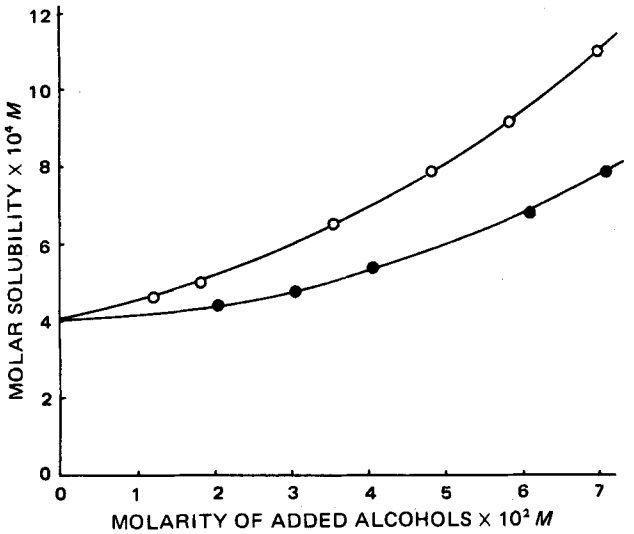

Figure 1—*Solubility of hydroquinone in carbon tetrachloride at 30° as a function of added alcohols. Key:* ○, *cyclohexanol; and* ●, *isobutanol.*

Equation 11 can be rewritten as:

$$[S_T] - S_0 = \alpha\{[L_T] - 2([S_T] - S_0)\} + \beta\{[L_T] - 2([S_T] - S_0)\}^2 \quad \text{(Eq. 14)}$$

Dividing both sides of Eq. 14 by $[L_T] - 2([S_T] - S_0)$ results in:

$$\frac{[S_T] - S_0}{[L_T] - 2([S_T] - S_0)} = \alpha + \beta\{[L_T] - 2([S_T] - S_0)\} \quad \text{(Eq. 15)}$$

Plots of the left side of Eq. 15 *versus* $[L_T] - 2([S_T] - S_0)$ give a straight line and both $K_{1:1}$ and $K_{1:2}$ can be calculated from the slope and the intercept. In using Eq. 15, all one needs are the easily obtainable values of $[S_T]$, S_0, and $[L_T]$.

It is also apparent from its derivation that Eq. 15, in contrast to Eq. 5, can be used even if the concentrations of the complex species (SL and SL_2) are very large. However, when $SL = L$, Eq. 15 cannot be applied to calculate $K_{1:1}$ and $K_{1:2}$. The standard Eq. 5 also cannot be used for these calculations unless one assumes that $SL \gg SL_2$.

The data of Chulkaratana (5) were analyzed according to Eq. 15 to demonstrate its utility. As shown in Fig. 1, the solubility of hydroquinone in carbon tetrachloride increases nonlinearly as a function of added isobutanol and cyclohexanol (5). This increased solubility of the phenolic compound was reported to be due to the formation of 1:1 and 1:2 complexes with the added alcohols. The values for $K_{1:1}$ and $K_{1:2}$ (Table I) were calculated using a lengthy manipulation of the data. When the same data were analyzed according to Eq. 15, Fig. 2 was obtained. The values of $K_{1:1}$ and $K_{1:2}$ were calculated from the intercepts and slopes of Fig. 2 and were in good agreement with literature values (Table I).

