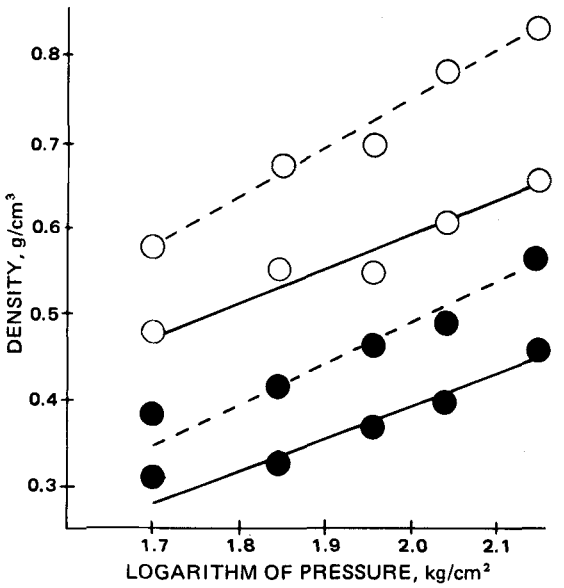

Figure 5—*Relationship of bulk and drop densities to logarithm of roller pressure. Key: ○, calcium carbonate; ●, magnesium carbonate; —, bulk density; and ---, drop density.*

crushing loads for lactose and dibasic calcium phosphate dihydrate were similar, the magnitude did not differ markedly from that of the materials. Thus, no correlation is apparent between the size of the material passed through the oscillating granulator and the crushing load of the compacted granules. In pharmaceutical processes, a property of a material is often influenced by the physicochemical nature of the material in addition to the process itself. Even more emphatically than the variation of the particle-size distribution, the effect of the nature of the material is demonstrated by phenacetin, which did not compact in the pressure range of 50–140 kg/cm^2.

As shown in Table III, the bulk density of the compacted granules was double to triple that of the bulk density of the powder for calcium carbonate, magnesium carbonate, and sulfadiazine. For acetaminophen, aminobenzoic acid, dibasic calcium phosphate dihydrate, and sulfisoxazole, the bulk density of the compacted granules was increased ~50% more than that of the bulk powder. Essentially the same relationship was demonstrated between the drop density of the compacted granules and that of the powder.

The primary effect of compaction was to increase the bulk density; little or no effect was shown on flowability. A decrease in the repose angle is intuitively associated with improved flowability. Although the repose angle for six materials was less after the materials were compacted, the compacted granules still possessed poor mobility. Only magnesium carbonate and sulfadiazine were discharged from the flowmeter, and their flow was a pulsating one. The repose angle increased for dibasic calcium phosphate dihydrate and hydrous lactose. For the hydrous lactose and granular dicalcium phosphate[1], which were marketed as readily flowable materials, the flow rate decreased from 44.6 and 66.8 g/sec, respectively, to a blockage in the flowmeter, indicating that compaction had destroyed the desired flowability.

Calcium carbonate and magnesium carbonate were selected to study the influence of compaction pressure on bulk density, because they had the smallest median diameters and lowest bulk densities. The bulk and drop densities of these materials compacted at five pressures are given in Table IV; clearly, the density increased as the compaction pressure increased. As shown in Fig. 5, there was a linear relationship between the bulk and drop density and the logarithm of compaction pressure.

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High-Pressure Liquid Chromatographic Determination of Chlorothiazide and Hydrochlorothiazide in Plasma and Urine: Preliminary Results of Clinical Studies

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Abstract □ High-pressure liquid chromatographic procedures were developed for the determination of chlorothiazide and hydrochlorothiazide in plasma and urine. The plasma assay incorporates a preextraction procedure that eliminates interference by endogenous substances. Chromatography is carried out on an octadecyl reversed-phase column. Mobile phases are 15% methanol in 0.01 *M* acetic acid for plasma and 4% acetonitrile in 0.01 *M* sodium perchlorate, adjusted to pH 4.6, for urine. At a flow rate of 2.5 ml/min, the retention times for chlorothiazide and hydrochlorothiazide are 3.5 and 4.6 min for plasma and 10.5 and 13.5 min for urine, respectively. Preliminary results of a clinical study in fasting male volunteers showed that the plasma levels and urinary excretion rate of chlorothiazide peaked at 1–2 hr following a 500-mg oral dose and subsequently declined irregularly. On the other hand, the plasma levels and urinary excretion rate of hydrochlorothiazide peaked at 2–3 hr following a 50-mg oral dose and subsequently declined in biphasic fashion. Urinary excretion rates of both chlorothiazide and hydrochlorothiazide closely resemble their concentration profiles in plasma.

