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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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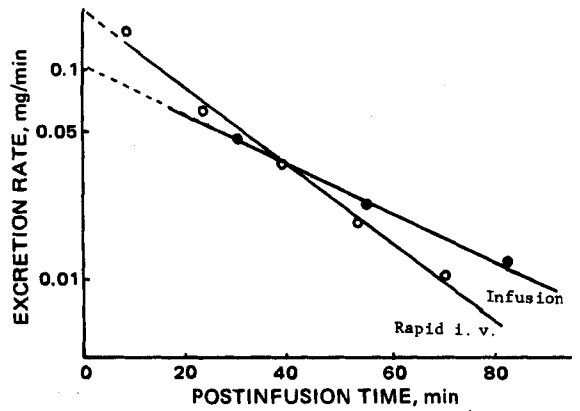

Figure 1—*Typical semilogarithmic urinary excretion plots obtained for I after its rapid intravenous administration of a single dose to a rat previously infused with normal saline for 1 hr (○) and after continuous intravenous infusion into a rat for 1 hr (●).*

0.061 cm o.d.). The inserted end of the cannula and the vein were tied together to keep the cannula in the vein. The surgically opened area was closed with clips using a clip applier[5].

The surgical procedure took ~20 min. The rat generally regained consciousness within 30 min after the cannulation. The rat then was placed in an acrylic restraining cage and usually was ready for infusion ~1 hr after cannulation.

Urinary Excretion Studies—The urinary excretion kinetics of I were studied under the following four conditions:

1. Compound I was infused into the rat for 1 hr at 0.2275 mg/0.13 ml/min. This infusion rate was chosen to produce, at the end of the 1-hr infusion, a blood concentration that would be close to the initial blood concentration produced following rapid intravenous administration of a single dose of 22 mg/kg as reported previously (1). Six rat studies were performed.

2. The rat was infused with normal saline for 1 hr at 0.092 ml/min, immediately followed by a rapid intravenous infusion of a single dose of 4.4 mg of I contained in 2 ml. This study served as a control for that described in Condition 1. Four rat studies were performed.

3. Compound I was infused into the rat for 1 hr at 0.2275 mg/0.13 ml/min in the presence of II. The infusion rate of II, which was contained in the same solution of I, was 4.55 mg/0.13 ml/min. The II infusion rate was chosen to produce, at the end of the 1-hr infusion, a blood concentration that would be similar to the initial blood concentration produced following rapid intravenous administration of a single dose of 100 mg/kg as reported previously (1). There was no need to conduct a separate single intravenous dose control study for this portion of the study (see *Discussion*). Five rat studies were performed.

4. The rat was infused first with normal saline at 0.13 ml/min for 50 min, followed by the I infusion at 0.5562 mg/0.13 ml/min for 10 min. The I infusion rate was chosen to produce, at the end of the 10-min infusion, a blood concentration that would be similar to that at the end of the 1-hr infusion as described in Condition 1. Five rat studies were performed.

All solutions used in Conditions 1–4 were isotonic and were adjusted to pH 7.4. Immediately after the prolonged or rapid infusion of I, the outer cannula was cut at a point ~1 cm from the skin, and the cannula opening was sealed with a flame. The rat then was transferred to a urine collection cage (9), and urine samples were collected according to the procedure described previously (9).

Blood Studies—The blood level kinetics of I were studied under the following conditions:

1. Compound I was infused into the rat for 1 hr at 0.2275 mg/0.13 ml/min. As described previously (8, 10), only one blood sample was obtained from a given rat upon its decapitation at a predetermined time. On a given day, altogether three blood I levels were determined from three different rats decapitated at 10, 20, and 30 min postinfusion. The procedure used for collecting blood samples was the same as described previously (8). Fifteen rat studies were performed. There was no need to conduct a separate single intravenous dose control study for this phase (see *Discussion*).

2. Compound I was infused into the rat for 1 hr at 0.2275 mg/0.13 ml/min in the presence of II. The infusion rate of II, which was contained in the same solution of I, was 4.55 mg/0.13 ml/min, as explained under the urinary excretion studies. The procedure for obtaining blood samples and the number of blood samples obtained from rats were identical to that described under Condition 1. Fifteen rat studies were performed. There was no need to conduct a separate single intravenous dose control study for this phase (see *Discussion*).

