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Urinary Excretion of Probenecid and Its Metabolites in Humans as a Function of Dose

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Abstract □ A GLC assay was used to study the excretion of probenecid and its metabolites in the urine of human subjects following oral doses of 0.5, 1, and 2 g. From 75 to 88% of the dose was found in the urine. The major metabolite, probenecid acyl glucuronide, accounted for 34–47% of the dose. Approximately equal amounts (10–15%) of the mono-*N*-propyl, secondary alcohol, and carboxylic acid metabolites were excreted in the unconjugated form with only traces in the conjugated form. The primary alcohol metabolite was not found in measurable amounts. The terminal half-lives for excretion of all metabolites were in the range of 4–6 hr, were independent of dose, and were limited by their rates of formation. A prolonged time course of excretion of the metabolites, particularly at higher doses, suggests that probenecid, being poorly soluble in water, precipitates from solution in the GI tract, forming a depot of drug from which absorption is dissolution rate limited. The urinary excretion of unchanged probenecid, which accounts for 4–13% of the dose, is dependent on both the pH and flow rate of urine.

Keyphrases □ Probenecid and metabolites—urinary excretion as a function of dose □ Excretion, urinary—probenecid and metabolites, effect of dose □ Uricosuric agents—probenecid and metabolites, effect of dose □ Metabolism—probenecid, urinary excretion as a function of dose

Probenecid, 4-[(dipropylamino)sulfonyl]benzoic acid, was introduced as an uricosuric agent in 1951. However, only recently has its metabolic fate in humans and animals been fully elucidated (1–4). Studies of the disposition of the drug generally have used ^{14}C-labeled probenecid, and studies in humans have been limited by the need for a convenient nonradioactive assay. A GLC assay of the methyl esters of probenecid and its metabolites was employed for studies in rats (1, 4). The GLC assay of the propyl esters was utilized in the present study of the urinary excretion of probenecid and its metabolites in humans.

The plasma half-life of probenecid has been reported to increase with increasing dose and has been attributed to a decreased rate of metabolism at higher doses (5, 6). Unfortunately, in the human study (5), the determinations of plasma half-lives were not conducted over a sufficiently long period to be certain that distributive equilibrium had been achieved. In the dog study (6), the period over which plasma levels were measured was not indicated. Therefore, it is not clear whether this reported dose-dependent plasma decline of probenecid is real or simply the artifactual result of an inadequate sampling protocol. Therefore, the effect of increasing doses of probenecid on the urinary excretion of the drug and its metabolites in humans was investigated.

EXPERIMENTAL

Method—Unconjugated probenecid and its unconjugated metabolites were extracted with methylene chloride from urine acidified with 5 *N* HCl, converted to the propyl esters with diazopropane, and quantitated by GLC. A stainless steel column (2.8 mm × 2 m) packed with 10% OV-1 on 80–100-mesh Chromosorb W-HP was used. The gas chromatograph[1] was fitted with a flame-ionization detector. Operating parameters were: column temperature, 250°; injection port temperature, 280°; nitrogen carrier gas flow, 23 ml/min; and sensitivity, 2.5×10^{-11} amp full scale. *N,N*-Dibenzyl-(2,5-dimethylbenzene)sulfonamide was used as the internal standard. Optimum column performance was maintained by occasional injection of a silylating mixture[2] followed by overnight conditioning of the column.

Total drug and metabolites were determined by heating the acidified urine for 2 hr at 100° before extraction, derivatization, and chromatography[3]. Metabolites were synthesized as reported earlier (1, 4).

Protocol—Two healthy male subjects (Subject 1, 27 years, 58 kg; Subject 2, 42 years, 61.4 kg) did not ingest other drugs or alcoholic beverages for 2 days prior and 3 days after ingestion of probenecid. After fasting overnight, the initial morning urine sample was taken for use as an analytical control. Since the absorption of many drugs having poor water solubility is known to be dissolution rate limited, the dose of probenecid (0.5, 1, and 2 g) was administered orally in solution. The drug[4] was dissolved in 200 ml of 2–3% sodium bicarbonate, the minimum quantity required for complete solution. In one case (2-g dose, Subject 2), the dose was dissolved in 200 ml of dilute

[1] Perkin-Elmer Mark II.
[2] Silyl-8, Pierce Chemical Co., Rockford, Ill.
[3] The analytical procedures were reported in detail (7).
[4] Pure drug powder, Merck Sharp and Dohme, West Point, Pa.

