of this dialysis study.

Dialysate Flow Measurement

The dependence of dialysis clearance on dialysate flow is evidenced by Eq. 5-6. If dialysate flow is slow, the concentration in the dialysate fluid within the dialyzer may build up and thus, decrease the concentration gradient across the dialyzer membrane. Dialysate flow is usually adjusted between 300 and 500 ml/min. In this study, flow rates of fluid out of the dialyzer were monitored at the end of each hour and were found to remain quite constant through the dialysis period (503.3, 510.6, 494.5 and 508.8 ml/min measured hourly).

Assay Procedure

Ethambutol concentrations in plasma, urine and dialysate samples were measured as previously described in Chapter 2.

RESULTS

Peak time, peak concentration, terminal half-life, renal clearance and percent excreted unchanged for EMB in tuberculosis patients with normal renal function (Class I) are given in Table 5-2. The peak times

Table 5-2

Some Pharmacokinetic Parameters of Ethambutol in Tuberculosis Patients with Normal Renal Function

Subject	Peak time (hr)	Peak conc. (μg/ml)	$t_{\frac{1}{2}}$ β (hr)	Renal clearance (ml/min)	% E.U.
CE[1]	2.5	3.55	3.05	371.8	50.67
PG[2]	2.0	3.75	4.93	409.7	35.51
MJ[3]	3.0	2.45	3.99	410.3	28.55

1 Single oral dose 15.8 mg/kg

2 Multiple oral doses 15 mg/kg/day

3 Multiple oral doses 25 mg/kg/day

ranged from 2 to 3 hrs and were similar to those found in normals. The peak concentrations ranged from 2.45 to 3.75 mcg/ml and were significantly lower than those found in normals. The peak concentration of 2.45 mcg/ml at 3 hr for subject MJ is particularly low considering the elevated dose of 25 mg/kg. Terminal half-lives (3.05-4.93 hrs) and renal clearance values (371.8-410.3 ml/min) were similar to those reported in our studies with normals. However, the percent of the unchanged drug excreted in the urine tends to be lower than that found in our previous studies with normal volunteers where values ranged from 54 to 67%.

Semilogarithmic plots of the plasma concentration versus time and the excretion rate versus time over a 12 hr period are shown for subject CE in Fig. 5-2. A distinct distribution phase was observed since this was the very first dose of EMB to the subject. Analysis of the terminal log linear phase from both curves by least squares regression yielded a half-life of about 3 hrs. The same type of plots are shown for subject MJ in Fig. 5-3, where the distribution phase appears diminished following multiple dosing. The half-life estimated from both curves approximated 4 hrs.

Similar plots were made for the Class II, renal failure subject WR in Fig. 5-4. Note the difference in time scale for the excretion rate plot in Fig. 5-4. The distribution phase essentially disappeared in this subject upon repeated dosing. The terminal half-life estimated from the plasma curve was 7 hrs and from excretion rate plot 6.8 hrs. The renal clearance was calculated to be 60 ml/min, a much smaller clearance value compared to that found in normals. The daily recovery of the administered dose in the urine was 30%.

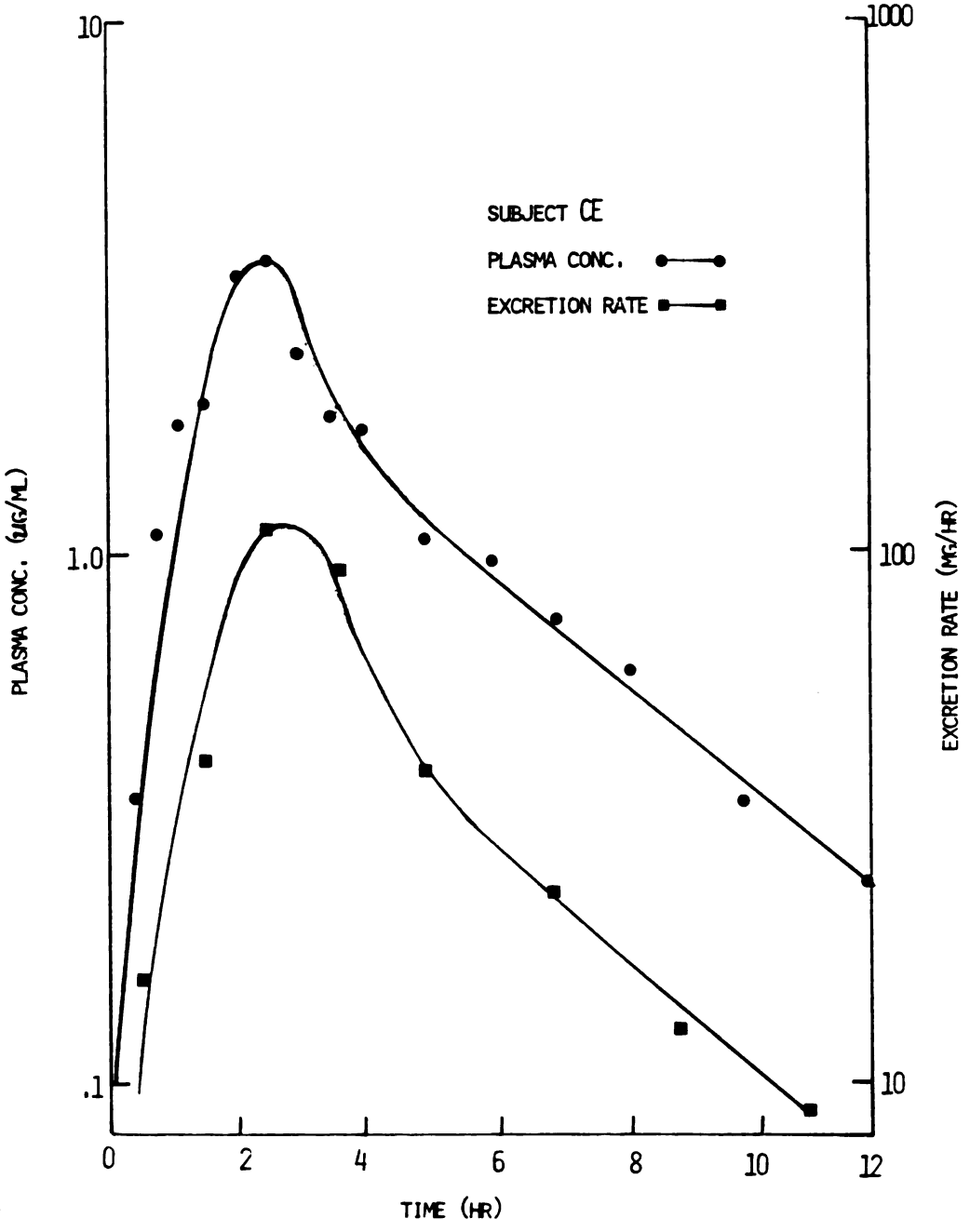

Fig. 5-2 Ethambutol plasma concentration vs. excretion rate plot, single oral dose 1000 mg.

