Rationale for Apparent Differences in Pharmacokinetic Aspects of Model Compounds Determined from Blood Level Data and Urinary Excretion Data in Rats

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Abstract □ Results of studies carried out in rats for model compounds, D-(−)-mandelic acid, benzoylformic acid, and some of their *para*-alkylated homologs, showed that their biological half-lives determined from the elimination phase of urinary excretion data were longer than those determined from the elimination phase of blood level data. With compounds that followed multicompartment open models, the initial distributive phase (α-phase) noted from the blood level data was not detected from the urinary excretion data. Based on the analysis of half-life data obtained in the absence and presence of DL-tropic acid (a competitive renal tubular secretion inhibitor of these compounds), it is proposed that, besides the shortness of the α-phase period, the factor accounting for these striking differences in the pharmacokinetic aspects of these compounds is their retention and/or detention in the renal tubular membranes during their tubular secretion. Furthermore, it is proposed that the renal tubular membranes do not constitute a part of the central or peripheral compartment. Examples are cited to show that, where studies are reported for the same drugs in the same human subjects in the same laboratory, drugs usually exhibit longer biological half-lives when urinary excretion data rather than blood level data are used.

Keyphrases □ Pharmacokinetics—model compounds, urinary excretion and blood level data compared, rats □ Elimination phase data—model compounds, urinary and blood level data compared, rats □ Biological half-lives—model compounds, determined from urinary excretion and blood level data and compared, rats

Upon comparing blood level data (1) with urinary excretion data (2–4) reported for the model compounds D-(−)-mandelic acid (I), D-(−)-*p*-methylmandelic acid (II), D-(−)-*p*-ethylmandelic acid (III), and D-(−)-*p*-isopropylmandelic acid (IV) in rats, striking differences became apparent with respect to two pharmacokinetic aspects.

1. The distributive phase noted from semilogarithmic plots of the blood concentration of the compound against time remained undetected in semilogarithmic plots of the urinary excretion rate of the compound against time. Accordingly, the two-compartment open model noted from blood level data reduced to a one-compartment open model when urinary excretion data were used.

2. The half-lives of the compounds, as determined from the elimination phase, were generally longer when determined from urinary excretion data rather than blood level data (Table I). Such apparent differences in these pharmacokinetic aspects were also observed for each compound in the presence of DL-tropic acid (V), a competitive inhibitor of renal tubular secretion of the compounds (2–6).

One purpose of this paper is to present the urinary excretion studies carried out in rats for additional model compounds, namely, benzoylformic acid (VI), *p*-methylbenzoylformic acid (VII), and *p*-ethylbenzoylformic acid (VIII). Their half-lives, as determined from the elimination phase, also were longer when calculated from urinary data rather than blood level data (1, 6), both in the absence and presence of V. Furthermore, the pharmacokinetics of VI, which were describable according to a three-compartment open model as evidenced from blood level studies (1, 6), reduced to a two-compartment open model when urinary excretion data were used. The pharmacokinetics of VII or VIII were describable according to a one-compartment open model (1, 6).

Another purpose of this report is to provide rationalization for the apparent differences noted in these pharmacokinetic aspects of I–IV and VI–VIII.

EXPERIMENTAL

Materials—The following were used: benzoylformic acid[1], mp 62–64°; *p*-methylbenzoylformic acid, mp 97–99°, synthesized by the method of Kindler *et al.* (7); *p*-ethylbenzoylformic acid, mp 65–67°, synthesized by the method of Kindler *et al.* (7); and DL-tropic acid, mp 118–120°.

Apparatus and Analytical Procedure—The GC procedure used for the quantitative determination of VI–VIII in rat urine was the same as described previously for mandelic acid and its *para*-alkylated homologs (2–4).

Methodology—The procedures for preparing rats and for urine collection following intravenous administration of VI–VIII were the same as described previously for I (2). Approximately 60 Sprague–Dawley male rats, 180–230 g (average, 200 g), were used repeatedly in all urinary excretion studies. The aqueous solution of the sodium salt of each compound used for intravenous administration was made isotonic, and the volume of the intravenous solution injected was 2 ml.

To determine the glomerular filtration rates[2] of the compound, the appropriate dose of the substrate compound (VI, VII, or VIII) and 3012 μmoles/kg of V as a renal tubular secretion inhibitor, both as their sodium salts contained in 2 ml, were injected intravenously to the rat. The intravenous dosage ranges used for VI, VII, and VIII were 166.65–333.3, 61.0–208.57, and 24.54–28.1 μmoles/kg, respectively[3].