Keyphrases □ Chlorothiazide—simultaneous high-pressure liquid chromatographic determinations with hydrochlorothiazide, plasma and urine □ Hydrochlorothiazide—simultaneous high-pressure liquid chromatographic determinations with chlorothiazide, plasma and urine □ High-pressure liquid chromatography—simultaneous determinations of chlorothiazide and hydrochlorothiazide, plasma and urine

Chlorothiazide and hydrochlorothiazide, two thiazide diuretics, are used commonly for treatment of hypertension, congestive heart failure, and other edematous conditions. The spectrophotometric methods originally used to measure these compounds in urine (1–4) are based on derivatizations involving the Bratton–Marshall reaction

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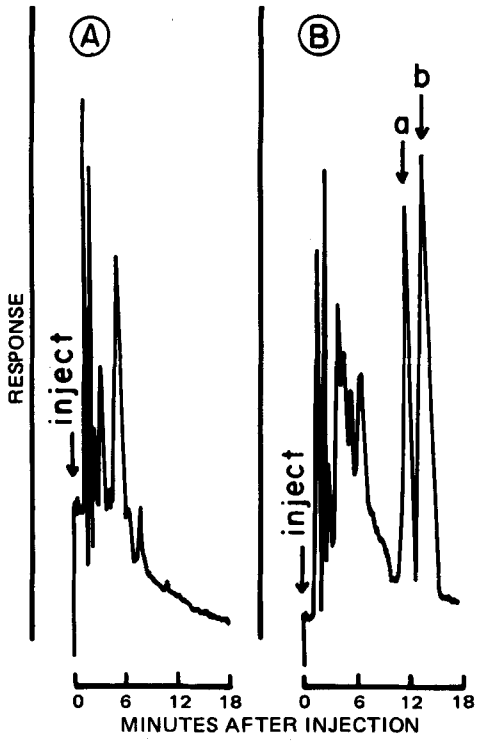

Figure 1—*Chromatograms from urine containing no drugs (A) and urine containing 15 μg of I/ml (a in B) and 18 μg of II/ml (b in B).*

and suffer from interference by endogenous urinary constituents (5, 6); TLC with direct fluorometric detection (7) and GLC procedures (8–10) are time consuming. The GLC procedures also involve complex extraction procedures and derivatization.

High-pressure liquid chromatographic (HPLC) assays were recently introduced for hydrochlorothiazide (11–13) and chlorothiazide (14). One method requires gel filtration to remove plasma components (11), while another uses no internal standard and requires injection of a sodium hydroxide solution into the liquid chromatograph, which can reduce the column life (12). None of the methods used the λ_{max} of 228 nm for plasma chlorothiazide and hydrochlorothiazide estimations, possibly because of interference by endogenous substances; therefore, maximum assay sensitivity has not been achieved. This limitation is particularly important for plasma where thiazide concentrations are low.

New rapid and sensitive assays for chlorothiazide and hydrochlorothiazide in plasma and urine, together with preliminary plasma level and urinary excretion data for these compounds in human volunteers, are reported in this paper.

EXPERIMENTAL

Extraction from Urine—To 1 ml of urine were added 60 μl of a 300-μg/ml aqueous solution of chlorothiazide or hydrochlorothiazide, each compound acting as the internal standard for the other, 1.5 ml of 0.01 *M* acetate buffer (pH 5.0), and 10 ml of ethyl acetate. After shaking for 10 min and centrifugation at 3000×*g* for 3 min, 8 ml of ethyl acetate was transferred to a clean tube and evaporated to dryness under nitrogen at room temperature. The residue was reconstituted in 75 μl of methanol by vortexing, and 20 μl was injected into the chromatograph.

