# Quantitative Investigation on Renal Handling of Drugs in Rabbits, Dogs, and Humans

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Received June 18, 1982 from the *Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto, 606, Japan.* Accepted for publication August 16, 1982.

**Abstract** □ A renal clearance method based on a computer analysis after administration of a single dose of drug was developed for measuring the renal handling of several drugs in rabbits, dogs, and humans. Secretion and reabsorption of sulfamethizole, sulfanilamide, cephalexin, and ampicillin in the nephron were analyzed quantitatively using the plasma concentration and the urinary excretion rate of the drugs. The validity of the proposed model was demonstrated. It appears that tubular secretion of sulfamethizole, cephalexin, and ampicillin depend on the active transport system which is described by Michaelis–Menten kinetics; the tubular reabsorption of these drugs is expressed by first-order kinetics. The maximum velocity of renal secretion per unit weight of these drugs was much higher in rabbits than in dogs or humans. Reabsorption showed similar values in dogs and humans. These findings suggest that an analysis of the renal handling of drugs in dogs might provide useful information when considering the appropriate therapeutic dose in humans.

**Keyphrases** □ Clearance, renal—of sulfamethizole, sulfanilamide, cephalexin, and ampicillin in the rabbit, dog, and human, determination using a computer analysis □ Sulfamethizole—determination of renal clearance using a computer analysis □ Sulfanilamide—determination of renal clearance using a computer analysis □ Cephalexin—determination of renal clearance using a computer analysis □ Ampicillin—determination of renal clearance using a computer analysis

The renal excretion of drugs is a complex phenomenon involving glomerular filtration, tubular secretion, and tubular reabsorption (1). Since the independent characterization of the transport kinetics of secretion and reabsorption is difficult, the quantitative analysis of the renal handling of drugs is not readily available, particularly in humans. Weiner *et al.* (2) described an analytical method for these processes; however, one needs a wide range of drug concentrations in plasma. Since limited plasma levels are obtained in humans, this method has little clinical applicability. Such methodology, however, is desirable when designing the drug-dosing schedule for patients with renal disease.

We have described a method that quantitatively analyzes the renal handling of drugs (3, 4). Using this method, the renal handling of sulfamethizole, sulfamethoxazole, sulfanilamide, and phenolsulfonphthalein was described in rabbits. In this study, the applicability of this method to dogs and humans was examined, and the renal handling of two widely used antibiotics (cephalexin and ampicillin) was investigated using plasma concentrations normally obtained with therapeutic doses.

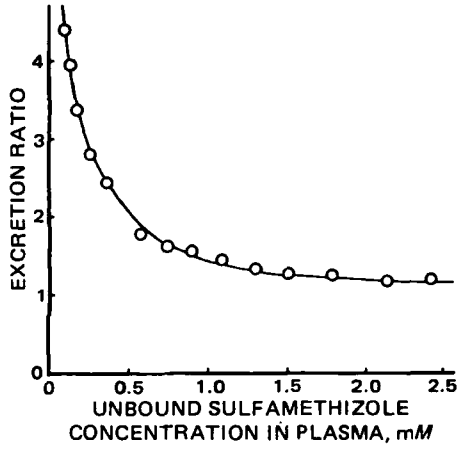


**Figure 1**—*Experimental renal clearance data (points) and computer-simulated curve (line) for sulfamethizole in the dog. The parameters obtained by this analysis are as follows:* $V_{max}$ = *20 μmoles/min,* $K_m$ = *0.16 mM, and* R = 0.13.

## EXPERIMENTAL

**Materials**—Sulfamethizole was JP IX grade[1]; sulfanilamide was reagent grade[2]. Cephalexin monohydrate[3], sodium ampicillin[3], and iodopyracet[4] were supplied. All other chemicals were reagent grade.

**Determination of Plasma Protein Binding**—Plasma protein binding was determined by the ultrafiltration technique using a membrane cone[5] for sulfamethizole and sulfanilamide and cellulose tubing[6] for cephalexin and ampicillin.

**Analytical Methods**—Plasma and urine samples were treated with the Somogyi deproteinizing reagent (5) and then analyzed for sulfamethizole and sulfanilamide using 2-dimethylaminoethyl-1-naphthylamine as the coupling reagent (6). Inulin was determined in samples deproteinized as described above using a modification of a previously described method (7). Cephalexin and ampicillin in plasma and urine were determined fluorometrically (8, 9); creatinine was determined using picric acid (10).

