Mechanistic Evaluation of Modifications of Distribution Pharmacokinetic Parameters of Model Organic Anions in Presence of a Model Renal Tubular Secretion Inhibitor in Rats

YASHWANT M. AMIN * and JANARDAN B. NAGWEKAR ˣ

Abstract □ The effects of DL-tropic acid (VIII) on the distribution pharmacokinetic parameters of the model compounds benzoylformic acid (I), *p*-methylbenzoylformic acid (II), *p*-ethylbenzoylformic acid (III), D-(−)-mandelic acid (IV), D-(−)-*p*-methylmandelic acid (V), D-(−)-*p*-ethylmandelic acid (VI), and D-(−)-*p*-isopropylmandelic acid (VII) were studied in rats. Since VIII is a competitive inhibitor of renal tubular secretion of I–VII and since all of these compounds (I–VIII) are negligibly bound to plasma proteins and are neither metabolized nor reabsorbed from the renal tubules, they were considered as model compounds. Therefore, changes observed in the values of the distribution pharmacokinetic parameters of I–VII were attributed to the influence of VIII on the transmembrane transport of the compounds between body compartments in rats. The decrease in the apparent volumes of the central compartments for I, IV, and VII, the increase in the apparent volumes of the peripheral compartments for IV–VII, the absence of change in the volumes of the central or peripheral compartments for the other compounds, and the increase in the ratios of the rate constants of the transfer of compounds from one compartment into another for I and IV–VII were explained in terms of the "aqueous pore" mechanism for the transmembrane transport of the anions of the compounds as well as the heteroporosity of the tissue membranes.

Keyphrases □ Pharmacokinetics—distribution parameters, mechanistic evaluation of modifications, model organic anions in presence of model renal tubular secretion inhibitor, rats □ Distribution—pharmacokinetic parameters, mechanistic evaluation, model organic anions in presence of model renal tubular secretion inhibitor, rats □ Transport—mechanistic evaluation of modifications of distribution pharmacokinetic parameters, model organic anions in presence of model renal tubular secretion inhibitor, rats

It was recently shown (1, 2) that a renal tubular secretion inhibitor such as probenecid increases the blood levels of penicillins not only by depressing their renal tubular secretion but also by decreasing the volumes of body central compartments for the drugs. The reason suggested (2) to account for the decrease in the volume of distribution was that the presence of probenecid caused an increase in the plasma protein binding of these antibiotics and/or limited their penetration or distribution into certain body compartments. It was further speculated that the higher rate constant of metabolism noted for the antibiotics in the presence of probenecid was due to the decrease in the apparent volume of distribution, which resulted in presenting a larger fraction of the drug at the site of metabolism.

If a drug is subjected simultaneously to metabolism, protein binding, and renal tubular secretion, it is difficult to isolate the effect of the renal tubular secretion inhibitor on its distribution pharmacokinetic parameters, especially if the renal tubular inhibitor can displace the drug from its protein binding sites or interfere with its metabolism. Therefore, if one intends to determine specifically the effect of the renal tubular secretion inhibitor on the distribution pharmacokinetic parameters of substrate compounds, it is advantageous to have compounds and their renal tubular secretion inhibitor that are neither bound to plasma proteins, metabolized, nor reabsorbed from the renal tubules. In addition, they should be completely recovered in the urine.

Since benzoylformic acid, D-(−)-mandelic acid, and their respective *para*-alkylated homologs possess such desirable properties (3–7) and DL-tropic acid, which is also a homolog of D-(−)-mandelic acid and possesses these ideal properties, is a competitive renal tubular secretion inhibitor of D-(−)-mandelic acid and its *para*-alkylated homologs (3–6) and of benzoylformic acid and its *para*-alkylated homologs (8), the purpose of this study was to determine in rats the effect of DL-tropic acid (VIII) on the distribution pharmacokinetic parameters of benzoylformic acid (I), *p*-methylbenzoylformic acid (II), *p*-ethylbenzoyl-

