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Tissue Distribution and Metabolism of Drugs. I. Quantitative Investigation on Renal Handling of Phenolsulfonphthalein and Sulfonamides in Rabbits[1,2]

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In order to analyze the renal handling of drugs under more suitable conditions to enable clinical application, renal clearance method based on computer analysis after administering a single dose of a drug was developed in rabbits. Phenolsulfonphthalein, sulfamethoxazole, sulfanilamide, and sulfamethizole which present characteristically different renal handling were studied, and it had been demonstrated that the results of analysis of our proposed model agreed well with the data obtained by combining the clearance experiments of constant-infusion technique with inhibitory experiments using iodopyracet. The order of reabsorption fraction was similar to that of the partition coefficient of these drugs. The renal tubular reabsorption of these drugs was dependent upon nonionic diffusion which could be explained by lipoid solubility of the drug, while tubular secretion could be explained by active transport that conformed to the Michaelis-Menten equation. Further, it was presumed that the tubular secretion of these drugs was dependent upon the unbound drug concentration in plasma. From these results, a comprehensive scheme which elucidated the renal handling of these four drugs was proposed. In this method as neither constant infusion nor massive dose loading of secretion inhibitors were used, the experiment was conducted under comparatively less harsh conditions than the standard renal clearance and inhibitory experiments. Thus, it is felt that this method can be safely applied to humans.

Keywords——renal handling of drugs in rabbits; renal clearance experiment of single-injection technique; computer analysis; Michaelis-Menten equation; active tubular secretion; passive tubular reabsorption; phenolsulfonphthalein; sulfonamides

The renal excretion of drugs is a major factor in determinating the effectiveness and safety of drugs, since it is closely related with alteration of the drug concentration in blood. Weiner and Mudge[4] carried out extensive physiological studies of renal excretion mechanisms, but investigation was not made on the quantitative relationship between secretion and reabsorption of drugs in the nephron, particularly in humans.

Recently, we introduced the analytical method of renal handling of sulfonamides in rabbits by means of inhibitory experiments and quantitatively determined movement of sulfonamides in the nephron,[5–7] but since the conditions involved are harsh, there is a limitation in the application of this method to humans. In a preliminary communication,[1] we proposed a new analytical method in which the renal excretion of sulfamethizole (SMZ) in rabbits was analyzed under more suitable conditions to enable clinical application to humans.

In order to determine whether the analytical method proposed by us can be widely applied to drugs in general, in this paper we have carried out observations on phenolsulfonphthalein (PSP), sulfamethoxazole (SMX), and sulfanilamide (SA) which are considered to follow a

1) Communication: R. Hori, K. Sunayashiki, and A. Kamiya, *J. Pharm. Sci.*, **65**, 463 (1976).
2) Presented in part at the 95th Annual Meeting of the Pharmaceutical Society of Japan, Nishinomiya, April 1975.
3) Location: *Kasumi 1-2-3, Hiroshima.*
4) I.M. Weiner and H. Mudge, *Am. J. Med.*, **36**, 743 (1964).
5) T. Arita, R. Hori, E. Owada, and K. Takahashi, *Chem. Pharm. Bull.* (Tokyo), **17**, 2526 (1969).
6) E. Owada, K. Takahashi, R. Hori, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), **22**, 594 (1974).
7) E. Owada, K. Takahashi, T. Arita, and R. Hori, *Chem. Pharm. Bull.* (Tokyo), **23**, 3215 (1975).

different renal handling. Comparison was made with the results of inhibitory experiments, and the possibility of applying it to humans was studied. Further, on the basis of these results, the renal excretion mechanisms of drugs including SMZ were discussed and a comprehensive scheme which elucidates the renal handling of these four drugs is proposed.

Experimental

Materials——Sulf methoxazole, sulfanilamide, and sulfamethizole were of J. P. IX grade while phenolsulfonphthalein and the other materials were of reagent grade.