Table I—Excretion of Probenecid and Its Metabolites in the Urine of Human Subjects[a]

Subject	Dose, mg	Total Excreted, % of Dose	Relative Amounts of Probenecid and Metabolites Excreted, % of Total Urinary Excretion				
			Probenecid	Probenecid Acyl Glucuronide	Mono-*N*-propyl Metabolite[b]	Carboxylic Acid Metabolite[b]	Secondary Alcohol Metabolite[b]
1	500	80.7	16.5	41.8	10.5	15.0	16.3
	1000	76.7	10.7	47.2	11.0	15.4	15.9
	2000	87.8	10.3	38.6	15.8	17.1	18.7
2	500	86.4	4.8	55.6	13.8	11.0	15.8
	500	87.3	8.9	54.3	10.1	11.6	15.1
	1000	87.2	4.5	50.8	14.4	13.2	17.0
	2000	84.2	5.4	51.1	15.3	14.0	14.2

[a] Amounts represent the total of each metabolite excreted over periods of approximately 48, 72, and 85 hr following the 0.5-, 1.0-, and 2.0-g doses, respectively. [b] Free form only; conjugated form amounted to less than 3% of dose.

sodium hydroxide, which gave a solution of pH 7. This change allowed an examination of the effect of the medium on the disposition of the drug.

No solid food or beverages were ingested for 2 hr after medication. To stimulate urine flow, 100 ml of water was taken each hour for 7 hr after medication. Urine was collected every 2 hr for the first 8 hr and then *ad libitum* until no drug or metabolites were detectable in urine (48–85 hr). The urine volume was measured and the pH was determined[5] immediately, and the samples were then stored in a freezer until analyzed. Periodic analysis of selected urine samples showed that the composition of urine remained unchanged during storage. At least a 2-week interval was allowed before administering a second dose to either subject.

RESULTS AND DISCUSSION

Excretion of Probenecid and Its Metabolites in Urine—It is apparent from Table I that 80–88% of orally administered probenecid was recovered from urine, mainly in the form of the conjugated drug and its oxidized metabolites. Perel *et al.* (2) also recovered a mean of about 75% of a 2.0-g oral dose of ^{14}C-labeled probenecid in the 0–48-hr urine of two subjects. The lack of complete recovery may suggest incomplete absorption and/or enterohepatic cycling of the drug. However, the consistent recovery over the range of doses from 0.5 to 2.0 g suggests that absorption is at least constant, if not complete, and, therefore, supports the idea that some drug is excreted in bile and eliminated in the feces because of incomplete reabsorption. Verification of biliary excretion would require intravenous administration of the drug, for which it is difficult to obtain the necessary permission using human subjects[6].

The results in Table I also show that the relative amounts excreted in the form of the various metabolites remains essentially unchanged at the three doses. These results, therefore, do not support the earlier speculation (5) that the rate of metabolism is slower at the higher (2.0

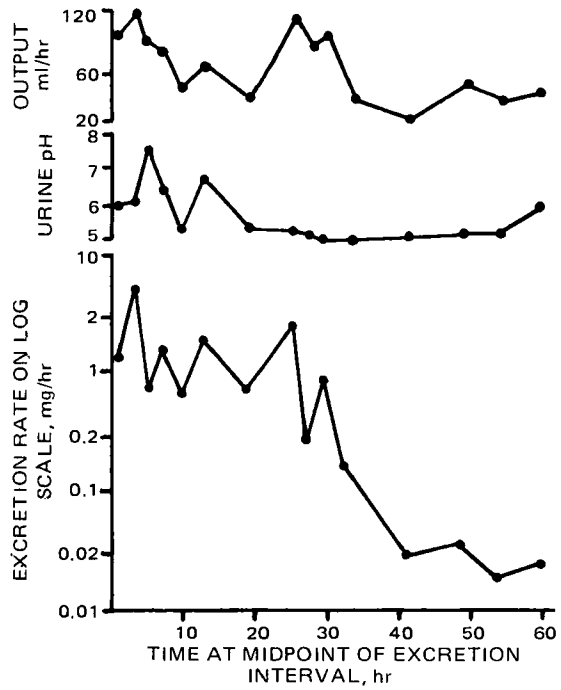

Figure 1—*Semilogarithmic plot of urinary excretion rate of probenecid and plots of urinary pH and urinary output against the time of midpoints of the excretion intervals for Subject 2 following a 1-g dose of probenecid.*