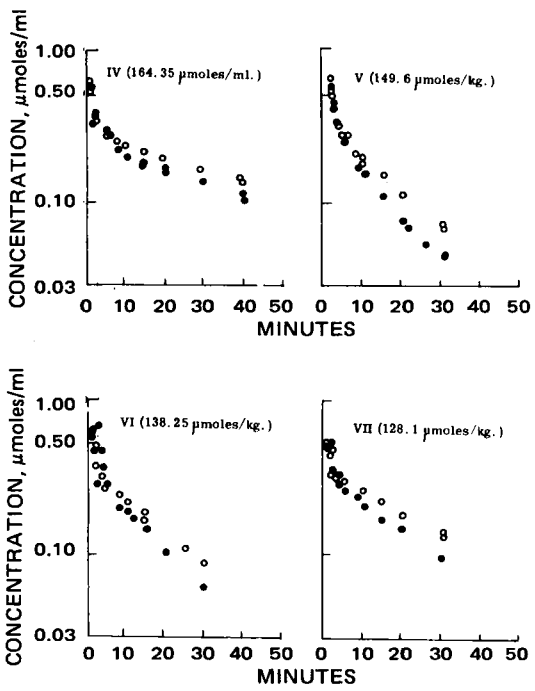

Figure 1—*Comparative semilogarithmic plots showing biexponential decline of blood levels of IV–VII in rats in the absence* (●) *and in the presence* (○) *of a 3012-μmoles/kg iv dose of VIII. The number in each parentheses refers to the intravenous dose of the respective compound. Each data point represents the concentration of the compound noted in a given rat, as explained in the text.*

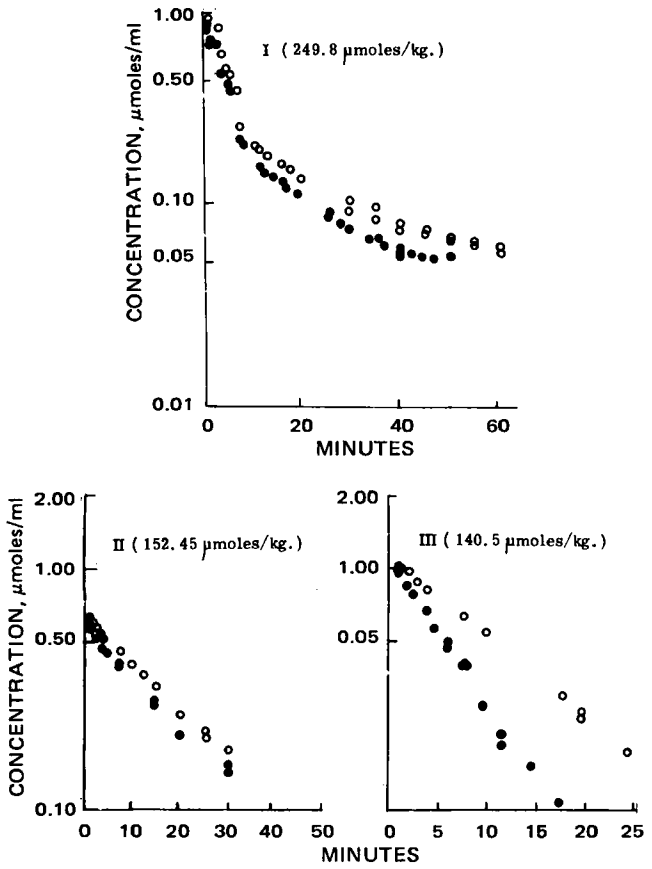

Figure 2—*Comparative semilogarithmic plots showing triexponential decline in blood levels of I and monoexponential decline in blood levels of II and III in rats in the absence (●) and in the presence (○) of a 3012-μmoles/kg iv dose of VIII. The number in parentheses refers to the intravenous dose of the respective compound. Each data point represents the concentration of the compound noted in a given rat, as explained in the text.*

formic acid (III), D-(−)-mandelic acid (IV), D-(−)-*p*-methylmandelic acid (V), D-(−)-*p*-ethylmandelic acid (VI), and D-(−)-*p*-isopropylmandelic acid (VII).

Furthermore, since the pKa values of these compounds are in the 3.2–4.2 range, these compounds are expected to remain in the systemic body fluids only in the anionic form. Consequently, any changes observed in the values of the distribution pharmacokinetic parameters of these anions in the presence of VIII (anionic) can be attributed to the influence of VIII on the transmembrane transport of these compounds between the body compartments in rats. As discussed previously (7), the distribution pharmacokinetic parameters considered for the compounds exhibiting a multicompartment open-model system are the apparent volumes of the central compartment (V_1), the apparent volumes of the peripheral compartments (V_2 and V_3), and the ratios of the apparent first-order rate constants of transport of compounds from one compartment into another. For those compounds that follow the one-compartment open model, the apparent volume of distribution (V_d) is considered.