[5] Autoclips and autoclip applier, Clay Adams, Division of Becton–Dickenson Co., Parsippany, N.J.

RESULTS

Urinary Excretion Kinetics of I after 1-hr Infusion—Since the urinary excretion of I is an apparent first-order process after a single intravenous dose in the 2.0–9.7-mg/rat range (1), and since, as shown later, I disappearance from the blood after the 1-hr infusion occurs monoexponentially, the urinary excretion of the compound after a 1-hr infusion can be expected to occur by a first-order process (Fig. 1). The data were treated according to:

$$\log(\Delta D_e/\Delta t) = \log k_{ex} D_0 - (k_{ex} t/2.303) \qquad \text{(Eq. 1)}$$

where $\Delta D_e/\Delta t$ is the urinary excretion rate of I at time t, the midpoint of the urine collection period; D_0 is the amount of I in the body at the time of discontinuation of infusion; and k_{ex} is a first-order rate constant for urinary excretion of the compound, which is essentially equivalent to the rate constant of I elimination since renal excretion is the only process by which I is eliminated intact from the body.

In constructing an individual infusion plot for each rat (Fig. 1), the first data point was ignored and is not shown since the urine sample collected within 10 min after the discontinuation of infusion very likely contained an amount of I greater than that expected. The reason for this belief was that the rat seldom urinated immediately prior to or after the discontinuation of infusion.

According to Eq. 1, the rate constant of urinary excretion was calculated from the slope ($-k_{ex}/2.303$) of the straight line of each plot obtained by least-squares regression. The urinary $t_{1/2}$ was calculated from $t_{1/2} = 0.693/k_{ex}$. The mean k_{ex} of I was 0.0281 ± 0.0042 min^{-1}, and the average urinary $t_{1/2}$ was 24.7 min.

Urinary Excretion Kinetics of I after Single Intravenous Dose in Control Study—Since the single intravenous dose urinary excretion kinetic studies of I conducted previously (1) did not involve continuous infusion of normal saline for 1 hr, those studies cannot be assumed (without evidence) to serve as the control for such studies after continuous infusion for 1 hr. Therefore, to provide an appropriate control, the urinary excretion kinetics of I were studied after administering a single intravenous dose to each rat, immediately after infusing the rat with normal saline for 1 hr. The typical urinary excretion data obtained in this control study are shown in Fig. 1. The mean k_{ex} of I was 0.0430 ± 0.0034 min^{-1}, which was significantly different ($p < 0.01$) from that obtained in the 1-hr infusion study. The average urinary $t_{1/2}$ of I was 16.2 min.

Urinary Excretion Kinetics of I after 1-hr Infusion in Presence of II—These excretion studies in the postinfusion period determined the urinary $t_{1/2}$ of I after blocking its renal tubular secretion with II. Both I and II were contained in the same solution, and the infusion rate of II or the concentration of II was 20 times greater than that of I to inhibit completely the renal tubular secretion of I. The typical apparent first-order urinary excretion data obtained for I are shown in Fig. 2. The mean

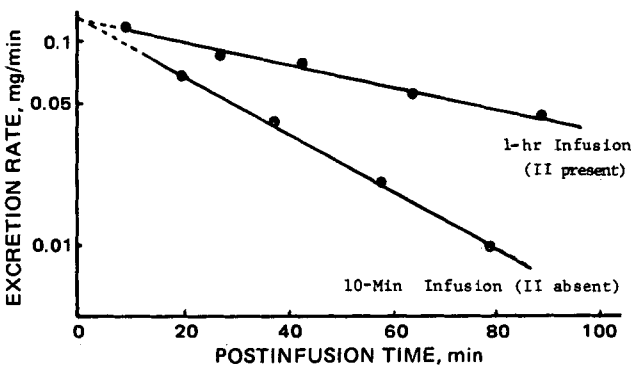

Figure 2—*Typical semilogarithmic urinary excretion plots obtained for I after its continuous intravenous infusion into a rat for 1 hr in the presence of II (●) and after its continuous intravenous infusion in the absence of II for 10 min into a rat previously infused with normal saline for 50 min (⊖).*