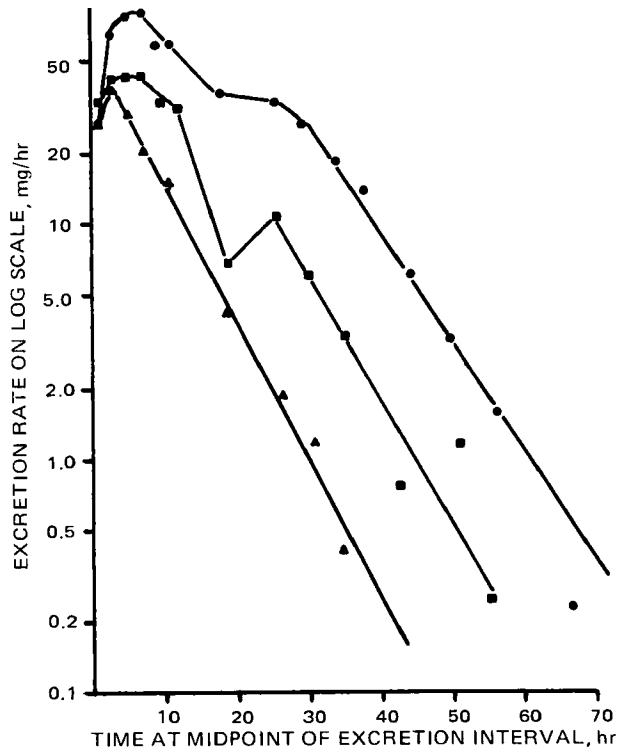

Figure 2—*Semilogarithmic plot of urinary excretion rate of probenecid metabolites against the time of midpoints of the excretion intervals for Subject 1 following 0.5- (▲), 1.0- (■), and 2.0- (●) g doses of probenecid. Excretion rate is expressed in terms of the amount (milligrams) of probenecid molecularly equivalent to the metabolites excreted.*

[5] Beckman Expandomatic pH meter, Beckman Instruments Inc., Fullerton, Calif.

[6] M. Gibaldi, State University of New York, Buffalo, N.Y., personal communication.

Table II—Terminal Half-Lives (in Hours) of Probenecid as Determined from Urinary Excretion Data for Different Metabolites at Various Doses[a]

Subject	Dose, mg	Mono-*N*-propyl Metabolite	Carboxylic Acid Metabolite	Secondary Alcohol Metabolite	Probenecid Acyl Glucuronide	Sum of Metabolites	
						Sigma-Minus	Excretion Rate
1	500	4.95	4.61	5.23	4.77	4.37	5.16
	1000	6.43	7.11	6.87	5.78	5.39	5.79
	2000	5.88	5.91	5.68	5.37	5.80	5.89
2	500	4.73	4.33	5.52	4.72	4.20	4.90
	500	4.27	5.32	4.61	4.94	4.91	5.43
	1000	4.71	5.12	5.52	4.55	5.95	5.44
	2000	4.04	4.37	4.58	4.08	5.51	5.36

[a] Obtained from slopes of the regression lines calculated for the apparently linear terminal portion of the plots. The *r* (coefficient of regression) values in all cases ranged from 0.92 to 0.99.

g) dose as compared to that at the lower dose (0.5 g). One would expect a decrease in the rate of metabolism at higher doses to result from the saturation of one or more metabolic pathways, thereby converting the apparent first-order kinetics observed for the particular pathway at lower doses to a zero-order process. This result would be reflected in an altered pattern of urinary metabolites at saturation doses as compared with lower doses, which was not observed.