It is anticipated that, in light of the mechanism suggested (7) for reversible transmembrane transport between the central and peripheral compartments, it

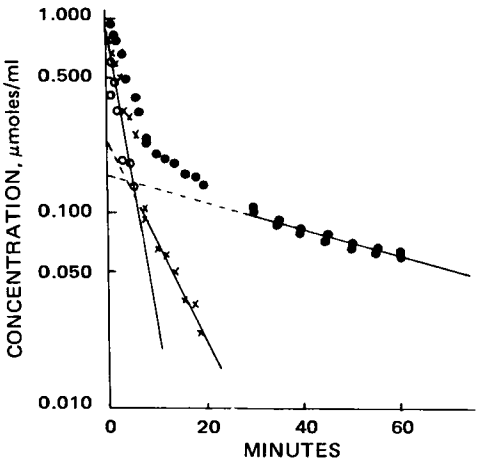

Figure 3—*Plot showing triexponential decline of blood levels of I following the administration of a 249.8-μmoles/kg iv dose per rat in the presence of a simultaneously administered 3012-μmoles/kg dose of VIII. Each data point represents the concentration of the compound noted in a given rat, as explained in the text. Key: ●, experimental data points; and ×, ○, data points obtained upon feathering.*

is possible to rationalize mechanistically the effect(s) of VIII that may be observed on the distribution pharmacokinetic parameters of I–VII.

EXPERIMENTAL

Materials—Compounds I–VII were the same as described previously (7). In addition, DL-tropic acid[1], mp 117–119°, was used.

Methodology—The methodology followed was described previously (7), except that 2 ml of the intravenous solution, containing an appropriate dose of one of the seven substrate compounds (I–VII), also contained 3012 μmoles/kg of VIII. Compound VIII served as a renal tubular secretion inhibitor of the substrate compounds.

The intravenous doses of the substrate compounds administered were 165.65[2], 249.8, and 333.33 μmoles/kg for I; 152.45 μmoles/kg[2] for II; 140.5 μmoles/kg[2] for III; 164.35 μmoles/kg[2] for IV; 149.6 μmoles/kg[2] for V; 138.25 μmoles/kg[2] for VI; and 128.1 μmoles/kg[2] for VII. Each compound (I–VIII) was injected as its sodium salt. The fact that a 3012-μmoles/kg iv dose[3] of VIII completely inhibits renal tubular secretion of I–III (7) and IV–VII (3–6) has been demonstrated already.

GLC Analysis—The equipment and procedure used to analyze I–VIII gas chromatographically in blood were the same as those described previously (7).

Binding of I to Whole Rat Blood and Plasma Proteins in Presence of VIII—The equilibrium dialysis procedure used for determining the binding of I to whole rat blood and plasma proteins was the same as that described previously (3–6), except that, along with 2 mg of I, the 4 ml of blood or plasma contained 40 mg of VIII. Since the binding of the other model compounds was considered negligible, no binding of those compounds was studied in the presence of VIII.

RESULTS AND DISCUSSION

Effect of VIII on Distribution Pharmacokinetic Parameters of I and IV and Their Respective Homologs—The blood level data obtained for the compounds in this study are presented in Figs. 1 and 2, along with those reported previously (7) for the same compounds in the absence of VIII. As illustrated in Figs. 3 and 4 for I and IV, respectively, the observed kinetic pattern of disappearance of each compound from the blood was the same as that noted in the absence of VIII. Initial estimates for the phar-

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] These doses represent 25 mg/kg.
[3] This dose represents 500 mg/kg.

Table I—Pharmacokinetic Parameters Estimated and Derived for the Three-Compartment Open Model by Nonlinear Least-Squares Fitting (NONLIN) of Whole Blood Concentration Data Obtained for I in Rats in the Presence of a Simultaneously Administered 3012-μmoles/kg iv Dose of VIII

Pharmacokinetic Parameters[a]	Average[a] of Three Studies in the 166.65–333.33-μmoles/kg Dosage Range
Estimated Parameters	
k_{12}, min^{-1}	0.0750
k_{21}, min^{-1}	0.1177
k_{13}, min^{-1}	0.1101
k_{31}, min^{-1}	0.0467
k_{el}, min^{-1}	0.0944
α, min^{-1}	0.3455
β, min^{-1}	0.0821
γ, min^{-1}	0.0163
V_1, ml/kg	181.45
Derived Parameters	
V_2, ml/kg	180.05
V_3, ml/kg	704.95
f_c	0.172
k_{12}/k_{21}	0.709
k_{13}/k_{31}	2.500
$t_{½\gamma}$, min	43.57

[a] Average of pharmacokinetic parameters determined for the data obtained at each dosage level study. The coefficients of determination (r^2) for the data at 166.65-, 249.80-, and 333.33-μmoles/kg doses were 0.997, 0.996, and 0.993, respectively; the correlation coefficients were 0.997, 0.997, and 0.994, respectively.

macokinetic parameters were obtained by use of appropriate equations as discussed previously. The data were found to fit very well to a three-compartment open model for I, to a two-compartment open model for IV and its *para*-alkylated homologs, and to a one-compartment open model for II and III, as indicated by the coefficients of determinations and correlation coefficients (Tables I–III).