Determination of Plasma Protein Binding——The degree of binding of PSP and three sulfonamides to rabbit plasma protein was determined by the membrane ultrafiltration technique using Centriflo membrane cone (CF-50A, Amicon Co.).[8]

Determination of Apparent Partition Coefficients——Apparent partition coefficients were determined by using chloroform or ethyl acetate as the lipid phase and aqueous phosphate buffer (pH 7.4) at 37°.

Analytical Methods——The plasma and urine samples were treated with Somogyi deproteinizing reagents,[9] and then analyzed as follows: (a) PSP by alkalization using NaOH; (b) sulfonamides by the procedure of Bratton and Marshall,[10] using 2-diethylaminoethyl-1-naphthylamine as the coupling agent; (c) inulin by a modification of the Dische and Borenfreund method.[11]

Clearance Methods——All of the experiments were carried out on male New Zealand albino rabbits weighing 1.8—2.8 kg anesthetized with sodium pentobarbital, 27 mg/kg *i.v.* Renal clearance experiment was performed by the single-injection technique, and also experiments with the combination of standard renal clearance and secretory inhibition using iodopyracet[5] were performed. Under the single-injection technique, PSP (700 mg/kg), SMX (250 mg/kg), SA (500 mg/kg), and SMZ (500 mg/kg) were administered intravenously. Urine samples were collected at 5 and 10 minutes intervals over a period of 2.5—3.0 hours, and blood samples were drawn at one minute before the midpoint of the urine collection periods. This one minute indicates the delay time required for PSP to appear in the urine after intravenous injection in rabbits based on experiments in humans.[12] Inulin was used to determine the glomerular filtration rate at the primary dose of 120 mg/kg, and was infused at the rate of 3 mg/min/body. The standard renal clearance and inhibitory experiments were performed in plasma using various drug concentrations. Details of the procedure of the clearance experiment were as described in the previous report.[5]

Computer Analysis——Generally, drugs in blood are filtered by the glomerulus and some of them are actively secreted into the proximal tubules from blood. Further, part of the drugs is reabsorbed from tubules into the blood, and the remainder is excreted in urine. By assuming that a drug is reabsorbed by nonionic diffusion and that the rate of active secretion of a drug conforms to the Michaelis-Menten equation and is dependent upon the unbound drug concentration in plasma, the clearance ratio corrected for plasma protein binding of the drug, ER (excretion ratio), can be expressed as;[1]

$$ER = \left(1+\frac{V_{max}}{(K_m+P_f)\cdot GFR}\right)(1-R) \qquad \text{(Eq. 1)}$$

where P_f is the unbound drug concentration in plasma, GFR is the glomerular filtration rate, and R, V_{max}, and K_m indicate the reabsorption fraction, the maximum velocity of secretion, and the Michaelis constant, respectively. On the other hand, if the rate of active secretion is dependent upon the total concentration of the drug in plasma (P), ER should be;

$$ER = \left(1+\frac{V_{max}}{(K_m+P)\cdot GFR}\cdot\frac{P}{P_f}\right)(1-R) \qquad \text{(Eq. 2)}$$

Equation 1 and 2 are programmed on the computer as model equations, Eq. 1 for Model A and Eq. 2 for Model B. In these equations, P, P_f, and GFR are determined by the clearance method after administration of a single dose and R, V_{max}, and K_m are parameters. Computers were used to analyze the renal clearance data applying the model equations. The initial values were obtained by analog computer, and then the best fitting parameters were determined using digital computer. The digital computer analysis was performed by least square regression analysis.

8) E. Owada, R. Hori, and T. Arita, *Yakuzaigaku*, **33**, 125 (1973).
9) M. Somogyi, *J. Biol. Chem.*, **86**, 655 (1930).
10) A.C. Bratton and E.K. Marshall, Jr., *J. Biol. Chem.*, **128**, 537 (1939).
11) Z. Dische and F. Borenfreund, *J. Biol. Chem.*, **192**, 583 (1951).
12) H.W. Smith, W. Goldring, and H. Chasis, *J. Clin. Invest.*, **17**, 263 (1937).