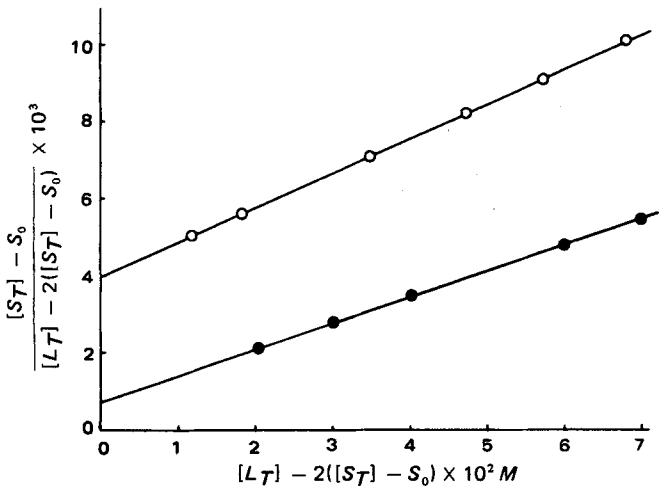

Figure 2—*Plots of Eq. 15 for determination of* $K_{1:1}$ *and* $K_{1:2}$ *for the hydroquinone–alcohol complex formation in carbon tetrachloride. Key:* ○, *cyclohexanol; and* ●, *isobutanol.*

Table I—Complexation Constants of Hydroquinone in Carbon Tetrachloride–Alcohol System

	Values from Eq. 15		Literature Values	
	$K_{1:1}, M^{-1}$	$K_{1:2}, M^{-2}$	$K_{1:1}, M^{-1}$	$K_{1:2}, M^{-2}$
Cyclohexanol	10.03	217.0	10.0	210.5
Isobutanol	1.83	169.5	1.75	167.5

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Plasma Area Method in Relative Bioavailability Evaluation of Drugs with Changing Biological Half-Lives

Keyphrases □ Bioavailability—method for drugs with changing biological half-lives, alternative calculations to plasma concentration–time curve method □ Drug absorption—relative bioavailability calculated for drugs with changing biological half-lives, compared to plasma concentration–time curve method □ Pharmacokinetics—relative bioavailability determined for drugs with changing biological half-lives, alternative method to plasma concentration–time curve method

To the Editor:

Both the rate and extent of absorption of a drug from dosage forms are important in biopharmaceutical and pharmacokinetic studies. An additional intravenous study often is needed to serve as a control and to obtain the disposition function of the drug (1–5). In the relative bioavailability (F) study of two dosage forms, the following total plasma (blood or serum) area method often is used without an intravenous study:

$$F = \frac{AUC_2\beta_2}{AUC_1\beta_1} \quad \text{(Eq. 1a)}$$

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$$F = \frac{AUC_2(t_{0.5})_1}{AUC_1(t_{0.5})_2} \quad \text{(Eq. 1b)}$$

where AUC_1 and AUC_2 are the estimated total areas (corrected to the same dose) under the plasma concentration–time curve from time zero to infinity obtained from dosage forms 1 and 2, respectively; β_1 and β_2 are the terminal exponential rate constants obtained from the studies on dosage forms 1 and 2, respectively; and $(t_{0.5})_1$ and $(t_{0.5})_2$ are the terminal biological half-lives from dosage forms 1 and 2, respectively. Equations 1*a* and 1*b* are commonly assumed to be theoretically correct and applicable to multicompartment systems.

The purpose of this communication is to consider theoretically the limitation of using Eq. 1*a* or 1*b* in a relative bioavailability study. Under certain conditions, such use might result in significant underestimations or overestimations of the relative bioavailability of the two dosage forms.

The total amount of drug absorbed into the general circulation up to infinite time (A_b) from a dosage can be calculated by (3):

$$A_b = AUCCl_{TB} = AUCk_{10}V_c = AUCV_{d\beta}\beta \quad \text{(Eq. 2)}$$

where Cl_{TB} is the total body drug clearance, k_{10} is the first-order elimination rate constant from the central compartment in the multicompartmental mammillary model system, V_c is the volume of the central compartment, $V_{d\beta}$ is the apparent volume of distribution during the terminal exponential phase, and β is the terminal exponential rate constant. Therefore, the relative bioavailability (F) from the two dosage forms when there is a difference in Cl_{TB} between the two studies in the same subject can be calculated theoretically by:

$$F = \frac{A_{b_2}}{A_{b_1}} \quad \text{(Eq. 3a)}$$

$$F = \frac{AUC_2Cl_{TB_2}}{AUC_1Cl_{TB_1}} \quad \text{(Eq. 3b)}$$

$$F = \frac{AUC_2(k_{10})_2}{AUC_1(k_{10})_1} \quad \text{(Eq. 3c)}$$

$$F = \frac{AUC_2(V_{d\beta})_2\beta_2}{AUC_1(V_{d\beta})_1\beta_1} \quad \text{(Eq. 3d)}$$

$$F = \frac{AUC_2(V_{d\beta})_2(t_{0.5})_1}{AUC_1(V_{d\beta})_1(t_{0.5})_2} \quad \text{(Eq. 3e)}$$