The comparative plots (Figs. 1 and 2) of concentration *versus* time for these compounds showed that VIII elevated the blood levels of each substrate compound, as expected. The comparative data concerning the distribution pharmacokinetic parameters for I–VII, both in the absence and presence of VIII, are presented in Tables IV and V. As noted (7) in the absence of VIII, no apparent dose-dependent pharmacokinetics were observed for I in the intravenous dosage range of 165.65–333.33 μmoles/kg in the presence of VIII (Fig. 5).

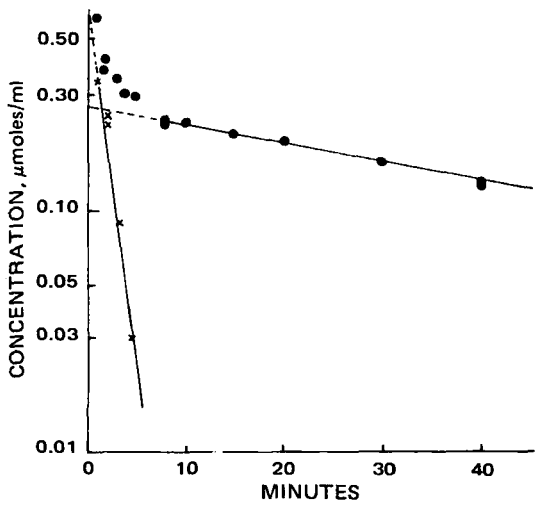

Figure 4—*Plot showing biexponential decline of blood levels of IV following the administration of a 164.35-μmoles/kg iv dose per rat in the presence of a simultaneously administered 3012-μmoles/kg dose of VIII. Each data point represents the concentration of the compound noted in a given rat, as explained in the text. Key:* ●, *experimental data points; and* ×, *data points obtained upon feathering.*

Table II—Pharmacokinetic Parameters Determined for II (152.45 μmoles/kg iv) and III (140.5 μmoles/kg iv) from Blood Level Data Obtained in Rats in the Presence of a Simultaneously Administered 3012.0-μmoles/kg iv Dose of VIII

	II	III
Pharmacokinetic Parameters		
C_b^0, μmoles/ml	0.6152	1.13
k_{el}, min^{-1}	0.0410	0.0737
$t_{½}$, min	16.9	9.4
V_d, ml/kg	246.80	125.20
Measures of Fit		
Coefficient of determination (r^2)	0.986	0.999
Correlation coefficient	0.993	0.999

Effect on IV and Its *para*-Alkylated Homologs—A comparison of the distribution pharmacokinetic parameter data (Table IV) for IV and its *para*-alkylated homologs shows that the volumes of the peripheral compartments and the ratios of k_{12}/k_{21} increased in the presence of a large dose of VIII. Furthermore, while the apparent volume of the central compartment slightly decreased (about 15–20%) for IV and VII, it remained practically unchanged for V and VI. These effects of VIII may be explained in terms of the aqueous pore diffusion mechanism previously advocated (7) for the transmembrane transport of these compounds.

It is reasonable to expect that VIII (pKa = 4.2), which is also a homolog of IV, should follow the same pathway for its transmembrane transport between the body compartments as do IV and its *para*-alkylated homologs. To facilitate comparison, the pharmacokinetics of VIII were studied following administration of 150.45 μmoles/kg iv. The blood level data obtained are presented in Fig. 6; the disappearance of the compound from the blood is also describable by a two-compartment open model. The initial estimates of the appropriate pharmacokinetic parameters of the compound were obtained and used to refine the values after fitting the data according to the two-compartment open model, using the NONLIN least-squares program (Table VI).