These results, therefore, suggest that no individual metabolic pathway is saturated, since it would be unreasonable to expect all pathways to exhibit an equal degree of saturation as a function of dose. A single rate-limiting step common to all metabolic pathways, which is saturable at high doses, might be postulated. For example, transport of the drug to the site(s) of metabolism might be capacity limited, but such a thesis is difficult to reconcile with present-day knowledge of drug metabolism.

The relative amounts of metabolites recovered in urine in this study are in excellent agreement with those reported by Perel *et al.* (2). They found that about 2% of the dose was excreted as the primary alcohol metabolite, which was not found in measurable quantities in the present study. This finding is consistent with *in vitro* studies where the primary alcohol metabolite was observed to be readily oxidized to the carboxylic acid (8).

Effect of Urine pH and Urine Flow Rate on Excretion of Unchanged Probenecid—While the fractions of the administered dose excreted in the forms of conjugated probenecid and its oxidation products were about the same for both subjects (Table I), there was a two- to three-fold difference in the fraction excreted as unchanged probenecid by the two subjects. It is well known that probenecid is almost totally reabsorbed from renal tubules, thus reducing its excretion in urine in unchanged form to trace amounts (2–5), particularly where the urine is acidic. In dogs and humans, where metabolic alkalosis was induced by intravenous infusion of sodium bicarbonate, the renal clearance of probenecid increased greatly (9, 10).

This pH-dependent elimination of probenecid explains the differences in the urinary excretion of the drug observed in the present study. The pH of blank urine samples collected from Subject 1 was close to neutral (pH 6–7); for Subject 2, the pH was generally about 1 unit lower. A corresponding difference in pH was noticeable following administration of probenecid, particularly in the first few hours. As a result of the administration of a considerable amount of sodium bicarbonate solution, the urine pH typically rose to about 8–8.5 in Subject 1; it rose to only 7–7.5 in Subject 2. Therefore, it is

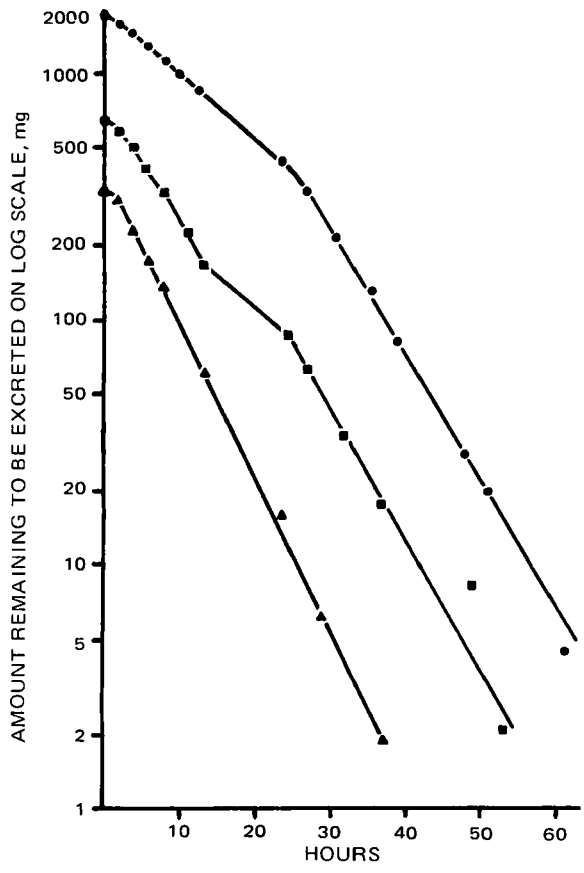

Figure 3—*Semilogarithmic plot of amount of probenecid metabolites remaining to be excreted (sigma-minus) against time for Subject 1 following 0.5- (▲), 1.0- (■), and 2.0- (●) g doses of probenecid. Amounts (milligrams) are expressed as probenecid molecularly equivalent to the metabolites.*