**Clearance Method**—*Rabbits*—Male New Zealand albino rabbits, weighing 2.0–2.5 kg, were used. The determination of renal clearance using a single injection technique and the standard renal clearance and secretory inhibition using iodopyracet was described previously (4).

*Dogs*—Male mongrel dogs, weighing 5–11 kg, were anesthesized with pentobarbital[7] (27 mg/kg). Renal clearance was determined in the same manner as described above for the rabbit.

*Humans*—Sulfamethizole (4 g of an aqueous solution), sulfanilamide

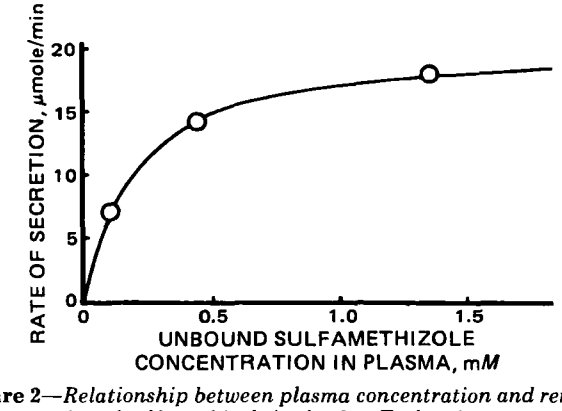


**Figure 2**—*Relationship between plasma concentration and renal tubular secretion of sulfamethizole in the dog. Each point represents the experimental data obtained by inhibitory experiments; the solid line represents the curve calculated using the proposed model. The parameters are as follows:* $V_{max}$ = *20 μmoles/min and* $K_m$ = *0.18 mM.*

[1] Eizai Co. Ltd.
[2] Nakarai Chemicals, Ltd.
[3] Toyo Jozo Co. Ltd.
[4] Daiichi Seiyaku Co. Ltd.
[5] Centriffo CF-50A, Amicon Co. Ltd.
[6] Visking Company, 8/32.
[7] Nembutal, Abbott Laboratories, North Chicago, Ill.



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**Table I—Renal Tubular Secretion and Reabsorption of Sulfamethizole and Sulfanilamide in Dogs**

| Parameter (Unit) | Sulfamethizole | Sulfanilamide |
|---|---|---|
| $V_{max}$ (μmole/min) | 20 ± 0.4[b] | 0 |
| $K_m$ (m$M$) | 0.18 ± 0.01 | — |
| $R$ | 0.18 ± 0.01 | 0.37 ± 0.03 |
| $R'$[a] | 0.19 ± 0.01 | 0.40 ± 0.05 |

[a] Reabsorption fraction obtained by inhibitory experiments. [b] Each value represents the mean ± $SE$ of three or four experiments.

**Table II—Renal Tubular Secretion and Reabsorption of Sulfamethizole in Humans**

| Subject | Age | $V_{max}$, μmole/min | $K_m$, m$M$ | $R$ |
|---|---|---|---|---|
| A(M)[a] | 24 | 91 | 0.09 | 0.10 |
| B(M) | 32 | 71 | 0.09 | 0.06 |
| C(F) (1st) | 23 | 110 | 0.16 | 0.13 |
| C(F) (2nd) | 23 | 110 | 0.16 | 0.33 |
| D(M) | 28 | 83 | 0.10 | 0.05 |
| E(M) | 46 | 80 | 0.09 | 0.10 |
| F(M) (1st) | 28 | 59 | 0.06 | 0.09 |
| F(M) (2nd) | 28 | 52 | 0.06 | 0.31 |

[a] (M) male, (F) female.

(2 g of an aqueous solution), cephalexin (500 mg of a powder), and ampicillin (500 mg of a powder) were administered orally to adult volunteers (22–46 years old, of average health and normal weight) following an overnight fast. Food was withheld for an additional 3 hr. Venous blood and urine samples were collected at intervals of 15 or 30 min up to 6 hr after drug administration. To ensure continuous flow of urine and to quantitate fluid intake, each subject received 50 ml of tap water every 30 min for the initial 4 hr postadministration. The volume and pH of each urine sample were measured and the samples were stored at 4° until analysis. The glomerular filtration rate was determined by measuring the clearance of endogenous creatinine.