Results and Discussion

Renal clearance data from the single-injection technique in rabbits were analyzed by Models A and B using analog and digital computers, and the renal excretion mechanisms of drugs were discussed. Four drugs, PSP, SMX, SA, and SMZ were used in the experiments.

Renal Handling of Phenolsulfonphthalein and Sulfonamides by the Single-injection Technique

It is known that PSP is secreted remarkably[13] by renal tubules, SMX slightly,[14] and SA not secreted at all.[5] The renal handling of these drugs was studied using the single-injection technique. The experiments of a single dose of PSP were analyzed by Models A and B and the results are shown in Fig. 1. When the parameters shown in Fig. 1 were selected, the simulated curve of Model A fitted the experimental data well indicating that active secretion was associated with renal tubular transport of PSP, but renal tubular reabsorption was not detectable. However, in Model B, a curve could not be fitted even by changing the values of the various parameters available. It is interesting that the simulation curve of Model A, rather than Model B, agreed well with the experimental data of PSP, in spite of the fact that PSP which has a comparatively large protein binding, is almost completely eliminated from the blood after only one passage through the kidneys. The results of the analyses by Model A for the other two drugs, SMX and SA, are shown in Fig. 2. When the parameters shown in the figure were selected, a good fit of the curve was achieved. This suggests that SA was not secreted, but SMX was secreted and reabsorbed by the renal tubules. These results are consistent with the previous data obtained by standard renal clearance experiments. However, in Model B, a curve that fits SMX could not be obtained

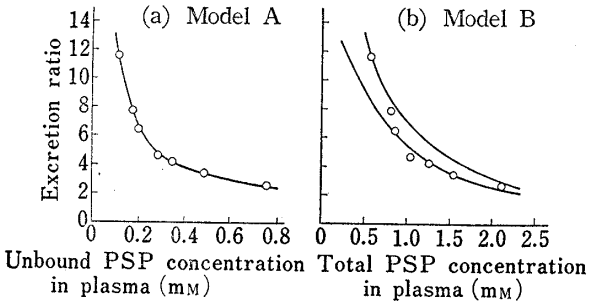

Fig. 1. Experimental Data Points and Simulated Computer Curves on Phenolsulfonphthalein (PSP)

Each point represents the renal clearance data. The solid line represents the computer-simulated curve. The upper solid line in Fig. 1-(b) is the simulation curve for the data of a low plasma level and the lower curve is for the data of a high plasma level of PSP. The parameters obtained by the analysis of Model A are as follows;

V_{max}=9.0 μmol/min
K_m=0.15 mM
R=0

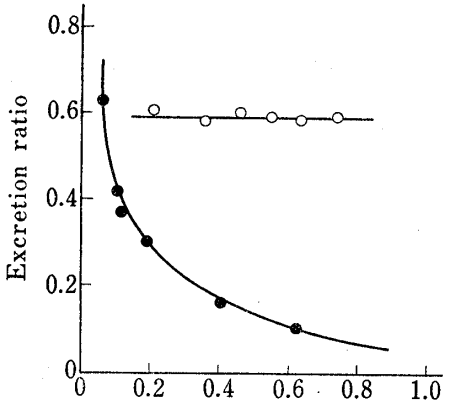

Fig. 2. Experimental Data Points and Simulated Computer Curves on Sulfamethoxazole (SMX) and Sulfanilamide (SA)

Each point represents the renal clearance data. The solid line represents the computer-simulated curve of Model A. The parameters are as follows;

	V_{max} (μmol/min)	K_m (mM)	R
SMX	2.1	0.04	0.91
SA	0	—	0.41

—●—: sulfamethoxazole.
—○—: sulfanilamide.