RESULTS AND DISCUSSION

Ideal Properties—As reported previously (1, 6), VI–VIII are neither significantly bound to plasma proteins, metabolized, nor expected to be reabsorbed from the renal tubules; additionally, virtually 100% of the administered dose of each compound was recovered in the urine in the unchanged form. These properties of the compounds are considered ideal properties, since they facilitate interpretation of the data. Therefore, these compounds (pKa 3.2–3.3) are also referred to as "model" organic anions in view of the objective of the study.

Treatment of Urinary Excretion Data—Semilogarithmic plots of the excretion rate *versus* the midpoint time of the urine collection period were prepared for data obtained for each compound. The biological half-lives reported in this paper are referred to as the elimination phase half-lives, because these values were determined from the slopes of the straight lines (least squares) based on the data points obtained in the elimination phase noted in the urinary excretion or

[1] Aldrich Chemical Co., Milwaukee, Wis.

[2] The purpose of this determination will be discussed in a separate paper.

[3] The reason for employing a lower dosage range for VIII than that used for VI or VII will be dealt with in a separate paper.

Table I—Biological Half-Lives Calculated from the Elimination Phase for the Compounds Using Urinary Excretion Data and Blood Level Data Obtained in the Absence and Presence[a] of V in Rats

Compound	Biological Half-Life (DL-Tropic Acid Absent), min		Biological Half-Life (DL-Tropic Acid Present), min	
	Urinary	Blood	Urinary	Blood
I	30.0[b]	34.7[c]	46.0[b]	36.1[c]
II	17.0[d]	10.6[c]	30.0[d]	14.9[c]
III	13.0[d]	13.8[c]	26.0[d]	16.8[c]
IV	23.0[e]	20.4[c]	35.0[e]	22.7[c]
VI	42.0	33.7[c]	47.1	43.5[c]
VII	16.7	14.1[c]	21.0	16.9[c]
VIII	13.4	5.2[c]	25.6	9.4[c]

[a] A fixed dose of 3012 μmoles/kg iv of V was administered simultaneously with the appropriate smaller doses of each compound. [b] From Ref. 2. [c] From Refs. 1 and 6. [d] From Ref. 3. [e] From Ref. 4.

blood level studies. Of course, for compounds that showed a monoexponential decline in their blood levels or urinary excretion rates, all data points were in the elimination phase.

Urinary Excretion Kinetics—*Compound VI*—The kinetics of urinary excretion of VI were studied using three different intravenous doses, namely 166.65, 249.8, and 333.33 μmoles/kg, and the numbers of individual rat studies carried out at these dosage levels were 7, 6, and 14, respectively. Semilogarithmic plots of excretion rates *versus* time for each study indicated a multiexponential decline in the excretion rate, as expected from the blood level studies where the disappearance of the compound from the blood occurred triexponentially. However, the data points obtained (which ranged from three to six points) in an individual study were not sufficient to characterize the triexponential nature of the curve. Therefore, composite semilogarithmic plots were prepared for the data obtained at each dose; such plots are shown for two doses in Fig. 1.

Since, from the blood level data (1), the α-, β-, and γ-phases were identified for the time periods of 0–8, 12–20, and 20 min onward, respectively, the urinary excretion rate data obtained at each dose were feathered on the basis of these time periods. However, as illustrated in Fig. 1 for one dose, the existence of the α-phase was not revealed nor was the β-phase well characterized at any dose. However, the elimination phase was well defined, which facilitated calculation of the elimination phase half-life. The values of the biological half-lives thus obtained at three intravenous doses were similar (41–43.7 min), with the average value being 42 min (Table I). The half-life determined from the urinary excretion data was longer than that determined from the blood level data.

Compound VII—The kinetics of urinary excretion of VII were studied at intravenous doses ranging from 61.0 to 208.6 μmoles/kg, and 33 individual rat studies were performed. Semilogarithmic plots prepared for the data of each study indicated a monoexponential decline in the excretion rates of the compound, as expected from the blood level studies where the disappearance of the compound from the blood occurred monoexponentially (1). The typical apparent first-order plots obtained for the compound in this dosage range are presented in Fig. 2. The average biological half-life of the compound determined from the 33 studies was 16.7 ± 1.8 min, which was longer than that determined previously (1) from blood level data.