**Computer Analysis**—As described previously (3), the excretion ratio (ER) can be expressed as:

$$ER = \left(1 + \frac{V_{max}}{(K_m + P_f)\,GFR}\right)(1 - R)$$

where $P_f$ is the unbound drug concentration in plasma, GFR is the glomerular filtration rate, $R$ is the reabsorption fraction, $V_{max}$ is the maximum velocity of secretion, and $K_m$ is the Michaelis constant. The computer analysis[8] using this equation was performed as previously described (3, 4).

## RESULTS

**Renal Handling of Sulfamethizole and Sulfanilamide in Dogs**—The renal clearance following a single injection of sulfamethizole in the dog was analyzed using the model equation (Fig. 1). As in the case of the rabbit, a curve was obtained which fit the experimental values quite well (see Table I for a list of parameters). To ascertain the validity of the values obtained by our model, a comparison of the rate of secretion and the reabsorption fractions was made using the renal clearance method in combination with secretory inhibition by the infusion of iodopyracet.

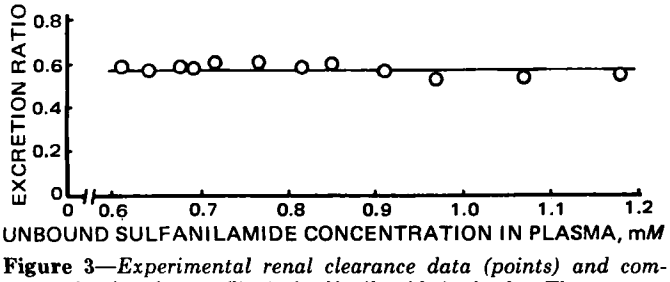


**Figure 3**—*Experimental renal clearance data (points) and computer-simulated curve (line) of sulfanilamide in the dog. The parameters obtained by this analysis are as follows:* $V_{max}$ = 0 *μmole/min and* R = 0.42.

[8] HITAC 8700, Hitachi Co. Ltd.

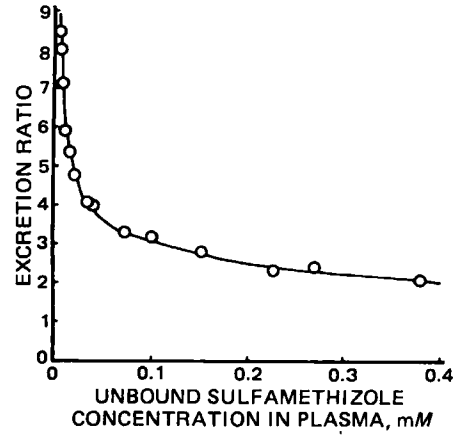


**Figure 4**—*Experimental renal clearance data (points) and computer-simulated curve (line) of sulfamethizole in the human. The parameters obtained by this analysis are as follows:* $V_{max}$ = 59 *μmoles/min,* $K_m$ = 0.06 m**M**, *and* R = 0.09.

First, a comparison was made of the rate of secretion obtained separately from inhibitory experiments and using $V_{max}$ and $K_m$ values obtained by our model. As shown in Fig. 2, a good agreement was achieved. Further, there was also good agreement of the reabsorption fractions: a value of 0.19 ± 0.01 was obtained from the inhibitory experiment compared with 0.18 ± 0.01 from the proposed model. Thus, it was found that the mechanism of the renal excretion of sulfamethizole in dogs was similar to that found in rabbits. That is, tubular reabsorption is dependent on passive transport, whereas tubular secretion involves active transport (which conforms to the Michaelis–Menten equation) and is dependent on the plasma concentration of unbound drug.

On the other hand, the renal clearance after a single injection of sulfanilamide is not dependent on its plasma concentration, and no active secretion process is involved (Fig. 3). Each renal clearance parameter for sulfanilamide is listed in Table I. In the inhibitory experiment for sulfanilamide, no change in renal clearance could be induced even with iodopyracet loading. As in the rabbit, there was no secretion in dogs; only reabsorption was observed. Further, the reabsorption fraction of 0.37 ± 0.03 agreed well with that obtained from the inhibitory experiment (0.40 ± 0.05). Therefore, the validity of this method has been demonstrated in dogs as well as rabbits.

**Renal Handling of Sulfamethizole and Sulfanilamide in Humans**—Our analytical method of renal handling of drugs was applied also to humans. The renal handling of sulfamethizole in humans is shown in Fig. 4. As in the case of rabbits and dogs, a curve was obtained which fit the experimental values quite well. This result indicates that the mechanism of sulfamethizole excretion in humans could be similar to that of other animals. The values of the parameters $V_{max}$, $K_m$, and $R$ for all human subjects are listed in Table II. These values are quite similar to those found with dogs, although some individual variation exists. The day-to-day variation of these parameters in the same subject was also examined for subjects C and F. There was no apparent change in the secretion parameter ($V_{max}$ and $K_m$), while variations were observed in the reabsorption fraction.