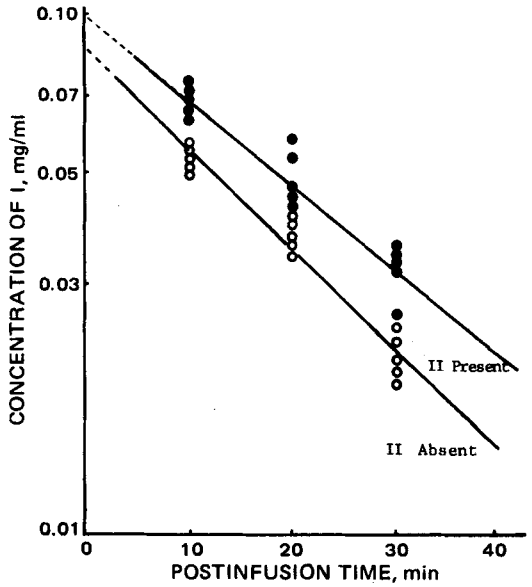

Figure 3—*Postinfusion blood levels of I in the absence (○) and presence (●) of II. Each data point represents the concentration of I noted in a given rat, as explained in the text.*

k_{ex} of I was 0.0122 ± 0.0012 min^{-1}, which was significantly lower ($p < 0.001$) than that observed in the absence of II in the 1-hr infusion study. The average urinary $t_{1/2}$ of I was 57.0 min.

Urinary Excretion Kinetics of I after 10-min Infusion—These excretion studies determined the effect of the infusion time on the urinary $t_{1/2}$ of I. The typical apparent first-order urinary excretion data obtained for I are shown in Fig. 2. The mean k_{ex} of I was 0.0399 ± 0.0049 min^{-1}, which was significantly greater ($p < 0.01$) than that observed in the 1-hr infusion study. The average urinary $t_{1/2}$ of I was 17.3 min.

Blood Level Kinetics of I after 1-hr Infusion in Absence of II—It was shown previously (8, 10) that the blood levels of I decline monoexponentially after a rapid intravenous administration of a single dose. The blood levels of I obtained in the postinfusion period also declined monoexponentially (Fig. 3), and these data are treated according to the following first-order equation:

$$\log C' = \log C'_0 - (K_{el}t/2.303) \qquad \text{(Eq. 2)}$$

where C' is the I concentration at time t in the postinfusion period, C'_0 is the I concentration at the time of discontinuation of infusion, and K_{el} is the first-order rate constant of elimination or excretion of I. The K_{el} of I was calculated from the slope ($-K_{el}/2.303$) of the least-squares straight line. The blood $t_{1/2}$ of the compound was calculated from $t_{1/2} = 0.693/K_{el}$.

The apparent volume of distribution, V_d, of I was calculated from:

$$V_d = \frac{k_0}{C'_0 K_{el}}(1 - e^{-K_{el}T}) \qquad \text{(Eq. 3)}$$

where k_0 is the infusion rate and C'_0 is the I concentration at time T of discontinuation of infusion. The value of C'_0 was obtained by extrapolating back to zero time the least-squares straight line obtained in Fig. 3. The values of the various pharmacokinetic parameters are listed in Table I.

Blood Level Kinetics of I after 1-hr Infusion in Presence of II—The apparent monoexponential decline of blood levels of I in the presence of II was observed (Fig. 3). The pharmacokinetic parameters calculated are listed in Table I.

DISCUSSION

Comparison of Urinary $t_{1/2}$ and Blood $t_{1/2}$ of I—In the previous single intravenous dose studies of I (1, 8, 10), the rats were not infused with normal saline for 1 hr; however, 5 ml of normal saline was injected intraperitoneally into each rat 20 min prior to intravenous I administration. Therefore, if any substantial difference were to occur between the I half-life determined in the single intravenous dose studies reported previously (1, 8, 10) and that determined in the present single dose studies of the saline-infused (control) rats, such a difference probably would be due to the surgical trauma and infusion procedure employed in the infusion studies.

Table I—Pharmacokinetic Parameters Determined for I from Postinfusion Blood Level Data Obtained in the Absence and Presence of II

	Without II	With II
Pharmacokinetic parameter		
C'_0, mg/ml	0.0842	0.0995
K_{el}, min^{-1}	0.0443	0.0373
$t_{1/2}$, min	15.6[a]	18.5[a]
V_d, ml/kg	281.1	273.0
Body clearance, ml min^{-1} kg^{-1}	12.5	10.2
Measures of fit		
Coefficient of determination (r^2)	0.996	0.996
Correlation coefficient	0.998	0.998

[a] For comparison, the blood $t_{1/2}$ values obtained for I in previous (8, 10) single intravenous dose studies in nonsurgical rats in the absence and presence of II were 14.1 and 16.9 min, respectively.