Extraction from Plasma—To 2 ml of plasma were added 100 μl of a 40-μg/ml aqueous solution of the internal standard hydroflumethiazide and 4 ml of toluene. After shaking for 10 min and centrifugation for 3 min, the toluene layer was aspirated and discarded. To the remaining solution were added 0.5 ml of 0.01 *M* acetate buffer (pH 3.8) and 5 ml of ethyl acetate. After shaking for 10 min and centrifugation for 3 min, 3.5–4.0 ml of ethyl acetate was transferred to a clean tube, evaporated to dryness, and reconstituted in 75 μl of methanol; then 20 μl was injected into the chromatograph.

Chromatography—The HPLC system consisted of a solvent pump[1], a fixed-volume (20-μl) sample injection valve[2], a 10-μm particle size reversed-phase octadecyl column[3] (30 cm × 4 mm), and a variable-wavelength UV detector[4] set at 228 nm for plasma and at 278 nm for urine. All chromatograms were recorded at a chart speed of 10 cm/hr.

Mobile phases were 4% acetonitrile in 0.01 *M* sodium perchlorate, adjusted to pH 4.6 with 0.01 *M* perchloric acid for urine, and 15% methanol in 0.01 *M* acetic acid for plasma. The flow rate was 2.5 ml/min in both assays. Concentrations were determined by the method of peak height ratios.

Clinical Study—Single doses of 500 mg of chlorothiazide[5] (I) and 50 mg of hydrochlorothiazide[6] (II) were administered to three healthy male volunteers[7]. Subjects fasted overnight before drug administration and continued fasting until 4 hr postdosing. All doses were administered at 8 am; the tablets were swallowed whole with 240 ml of water. Blood samples (10 ml) were taken from a forearm vein into heparinized tubes[8] immediately before dosing and serially through 48 or 34 hr postdosing for I or II, respectively. Urines were collected quantitatively through 48 or 60 hr postdosing for I or II, respectively. The blood and urine collection times conformed to larger clinical protocols for both compounds[7]. Plasma and urine samples were stored at −20° until they were assayed, usually within 1 week.

Reagents—Human plasma for assay development and standardization was purchased[9]. Human drug-free urine was obtained from male donors as required. Chlorothiazide[10] (I), hydrochlorothiazide[10] (II), and hydroflumethiazide[11] (III) of reference standard quality and methanol[12], acetonitrile[12], acetic acid[13], sodium perchlorate[14], and perchloric acid[15] of analytical grade quality were used as received.

Table I—Day-to-Day Reproducibility of the Assay for Chlorothiazide and Hydrochlorothiazide in Urine [a]

Concentration in Urine, μg/ml	Found, μg/ml	Recovery, %	*CV*, %
	Chlorothiazide		
2	2.04	102	6.7
5	5.04	101	5.6
10	10.0	100	4.9
20	20.2	101	6.2
50	51.2	102	4.7
100	102.3	102	6.3
	Hydrochlorothiazide		
2	2.01	101	5.9
5	4.95	99	9.1
10	9.7	97	4.3
20	19.0	95	5.5
50	51.8	104	8.4
100	100.5	101	6.5

[a] n = 10 for all concentrations.

RESULTS AND DISCUSSION

Assays—Both chlorothiazide (I) and hydrochlorothiazide (II) are excreted from the body extensively as unchanged drug in urine (14, 15), and the urinary concentration of both compounds is higher than in plasma. Urine analysis requires specific methods to distinguish the

[1] Model 110, Altex Scientific, Berkeley, Calif.
[2] Model 210, Altex Scientific, Berkeley, Calif.
[3] MCH-10 Micropak, Varian Associates, Palo Alto, Calif.
[4] Hitachi model 100-10, Altex Scientific, Berkeley, Calif.
[5] Lot 559-197, 250-mg tablets, Lederle, Pearl River, N.Y.
[6] Lot B 0686, Hydrodiuril, 50-mg tablets, Merck Sharp and Dohme, West Point, Pa.
[7] These dosages were part of more extensive bioavailability studies, which will be reported separately.
[8] Vacutainer, Becton–Dickinson, Rutherford, N.J.
[9] American Red Cross, Madison, Wis.
[10] Merck Sharp and Dohme, West Point, Pa.
[11] Bristol Laboratories, Syracuse, N.Y.
[12] Burdick & Jackson, Muskegon, Mich.
[13] Allied Chemical Corp., Morristown, N.J.
[14] Fisher Scientific Co., Fair Lawn, N.J.
[15] J. T. Baker Chemical Co., Phillipsburg, N.J.