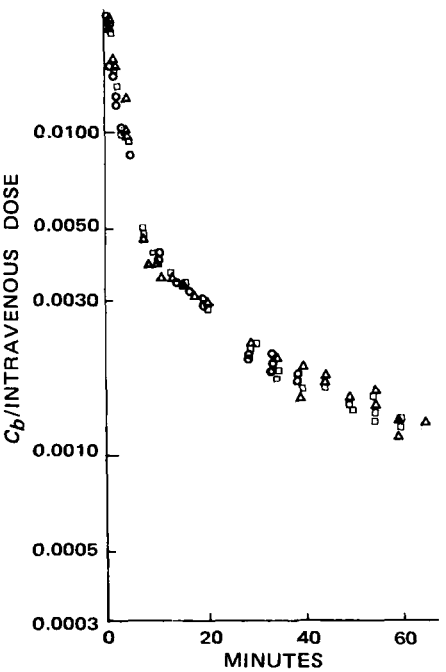

Figure 5—*Superposition plot of* C_b*/intravenous dose* versus *time indicating apparent absence of dose-dependent pharmacokinetics for I in the 166.65–333.33-μmoles/kg dosage range when simultaneously administered to rats in the presence of a 3012-μmoles/kg dose of VIII. Key:* ○, *166.65-μmoles/kg iv dose;* □, *249.80-μmoles/kg iv dose; and* △, *333.33-μmoles/kg iv dose.*

Table III—Pharmacokinetic Parameters Estimated and Derived for the Two-Compartment Open Model by Nonlinear Least-Squares Fitting (NONLIN) of Whole Blood Concentration Data Obtained for IV and Its *para*-Alkylated Homologs in the Presence of a Simultaneously Administered 3012-μmoles/kg iv Dose of VIII

Pharmacokinetic Parameters	IV (164.35 μmoles/kg)	V (149.60 μmoles/kg)	VI (138.25 μmoles/kg)	VII (128.10 μmoles/kg)
Estimated Parameters				
C_b^0, μmoles/ml	1.0300	0.8994	0.8623	0.8521
	(0.036)[a]	(0.028)	(0.025)	(0.028)
k_{12}, min^{-1}	0.5282	0.2601	0.2506	0.5133
	(0.027)	(0.020)	(0.018)	(0.030)
k_{21}, min^{-1}	0.0648	0.1217	0.1028	0.0707
	(0.002)	(0.004)	(0.004)	(0.005)
k_{el}, min^{-1}	0.2417	0.2071	0.2090	0.4203
	(0.011)	(0.018)	(0.018)	(0.028)
α, min^{-1}	0.8155	0.5423	0.5212	0.9737
β, min^{-1}	0.0192	0.0465	0.0412	0.0305
Derived Parameters				
V_1, ml/kg	159.55	166.30	160.15	150.35
V_2, ml/kg	1301.40	355.45	390.45	1092.20
k_{12}/k_{21}	8.15	2.14	2.44	7.27
f_c	0.079	0.224	0.197	0.072
$t_{1/2\beta}$, min	36.10	14.90	16.80	22.70
Measures of Fit				
Coefficient of determination (r^2)	0.997	0.998	0.998	0.999
Correlation coefficient	0.999	0.997	0.996	0.996

[a] The number in parentheses refers to the standard deviation of the corresponding parameter.

Table IV—Distribution Pharmacokinetic Parameters Obtained for IV–VII in Rats in the Absence and Presence of VIII

	IV		V		VI		VII	
Pharmacokinetic Parameters	VIII Absent[a]	VIII Present	VIII Absent[a]	VIII Present	VIII Absent[a]	VIII Present	VIII Absent[a]	VIII Present
V_1, ml/kg	192.5	159.5	164.9	166.3	149.9	160.1	188.2	150.3
V_2, ml/kg	1121.2	1301.4	217.7	355.4	283.6	390.4	428.9	1092.2
k_{12}/k_{21}	5.8	8.1	1.3	2.1	1.8	2.4	2.2	7.2

[a] Data obtained from Ref. 7.

Since the molecules of VIII are expected to follow the same pathway for their transport from the central compartment to the peripheral compartment, it is conceivable that these molecules would compete for the same sites of adsorption in the aqueous pore lining as do IV and its *para*-alkylated homologs, especially when a large dose of VIII is administered along with a much smaller dose of each substrate compound. In this event, it is not unlikely that the substrate molecules of IV or any of its homologs would find less resistance for their transport through the aqueous pores and, consequently, exhibit higher values not only of the volumes of peripheral compartments but also of the ratios of the distribution rate constants (k_{12}/k_{21}). Another factor that may be expected to contribute to the increased volume of the peripheral compartment is the increase in the $t_{1/2\beta}$ (Table III), which would tend to favor an increase in time to allow further distribution or penetration of the compound into the peripheral compartment.