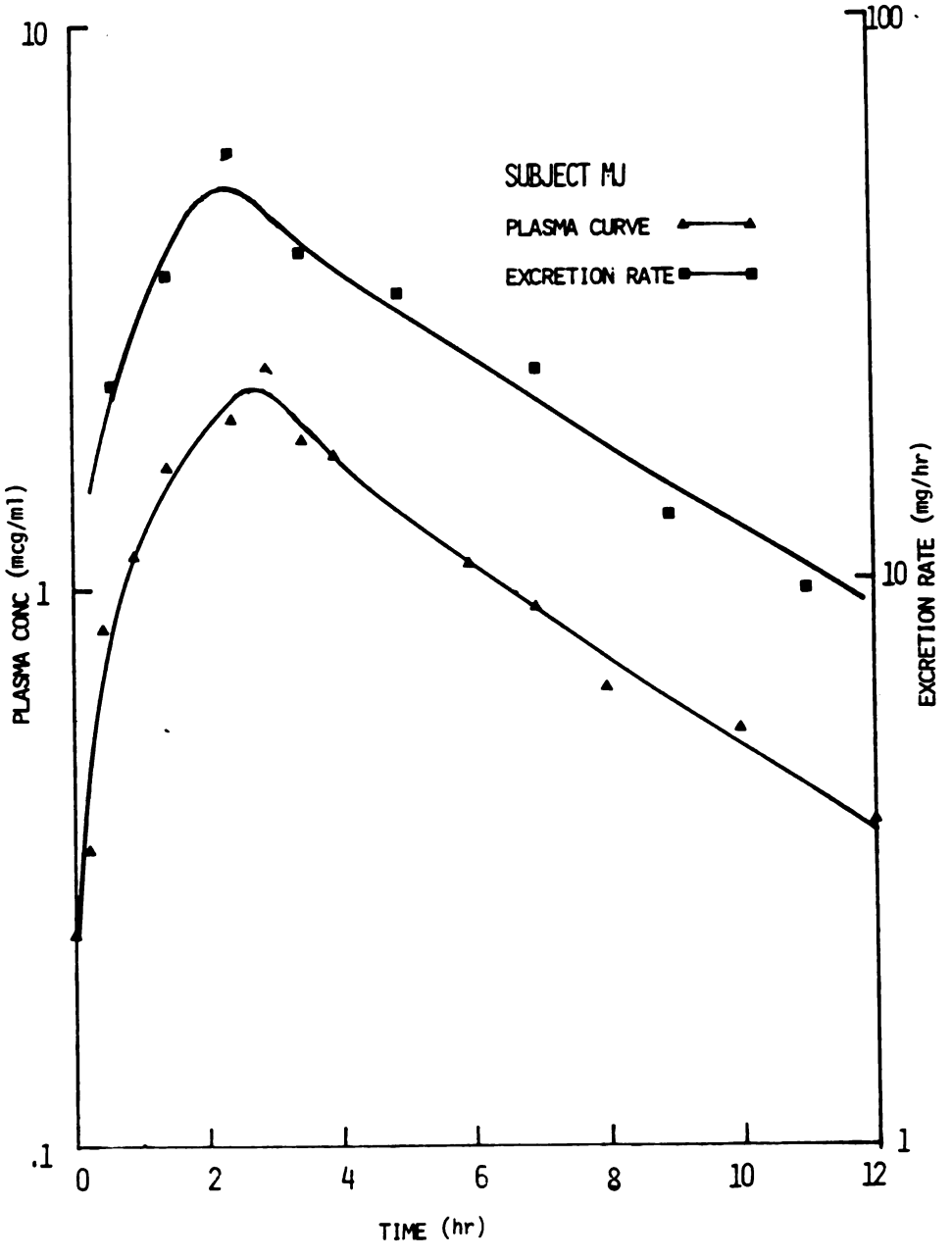

Fig. 5-3 Plasma concentration vs. time and excretion rate vs. time plots in one tuberculosis patient with normal renal function.