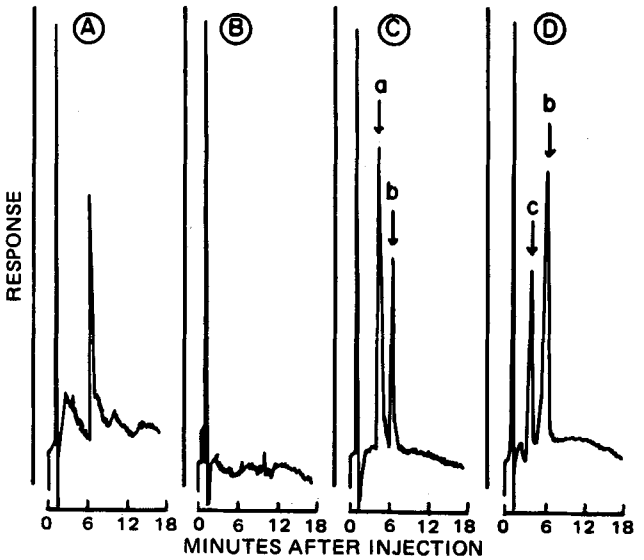

Figure 2—*Chromatograms from plasma containing no drugs and assayed without a toluene preextraction step. (A), plasma containing no drugs and assayed with a toluene preextraction step (B), plasma containing 400 ng of II/ml (a in C) and 2 μg of III/ml (b in C), and plasma containing 200 ng of I/ml (c in D) and 2 μg of III/ml (b in D).*

thiazide from interfering substances (5, 6). In addition to specificity, plasma analysis requires high assay sensitivity to measure the low drug concentrations. The assays described here are specific and sensitive for I and II in human urine and plasma following single or repeated doses.

Typical chromatograms obtained from blank urine and from urine containing I and II are shown in Fig. 1. Retention times for I and II from urine were 10.5 and 13.5 min, respectively, and the response was linear for both compounds in the range of 2–100 μg/ml, with a coefficient of variation of <10%. Standard curves obtained from repeated determinations were $y = 0.040 + 0.0598x$, $r = 0.999$, $n = 35$, for I and $y = 0.024 + 0.0712x$, $r = 0.996$, $n = 35$, for II. The day-to-day reproducibility of the urine assays, determined on 10 occasions during 2 months, is shown in Table I.

Typical chromatograms obtained from blank plasma, with and without toluene preextraction, and from plasma containing I, II, and hydroflumethiazide (III), are given in Fig. 2. Retention times for I, II, and III from plasma were 3.5, 4.6, and 6.0 min, respectively, and the response was linear for I and II concentrations of 10–750 ng/ml, with a coefficient of variation of <10%. Standard curves obtained from repeated determinations were $y = 0.007 + 0.0032x$, $r = 0.999$, $n = 35$, for I and $y = 0.023 + 0.004x$, $r = 0.999$, $n = 35$, for II. The day-to-day reproducibility of the plasma assays determined on 10 occasions during 2 months is shown in Table II. In the urine assays, I and II could be separated from each other by using 4% acetonitrile in 0.01 *M* sodium perchlorate (pH 4.6) as the mobile phase. Therefore, they were used as internal standards for each other. Interference by endogenous substances was not a major problem with the urine assays, and clear chromatograms were obtained with the detector set at 278 nm.

Table II—Day-to-Day Reproducibility of the Assays for Chlorothiazide and Hydrochlorothiazide in Plasma [a]

Concentration in Plasma, ng/ml	Found, ng/ml	Recovery, %	*CV*, %
	Chlorothiazide		
10	9.9	99	5.8
25	24.3	97	5.5
50	49.0	98	6.0
100	99.6	100	6.2
500	508	102	5.7
750	746	99	4.4
	Hydrochlorothiazide		
10	10.0	100	6.7
20	19.3	96	6.7
50	50.6	101	6.9
100	99.0	99	6.2
250	254	102	5.4
500	498	100	4.2
750	750	100	1.7

[a] $n = 10$ for all concentrations.