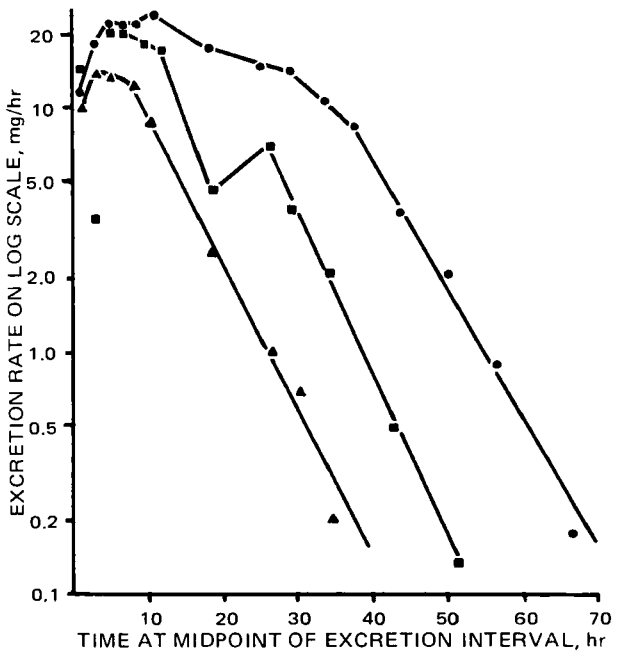

Figure 4—*Semilogarithmic plot of urinary excretion rate of probenecid acyl glucuronide against the time of midpoints of the excretion intervals for Subject 1 following 0.5- (▲), 1.0- (■), and 2.0- (●) g doses of probenecid.*

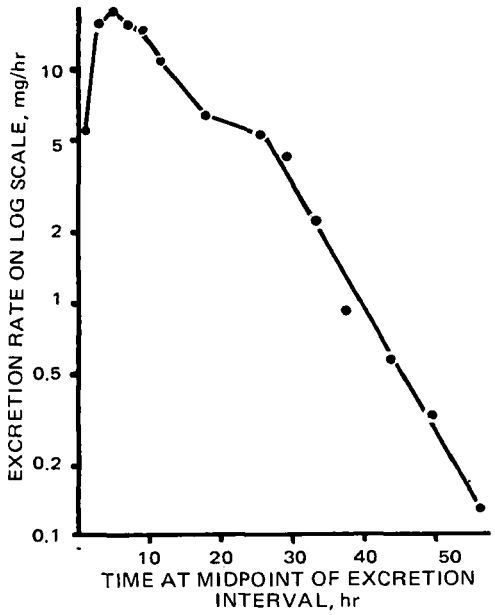

Figure 5—*Semilogarithmic plot of urinary excretion rate of the secondary alcohol metabolite against the time of midpoints of the excretion intervals for Subject 1 following a 2.0-g dose of probenecid. Excretion rate is expressed in terms of the amount (milligrams) of probenecid molecularly equivalent to the metabolites excreted.*

not surprising that a larger fraction of the dose was excreted unchanged in Subject 1 as compared to Subject 2.

There was no direct correlation between urinary pH and renal excretion of probenecid in this study. Probenecid is a weak acid with a pKa of 3.4 and is, therefore, completely ionized (>99.9%) at pH values of 6.4 and greater. Even at pH 5.0, the lowest urine pH observed in this study, 97.6% of the drug would exist in the ionized form. The present results indicate that the tubular reabsorption of probenecid is essentially complete even when the urine pH is well above the pKa of the drug. A completely quantitative explanation for this observation

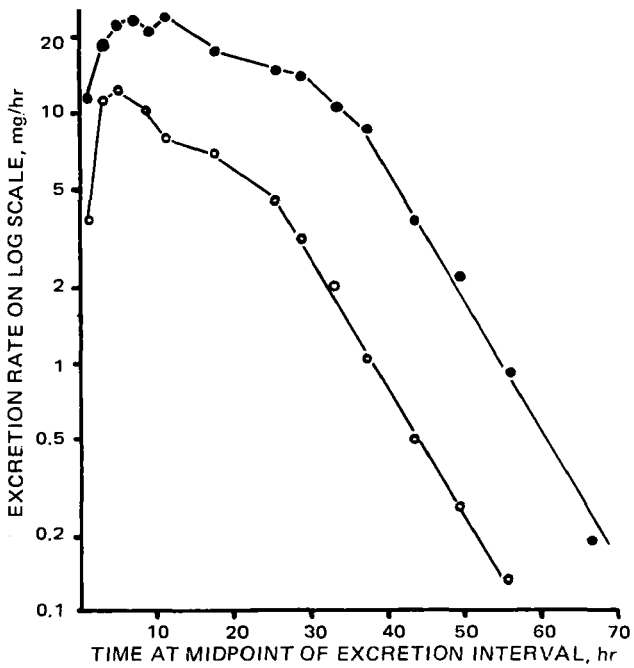

Figure 6—*Semilogarithmic plot of urinary excretion rate of probenecid acyl glucuronide (●) and the mono-N-propyl metabolite (○) in Subject 1 following a 2.0-g dose of probenecid. Excretion rate is expressed in terms of the amount (milligrams) of probenecid molecularly equivalent to the metabolites excreted.*