13) J.A. Shannon, *Am. J. Physiol.*, **113**, 602 (1935).
14) T. Arita, R. Hori, M. Takada, S. Akuzu, and A. Misawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 570 (1972).

by this analysis. Therefore, it seems that Model A is more appropriate for these drugs. From the results of PSP, SMX, and SMZ,[1] it is presumed that the active secretion of drugs in the renal tubule is dependent upon the unbound drug concentration in plasma.

Comparison of the Rate of Secretion and Reabsorption between Computer Analysis and Inhibitory Experiments

In order to confirm the validity of the analysis by Model A, inhibitory experiments were performed at various drug concentrations in plasma. The rates of secretion determined by inhibitory experiments were compared with those calculated using the parameters obtained by this analysis. Iodopyracet was used in the inhibitory experiment. It is known that iodopyracet is actively secreted by anion transport mechanism and that it competes with *p*-aminohippurate (PAH) secretion. Renal excretion of PSP and SMX was inhibited by iodopyracet infusion. One such experiment of PSP is shown in Table I. *ER* was markedly diminished by iodopyracet loading. The rates of secretion (*S*) were determined by inhibi-

Table I. Renal Clearance Data of Phenolsulfonphthalein before and after Blockade of Tubular Secretion by Iodopyracet

	Time (min)	*GFR* (ml/min)	*P* (μM)	P_f (μM)	$U \cdot V$ (μmol/min)	*ER*
Control period	−30 to −20	9.59	198	17.3	2.01	12.1
	−20 to −10	9.59	196	15.2	1.93	13.2
	−10 to 0	9.21	181	13.1	1.62	13.5
		Tubular secretion blockade				
Inhibition period[a]	15—25	9.20	162	16.9	0.157	1.01
	25—35	9.25	139	15.9	0.146	0.99
	35—45	9.29	130	14.6	0.137	1.01

a) Iodopyracet infusion period.
Abbreviation in this table: *GFR*, glomerular filtration rate; *P*, total concn. in plasma; P_f, unbound concn. in plasma; $U \cdot V$, urinary excretion rate; *ER*, excretion ratio.

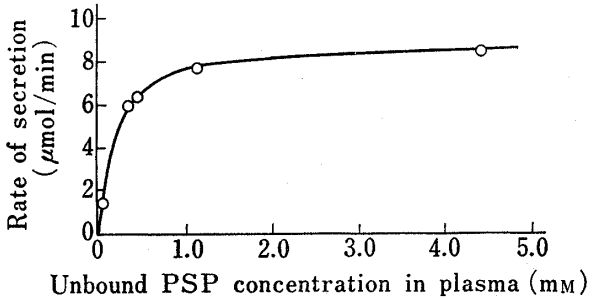

Fig. 3. Relationship between Plasma Concentration and Renal Tubular Secretion of Phenolsulfonphthalein (PSP)

Each point represents the experimental data obtained by inhibitory experiments. The solid line represents the calculated curve using the parameters obtained by the analysis of Model A. The parameters are as follows; inset.

V_{max}=9.0 μmol/min
K_m=0.15 mM

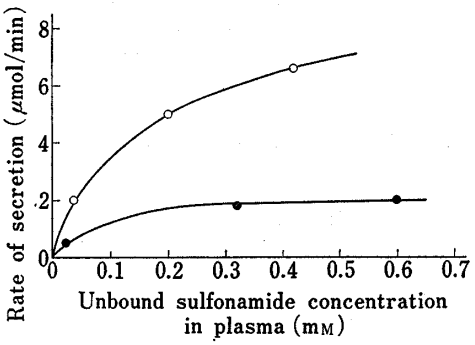

Fig. 4. Relationship between Plasma Concentration and Renal Tubular Secretion of Sulfamethoxazole (SMX) and Sulfamethizole (SMZ)

Each point represents the experimental data obtained by inhibitory experiments. The solid line represents the calculated curve using the parameters obtained by the analysis of Model A. The parameters are as follows;