The results of the renal clearance of sulfanilamide in humans (Fig. 5) indicated that this drug was excreted by a mechanism similar to that found in rabbits and dogs. It is evident that there was no secretion, only reabsorption.

**Renal Handling of Cephalexin and Ampicillin**—Since we were

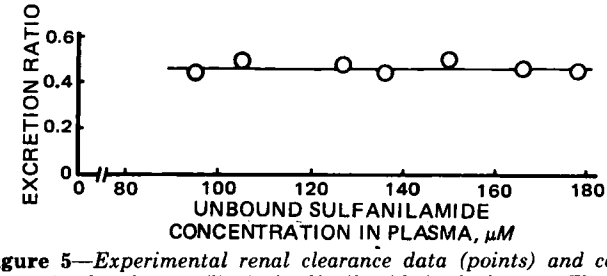


**Figure 5**—*Experimental renal clearance data (points) and computer-simulated curve (line) of sulfanilamide in the human. The parameters obtained by this analysis are as follows:* $V_{max}$ = 0 *μmole/min and* R = 0.52.

**Table III—Parameters for Renal Tubular Secretion and Reabsorption of Drugs in Humans, Dogs, and Rabbits [a]**

| Parameter (unit) | Sulfamethizole | | | Cephalexin | | | Ampicillin | | |
|---|---|---|---|---|---|---|---|---|---|
| | Human | Dog | Rabbit | Human | Dog | Rabbit | Human | Dog | Rabbit |
| $V_{max}$ (μmole/min/body) | 82 ± 7.6 | 20 ± 0.4 | 33 ± 4 | 14 ± 2 | 0.75 ± 0.08 | 2.1 ± 0.2 | 2.6 ± 0.4 | 1.1 ± 0.1 | 0.33 ± 0.03 |
| $V_m'$ (μmole/min/kg) | 1.4 ± 0.1 | 2.3 ± 0.1 | 15 ± 1 | 0.24 ± 0.03 | 0.14 ± 0.01 | 1.0 ± 0.1 | 0.04 ± 0.01 | 0.06 ± 0.01 | 0.14 ± 0.01 |
| $K_m$ (μM) | 110 ± 40 | 180 ± 10 | 1700 ± 110 | 17 ± 3 | 16 ± 1 | 36 ± 3 | 10 ± 1 | 31 ± 5 | 29 ± 5 |
| $R$ | 0.15 ± 0.04 | 0.18 ± 0.01 | 0.20 ± 0.02 | 0.47 ± 0.03 | 0.49 ± 0.02 | 0.33 ± 0.03 | 0.01 ± 0.01 | 0.00 ± 0.01 | 0.00 ± 0.01 |

[a] Each value represents the mean ±*SE*.

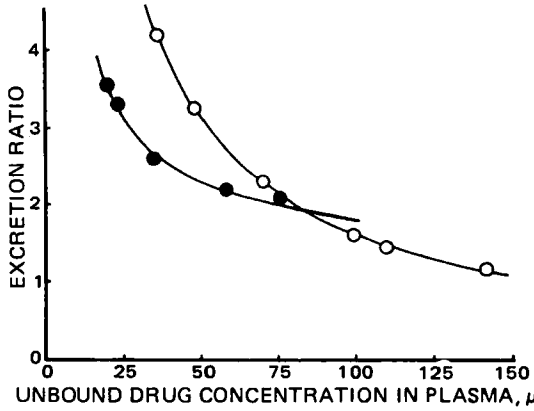


**Figure 6**—*Experimental renal clearance data (points) and computer-simulated curve (line) of cephalexin and ampicillin in the rabbit. Key: (○) cephalexin and (●) ampicillin.*

successful in elucidating the mechanism of the renal handling of sulfamethizole and sulfanilamide through the use of this model, we attempted to apply this methodology to other drugs using cephalexin and ampicillin. As shown in Figs. 6 (rabbit), 7 (dog), and 8 (human), good agreement was obtained between the experimental data and theoretical curves calculated using the appropriate parameters (Table III) obtained in rabbits, dogs, and humans. The data suggested that cephalexin and ampicillin were excreted from the kidney in a process similar to the excretion of sulfamethizole. Welles *et al.* described the renal handling of cephalexin in rabbits and dogs, and demonstrated that secretion and reabsorption were extensively involved in renal excretion (11). Secretion has also been reported for ampicillin (12). The results presented here support the findings that both secretion and reabsorption contribute significantly to the excretion of cephalexin, while the contribution is very small in these three species in the case of ampicillin.