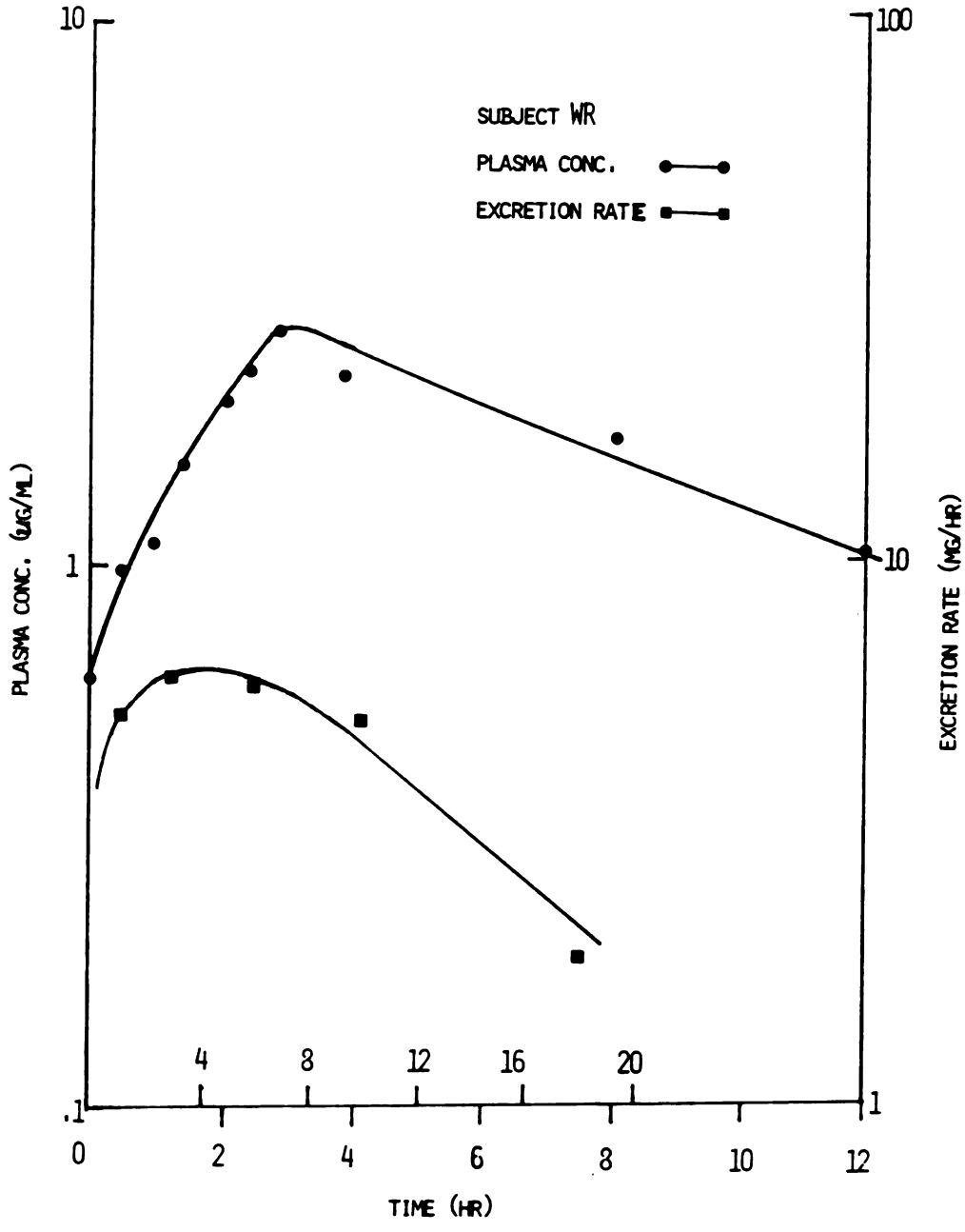

Fig. 5-4 Ethambutol plasma concentration vs. excretion rate plot, repeated oral dose 300 mg. Upper time scale for excretion rate and lower one for plasma concentration.

Concentrations of EMB in plasma entering and leaving the dialyzer before and during hemodialysis and drug concentrations in the momentary dialysate are recorded for the Class III, hemodialysis subject in Table 5-3.

Chronic dialysis patients have positive water and sodium balance ranging from 0 to several liters during the interdialytic interval which must be removed by ultrafiltration during dialysis. Gotch et al. (62) have extensively discussed the ultrafiltration rate as a function of membrane hydraulic pressure, active membrane area and the effective transmembrane pressure. In this study, ultrafiltration was determined by loss in body weight, measured before and after hemodialysis. A loss of 2.8 kg in body weight during the 4 hr dialysis period was calculated to give an ultrafiltration rate of 0.7 L/hr or approximately 12 ml/min. From the data in Table 5-3 dialysis clearance can be calculated using Eqs. 5-9 and 5-10. The cumulative dialysate volume, the drug concentration in the hourly dialysate and the rate of hourly removal are recorded in Table 5-4. From these data values of dialysis clearance can be calculated using Eq. 5-11. The dialysis clearance, calculated for separate measurements and using different formulas are compared in Table 5-5. The dialysis plasma clearance ranged from 48.4 to 61.5 ml/min and averaged 54.6 ml/min. Two dialysis blood clearance values were obtained with an average of 57.7 ml/min.

From the dialysis study, we found an off-dialysis half-life of 9.6 hrs and an on-dialysis half-life of 5.7 hrs (Fig. 5-5) indicating a 40% decrease in half-life during dialysis.

The plasma volume of distribution for EMB in this patient was calculated using both Eqs. 5-13 and 5-14. The average dialysis plasma

Table 5-3

Plasma and Dialysate Data

Time (hr)	Ethambutol plasma concentration (mcg/ml)		Momentary dialysate concentration (mcg/ml)	Dialysate flow (ml/min)
	into dialyzer	out of dialyzer		
0	2.72	--	--	--
.5	2.54	1.91	.278	503.3
1.5	2.06	--	--	--
2.5	1.93	1.47	.24	494.5
3.5	1.82	--	--	--
4.0	1.61	--	--	--

Table 5-4

Removal Rate of Ethambutol from a Hemodialysis Patient during a Four Hour Period

Time (hr)	Dialysate Volume (liter)	Concentration (mcg/ml)	Removal Rate (mg/hr)
0 - 1	30.2	.25	7.55
1 - 2	30.6	.25	7.51
2 - 3	29.6	.21	6.08
3 - 4	31.0	.17	5.28

Total Drug Removal = 26.42 mg

Table 5-5

Comparison of Dialysis Clearance

Time	Data	Formula	Dialysis Clearance (ml/min) Cl_{Hb}	Cl_{Hp}
0.5 hr	Q_{Bi}, C_{Pi}, C_{Po}	5-9	58.6	
2.5 hr	"	"	56.8	
0.5 hr	Q_{Do}, C_{Do}	5-10		55.1
2.5 hr	"	"		61.5
0-1	Drug removal rate, $C_{Pi,mid}$	5-11		49.5
1-2	"	"		60.7
2-3	"	"		52.5
3-4	"	"		48.4
		Mean Clearances:	57.7 ± 1.3	54.6 ± 5.5