The decrease in the volume of the central compartment for IV and VII in the presence of VIII may be explained on the basis of the heteroporosity of the membrane of the tissues of the central compartment (7). As pointed out previously (7), a substantial percentage of the total pores of these membranes may be about 8 Å in diameter, which probably would allow passage of only one molecule at a time through a pore. Therefore, when a large excess of the molecules of VIII are present in the central compartment, these molecules would predominantly occupy the small size pores and prevent access to the substrate molecules of IV or any of its *para*-alkylated homologs, thus diminishing the volume of the central compartment for the substrate compound. Since the molecules of V and VI are indicated to have little or no access through the small

Table V—Distribution Pharmacokinetic Parameters Obtained for I in Rats in the Absence and Presence of VIII

Pharmacokinetic Parameters	VIII Absent[a]	VIII Present
V_1, ml/kg	227.40	181.55
V_2, ml/kg	110.00	180.10
V_3, ml/kg	792.80	705.00
$V_2 + V_3$, ml/kg	902.80	885.00
k_{12}/k_{21}	0.418	0.709
k_{13}/k_{31}	2.067	2.500

[a] Data obtained from Ref. 7.

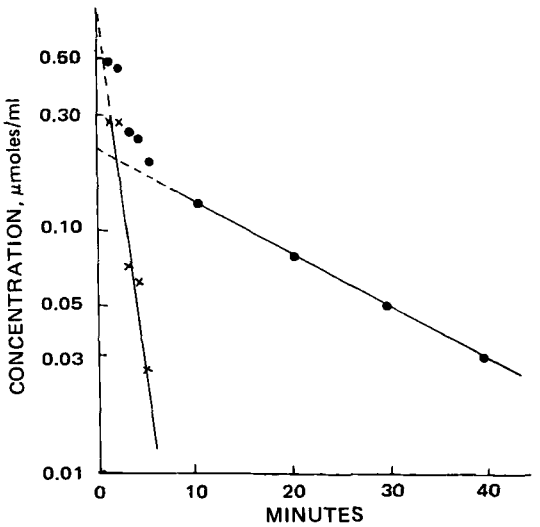

Figure 6—*Plot showing biexponential decline of blood levels of VIII following the administration of a 150.45-μmoles/kg iv dose per rat. Each data point represents the concentration of the compound noted in a given rat, as explained in the text. Key:* ●, *experimental points; and* ×, *data points obtained upon feathering.*

Table VI—Pharmacokinetic Parameters Estimated and Derived for the Two-Compartment Open Model by Nonlinear Least-Squares Fitting (NONLIN) of Whole Blood Concentrations Data Obtained for VIII in Rats

Pharmacokinetic Parameters	Dose (150.45 μmoles/kg iv)
Estimated Parameters	
C_b^0, μmoles/ml	0.7422 (0.067)[a]
k_{12}, min^{-1}	0.2508 (0.026)
k_{21}, min^{-1}	0.1396 (0.021)
k_{el}, min^{-1}	0.1691 (0.040)
α, min^{-1}	0.5135
β, min^{-1}	0.0460
Derived Parameters	
V_1, ml/kg	202.7
V_2, ml/kg	364.0
k_{12}/k_{21}	1.8
f_c	0.272
$t_{½\beta}$, min	15.1
Measures of Fit	
Coefficient of determination (r^2)	0.982
Correlation coefficient	0.987

[a] The number in parentheses refers to the standard deviation of the corresponding parameter.

size pores previously mentioned, the volume of the central compartment for these compounds in the presence of an excessively large number of molecules of VIII can be expected to remain unchanged.

Effect on I—Comparison of the distribution pharmacokinetic parameter data (Table V) for I in the presence of VIII shows that the volume of the central compartment, the volume of peripheral compartment 1 (V_2), and the volume of peripheral compartment 2 (V_3) decreased, increased, and practically remained unchanged, respectively. The decrease in the volume of the central compartment for I can be explained on the same basis (*i.e.*, heteroporosity of the membranes of the tissues of the central compartment) as the decrease in the volume of the central compartment for IV and VII. Accordingly, when present in excessively large numbers in the central compartment, the molecules of VIII would predominantly occupy the small size pores of the membranes of the tissues of the central compartment and prevent access to the molecules of the substrate compound, I, thereby diminishing V_1 for I. The decrease in the volume of the central compartment in the case of I (20%) is similar to that noted in the case of IV (18%).