The low levels of I and II that occur in plasma compared to urine required some procedural modifications to obtain the required assay sensitivity and selectivity. A toluene preextraction step removed interfering peaks due to endogenous plasma components but did not remove I–III, which are sparingly soluble in toluene. Assay sensitivity was improved further by setting the detector at 228 nm, the λ_{max} for both I and II, and also by using methanol–acetic acid as the mobile phase. Both I and II absorb UV light at 228 nm to a greater extent in this solvent than in acetonitrile–sodium perchlorate. Effective separation of I and II could not

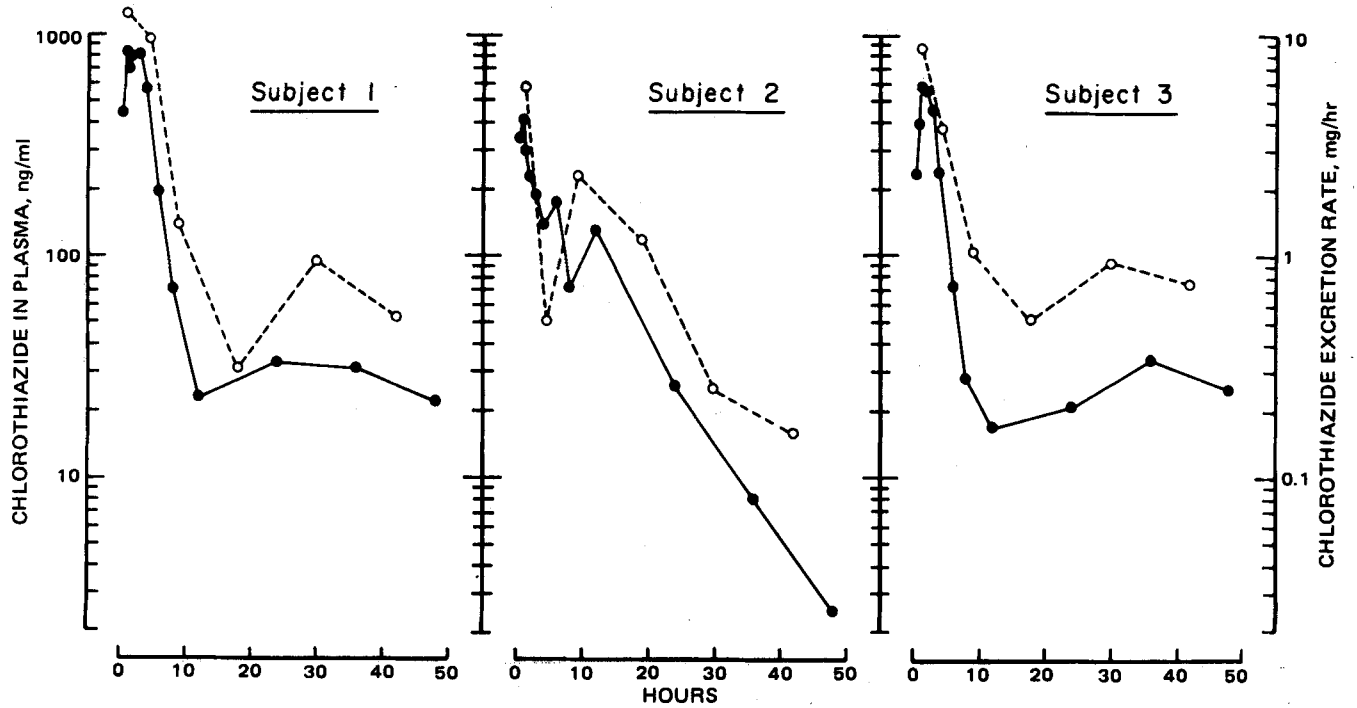

Figure 3—*Plasma levels (●) and urinary excretion rates (○) for I in three subjects following a single 500-mg oral dose.*