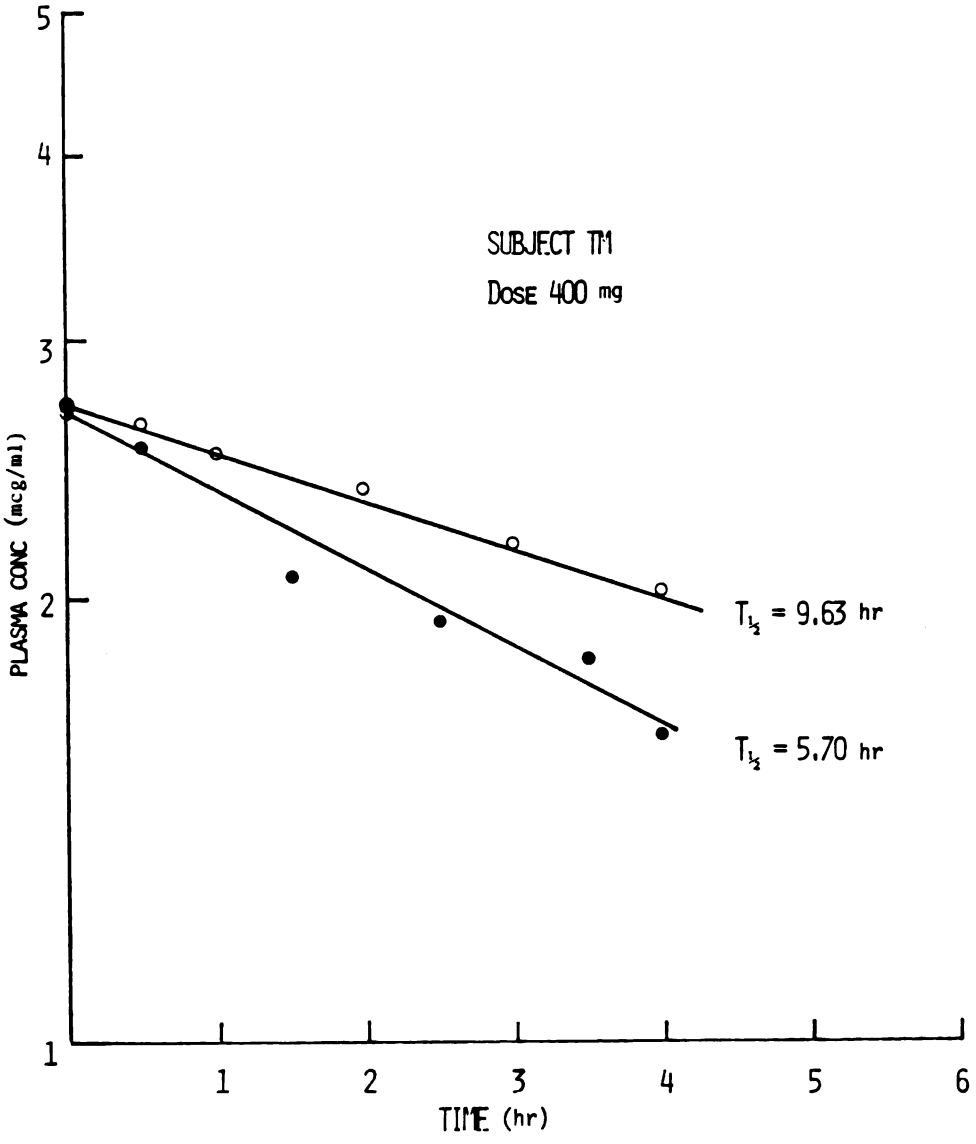

Fig. 5-5 Plasma concentration decay curve off (○——○) and on (●——●) dialysis.

clearance (54.6 ml/min) and plasma concentration were used for the calculation. A volume of 66.3 L was obtained using Eq. 5-13 and 58.5 L using Eq. 5-14. These two values are quite comparable and approximate 80% of the body weight in this patient.

DISCUSSION

In previous oral studies peak plasma EMB concentrations ranged from 3.35-6.0 mcg/ml. Our results in tuberculosis patients indicate lower peak concentrations at the same (or even higher) dose. This observation is consistent with the fact that the percent of the drug excreted unchanged is also considerably lower in these tuberculosis patients. One of these patients, MJ, has been defined by his doctor as "drug resistant." The resistance in this particular subject may actually result from poor drug bioavailability instead of from resistance developed by the microorganism. Alterations of pharamcokinetics in disease states have been extensively discussed (63). In our study with tuberculosis patients, the lower peak concentraions could also be attributed to an enlarged volume of distribution. In some disease states, volumes of distribution increase as a function of decreased plasma protein binding (64). However, this is improbable for EMB since plasma protein binding is not a significant value even in normals. The volume of distribution in tuberculosis patients can not be determined until I.V. dosing studies are carried out.

The decreased recovery of unchanged EMB in the urine for the TB patients with normal renal function may be due to enhanced drug metabolism in the disease state. The quantitation of the urinary metabolite(s)

would serve as a direct proof. If availability was similar to that seen in normals (see Table 4-10) metabolic elimination would have to approximate renal elimination, i.e., plasma clearance ≈ renal clearance. If volume of distribution remained constant, then plasma half-life would decrease as metabolism became more pronounced. The terminal half-lives in these 3 subjects (see Table 5-2) remained similar to those found in normals (see Table 4-4) indicating little possibility of enhanced drug metabolism if volume of distribution remains constant. However, an increase in volume of distribution could possibly counteract changes in the metabolic and renal rate constants yielding approximately the same half-life as seen in normals. Thus, although we speculate that the decreased plasma levels and percent of drug excreted unchanged in the TB patients is due to decreased bioavailability, confirmation of this must wait further studies following intravenous dosing.

The terminal half-lives in renal failure have been reported to range from 7.2 for anephric to 9.6 hrs for anuric patients (24, 25). These half-lives are 1.7 to 3 times longer than those of normals. Our results in one patient with renal insufficiency (creatinine clearance 14 ml/min) indicated a terminal half-life of 7 hrs which is of the same order of magnetude as that reported by others. The pharmacokinetic study in this patient resulted in a low urinary recovery of EMB (30.3% of dose) and decreased renal clearance (60 ml/min). If we assume that metabolic clearance in this patient was similar to that found in normals (100 ml/min) then the percent of the absorbed dose excreted unchanged should be equal to renal clearance divided by total clearance (60/160) or 38%. The 30.3% of the dose found in the urine for this patient indicates that the extent of bioavailability of EMB would be 81% if our assumptions

concerning metabolic clearance are correct.

It is interesting to note that EMB could be more available for absorption to the TB patient with renal failure than to the 3 TB patients with normal kidney function. Confirmation of this must wait further studies following intravenous dosing.