Although, in the presence of VIII, V_2 for I substantially increased and V_3 slightly decreased, the total volume ($V_2 + V_3$) of the peripheral compartments remained practically unchanged (Table V). The rationalization for these observations follows. In the preceding paper (7), it was indicated that the binding of I with the biopolymers of the intracellular fluids of the tissues of the peripheral compartments restricts its diffusion into only limited regions of the tissues of the peripheral compartments as compared to that of IV. Even when the concentration of VIII was several times greater than that of I, it did not affect the binding of I to plasma proteins, as determined from *in vitro* equilibrium dialysis studies, thus leading to the assumption that VIII does not interfere *in vivo* with the binding of I to the biopolymers of intracellular fluid of the tissues of the peripheral compartments. Therefore, it may not be too surprising that the total volume ($V_2 + V_3$) of the peripheral compartments for I remained practically unchanged in the presence of VIII.

However, in accordance with the other idea proposed previously (7), that the two peripheral compartments are identified for I because of the differences in the extent of hydrogen bonding of its molecules with the constituents (proteins and/or phospholipids) of the aqueous pores of membranes of the tissues of the peripheral compartments, the diffusion of molecules of I through the aqueous pores may be affected to different degrees in the presence of the molecules of VIII to reflect a substantial increase in V_2 and a very slight decrease (which may even be discounted as being within experimental error) in V_3 for I, as noted in this study. The apparent increase in V_2 may also be attributed to the increased $t_{1/2\gamma}$ of I in the presence of VIII.

The fact that the ratios of the distribution rate constants (k_{12}/k_{21} and k_{13}/k_{31}) observed for I increased in the presence of VIII also supports the interpretation (made earlier in connection with the increase in k_{12}/k_{21} value of IV) that the substrate molecules of I find less resistance for their transport through the larger aqueous pores in the presence of a large excess of the molecules of VIII.

Table VII—Distribution Pharmacokinetic Parameters Obtained for I in Rats in the Absence and Presence of VIII

	II		III	
Pharmacokinetic Parameter	VIII Absent[a]	VIII Present	VIII Absent[a]	VIII Present
V_d, ml/kg	247.8	248.0	123.5	124.2

[a] Data obtained from Ref. 7.

Effect on II and III—The volume of distribution of II as well as III remained unchanged in the presence of VIII (Table VII). This finding suggested that the diffusion of these *para*-alkylated compounds through the small size aqueous membrane pores is not involved, as was inferred in the case of V and VI, the *para*-alkylated (methyl and ethyl) homologs of IV. This finding also confirmed that the V_d of III is indeed as low as 125 ml/kg, as noted previously (7).

The increase in the biological half-lives of these compounds did not tend to increase their V_d, as was implicated with the compounds following a two- or three-compartment open model. This finding suggests that, in the case of compounds (organic anions) whose distribution is primarily restricted to well-perfused tissues, the increase in their residence time in the body (because of their increased biological half-lives) brought about by the renal tubular secretion inhibitor would probably not increase their V_d.

CONCLUSIONS

It is recognized that a majority of drugs do not possess the ideal properties described here for the model compounds; most drugs are metabolized, bound to plasma proteins, may or may not be secreted or reabsorbed in the renal tubules, or may remain in the systemic body fluids partially or completely ionized or unionized. It is also recognized that the renal tubular secretion inhibitor of substrate drugs may or may not interfere with these processes of the substrate drugs. However, in the event that differences are observed in the distribution pharmacokinetic parameters of a drug in the presence of its renal tubular secretion inhibitor, it is desirable to know what factors are primarily responsible for such differences. Unless one is cognizant of all possible effects each factor can produce, the task of proposing mechanisms to account for the changes observed becomes difficult and highly speculative.

The literature survey indicated that either no deliberate efforts have been made to determine the effect of renal tubular secretion inhibitors on the distribution pharmacokinetic parameters of the substrate compounds or, if such efforts have been made, the reasons offered have remained highly speculative. Therefore, it is extremely desirable to build up a body of knowledge, based on model studies where the effect of each factor is exclusively determined, which will allow more realistic speculation. Although the compounds employed in the study are not used as drugs, except for D-(−)-mandelic acid, they served as model compounds to reveal not only features involved in the transmembrane transport process of organic anions but also to illustrate the influence of a renal tubular secretion inhibitor on the distribution pharmacokinetic parameters of these substrate organic anions.