It is obvious that Eqs. 3*b*–3*e* are different from Eqs. 1*a* and 1*b*. In the absence of known causes, it is reasonable to assume that in multicompartmental mammillary models, the change in β during the two absorption studies is attributed entirely to the change in k_{10} and that both the intercompartmental distribution rate constants and V_c remain the same (2–6). If one accepts these assumptions, then Eqs. 1*a* and 1*b* are theoretically incorrect. The validity of Eqs. 1*a* and 1*b* is based on the assumption that the $(V_{d\beta})_1$ and $(V_{d\beta})_2$ are the same in spite of the change in β.

A hypothetical example based on reported ampicillin pharmacokinetic data (7) will be used to illustrate the influence of the change in β on $V_{d\beta}$ and thus the relative bioavailability data. The biexponential disposition function ($A_1e^{-\alpha_1 t} + B_1e^{-\beta_1 t}$) of ampicillin was calculated from the average data in nine subjects following an intravenous bolus dose of 615 mg (7):

$$C_p(\mu g/ml) = 120.4e^{-2.4t} + 12.12e^{-0.39t} \quad \text{(Eq. 4)}$$

The $(V_{d\beta})_1$ value for ampicillin based on Eq. 4 was calculated by the standard method [*i.e.*, $(V_{d\beta})_1$ = dose/$AUC_1\beta_1$]. The new $V_{d\beta}$ value for every change in β (±10, 20, 30, 40, 60, and 80% from the original value) was calculated by the reported method (2–5), assuming no changes in the central compartment volume and intercompartmental transfer rate constants. The new disposition function ($A_2e^{-\alpha_2 t} + B_2e^{-\beta_2 t}$) corresponding to the change in β was calculated first. The new $V_{d\beta}$ or $(V_{d\beta})_2$ value then was calculated with:

$$(V_{d\beta})_2 = \frac{\text{dose}}{AUC_2\beta_2} \quad \text{(Eq. 5)}$$

The details for calculating the new $V_{d\beta}$ value are shown in the *Appendix*. The results of these theoretical studies are summarized in Table I. In these studies, the dose was assumed to be absorbed completely into the general circulation. The *AUC* should be independent of the administration route and absorption kinetics if the same total amount of drug is absorbed into the general circulation.

In contrast to the common notion, the data in Table I show that $V_{d\beta}$ might change markedly owing to the change in β. For example, when β was increased by 30%, $V_{d\beta}$ increased by 134%. In the literature, a change in $V_{d\beta}$ due to a change in β often was assumed to result from the "real" physiological change in the distribution characteristics of the drug. In these analyses, both the central compartment volume (or the apparent initial volume of distribution) and the apparent steady-state volume of distribution were assumed to remain the same despite the change in β.

The implication of these results is that the use of Eq. 1*a* or 1*b* for calculating relative bioavailability might overestimate or underestimate the true F value. If it is assumed that all of the drug is absorbed from the two dosage forms, all F values calculated based on Eq. 1*a* or 1*b* should be expected to be equal to 1.0 according to conventional understanding. However, this is not the case, as shown in Table I (F values ranged from 0.429 to 1.919). The effect of the change in β on $V_{d\beta}$ was reported earlier (4, 6). Nevertheless, its implication in the evaluation of relative bioavailability has not been investigated fully.