There has been no adequate study performed to determine the dosage requirements of EMB in patients with renal failure (Table 1-4). Bailey's recommendation (26) of a normal dose in renal failure is not justified. Due to the large percent of drug excreted unchanged in the urine, dosage in renal failure should be reduced. In the two patients that we have studied, reduced EMB doses were administered, 300 mg daily in subject WR (5.4 mg/kg) and 400 mg daily in subject TM (4.9 mg/kg).

If patient WR was given a normal dose of 25 mg/kg as suggested by Bailey, the resulting trough plasma concentration would be 2.7 mcg/ml and peak plasma concentration 16.3 mcg/ml. The maintenance of such high levels may cause ocular toxicity and hence is unacceptable.

Dume et al. (24) suggested that patients with severe renal failure be given EMB doses of 17.9 mg/kg. It is difficult to understand how they arrived at this number. One possible way is to use the equation of Tozer (65) where corrections can be made for changes in renal failure. Substituting values given by Dume et al. ($t_{\frac{1}{2},n}$ = 4.2 hr, $t_{\frac{1}{2},f}$ = 7.2 hr, fe = 0.46 and K.F. = 0) a dose of 14 mg/kg would be calculated for the renal failure patient if 25 mg/kg was the dose given to normals. This calculated dose is probably too high since fe, the fraction excreted unchanged in normals, 0.46, was surly underestimated by Dume et al. (24).

Strauss and Erhardt (32) recommended that dose in patients with creatinine clearances below 70 ml/min be adjusted to less than 15 mg/kg.

Since the study was based on a relatively poor assay, it is difficult to comment on the relevancy of this recommendation.

The only suggestion made using a pharmacokinetic basis was that of Christopher et al. (25). Although ^{14}C-EMB was used in their kinetic studies, their recommendation is consistent with our pharmacokinetic measures. In our studies in 2 TB patients with renal failure, 4.9 and 5.4 mg/kg doses were administered daily. A similar daily dose of 5 mg/kg was suggested by Christopher et al. for EMB in renal failure.

There is very little information on the pharmacokinetics of EMB during dialysis. Bailey (36) states that EMB is not dialyzable but no specific data is given in support of this statement. According to Dume et al. (14), peritoneal dialysis was not an efficient procedure for removing EMB while hemodialysis was so effective that a new dose was suggested at the end of a 8 hr dialysis period. From our kinetic study in one patient with EMB during dialysis, a 40% replacement of the dose pre- or post-dialysis was suggested while a slightly smaller adjustment (35.7%) was recommended by Christopher et al. (25).

Questions are often raised in pharmacokinetic analyses as to whether blood or plasma drug concentrations should be measured (66). This is especially critical when clearance measurements are being interpreted in terms of body processes. Most difficulties can be overcome when mass balance of the drug is considered. The differences in plasma and blood clearance measurements were discussed earlier in this chapter. Plasma or blood clearance may be determined directly when dialysate is measured in addition to incoming concentrations of arterial plasma or blood (Eqs. 5-10 and 5-11). However, when only arterial and venous blood or plasma are measured, only blood clearance can be calculated (Eq. 5-9) directly

from these measurements as demonstrated in the first part of this chapter. Even then, these calculations (Eqs. 5-9, 5-10, or 5-11) assume that drugs equilibrate extremely rapidly between the erythrocytes and plasma. Thus, as drug passes from plasma water into the dialyzer, the remaining drug in the blood reequilibrates between plasma and red blood cells. In other words, transfer across the erythrocyte membrane does not rate limit drug distribution. Therefore, when relating clearance to body flows, blood flow and not plasma flow to the dialyzer must be used and the resulting clearance is blood clearance (Cl_{db}). However, blood clearances may be converted to plasma clearances and vice versa if both the hematocrit and the RBC to plasma ratio are known (see Appendix 5-4).

In our dialysis study, the dialysis blood clearance averaged 57.7 ml/min and the dialysis plasma clearance determined at the same times (0.5 and 2.5 hrs) averaged 58.3 ml/min indicating that drug was cleared not only from plasma but also from red blood cells. Knowing the hematocrit of the patient (H = 0.29), the C_{RBC}/C_P ratio can be retrospectively calculated according to Equation d in Appendix 5-4. This ratio was found to be 1.04, a value slightly lower than our previous findings in normal subjects (Table 4-12).

A volume of distribution derived from a dialysis experiment should be identified as either a blood volume of distribution or a plasma volume of distribution. The latter is generally used in pharmacokinetics. In this case, where the RBC to plasma ratio is approximately one, the plasma and blood volumes of distribution will be identical. Calculation by Eq. 5-13 using the average dialysis plasma clearance of 54.6 ml/min resulted in a blood volume of distribution of 66.3 liter. A similar calculation using the average dialysis blood clearance of 57.7 ml/min

resulted in a blood volume of distribution of 70.1 liter.

The effectiveness of drug removal by hemodialysis has been discussed by Tozer et al. (67). It was suggested that the usefulness of hemodialysis should be evaluated in terms of whether significant amounts of the drug would be removed within a reasonable period of dialysis. Although only 26 mg of drug from a 400 mg dose of EMB was eliminated during a 4 hr dialysis period in subject TM, the removal should be considered in terms of drug in the body at the beginning and end of dialysis.

Appling Fick's Law of Diffusion, Wolf et al. (68) has proposed that the effectiveness of solute transport is proportional to the concentration gradient across the dialyzer membrane. The starting concentration gradient upon dialysis is a function of the dose administered and the administration time. Since hemodialysis in the study reported here was not initiated until 10 hrs after oral dosing, the concentration gradient is low to begin with and therefore only a small fraction of the dose was removed. However, if another criteria is considered, i.e., clearance by dialysis relative to total body clearance of the drug, the dialysis clearance contributes 40.8% to the total body clearance (Appendix 5-5). This significant contribution was also reflected by the on and off dialysis half-lives (Fig. 5-5).