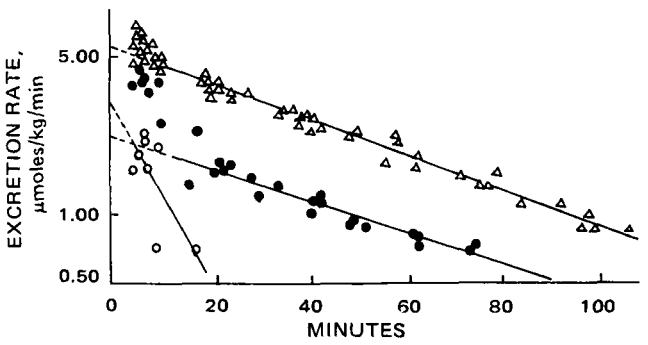


Figure 1—*Composite semilogarithmic plots of urinary excretion rate* versus *time constructed for data obtained for VI following fixed doses of 166.65 (●) and 333.33 (△) μmoles/kg iv to rats. The data points (○) obtained upon feathering are shown for the 166.65-μmoles/kg dose only.*

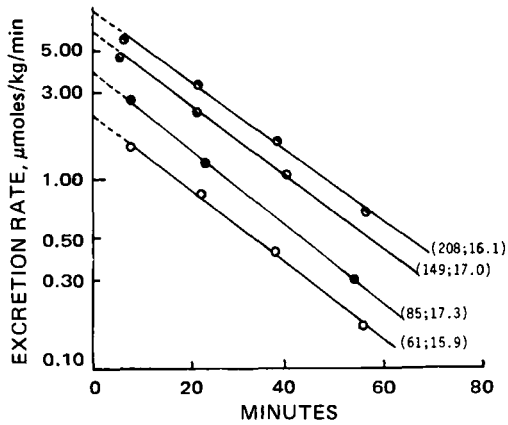


Figure 2—*Typical apparent first-order plots of log urinary excretion rate* versus *time obtained for VII following intravenous administration of various doses to rats. The numbers in parentheses refer to the intravenous dose of VII in micromoles per kilogram and its biological half-life in minutes, respectively.*

Compound VIII—The kinetics of urinary excretion of VIII were studied at the intravenous dose of 1 mg/rat. Depending on the body weight of the rats, this dose ranged from 25.54 to 28.1 μmoles/kg; only four individual rat studies were performed. Semilogarithmic plots prepared for the data of each study also indicated a monoexponential decline in the excretion rates of the compound, as expected from the blood level studies where the disappearance of the compound from the blood occurred monoexponentially even at an intravenous dose as high as 140.5 μmoles/kg (1). The typical apparent first-order plots obtained for the compound are shown in Fig. 3. The average biological half-life was 13.4 ± 1.6 min, which was longer than that determined previously (1) from blood level data.

Urinary Excretion Kinetics of VI–VIII in Presence of V—The original purpose of studying the kinetics of urinary excretion of VI, VII, or VIII in the presence of a simultaneously administered large intravenous dose (3012 μmoles/kg) of V was to determine the initial glomerular filtration rates of these compounds, which were necessary in the determination of the Michaelis–Menten kinetic parameters of renal tubular secretion of the compounds according to the methodology described previously (2–4). This objective was achieved for VII but not for VI and VIII.

Compound VII—The kinetics of urinary excretion of VII were studied following its intravenous administration at 55–192 μmoles/kg in the presence of 3012 μmoles/kg iv of V. This dose of V was employed because it was sufficient to inhibit the renal tubular secretion of I and its *para*-alkylated homologs (3, 4); 25 individual rat studies were carried out in this dosage range. As expected, the semilogarithmic plots prepared for the data of each study also indicated a monoexponential decline in the excretion rates of the compound; the typical apparent first-order plots obtained in this dosage range are presented in Fig. 4.