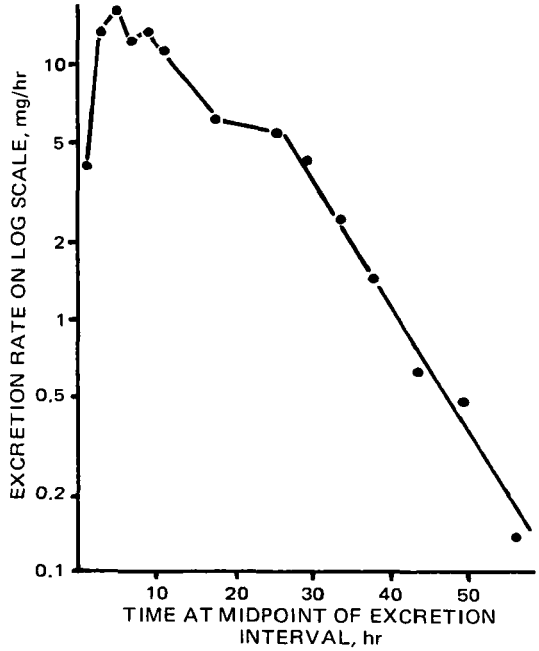

Figure 7—*Semilogarithmic plot of urinary excretion rate of the carboxylic acid metabolite against the time of midpoints of the excretion intervals for Subject 1 following a 2.0-g dose of probenecid. Excretion rate is expressed in terms of the amount (milligrams) of probenecid molecularly equivalent to the metabolites excreted.*

is difficult to formulate, since renal tubular reabsorption of a drug is dependent on both its physicochemical properties (pKa and lipid solubility) and the urine flow rate. The task is further complicated by the lack of information relating the pH of the tubular fluids at various segments of the nephron to the final urine pH.

It was reported that the urinary excretion of probenecid by dogs is flow rate dependent (9). A dependence on flow rate was also apparent in both subjects in the present study. A typical illustration is shown in Fig. 1. Over the interval from 20 to 55 hr, where the pH remained constant, the excretion pattern of probenecid mimicked the pattern for urine flow rate.

In view of the dependence of probenecid elimination on both urine pH and urine flow rate and because of the prolonged period required for absorption of the orally administered drug, a meaningful elimination half-life for probenecid cannot be obtained from plots, such as Fig. 1, of the unchanged drug excreted in urine.

Time Course of Urinary Excretion of Probenecid and Its Metabolites—The urinary excretion data were analyzed by the techniques suggested by Martin (11). Excretion rate plots and sigma-minus plots for the total drug excreted by Subject 1, as expressed by the sum of probenecid metabolites, are presented in Figs. 2 and 3. An excretion rate plot for probenecid acyl glucuronide is presented in Fig. 4. Figures 5–7 show the excretion rate plots for individual metabolites in Subject 1 following the 2.0-g dose. Corresponding plots of the data for Subject 2 were very similar. Half-lives for the excretion of individual metabolites for both subjects were estimated from regression lines calculated for the terminal portions of the plots and are summarized in Table II.

The results in Table II and Figs. 5–7 show that the half-lives for excretion of each metabolite do not differ significantly. Figures 5–7 show that the shapes of the excretion curves are similar for all metabolites, suggesting that each metabolite does not affect excretion of the others. The last two columns in Table II show that the half-lives calculated from the total dose excreted, as measured by the sum of all metabolites, are also in good agreement with those based on excretion of individual metabolites. The apparent half-lives calculated from the terminal portions of these curves for all doses lie in the range of about 4–7 hr. However, at any one dose, the variation in half-lives for individual metabolites is much less.