However, the average urinary $t_{1/2}$ (16.2 min) of I determined in the control rats was similar to that (16.7 min) reported previously (1) in the nonsurgical rats after administration of a single intravenous dose of I. Furthermore, the urinary $t_{1/2}$ (17.3 min) of I determined after infusing it for 10 min into rats, which were already infused with normal saline for 50 min, was not substantially greater than that determined in the previous single intravenous dose studies (1) performed in the nonsurgical rats. Additionally, the blood $t_{1/2}$ (15.6 min) and the body clearance (12.5 ml/min/kg) of I determined in the infusion studies also were similar (14.1 min; 12.1 ml/min/kg) to those noted for I in the previous (8) single intravenous dose studies in nonsurgical rats.

These observations suggested that the surgery and infusion procedures have little or no effect on the I half-lives and that the values of the urinary and blood half-lives obtained for I in the previous single intravenous dose studies in the nonsurgical rats can be used as the control values for the present study.

Comparison of Ratios of Urinary $t_{1/2}$ and Blood $t_{1/2}$ Values of I in Absence and Presence of II—The average blood $t_{1/2}$ or urinary $t_{1/2}$ of I in the presence of II was greater than that determined in the absence of II in the single intravenous dose study and in the infusion study. However, while the increase in blood $t_{1/2}$ of I observed in the infusion study with II was similar to that noted in the single intravenous dose study (Table I), the increase in the urinary $t_{1/2}$ of I observed in the presence of II in the infusion study was 1.8 times greater than that noted in the single intravenous dose study. Therefore, the increase observed in the blood $t_{1/2}$ of I in the presence of II either in the single intravenous dose study or in the infusion study primarily reflects the effect of inhibition of renal tubular secretion of I by II. However, the greater increase observed in the urinary $t_{1/2}$ of I in the presence of II in the infusion study, as compared to that observed in the single intravenous dose study, cannot be attributed merely to the inhibition of renal tubular secretion of I by II. Other factors such as retention or detention of I in the renal tubular membranes (1) and its distribution into the deeper tissues of the kidney (5, 6) may be responsible for this phenomenon.

Therefore, to reveal the role of these factors, although indirectly, the various ratios of the I half-lives were evaluated. Previous studies (1) with I and other model organic anions showed that the values of all four ratios listed in Table II were greater than unity for the organic anions. This

Table II—Various Ratios of Urinary $t_{1/2}$ and Blood $t_{1/2}$ Values Obtained in Infusion Studies and Single Intravenous Dose Studies of I in the Absence and Presence of II

Ratio	Infusion	Single Intravenous Dose
$\left(\frac{\text{urinary } t_{1/2}}{\text{blood } t_{1/2}}\right)_{\text{II absent}}$	1.58	1.18
$\left(\frac{\text{urinary } t_{1/2}}{\text{blood } t_{1/2}}\right)_{\text{II present}}$	3.08	1.24
$\frac{(\text{urinary } t_{1/2})_{\text{II present}}}{(\text{urinary } t_{1/2})_{\text{II absent}}}$	2.26	1.25
$\frac{(\text{blood } t_{1/2})_{\text{II present}}}{(\text{blood } t_{1/2})_{\text{II absent}}}$	1.19	1.20

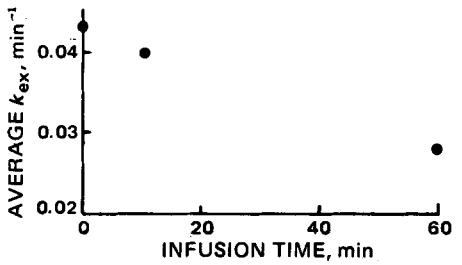

Figure 4—*Plot showing the effect of continuous intravenous infusion time on the postinfusion* k_{ex} *of I in rats.*

analysis led to the proposal (1) that once the organic anions entered the renal tubular membranes, they probably did not diffuse back into the blood compartment and that the renal tubular membranes did not constitute a part of the blood compartment or the peripheral tissue compartment for anions associated with multicompartment disposition characteristics.