The apparent initial excretion rates obtained (from intercept values obtained upon extrapolating the least-squares straight lines such as those shown in Fig. 4) were plotted against the corresponding intravenous doses of the compound (Fig. 5). As seen in this figure, the ap-

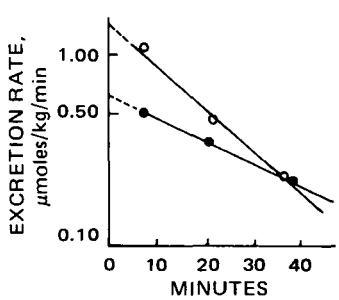


Figure 3—*Typical apparent first-order plots of log urinary excretion rate* versus *time obtained in rats for VIII following administration of 27.4 μmoles/kg iv in the absence of V (○) and 32.11 μmoles/kg iv in the presence of simultaneously administered 3012 μmoles/kg iv of V (●).*

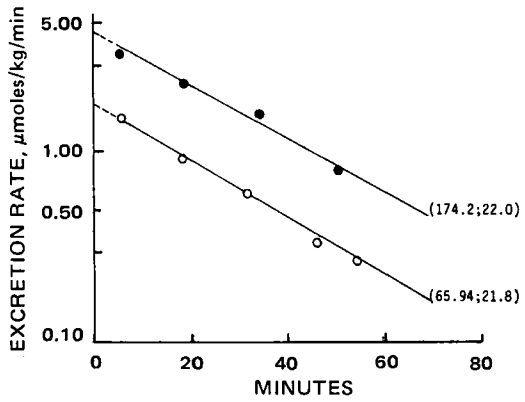


Figure 4—*Typical apparent first-order plots of log urinary excretion rate* versus *time obtained in rats for VII in the presence of simultaneously administered 3012 μmoles/kg iv of V. The numbers in parentheses refer to the intravenous dose of VII in micromoles per kilogram and its biological half-life in minutes, respectively.*

parent initial excretion rate of VII was directly proportional to its intravenous dose, indicating that the administered dose of V practically completely inhibited the renal tubular secretion of VII and that the apparent initial excretion rates of VII were due to its glomerular filtration rates, as shown for other compounds (3–5). On the basis of these data as well as the Michaelis–Menten kinetic parameters of renal tubular secretion[4] determined for VII, it was concluded that VII and V share the same pathway for their renal tubular secretion (3–5). The mean biological half-life of VII, owing to its elimination occurring by only the glomerular filtration process, was 21 min. In the presence of V, the half-life of the compound determined from urinary excretion data was indeed longer than that determined from blood level data (Table I).

Compound VI—The kinetics of urinary excretion of VI were studied at 166.65-, 249.8-, and 333.33-μmoles/kg doses, each in the presence of simultaneously administered 3012 μmoles/kg iv of V (six individual rat studies at each dose). For the reason stated previously, composite semilogarithmic plots were prepared for the data obtained at each dose; such plots are shown for two doses in Fig. 6. As can be seen upon feathering the data, as illustrated for one dose, the α-phase noted from the blood level data (5) remained undetected and the decline in the excretion rate appeared to occur biexponentially.

In contrast to the data obtained in the absence of V (Fig. 1), the distributive phase (Fig. 6) appeared to be fairly well defined in these studies. Since the initial excretion rates of the compound in the absence of V (Fig. 1) could not be determined, there was no interest or concern to determine the initial excretion rates of the compound in the presence of V (which actually are the initial glomerular filtration rates of the compound). The half-life values of the compound determined from the elimination phase at various doses were similar (average of 47.14 min). Since the half-life obtained in the presence of V was longer than that obtained in the absence of V, V probably inhibited the renal tubular secretion of VI and probably shared the same renal tubular secretion mechanism available for V (2). The half-life of the compound determined from the urinary excretion data was longer than that determined from the blood level data.

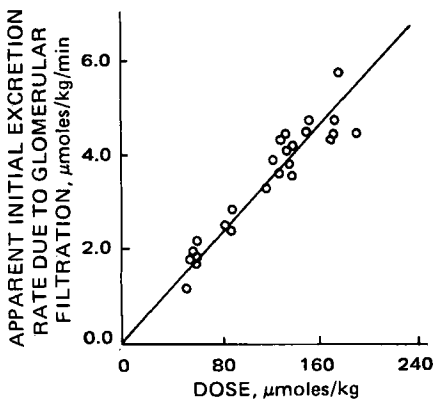


Figure 5—*Linear relationship between the apparent initial excretion rate due to glomerular filtration and the intravenous dose of VII.*

[4] To be discussed in a separate paper.