These results strongly suggest that the overall rate of metabolism is much slower than the rate of excretion in the dose range studied. In addition, negligible amounts of probenecid are excreted during the terminal phase where linear elimination kinetics are observed.

Therefore, the half-life of elimination by metabolism, obtained from the urinary excretion plots, represents the half-life for elimination of probenecid during the terminal excretion phase and is represented as such in Table II.

It can be seen from Figs. 2 and 3 that the time required for elimination of the dose is extended appreciably as the dose is increased. This appears to be due to a prolonged absorption of the drug. The apparent absorptive phase was about 10 hr for the 0.5-g dose and increased to 30–40 hr for the 2.0-g dose. The curvature in the sigma-minus plots at the 2.0-g dose suggests some type of saturation. In this connection, it is interesting to examine the excretion of the major metabolite, probenecid acyl glucuronide.

The maximum excretion rate was roughly constant at about 20 mg/hr over 5–30 hr for the 2.0-g dose for both subjects. Data from Subject 1 are presented in Fig. 4. For the 1-g dose, the maximum excretion rate was also about 20 mg/hr over 5–20 hr. For the 0.5-g dose, the excretion rate reached a maximum of approximately 15 mg/hr about 5 hr after medication and immediately began to decline.

The other metabolites exhibited similar plateaus in their excretion rate plots as shown in Figs. 5–7, where the excretion rates of the mono-*N*-propyl, carboxylic acid, and secondary alcohol metabolites are also seen to plateau at about 10–15 mg/hr. This parallel can be seen in Table I, which demonstrates that the fractions of the drug recovered in urine in the form of these metabolites are about equal. This apparent saturation could result from a single rate-limiting step in a sequential process of metabolism as mentioned earlier. Saturation of urinary excretion offers another possible explanation, but the results of Perel *et al.* (2) indicate that urinary excretion is not saturated.

The poor solubility of probenecid in water suggests a more likely explanation. The solubility of probenecid in water at 37° was determined to be about 50 μg/ml. Therefore, higher doses of the drug administered in solution possibly could have precipitated in the GI tract. The presence of insoluble probenecid in the GI tract would cause the absorption process to be dissolution rate limited (zero order), making the drug available for metabolism at a constant rate until all precipitated drug redissolved. Blood level data, as well as excretion data following intravenous administration of probenecid, would be useful in further resolving the mechanism of absorption, distribution, and elimination of the drug.

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Rapid Determination of Theophylline in Human Plasma by High-Pressure Liquid Chromatography

ORVILLE H. WEDDLE ˣ and WILLIAM D. MASON

Abstract □ A rapid, specific, high-pressure liquid chromatographic method for the determination of theophylline in plasma was developed. The procedure is fast enough (21 min from receipt of blood to reporting value) to be used for emergency determinations. The sensitivity, precision, and accuracy are sufficient for routine monitoring of therapeutic levels in patients. The assay is specific enough to be valid in the presence of caffeine and theobromine. Metabolites of theophylline as well as a number of drugs do not interfere with the assay.

Keyphrases □ Theophylline—high-pressure liquid chromatographic analysis, plasma □ High-pressure liquid chromatography—analysis, theophylline, plasma □ Relaxants, smooth muscle—theophylline, high-pressure liquid chromatographic analysis, plasma

Many patients receive theophylline for the prevention and treatment of asthmatic attacks. Several investigations showed good correlation between the plasma concentrations of theophylline and the improvement of pulmonary function in asthmatic patients (1–3). Recent reviews of pharmacokinetic control of drug therapy (4, 5) suggested that monitoring of blood theophylline levels can contribute significantly to the control of therapy of asthmatics. Unfortunately, these benefits are of limited application to the emergency care situation. Present methods of analysis either lack specificity or are too time consuming to contribute to the rapid adjustment of theophylline levels in the patient admitted for acute asthmatic attack where a subtherapeutic theophylline level may or may not be a contributing cause.

The method of theophylline analysis most widely used at present is that of Schack and Waxler (6). This procedure is not suited to monitoring theophylline therapy in many emergency situations since caffeine, theobromine, and other dietary xanthines that may be present in the patient are not distinguished from theophylline. It is used where patients can be placed on