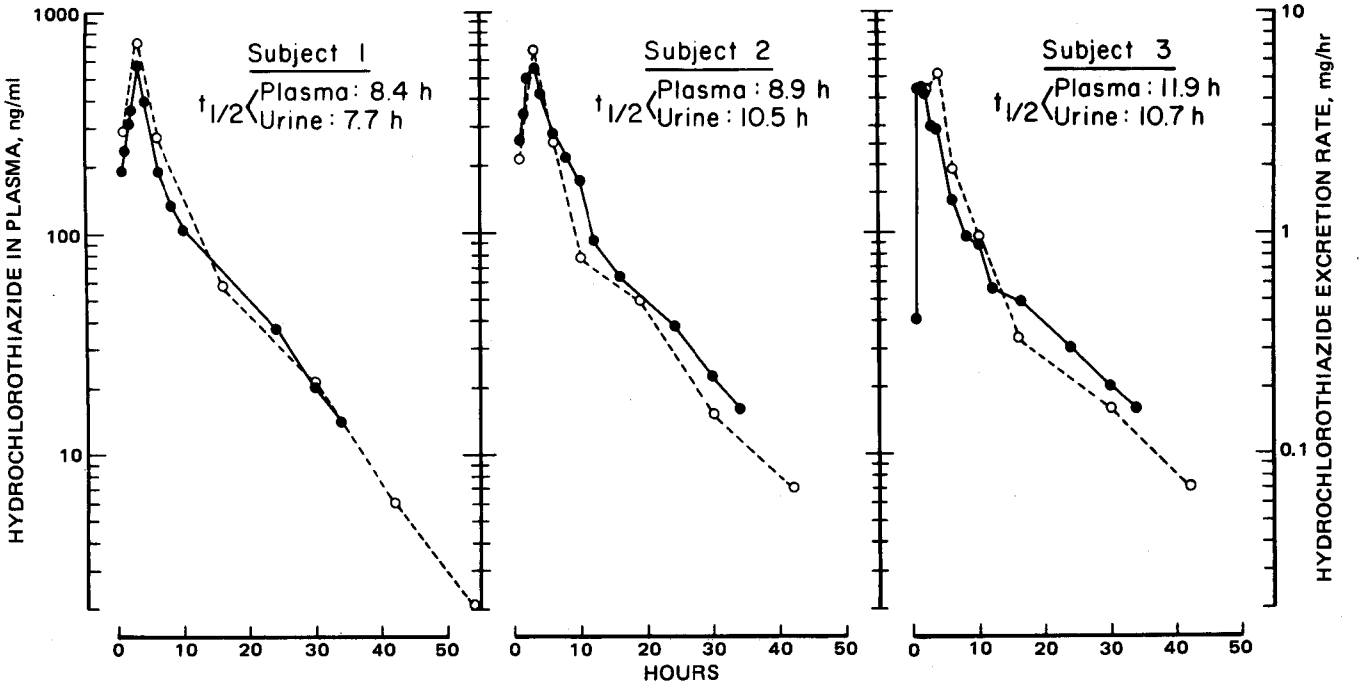

Figure 4—*Plasma levels (●) and urinary excretion rates (○) for II in three subjects following a single 50-mg oral dose.*

be achieved using methanol–acetic acid, but both compounds did separate from III. Therefore, III was used as the internal standard in the assays for I and II in plasma.

Clinical Studies—Individual plasma levels of I and II, each drug being administered to three subjects, are shown in Figs. 3 and 4, respectively.

The I profiles were similar in the three individuals. Peak plasma I levels of 420–850 ng/ml were achieved 1–2 hr following a single 500-mg dose. Drug levels then declined rapidly until 8–12 hr postdosing, at which time they became irregular, exhibiting a saw-toothed effect in two subjects while declining to lower levels in the other subject.

The II profiles were also similar in the three individuals but quite different from the plasma profiles obtained with I. Peak plasma II levels of 450–550 ng/ml were achieved 2–3 hr following a single 50-mg dose. Drug levels then declined rapidly until 10–12 hr postdosing and continued to decline at a slower monoexponential rate. The terminal plasma half-lives, which were measurable for II, were 8.4, 8.9, and 11.9 hr in the three subjects.