These results and discussion suggest that caution should be used in the interpretation of experimental data using Eq. 1*a* or 1*b*. These two equations are theoretically valid if the disposition kinetics of a drug can be described adequately by the one-compartment open model. The results of simulation studies with other drugs indicate that the greater the distribution phase of a drug, the less the accuracy of these equations.

Table I—Pharmacokinetic Analysis of Ampicillin[a] Resulting from Changes in β

Percent Change in β from β_1	AUC_2, (hr μg)/ml	$(V_{d\beta})_2$, liters	F^b
0[c]	81.2[d]	19.4[e]	1.000
+10	61.3	23.4	0.830
+20	43.4	30.3	0.641
+30[f]	26.8	45.3	0.429
−10	104.1	16.8	1.154
−20	131.3	15.0	1.293
−30	164.8	13.7	1.420
−40	208.1	12.6	1.537
−60	353.9	11.1	1.743
−80	779.4	10.1	1.919

[a] The disposition function from the 615-mg iv dose study (7); C_p (μg/ml) = $120.4e^{-2.4t} + 12.12e^{-0.39t}$. [b] Based on Eq. 1*a*. [c] That is, $\beta = \beta_2 = 0.39\ hr^{-1}$. [d] That is, $AUC_1 = AUC_2 = 81.2$ (hr μg)/ml. [e] That is, $(V_{d\beta})_1 = (V_{d\beta})_2 = 19.4$ liters. [f] A 40% or greater change resulted in unrealistic data.

Since it is difficult to calculate $V_{d\beta}$ accurately based on plasma data from absorption studies, the use of the more correct equations, Eqs. 3*b*–3*e* is virtually impossible from a practical point of view. Therefore, Eqs. 1*a* and 1*b* can be used routinely with confidence as an approximate method for drugs whose disposition kinetics can be approximated by the one-compartment model (*e.g.*, the distribution phase accounts for <5–10%) and also for "high distribution" drugs whose changes in β between the absorption studies are <5–10%.

These limitations might be more critical in studies involving only one or a few subjects. The errors in the relative bioavailability evaluation (overestimation and underestimation) due to the change in β might cancel out when a larger population of subjects is used. Therefore, the use of Eq. 1*a* or 1*b* might be satisfactory under this circumstance.

The analyses (Table I) were based on the assumption that the elimination rate of the drug was proportional to its concentration in the venous plasma (almost exclusively used in bioavailability studies in humans). Such an assumption might be invalid since our recent studies showed marked arteriovenous plasma concentration differences for many drugs in dogs and rabbits (8). From a physiological point of view, the elimination rate of a drug from the body in linear pharmacokinetics should be generally proportional to its concentration in the arterial plasma and not in the venous plasma (especially for renal elimination).

The question of the theoretical validity of using venous plasma data to calculate $V_{d\beta}$ and the steady-state volume of distribution (V_{dss}) based on the conventionally used equations has been raised (8, 9). The implication of arteriovenous plasma concentration differences in the bioavailability evaluation of drugs with a changing terminal half-life remains to be explored. Based on the venous plasma data from absorption studies alone, there is virtually no way to characterize accurately the change in the volume of distribution of a drug in the body when there is a change in β between the two studies. The uncertainty in the dose absorbed also contributes to the difficulty in estimating the volume of distribution.

It is of interest to note from Eq. 2 that as long as Cl_{TB} remains unchanged, the dose absorbed is proportional to the AUC in spite of any change in the terminal biological half-life or the apparent volume of distribution (used in a broad sense). In normal bioavailability studies in healthy subjects with strict protocols, it seems difficult to conceive that there will be any significant physiological or biochemical changes in the body that would result in a significant change in the real drug distribution characteristics and cause a noticeable change in the AUC or the biological half-life. The apparent steady-state volume of distribution is a reflection of the equilibrium partition properties of the drug between various tissues and plasma. Such properties probably would not change significantly during a relatively short period.

Determination of renal clearance has been proposed as a means of estimating the change in the total body clearance in the relative bioavailability study (10). This method is particularly valuable if a drug is extensively excreted unchanged in the urine.