Another pharmacokinetic parameter which governs drug dialyzability is the volume of distribution. A cut-off volume of distribution (250 L) has been arbitrarily proposed (69). If the volume of distribution is larger than 250 L, little drug will be available in the blood for elimination by dialysis, and hence the effect of hemodialysis will be minimal. In the patient studied, the volume of distribution approximates

66 L and thus favors dialyzability. The small molecular weight of EMB (M. W = 204) also favors effective dialysis.

Drug elimination form the body and appearance in the dialysate is frequently reported in the clinical dialysis literature in terms of "dialysance"

$$D = Q_B \frac{C_{Bi} - C_{Bo}}{C_{Bi} - C_{Do}}$$

where C_{Di} denotes the drug concentration in the recirculating dialysate flow. The use and misuse of this parameter has been discussed by Gotch et al. (61). The dialysis system in this study ultilized a single pass Dow-Cordis hollow fiber kidney. Since no recirculation of the dialysate flow takes place ($C_{Di} = 0$), dialysis clearance is equal to dialysance.

Since our kinetic study in one patient with EMB during both an on and off dialysis period indicated a 40% reduction in terminal half-life (Fig. 5-5), we would suggest a 40% increase of predose or replace 40% of the dose postdialysis. However, this suggestion is only valid when dialysis starts 2-4 hrs after oral administration or immediately following intravenous infusion.

APPENDIX 5-1

The following set of conditions are assumed:

$Q_B = 300$, $C_{Bi} = 8$, $C_{Pi} = 10$, $H = 40\%$

$Q_D = 1000$, $Q_F = 0$, and $Cl_{db} = 100$

since

$$Cl_{db} = Q_B \frac{C_{Bi} - C_{Bo}}{C_{Bi}} \qquad \text{(Eq. 5-5)}$$

and $$Cl_{db} = \frac{Q_D \cdot C_{Do}}{C_{Bi}} \qquad \text{(Eq. 5-7)}$$

C_{Bo} can be retrospectively calculated using Eq. 5-5

$$C_{Bo} = 5.33$$

and C_{Do} can also be retrospectively calculated from Eq. 5-7

$$C_{Do} = 0.8$$

Knowing $\frac{C_B}{C_P} = 0.8$ and $C_{Bo} = 5.33$, C_{Po} can be easily calculated

$$C_{Po} = 6.66$$

Thus, when Cl_{dp} is calculated using Eq. 5-12

$$Cl_{dp} = (1 - 0.4) \times 300 \times \frac{10 - 6.66}{10}$$

$$= 60$$

while calculated by Eq. 5-10

$$Cl_{dp} = \frac{1000 \times 0.8}{10}$$

$$= 80$$

Equation 5-12 underestimates the actual Cl_{dp}.

APPENDIX 5-2

Derivation for Equation 5-13

Designate Cl_T, Cl_M and Cl_d as total clearance, "metabolic clearance" and dialysis clearance, respectively. Metabolic clearance is defined here as the sum of all clearance processes for drug when the patient is not on dialysis. This can include any residual urinary clearance which the patient may have. The following equation can be written

$$Cl_T = Cl_M + Cl_d \qquad \text{or} \qquad Cl_d = Cl_T - Cl_M$$

By definition

$$Cl_T = 0.693\ V_d/t_{\frac{1}{2},on}$$

$$Cl_M = 0.693\ V_d/t_{\frac{1}{2},off}$$

Upon substitution

$$Cl_d = 0.693\ V_d/t_{\frac{1}{2},on}$$

$$Cl_d = 0.693\ V_d\ \left(\frac{1}{t_{\frac{1}{2},on}} - \frac{1}{t_{\frac{1}{2},off}}\right)$$

and

$$V_d = \frac{Cl_d}{0.693\ \left(\frac{1}{t_{\frac{1}{2},on}} - \frac{1}{t_{\frac{1}{2},off}}\right)} \qquad \text{(Eq. 5-13)}$$

where Cl_d can be obtained from Eq. 5-10 or Eq. 5-11.

APPENDIX 5-3

Derivation for Equation 5-14

Designate Cl_T and Cl_M as total clearance and "metabolic clearance", respectively. By defination

$$Cl_T = 0.693\ V_d/t_{\frac{1}{2},on}$$

$$Cl_M = 0.693\ V_d/t_{\frac{1}{2},off}$$

The fraction of the drug removed by dialysis

$$f_D = \frac{Cl_T - Cl_M}{Cl_T}$$

$$f_D = 1 - \frac{Cl_M}{Cl_T}$$

$$f_D = 1 - \frac{t_{\frac{1}{2},on}}{t_{\frac{1}{2},off}}$$

Apparent concentration decrease during dialysis, ΔC may be defined as

$$\Delta C = C_P^o - C_P^t$$

Of this decrease, f_D is the fraction due to removal by the dialyzer. Therefore, the dialyzer contributes $f_D \cdot \Delta C$ to the apparent concentration decrease. By definition, volume of distribution is a volume term used to balance the mass and the concentration.

Hence,

$$\text{Total Drug Removal (by dialyzer)} = (f_D \cdot \Delta C) \cdot V_d$$

and $V_d = \text{Total Drug Removal}/f_D \cdot \Delta C$

or

$$V_d = \frac{\text{Total Drug Removal}}{(C_P^o - C_P^t)\left(1 - \frac{t_{\frac{1}{2},on}}{t_{\frac{1}{2},off}}\right)} \qquad \text{(Eq. 5-14)}$$

APPENDIX 5-4

Let Cl_{db} and Cl_{dp} represent dialysis blood and plasma clearance, respectively, and C_B and C_P drug blood and plasma concentration, respectively. The following equation will satisfy the law of mass balance

$$Cl_{db} \times C_B = Cl_{dp} \times C_P \qquad (a)$$

That is, drug removal rates will be equal no matter whether clearance is expressed in terms of blood or plasma.

The relation between blood and plasma concentration may be expressed as:

$$C_B = C_P(1 - H) + C_{RBC}H \qquad (b)$$

where C_{RBC} is the drug concentration in red blood cells and H is the hematocrit. Equation b is just a rearrangement of the mass balance statement that the total amount of drug in the blood will equal the amount in the plasma plus the amount in the red blood cells. Multiplying each side of Eq. b by blood volume gives this mass balance.

$$\frac{C_B}{C_P} = (1 - H) + \frac{C_{RBC}}{C_P} H \qquad (c)$$

Define $\frac{C_{RBC}}{C_P} = K$ and from Equation a

$$Cl_{dp} = Cl_{db} \times \frac{C_B}{C_P}$$

$$Cl_{dp} = Cl_{db} \times (1 - H) + KH \qquad (d)$$

APPENDIX 5-5

The following notations are defined as before.