The essential features revealed for the compounds exhibiting multicompartment open-model systems are that: (*a*) the organic anions most probably diffuse through the aqueous pores of the membranes; (*b*) the permeating molecules are subjected to electrostatic forces, hydrogen bonding, and hydrophobic bonding in the aqueous pores; and (*c*) the heteroporosity of the membranes may influence the volume of a given body compartment. The feature revealed by the compounds that followed a one-compartment open model was that the volume of distribution of the substrate organic anions would not necessarily change in the presence of their renal tubular secretion inhibitor.

It is hoped that the insight gained into the pharmacokinetic be-

havior of the model organic anions employed in this study will provide further understanding, not only of the design and modification of drug molecules but also of the effects of the competitive renal tubular secretion inhibitor on the distribution pharmacokinetic parameters of the substrate compounds.

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Behavior of Erythrocytes in Ternary Solvent Systems

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Abstract □ The effect of ternary solvent systems on erythrocytes was investigated. Hemolysis experiments were run at 37° in solutions containing various amounts of water, two nonaqueous solvents, and 0.9% sodium chloride. The nonaqueous solvents were propylene glycol, polyethylene glycol 400, dimethyl sulfoxide, dimethylformamide, and tetramethylurea. Ternary diagrams based on the critical hemolytic compositions of the various ternary systems are presented.

Keyphrases □ Erythrocytes—behavior in ternary solvent systems, ternary diagrams, critical hemolytic compositions □ Solvents—effect of ternary solvent systems on erythrocytes, ternary diagrams, critical hemolytic compositions □ Blood—behavior of erythrocytes in ternary solvent systems, ternary diagrams, critical hemolytic compositions □ Intravenous preparations—behavior of erythrocytes in ternary solvent systems

To prepare a safe and efficacious injection, it is sometimes necessary to employ a mixed solvent system consisting of water and a nonaqueous solvent. The behavior of rabbit and human erythrocytes in aqueous solutions of propylene glycol and liquid polyethylene glycol (1–3) and the behavior of human erythrocytes in aqueous solutions of dimethyl sulfoxide, liquid amides, tetramethylurea systems, and monohydric alcohols (4–7) were reported in previous papers in this series.

Binary solvent systems are not the only systems that can be used for intravenous preparations. Since aqueous solutions containing two or more nonaqueous solvents might be necessary to prepare a suitable solution, the effects of some ternary solvent systems on red blood cells were studied. Experiments were conducted to determine the behavior of human erythrocytes in systems containing two nonaqueous solvents in 0.9% aqueous saline.

The nonaqueous solvents selected were propylene glycol, polyethylene glycol 400, dimethyl sulfoxide, dimethylformamide, and tetramethylurea. Critical hemolytic concentrations were determined for the various ternary solvent systems, and the data were used to construct ternary diagrams depicting the various concentrations of water and two nonaqueous solvents, mixed together as ternary solvent blends, which are compatible with human red cells.

EXPERIMENTAL

Materials—Propylene glycol[1] USP, polyethylene glycol 400[2] (average mol. wt. 380–420), tetramethylurea[3], and reagent grades of dimethylformamide[4] and sodium chloride[2] were used.

Preparation of Solutions—All solvent systems were volume-

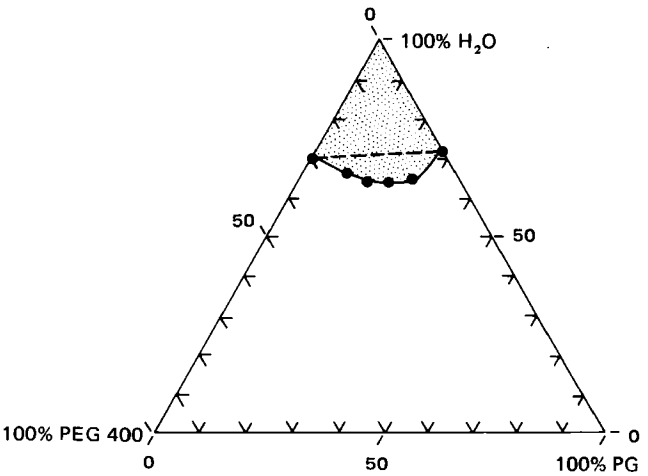

Figure 1—*Ternary diagram for aqueous propylene glycol (PG)–polyethylene glycol 400 (PEG 400) solutions containing 0.9% sodium chloride at 37°.*

[1] Fisher Scientific Co.
[2] J. T. Baker Chemical Co.
[3] Aldrich Chemical Co.
[4] Mallinckrodt Chemical Works.