In view of the potential arteriovenous plasma concentration differences, it was pointed out that the timed interval method for the renal clearance measurement following a single dose might result in significant overestimation or underestimation of the true renal clearance of the drug (8, 9, 11).

Recently, a unique statistical approach for evaluating relative bioavailability studies involving a change in the terminal half-life was presented (12, 13). Perhaps, a combination of the statistical (12, 13) and renal clearance (10) approaches and the discussions presented in this communication might serve as the basis for further studies on this subject.

Appendix—This discussion concerns the relationship between the new disposition function ($A_2e^{-\alpha_2 t} + B_2e^{-\beta_2 t}$) and the original disposition function ($A_1e^{-\alpha_1 t} + B_1e^{-\beta_1 t}$) assuming that there is only a change in the first-order elimination rate constant from the central compartment in the two-compartment open model system when the terminal exponential rate constant changes from β_1 to β_2.

The pharmacokinetic parameters from the original disposition function obtained after intravenous dosing can be calculated by (3–5):

$$k_{21} = \frac{A_1\beta_1 + B_1\alpha_1}{A_1 + B_1} \quad \text{(Eq. A1)}$$

$$(k_{10})_1 = \frac{\alpha_1\beta_1}{k_{21}} \quad \text{(Eq. A2)}$$

$$k_{12} = \alpha_1 + \beta_1 - k_{21} - (k_{10})_1 \quad \text{(Eq. A3)}$$

$$V_c = \frac{\text{dose}}{A_1 + B_1} \quad \text{(Eq. A4)}$$

The new elimination rate constant, $(k_{10})_2$, and the new disposition function can be obtained by (3–5):

$$(k_{10})_2 = \frac{\beta_2^2 - (k_{12} + k_{21})\beta_2}{\beta_2 - k_{21}} \quad \text{(Eq. A5)}$$

$$\alpha_2 = (k_{10})_2 + k_{12} + k_{21} - \beta_2 \quad \text{(Eq. A6)}$$

$$A_2 = \frac{\text{dose}(\alpha_2 - k_{21})}{V_c(\alpha_2 - \beta_2)} \quad \text{(Eq. A7)}$$

$$B_2 = \frac{\text{dose}(k_{21} - \beta_2)}{V_c(\alpha_2 - \beta_2)} \quad \text{(Eq. A8)}$$

where β_2 is obtained from the terminal exponential phase during the absorption study.

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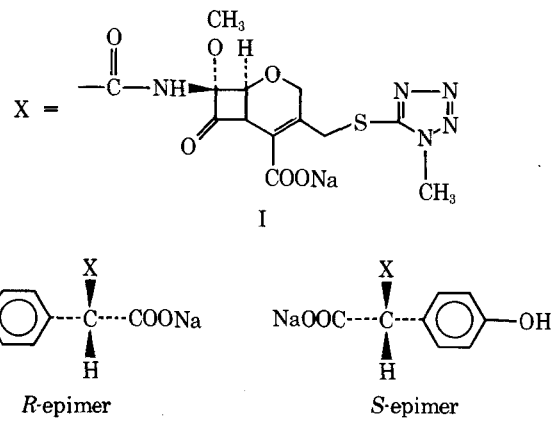
Plasma Protein Binding and Urinary Excretion of *R*- and *S*-Epimers of an Arylmalonylamino 1-Oxacephem I: In Humans

Keyphrases □ Stereoisomers, arylmalonylamino 1-oxacephem—new β-lactam antibacterial agent, renal clearance, plasma protein binding, humans □ Renal clearance—arylmalonylamino 1-oxacephem stereoisomers, effect of plasma protein binding, humans □ Protein binding, human plasma—arylmalonylamino 1-oxacephem stereoisomers, effect on renal clearance

To the Editor:

A new antibacterial agent, 7β-[2-carboxy-2-(4-hydroxyphenyl)acetamido]-7α-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-1-oxa-1-dethia -3- cephem-4-carboxylic acid disodium salt (I), is an arylmalonylamino 1-oxacephem derivative that was discovered and is being developed in our laboratories (1, 2). It consists of the *R*- and *S*-epimers in about a 1:1 ratio. This drug is not metabolized and almost all of the dose, ~90% or more, is excreted into the urine in humans (3, 4), ~80% is excreted into the urine in rats, and 86–90% is excreted into the urine in dogs (5, 6) when assayed by agar diffusion using *Escherichia coli* 7437 (2). This study was carried out to elucidate the behavior of the *R*- and *S*-epimers of I in the human body.