From the dialysis data: V_d = 66.3 L; $t_{\frac{1}{2},on}$ = 5.7 hr

$$Cl_T = 0.693\ V_d/t_{\frac{1}{2},on}$$

$$= 0.693 \times 66.3/5.7$$

$$= 8.06\ L/hr$$

$$Cl_d = 54.6\ ml/min$$

$$= 3.27\ L/hr$$

$$\frac{Cl_d}{Cl_T} = \frac{3.27}{8.06}$$

$$= 40.6\%$$

Hence, the dialysis clearance contributes 40.6% to the total body clearance.

CHAPTER 6

SUMMARY AND CONCLUSIONS

Previously available gas chromatographic methods for ethambutol may only be used to measure quantities of drugs much greater than those found in biologic fluids such as plasma and dialysate. Pharmacokinetic studies of ethambutol require a sensitive and specific method of measuring the unchanged compound. For these reasons we have developed a gas chromatographic method for ethambutol involving derivatization with trifluoroacetic anhydride and subsequent quantitation using an electron capture detector. The electron capture method of detection is required for samples such as blood, plasma and in particular, dialysate in which low levels of ethambutol are anticipated. A second GLC method involving derivatization with bis(trimethylsilyl)trifluoroacetamide and subsequent quantitation using a flame ionization detector was described. The flame ionization method is suitable for urine samples or for dosage form analysis in which high levels of ethambutol are anticipated. A dual column and dual detector gas chromatograph was used for simultaneous micro (plasma, blood and dialysate) and macro (urine, dosage form) determinations of ethambutol.

Following the development of the analytical method, three rhesus monkeys were studied. The monkey plasma and urine data were fitted with the NONLIN program to a two compartment open body model. The α phase in these monkeys had a mean half-life of 9.8 min while the half-life of the β phase ranged from 2.4 to 3.4 hr. The mean values for the volume of the central pool and the steady-state volume of distribution for this drug were 0.36 and 1.58 L/kg, respectively. The fraction of

the dose administered that was eliminated by the urinary route varied from 0.65 to 0.80. Renal clearances were 6.7 to 9.4 ml/min·kg indicating an active secretion mechanism. Deconvolution of oral plasma curves indicated that the absorption in the monkey may be described by two consecutive first-order processes each having approximately the same half-life of 30 min, followed by a very slow release.

Human studies were initiated at the completion of the animal study. Six healthy adult volunteers completed the study. Each subject received intravenous and oral ethambutol doses of approximately 15 mg/kg. Following termination of intravenous infusion, ethambutol plasma concentrations and excretion rates showed multiexponential decay. Preliminary graphical analysis indicated four disposition phases following drug infusion and therefore a four compartment body model was used for the I.V. plasma data analysis. The α, β, γ and δ phases in these subjects had a mean half-life of 8.6, 40.1, 135 and 924 min, respectively. The corresponding areas under the four phases are 21.5, 17.8, 31.3 and 29.3%, respectively. The mean values for the volume of the central pool and the steady-state volume of distribution for ethambutol were 0.42 and 4.02 L/kg, respectively. The large extravascular volume reflects the significance of the longer γ and δ phases in estimating total amounts of drug in the body. The fractional area calculated under the plasma concentration time curve after 12 hrs ranged from 15 to 28% with an average of 21.3 $\pm$ 4.8%. This relatively large area under the curve from the 12-hr half-life indicates that there should be approximately a 20% increase in steady-state levels upon multiple dosing as opposed to levels predicted using the shorter half-lives. The total body clearance and renal clearance were 8.5 and 6.7 ml/min·kg, respectively,

indicating active renal secretion in man as well as in monkey. These clearance values are consistent with those calculated using a model independent method suggesting that a four compartment model is a reasonable description of the disposition kinetics of ethambutol.

In previous reports, peak plasma ethambutol concentrations varied significantly between different studies at the same dose. Our results indicated only minor variation among individuals. In contrast to the reported large variation of the percent of the oral dose excreted unchanged in the urine, we found approximately 54 to 67% of the dose was excreted unchanged after a single oral dose. Because of the high renal clearance of ethambutol and the large amount excreted unchanged in the urine, changes in renal function would predictably alter the kinetics of the drug. Since it appears that ethambutol is actively secreted, there is also a potential for importantly affecting the renal clearance of ethambutol by co-administration of drugs that might compete for secretory pathways.

In the present study, absolute bioavailabilities were estimated from area under the plasma curve and 72 hr urinary excretion data. We conclude that approximately 75% of the oral dose is available to the systemic circulation. No significant difference was found in bioavailability of ethambutol between solution and tablet. The oral human data exhibited an unusual absorption pattern which could not be analyzed assuming two first-order processes. Absorption rate plots seem to suggest that ethambutol absorption may be described by three first-order processes in these subjects. Confirmation of this must wait further treatment of the data. The unusual absorption of ethambutol in man and monkey might be explained by binding in the absorption process. This

hypothesis is based on the speculation that ethambutol, an EDTA analog, might be expected to bind or chelate with a number of molecules in the G.I. fluids/or tissues.

Three patients with tuberculosis and normal kidney function were then studied. The peak times, terminal half-lives and renal clearance values were similar to those reported in our studies with normals. However, the percent of the unchanged drug excreted in the urine tends to be lower than that found in our previous studies with normal volunteers. This observation is consistent with the fact that the peak plasma concentrations are also considerably lower in these tuberculosis patients. We speculate that the decreased plasma levels and percent of drug excreted unchanged in tuberculosis patients is due to decreased bioavailability. Other possibilities such as enlarged volume of distribution and enhanced drug metabolism in the disease state can not be completely ruled out until further studies following intravenous dosing are undertaken.

The pharmacokinetic study in one patient with tuberculosis and renal insufficiency (creatinine clearance = 14 ml/min) resulted in a low urinary recovery of ethambutol (30.3% of dose) and decreased renal clearance (60 ml/min). Assuming a metabolic clearance of 100 ml/min which was found in normals then the percent of the absorbed dose excreted unchanged was calculated to be 38%. The 30.3% of the dose found in the urine for this patient indicates that the extent of bioavailability of ethambutol would be 81%. It is interesting to note that ethambutol could be more available for absorption to the tuberculosis patient with renal failure than to the three patients with normal kidney function.