While the reason for the different behavior of I and II in human plasma is not known, the types of profiles reported here have been observed routinely in subjects receiving these drugs in this laboratory[16] and have been reported by others. The rapid initial loss of orally dosed I from plasma followed by variable drug levels was reported recently (14), while biphasic elimination of plasma II with an apparently prolonged drug distribution phase was reported at dose levels similar to those used in this study (15).

The cumulative 48-hr urinary recovery of I accounted for only 10.4, 19.0, and 13.9% of the dose in the three subjects, while 87.8, 79.3, and 74.4% of II were recovered in 60-hr urine. High urinary recovery of II and low recovery of I also are consistent with previous observations (14, 15).

To compare the kinetics of urinary recovery with the rate of drug loss from plasma, the individual urinary excretion rates of both compounds were calculated at the midpoint of each urine collection interval; the values were plotted on a semilogarithmic scale along with the plasma drug profiles in Figs. 3 and 4.

Comparison of the data indicates similarity in the individual plasma levels and urinary excretion rates for both I and II. Details of plasma levels and urinary excretion of these two compounds in a larger number of subjects will be reported in detail, together with pharmacokinetic analysis. However, these preliminary observations are of interest in view of the limited pharmacokinetic information available for the thiazide diuretics and their identification as compounds with actual or potential bioequivalence problems (16). These results are important also in view of the nonproportionality that has been reported between urinary recovery of I and the administered dose (14, 17) and the good correlations reported between dose size and resulting plasma II levels (15). There is clearly a high correlation between plasma levels and urinary excretion kinetics of both I and II, and these relationships are being examined further at different dose levels.

The methods described here are sufficiently sensitive to detect plasma levels of I for 48 hr and II for 34 hr and to measure urinary excretion of both compounds for 48–60 hr after oral doses of 500 and 50 mg of I and II, respectively. The procedure for plasma has marked advantages over previous methods (11–13) in both sensitivity and specificity. Degradation of II, which may occur in other procedures (12, 13) where sodium hydroxide solutions are used for back-extraction or reconstitution, is avoided by eliminating the use of alkaline solutions. Although the procedures are different in urine and plasma, the use of identical extraction and chromatographic conditions for both compounds in plasma and urine makes the methods simple, accurate, and convenient for pharmacokinetic studies.

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Pharmacokinetic Evidence for Possible Renal Accumulation of Model Organic Anions in Rats

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Abstract □ The urinary excretion and blood level kinetics of *p*-methylbenzoylformic acid (I) after intravenous infusion for 1 hr were studied in rats. The determined first-order half-lives were compared with those determined in studies in which a single intravenous dose of I was administered rapidly to rats that previously were infused with normal saline for 1 hr. While the blood $t_{1/2}$ or body clearance of I determined in the 1-hr infusion studies was similar to that determined in the single intravenous dose studies, the urinary $t_{1/2}$ of I determined in the 1-hr infusion studies was significantly greater than that determined in the single intravenous dose studies. In infusion studies where the half-lives of I were determined in the presence of renal tubular secretion inhibitor, DL-tropic acid (II), the ratio of (urinary $t_{1/2})_{\text{II present}}$/(urinary $t_{1/2})_{\text{II absent}}$ was almost twice the ratio of (blood $t_{1/2})_{\text{II present}}$/(blood $t_{1/2})_{\text{II absent}}$. The urinary $t_{1/2}$ value of I determined after infusion for only 10 min was intermediate between values obtained in the single intravenous dose studies and the 1-hr infusion studies. These data provide pharmacokinetic evidence to support the hypothesis that I and other organic anions temporarily accumulate in the surface-lying renal tubular cells after a single intravenous dose, but they tend to penetrate into the deeper renal tubular cells upon intravenous infusion, with depth of penetration increasing with increasing infusion time.