Plasma and urine samples were collected from four healthy volunteers after intravenous injection of 1 g of I. Concentrations of *R*- and *S*-epimers in the samples were determined separately by high-performance liquid chromatography [Nucleosil $10C_{18}$, using pH 6.0 ammonium acetate buffer–methanol (11.5:1) for the plasma samples and pH 6.0 phosphate buffer containing 0.005 *M* tetra-*n*-butylammonium hydroxide–methanol (75:25) for the urine samples][1]. Plasma concentration–time curves for the *R*- and *S*-epimers indicated that the *R*-epimer was eliminated faster than the *S*-epimer (Fig. 1). The time course of the concentration ratio between the *R*- and *S*-epimers in urine also showed that the excretion of the *R*-epimer was faster than that of the *S*-epimer[1]. The renal clearances calculated from these data were 65.5 ± 3.4 and 43.5 ± 3.1 ml/min/1.48 m^2 for the *R*- and *S*-epimers, respectively.



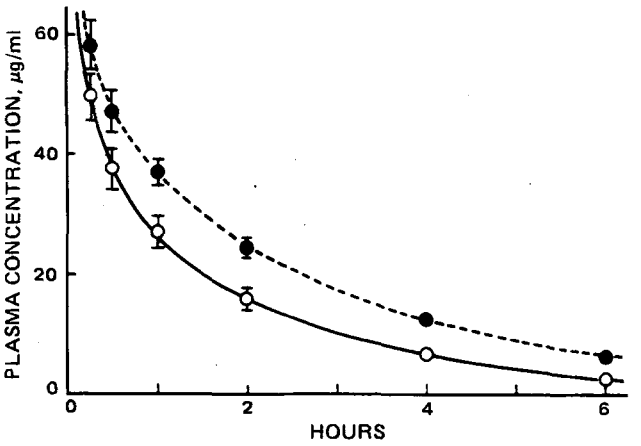


Figure 1—*Plasma concentration of* R- (○) *and* S- (●) *epimers after intravenous administration of I. Each data point gives the mean and standard deviation of four healthy volunteers.*

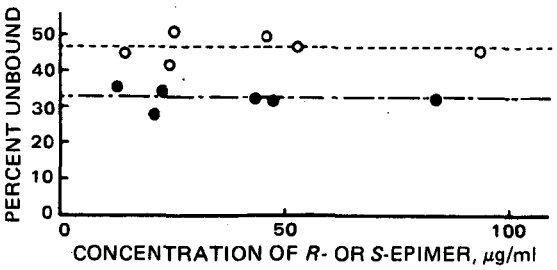


Figure 2—*Human plasma protein binding of* R- (○) *and* S- (●) *epimers of I. The epimer concentration includes both bound and unbound epimer.*

To investigate this difference, protein binding of these epimers was examined by ultrafiltration[2] of fresh human plasma containing I at 37°. The results indicated that the fraction of the unbound *R*-epimer was higher than that of the *S*-epimer (Fig. 2). The mean unbound ratio of the *R*-epimer was 47%, and that of the *S*-epimer was 33%.

The renal clearance of unbound *R*- and *S*-epimers calculated from these results was 140.2 ± 8.1 and 132.2 ± 7.0 ml/min/1.48 m^2, respectively. These two values were not significantly different.

These results suggest that the faster excretion of the *R*-epimer of I compared to the excretion of the *S*-epimer can be explained by the larger unbound fraction of the former in human plasma.

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