Finally, one anuric patient with tuberculosis undergoing hemodialysis was studied. A loss of 2.8 kg in body weight during the 4 hour

dialysis period was calculated to give an ultrafiltration rate of 12 ml/min. The dialysis plasma clearance ranged from 48.4 to 61.5 ml/min and averaged 54.6 ml/min. Two dialysis blood clearance values were obtained with an average of 57.7 ml/min. The plasma volume of distribution for ethambutol in this patient averaged 62.4 L and approximated 80% of his body weight.

The effectiveness of drug removal by hemodialysis is judged by the relative contribution of dialysis clearance to the total body clearance of the drug. In the patient studied, the dialysis clearance contributes 40.6% to the total body clearance. This significant contribution was also reflected by the on and off dialysis half-lives ($t_{\frac{1}{2},on}$ = 5.7 and $t_{\frac{1}{2},off}$ = 9.6 hr).

Since our kinetic study in this patient during both an on and off dialysis period indicated a 40% reduction in terminal half-life, we would suggest a 40% increase or replace 40% of the dose postdialysis. However, this suggestion is only valid when dialysis starts 2-4 hrs after oral administration or immediately following intravenous infusion.

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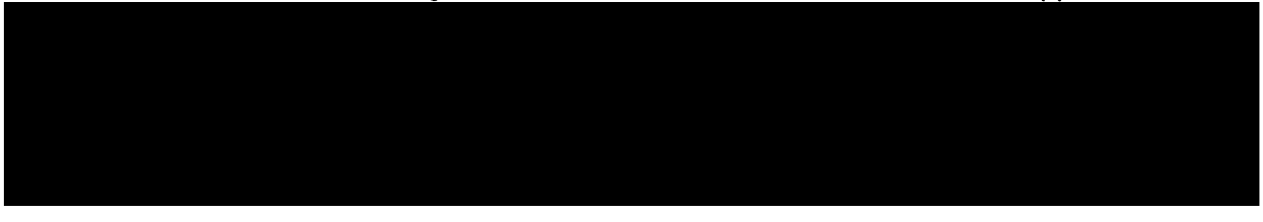

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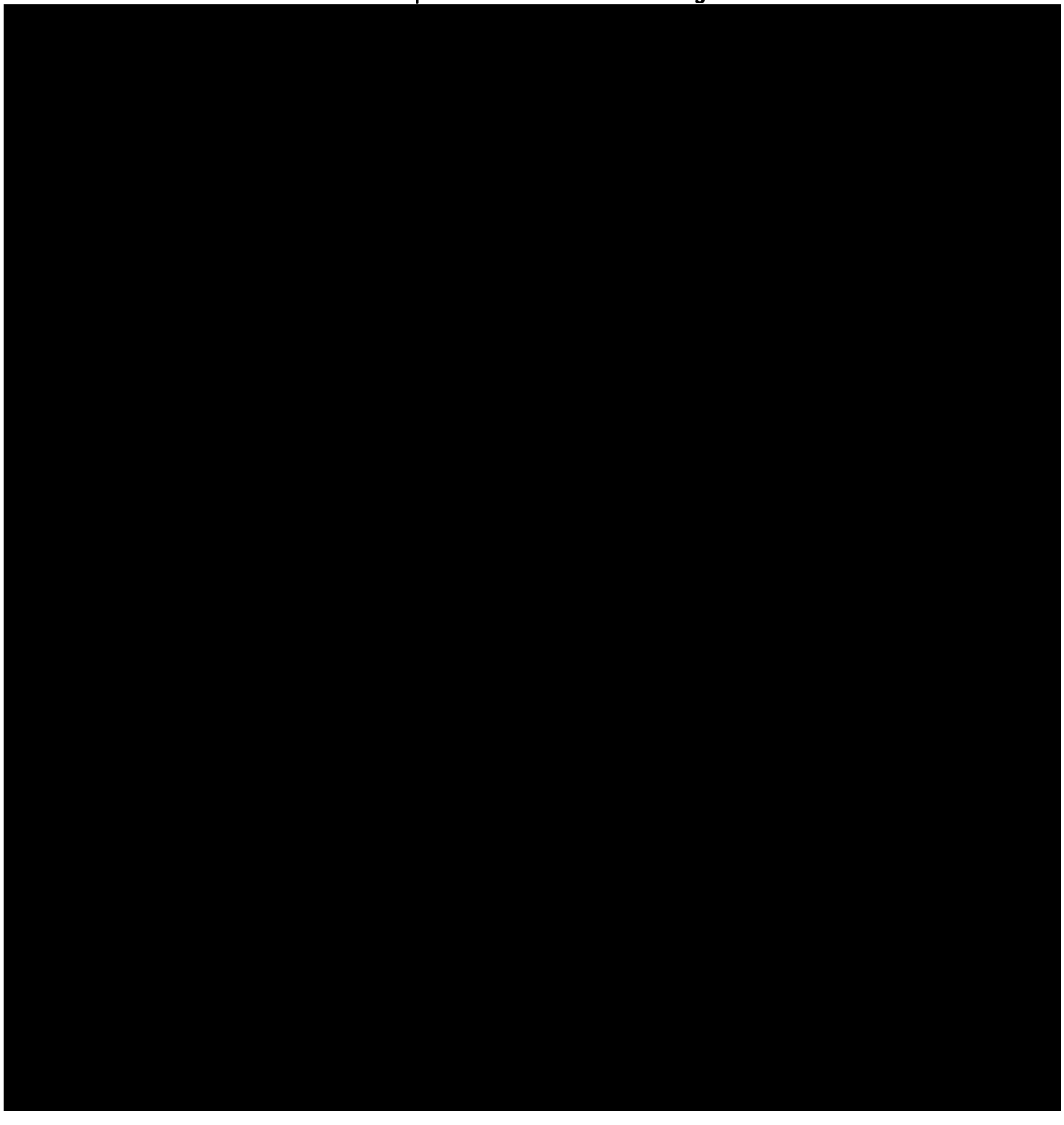

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