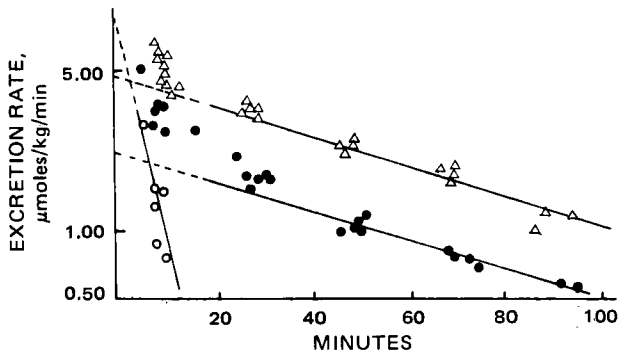


Figure 6—*Composite semilogarithmic plots of urinary excretion* versus *time for the data obtained in rats for VI following fixed doses of 166.65 (●) and 333.33 (△) μmoles/kg iv in the presence of simultaneously administered 3012 μmoles/kg iv of V. The data points (○) obtained upon feathering are shown for the 166.65-μmoles/kg dose of VI only.*

Compound VIII—The kinetics of urinary excretion of VIII were studied in the presence of V (3012 μmoles/kg) at one intravenous dose (1 mg/rat) only; three individual rat studies were performed. As expected, the decline in the excretion rate occurred monoexponentially (Fig. 3), and the half-life (25.6 min) was longer than that observed in the absence of V. This finding indicated that the compound probably shared the same renal tubular secretion mechanism that was available for V. Furthermore, the half-life of the compound determined from urinary excretion data was longer than that determined from blood level data.

Rationale for Apparent Differences Observed in Pharmacokinetic Aspects—The most probable reasons for the distributive phases to remain undetected in the urinary excretion studies are as follows.

First, as determined from the blood level data (1), the distributive phases for I–IV (which follow a two-compartment open system) lasted for 4–8 min. Therefore, unless at least three or four urine samples are collected during this period, the distributive phase cannot be characterized. Since the first urine samples that can be conveniently obtained from the rats without catheterizing their ureters are generally 10–12 min after the intravenous administration of the compounds, only one urine sample can be obtained from each rat to reflect partly the excretion rate during the distributive phase. Consequently, such a data point is likely to be equally influenced by elimination phase kinetics of the compound; subsequent urine samples would undoubtedly represent the data points due to the elimination phase. Therefore, it was not surprising that Nagwekar and coworkers (2–5) did not detect the distributive phase from the urinary excretion rate studies of I–IV in rats and treated urinary excretion data according to a one-compartment open model.

If urine samples are obtained at 1-min intervals by catheterizing the ureter of a rat, it might be possible to characterize the distributive phase. But this may not be possible if there is a delay time up to 4 min from glomerular filtration to the appearance of drug in the collecting vessel, as demonstrated (8) in rats for inulin and urea. When urine samples were collected from catheterized rats at 2-min intervals following the administration of inulin or urea, the peak rates of excretion of these compounds were noted in the third urine sample (at the 6th min) (8). In view of this observation made by Bourne and Barber (8), the distributive phase for a compound probably cannot be characterized from urinary excretion rate studies, especially when the duration of the distributive phase is 4–8 min as observed for I–IV from the blood level data.

For the case following a three-compartment open model, the duration of the α-phase was up to 8 min and the duration of the β-phase was noticeable from 12 to 20 min following the intravenous administration of the compound. Since the α-phase lasted for only 8 min, this phase, as explained for I–IV, could not be detected from the urinary excretion studies. Therefore, as seen in Figs. 1 and 6, the

Table II—Ratios of Elimination Phase Biological Half-Lives ($t_{1/2}$) of the Compounds Based on Data Presented in Table I

Compound	Urinary $t_{1/2}$ / Blood $t_{1/2}$ (V Present)	Urinary $t_{1/2}$ / Blood $t_{1/2}$ (V Absent)	(Urinary $t_{1/2}$) V Present / (Urinary $t_{1/2}$) V Absent	(Blood $t_{1/2}$) V Present / (Blood $t_{1/2}$) V Absent
I	1.27	0.90	1.35	1.04
II	2.01	1.60	1.76	1.40
III	1.54	0.94	2.00	1.21
IV	1.54	1.13	1.52	1.11
VI	1.08	1.25	1.12	1.29
VII	1.24	1.18	1.25	1.20
VIII	2.72	2.57	1.92	1.80

urinary excretion data appear to be describable by a two-compartment open model.