Keyphrases □ Renal accumulation—*p*-methylbenzoylformic acid, pharmacokinetics, rats □ *p*-Methylbenzoylformic acid—model organic compound used for determination of renal accumulation, pharmacokinetics □ Pharmacokinetics—renal accumulation of model organic anions, rats

Recent studies (1) in rats showed that the biological half-lives of mandelic acid, benzoylformic acid, and several of their *para*-alkylated homologs, including *p*-methylbenzoylformic acid (I), determined from urinary excretion data are longer than those determined from blood level data following a single intravenous dose. In the presence of a simultaneously administered intravenous dose of DL-tropic acid (II), a competitive inhibitor of renal tubular secretion of these compounds, the urinary half-lives were disproportionately longer than the blood half-lives. The ratios of (urinary $t_{1/2}$/blood $t_{1/2}$) determined for these compounds in the presence of II were greater than such ratios determined in the absence of II. The longer urinary half-lives were attributed to the retention or detention of these compounds in the renal tubular membranes during secretion.

BACKGROUND

Several reports (2–4) indicated that cellular trapping of organic anions, such as *p*-aminohippurate and phenolsulfonphthalein, occurs during their renal tubular secretion in mammals. Recent *in vitro* rat kidney slice uptake studies (4, 5) showed that *p*-aminohippurate accumulates mainly in the proximal renal tubules, and the volume of renal tubules that absorb the compound increases with incubation time and its concentration in the medium. Also, in the presence of renal tubular secretion inhibitors, a redistribution of accumulation of *p*-aminohippurate occurred and further uptake took place in the deeper proximal renal tubules (6).

The purpose of this study was to obtain pharmacokinetic evidence in support of the proposed hypotheses concerning the retention/detention and distribution of organic anions in the renal tubules by studying blood level kinetics and urinary excretion kinetics of I in rats after intravenous infusion for 1 hr, both in the absence and presence of II. Therefore, the specific objectives of the study were to demonstrate that:

1. The (urinary $t_{1/2})_{\text{infusion}}$ of I determined in the infusion study is longer than its (urinary $t_{1/2})_{\text{single iv dose}}$ determined in the single intravenous dose study.
2. The (blood $t_{1/2})_{\text{infusion}}$ of I is similar to its (blood $t_{1/2})_{\text{single iv dose}}$.
3. The ratio of (urinary $t_{1/2}$/blood $t_{1/2})_{\text{infusion}}$ of I obtained in the presence of II is greater than that obtained in the absence of II.
4. The urinary $t_{1/2}$ determined after infusing I for 1 hr is longer than that determined after infusing it for 10 min.

Compound I (pKa 3.3) was used as a model organic anion because it is neither metabolized, bound to plasma proteins, nor reabsorbed from the renal tubules of rats (1). Furthermore, the entire administered dose of I is eliminated renally (which includes renal tubular secretion), and it follows one-compartment open model kinetics (1).

EXPERIMENTAL

Materials—*p*-Methylbenzoylformic acid, mp 97–99°, was synthesized by the method of Kindler *et al.* (7). DL-Tropic acid[1], mp 116–118°, and pentobarbital sodium[2] (USP grade) were also used.

Apparatus and Analytical Procedure—The GLC[3] procedures used for the quantitative determination of I in rat urine and blood were essentially the same as described previously (1, 8).

Surgical Preparation of Rats—Sprague–Dawley male rats, 170–230 g (a majority weighed ~200 g), were used in the blood level kinetic studies and urinary excretion kinetic studies. Food, but not water, was withheld from the rats 12–14 hr prior to the study and during it. Each rat was prepared surgically for infusion of the isotonic solution of I (1.75 mg/ml), with or without II, *via* a femoral vein.

A rat was anesthetized with pentobarbital sodium (40 mg/kg ip) and placed on an animal board. A small amount of ether was used to anesthetize the rats completely. The limbs were tied to the animal board, and hair between the left leg and abdomen was shaved off with an electric clipper; the shaved part was cleaned with 70% (v/v) ethanol. The skin between the left leg and the midline was cut open to expose a femoral vein, which was isolated from the femoral artery and femoral nerve. The femoral vein was cannulated with polyethylene tubing[4] (0.028 cm i.d.,

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] Merck & Co., Rahway, N.J.
[3] Hewlett-Packard model 5720A gas chromatograph.
[4] Intramedic (PE-10), Clay Adams, Division of Becton–Dickenson Co., Parsippany, N.J.

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