The data for I (Table II) clearly indicate that, while the ratios of (blood $t_{1/2}$ with II/blood $t_{1/2}$ without II) observed in the infusion studies was practically identical to that noted in the single intravenous dose studies, the ratio of (urinary $t_{1/2}$ with II/urinary $t_{1/2}$ without II) observed in the infusion studies was almost two times greater than that in the single intravenous dose studies. Therefore, the possible accumulation of I in the renal tubular membranes was not only more pronounced when administered by continuous infusion but such retention occurred only in the eliminating organ, the kidney. The urinary $t_{1/2}$ data in Table II also prompted the suggestion that I diffuses into the deeper tissues in the kidney. This hypothesis appears to be supported by the fact that the ratio of the ratios expressed as (urinary $t_{1/2}$/blood $t_{1/2})_{\text{infusion}}$/(urinary $t_{1/2}$/blood $t_{1/2})_{\text{single iv dose}}$ observed for I in the presence of II was almost two times greater than that observed in the absence of II.

Effect of Infusion Time on k_{ex} of I—As pointed out earlier, the k_{ex} value of I in the 10-min infusion study was 0.0399 ± 0.0049 min^{-1}, which was significantly ($p < 0.01$) greater than that observed in the 1-hr infusion study. However, this k_{ex} value was significantly smaller than that of I determined in the single intravenous dose study only at a 75% confidence limit. There appears to be a trend to suggest that k_{ex} of I decreases with increasing infusion time (Fig. 4). Since the excretion rate constants of I were not determined at additional infusion times, it is not known whether a relationship between k_{ex} of I and its infusion time is linear over the infusion time of 0–60 min.

Possible Mechanism—The effect of length of the infusion period on the urinary $t_{1/2}$ or k_{ex} of I observed in the present study is parallel to the effect of length of the incubation period on the depth of uptake of *p*-aminohippurate by the rat kidney slice studies of Wedeen and Weiner (4–6). The mechanism they proposed, which accounts for the intrarenal distribution and localization of tritiated *p*-aminohippurate in the rat kidney slices, was that the uptake of this anion occurs primarily in the surface-lying tubules during the shorter incubation period but that the uptake occurs in the tubules in the deeper tissue region during prolonged incubation. It also was demonstrated (6) that, in the presence of even the nonmetabolic competitive inhibitors, although no uptake of *p*-aminohippurate occurred in the surface-lying tubules of the cortical slices, its uptake proceeded into the deeper proximal tubules.

In view of the proposed mechanisms (4–6), it seems reasonable to assume that I penetrates into the deeper lying proximal renal tubules upon its intravenous infusion for 1 hr and, because of its increased residence time in the kidney, results in a prolonged urinary $t_{1/2}$. Furthermore, in the presence of II, I probably is able to gain access to further deeper lying renal tubules, resulting in a prolonged urinary $t_{1/2}$.

Therefore, it may be hypothesized that I tends to accumulate temporarily in the surface-lying renal tubular cells after the administration of a single intravenous dose but that it tends to penetrate into the deeper renal tubular cells upon intravenous infusion, with the depth of cortical tissue involved in its uptake increasing with increasing infusion time. Consequently, uptake of the compound in the deeper lying tubules, together with its temporary retention in the renal tubular membranes during secretion, is reflected in the longer apparent half-life of the compound when determined from the urinary excretion data than when determined from the blood level data.

Practical Significance of Study—As noted previously (1), the apparent biological half-lives of drugs sometimes are longer in humans when determined from urinary excretion data than when determined from blood level data. The previous (1) and present studies with model organic anions suggest that if drugs that are subject to renal tubular secretion exhibit a greater urinary $t_{1/2}$ than blood $t_{1/2}$, it may be due to their accumulation in the renal tubular membranes and penetration into the deeper lying cortical renal tubules, especially in chronic drug therapy. Such pharmacokinetic disposition of some drugs may result in nephrotoxicity caused by interference by drug molecules in the integrity of the membrane structure and normal metabolic functions of tubular cells. Therefore, if a drug is suspected to be nephrotoxic, comparison of its urinary $t_{1/2}$ and blood $t_{1/2}$ values in test subjects may help to detect this possibility.

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