The second possible reason that may explain the absence of the distributive phase with I–IV and VI, as noted from urinary excretion data, is that there might be detention and/or retention of the compounds in the renal tubular membrane during their renal tubular secretion. The renal tubular secretion process mediated by the component of the tubular membrane that serves as a secretion carrier may be described by Scheme I, where D^- is the negatively charged compound, and C^+ is a positively charged carrier of the renal tubular membrane which complexes with the drug within the membrane to yield the complex D^-C^+. This complex diffuses across the membrane to the inner surface of the membrane.

The molecules of all compounds are expected to remain in the biological fluid in the ionic form, D^-, because their pKa values are low (3.2–3.7). In the transmembrane transport of the compounds, the role of proteins and/or phospholipids has been implicated (4, 5, 9, 10). Since the outer surface of the membrane is covered by proteins, the permeant molecules possibly could be adsorbed by the proteins, followed by desorption into the renal tubular membrane where the permeant molecules could complex with the positively charged carrier, possibly the phospholipids. The drug–carrier complex then diffuses to the inner surface of the membrane where the dissociation of the complex occurs. The permeant molecules thus released are adsorbed by the inner layer of protein and eventually desorbed into the tubular lumen fluid. The rate-determining step assumed in this scheme is the formation of the D^-C^+ complex.

The retention of the compounds in the renal tubular membrane, in the presence of an inhibitor (V), can be explained by Scheme II, where I^- is the competitive inhibitor of D^-, and I^-C^+ is the inhibitor–carrier complex. According to this scheme, the presence of a large concentration of the inhibitor (V) competitively inhibits the renal tubular secretion of the substrate compound, D^-.

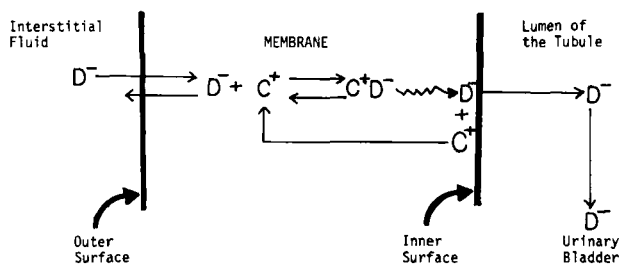
As noted in Table I, in studies involving V, the biological half-lives of the substrate compounds determined from urinary excretion data were longer than those determined from blood level data. Similarly, in studies carried out in the absence of V, the biological half-lives of the compounds, except I and III, determined from urinary excretion data were longer than those determined from blood level data. These effects can be explained also in terms of the two factors discussed previously to account for the disappearance of the distributive phases of these compounds.

Schemes I and II lead to the assumption that, for these compounds, the renal tubular membranes are part of neither the central compartment nor the peripheral compartment(s) of the body. However, this statement does not imply that the rest of the kidney organ is also not a part of the central or peripheral compartments. This situation is analogous to the fact that drug present in the GI fluid is considered neither a part of the central compartment nor the peripheral compartment of the body. Therefore, the molecules of the compounds, appearing in the renal tubular membranes after desorption from the proteins of the outer surface of the membranes, are considered to be lost from the body. Furthermore, since these molecules are retained and/or detained temporarily within the membrane as a result of their relatively slower rates of entering the lumen of the tubules, the biological half-life of the compound can be expected to be shorter when determined from blood level data rather than urinary excretion data. This hypothesis gains support from the fact that the ratios of "urinary" biological half-life to "blood" half-life of the compounds calculated on the basis of data obtained in the presence of V are, for all practical purposes, greater than such ratios calculated for the compounds on the basis of data obtained in the absence of V (Table II).