**Species Variation in Renal Handling of Sulfamethizole, Cephalexin, and Ampicillin**—The parameters for these three drugs in rabbits, dogs, and humans are shown in Table III. The reabsorption fraction, $R$, in all species was almost the same, but there were differences in the secretion parameters $V_{max}$ and $K_m$. Comparison of the $V_{max}$ values per unit weight ($V_m$) of sulfamethizole, cephalexin, and ampicillin in the three species showed that rabbits had the highest values with all three drugs, while the values for dogs and humans were approximately the same. Petitpierre *et al.* reported that the renal function of beagle dogs resembles that of humans (13). Our data confirmed quantitatively that the two are similar. Accordingly, it is possible that the renal handling of a drug in humans can be predicted quantitatively by initially performing experiments in dogs.

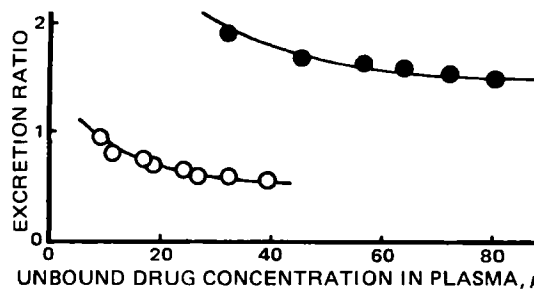


**Figure 7**—*Experimental renal clearance data (points) and computer-simulated curve (line) of cephalexin and ampicillin in the dog. Key: (○) cephalexin and (●) ampicillin.*

## DISCUSSION

In previous reports (3, 4), we proposed a quantitative analytical method to determine the renal handling of such drugs as sulfamethizole, sulfamethoxazole, sulfanilamide, and phenolsulfonphthalein in rabbits. In this study, the methodology was applied to dogs and humans. The renal handling of two widely used antibiotics (cephalexin and ampicillin) was investigated after a single oral dose. It was demonstrated that our method can be applied to dogs and humans as well as rabbits. Some reports are available concerning the urinary excretion of such drugs as inulin (14) and phenolsulfonphthalein (15) in humans; however, few studies quantitate separately the processes of tubular secretion and reabsorption. It was also demonstrated that the tubular secretion of these drugs can be explained by an active transport process which conforms to the Michaelis–Menten equation, while tubular reabsorption can be explained by a first-order process. As our equation fit the experimental data obtained from rabbits, dogs, and humans, it can be assumed that the tubular secretion of these drugs is dependent on the unbound drug concentration in the plasma for each of these species. Levy *et al.* reported that the renal tubular secretion rate in the rat is proportional to the total concentration of sulfisoxazole in plasma rather than the free drug concentration (16, 17). Although further studies are needed, this investigation using sulfamethizole, cephalexin, and ampicillin in three different species shows that tubular secretion correlates with unbound drug concentration.

The renal handling of sulfamethizole, phenolsulfonphthalein, sulfamethoxazole, and sulfanilamide in rabbits was reported previously (4). In this study, the renal handling of cephalexin and ampicillin in rabbits, dogs, and humans was described. It was demonstrated that cephalexin belonged to the secretion and reabsorption type and ampicillin belonged to the secretion type in all three species. Although both drugs have very low lipid solubility, cephalexin showed a relatively high reabsorption. This suggests the possibility of an active-like reabsorption process for cephalexin. The renal clearance values *versus* the unbound plasma concentration curve of cephalexin in all three species did not show the

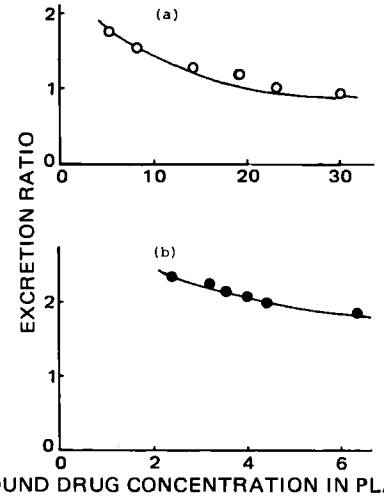


**Figure 8**—*Experimental renal clearance data (points) and computer-simulated curve of cephalexin (a) and ampicillin (b) in the human.*