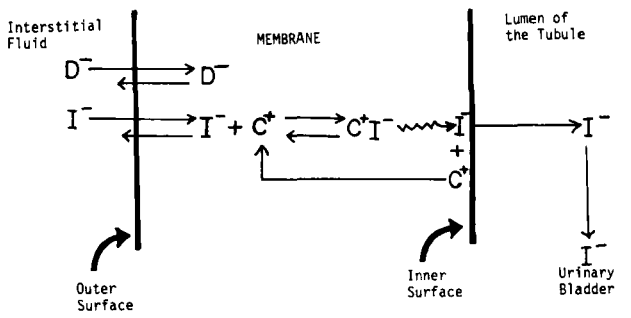
Furthermore, for these compounds, the ratios of urinary biological half-life in the presence of V to that in the absence of V are, for all practical purposes, greater than the ratios of blood biological half-life in the presence of V to that in the absence of V (Table II). This observation also may be explained in light of the proposed theory that the competition of V with the substrate compounds for the carrier molecules within the renal tubular membrane can be expected to be greater than for the adsorption sites at the outer surface of the renal tubular membrane, since the number of carrier molecules is much more limited than that of the adsorption sites. The inherent suggestions made here are that: (*a*) the competition offered by V (because of its higher concentration) to the substrate compounds for gaining access from the central compartment to the renal tubular membrane is probably much less (and for some compounds insignificant) than to their association with the renal tubular secretion carrier molecules present within the tubular membrane, and (*b*) the substrate compound accumulated within the membrane does not readily diffuse back into the central compartment to reflect prolongation in the half-life of the compound when determined from blood level data to the same degree as when determined from urinary excretion data.

Examples from Literature—Although scientific literature is deluged with reports of pharmacokinetic studies of drugs in humans and animals, the half-lives of drugs usually were determined from either blood level data or urinary excretion data. However, in some studies the half-lives of the same drugs were determined in the same subjects in the same laboratory from both urinary excretion data and blood level data and, therefore, allow comparison of the biological half-lives determined from these two kinds of data (Table III). These data for the drugs were, of course, obtained in the absence of other drugs.

According to Table III, the biological half-lives (elimination phase) of these drugs tend to be longer when determined from urinary excretion data than from blood level data. Because these drugs do not



Scheme I



Scheme II

Table III—Ratios of Urinary Biological Half-Life to Blood Biological Half-Life Calculated for Drugs Based on Human Data[a] Reported in the Literature

Drug	Urinary $t_{1/2}$ / Blood $t_{1/2}$	Reference
Oxacillin	1.42	11
Ampicillin	1.10	11
Penicillin G	1.14	11
Methicillin	1.16	11
Dicloxacillin	1.07	11
Chlorpropamide	1.08	12
Acetylstrophanthidin	1.08	13
Pindolol	1.17	14
Pindolol (oral)	1.48	14
Dextroamphetamine (oral)	1.1	15

[a] Unless indicated otherwise, the route for drug administration used in these studies was intravenous.

possess the "ideal properties" described previously, one or several factors might contribute to yield longer biological half-lives when determined from urinary excretion data. However, since most of these drugs are secreted by renal tubules, the differences noted in the biological half-lives for these drugs may be explained in terms of the theory proposed here.

Significance of the Study—Other investigators have pointed out that pharmacokinetic analyses of drugs based on their urinary excretion data may not necessarily reflect their time course in the blood, especially at the initial time period, due to such factors as the metabolism of drugs in the kidney (16), insufficient frequency of collecting urine samples at the initial period following intravenous drug administration (17), overlooking of an important anatomical fact concerning the kidney with regards to kinetics of renal tubular secretion of drugs (18), and significant "lag time" for drug to appear in the urine (16, 19). The additional factor proposed from the analysis of the data presented in this paper is that, for drugs secreted by renal tubules, the accumulation of the drug may occur in the renal tubular membrane (which may not constitute a part of the central compartment or the peripheral compartment) and thereby cause the biological half-lives to be longer than those determined from blood level data. This interpretation is facilitated primarily because the compounds used in the study possess ideal properties, as described previously.

This study also shows that, if accumulation of drugs secreted by renal tubules occurs in the renal tubular membrane, especially in the presence of a renal tubular secretion inhibitor of the drugs, their chronic use in patients might result in nephrotoxicity due to interference by drug molecules in the integrity of the membrane structure (in terms of membrane porosity, *etc.*) and normal metabolic functions of tubular cells. Therefore, the optimum dosage regimen for certain drugs known or suspected to be nephrotoxic should be designed by considering their half-lives determined from urinary excretion data as well as blood level data in addition to other pharmacokinetic parameters.

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ACKNOWLEDGMENTS AND ADDRESSES

Received August 11, 1975, from the *Pharmaceutics Division, College of Pharmacy and Allied Health Professions, Wayne State University, Detroit, MI 48202*

Accepted for publication November 21, 1975.

Abstracted in part from a dissertation submitted by Y. M. Amin to the Graduate Division, Wayne State University, in partial fulfillment of the Doctor of Philosophy degree requirements.

* Recipient of a Wayne State University Graduate Fellowship, 1973–1974.

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