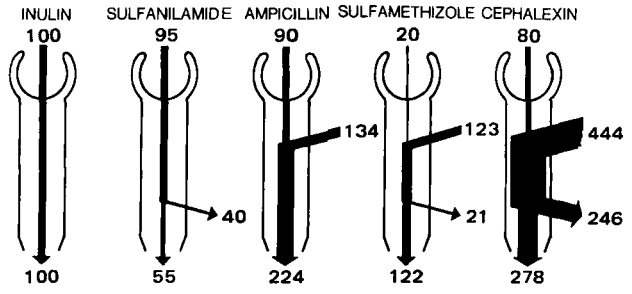


**Figure 9**—*Schematic representation of renal handling of drugs in the human. The parameters in Table III are used; detailed information can be found in the text.*

typical active reabsorption pattern such as that found for glucose (18). Therefore, the contribution of a nonlinear reabsorption process would not be significant under the conditions used here even if an active-like process were to be included in its reabsorption.

Figure 9 shows a schematic representation of the renal handling of these drugs in humans compared with that of inulin. The numbers given, calculated using a plasma drug concentration of 5 $\mu M$ and glomerular filtration rate of 120 ml/min, represent the rate (in percent) of the designated transport process (*i.e.*, filtration, secretion, reabsorption, and urinary excretion) normalized for inulin glomerular filtration (600 nmoles/min). Plasma concentration of drugs were corrected by protein-binding percentage. Thus, for example, since sulfanilamide is 5% protein-bound in plasma, its filtration rate is 95% that of inulin (95% of 600 = 570 nmoles/min). Similarly, reabsorption of sulfanilamide occurs at a rate 40% of inulin filtration, *i.e.*, at 40% of 600 = 240 nmoles/min. It is evident that tubular secretion and reabsorption would be the most important process in regulating the urinary excretion of sulfamethizole, cephalexin, and ampicillin. If renal functions, such as renal secretion, were decreased by renal failure, the renal excretion of sulfamethizole, cephalexin, and ampicillin would be reduced greatly resulting in a high concentration in the blood and target organ. The impact of renal failure on the process of filtration, secretion, and reabsorption should be known in order to provide optimal treatment and protection from adverse reactions in patients with this disease state. We have applied the method described in this paper to the analysis of renal handling of drugs in patients with renal failure, the results of which will be discussed in an ensuing manuscript.

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# Thermodynamics of Distribution of *p*-Substituted Phenols Between Aqueous Solution and Organic Solvents and Phospholipid Vesicles

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**Abstract** □ The distribution of *p*-substituted phenols between 0.15 *M* NaCl and a range of organic solvents (including 1-octanol) was examined over a range of temperatures. The thermodynamic parameters of transfer, $\Delta G$, $\Delta H$, and $\Delta S$, were determined and the values examined in the light of Hildebrand and Scott's solubility parameter theory, and the collision complexes between solute and organic solvent. $\Delta H$ of transfer was positive for nonpolar solvents and negative for 1-octanol; the transfer processes were entropy and enthalpy dominated, respectively. The distribution of the phenols into phospholipid vesicles was examined below the phase-transition temperature. Although $\Delta G$ of transfer for vesicle–water systems was similar to that for octanol–water systems, the full thermodynamic analysis indicated that the two systems were dissimilar. The use of vesicle distribution data in structure–activity studies is discussed.

**Keyphrases** □ *p*-Substituted phenols—partitioning between water and organic solvents, phospholipid vesicles, thermodynamics □ Phospholipid vesicles—partitioning of *p*-substituted phenols, water and organic solvents, thermodynamics □ Thermodynamics—*p*-substituted phenols, partitioning, water and organic solvents, phospholipid vesicles

The importance of hydrophobicity in drug absorption, drug binding, and drug-receptor site interactions is well known (1, 2). Some measure of the hydrophobicity of a solute is given by the distribution (partition) coefficient between water and a suitable organic solvent (3). Usually the choice of the organic phase has been 1-octanol; however, Rytting *et al.* (4) have argued from a thermodynamic standpoint that a nonpolar inert solvent such as isooctane or cyclohexane would be a more appropriate solvent for distribution studies.

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