	V_{max} (μmol/min)	K_m (mM)
SMX	2.1	0.04
SMZ	33	1.7

—●—: sulfamethoxazole.
—○—: sulfamethizole.

tory experiments,[5] and also calculated by Eq. 3[1] using the parameters obtained by the analysis of Model A.

$$S = \frac{V_{max} \cdot P_f}{K_m + P_f} \qquad \text{(Eq. 3)}$$

Comparison of these S of PSP are shown in Fig. 3. The calculated curve of Model A agreed well with the experimental data obtained by inhibitory experiments. The results of SMX are shown in Fig. 4 together with those of SMZ. There was good agreement between the respective theoretical curves and experimental data for these two drugs. On the other hand, as there was no secretion of SA, inhibition by iodopyracet was not observed. Thus, it is considered PSP and SMX are actively secreted by anion transport mechanism. The reabsorption fractions agreed well for all four drugs (Table II). From these findings it has been demonstrated that the results of analysis of Model A by the renal clearance experiments of the single-injection technique agreed well with the data obtained by the clearance experiments of constant-infusion technique for secretion and reabsorption, and that this method can be widely applied to the quantitative analysis of renal handling of drugs. Further, as neither constant infusion nor massive dose loading of secretion inhibitors were used in this method, the experiment was conducted under comparatively less harsh conditions than standatd renal clearance and inhibitory experiments. Thus, it is felt that this method can be safely applied to humans.

Table II. Comparison of Reabsorption Fractions Obtained by the Analysis of Model A and Inhibitory Experiments

	Reabsorption fraction (R)	
	Model A[a]	Inhibitory experiments[b]
Phenolsulfonphthalein	0	0
Sulfamethizole	0.20±0.02	0.22±0.02
Sulfanilamide	0.41±0.03	0.38±0.05
Sulfamethoxazole	0.91±0.08	0.89±0.09

a) The data were obtained by the analysis of Model A after administration of a single dose.
b) The data were obtained by combining the clearance experiments of constant-infusion technique with inhibitory experiments using iodopyracet.
Each value represents the mean ± S.E. from three or four animals.

Renal Tubular Transport Mechanisms of Phenolsulfonphthalein and Sulfonamides

Schematic representation of these analytical data are shown in Fig. 5. The order of reabsorption fraction was SMX>SA>SMZ>PSP=0. This was similar to the order of the partition coefficients of these drugs between chloroform or ethyl acetate as the lipid phase and aqueous phosphate buffer, pH 7.4 (Table III). Thus it is evident that reabsorption of these drugs is dependent upon the lipoid solubility of the drug. Further, the assumption advanced regarding reabsorption, that is, reabsorption is dependent upon nonionic diffusion, was supported. The order of maximum velocity of secretion (V_{max}) was SMZ>PSP>SMX >SA=0. Michaelis constant of secretion (K_m) also presented a similar relationship.

Summarizing these results, the renal excretion pattern of the drug used in this experiment can be classified into three types. PSP belongs to the no reabsorption type, SA to the no secretion type, and SMZ and SMX to the secretion and reabsorption type (Fig. 5). It is considered that the rate of secretion of SMZ, PSP, and SMX will fluctuate complicatedly dependent upon their respective concentrations in blood. Further, the urinary excretion of such drugs as SA is markedly reduced due to decreased glomerular filtration rate, while that of such drugs as SMZ is the result of reduction in renal tubular function. Therefore, in drugs which present different excretion patterns, it is presumed that the elimination rate will be

affected by the type of renal failure. Thus, it is felt that valuable basic knowledges have been obtained in the administration of drugs in diseased state. PSP was used clinically for renal function tests because the amount of secretion was remarkable, but this analysis revealed that the rate of SMZ secretion was greater than that of PSP. However, as SMZ was reabsorbed, it was not possible to analyze it by routine procedures to determine renal handling, but by using this method it is considered that more information can be obtained through a single renal function test. Thus, it is suggested that SMZ may be used as a reagent in the renal function test.

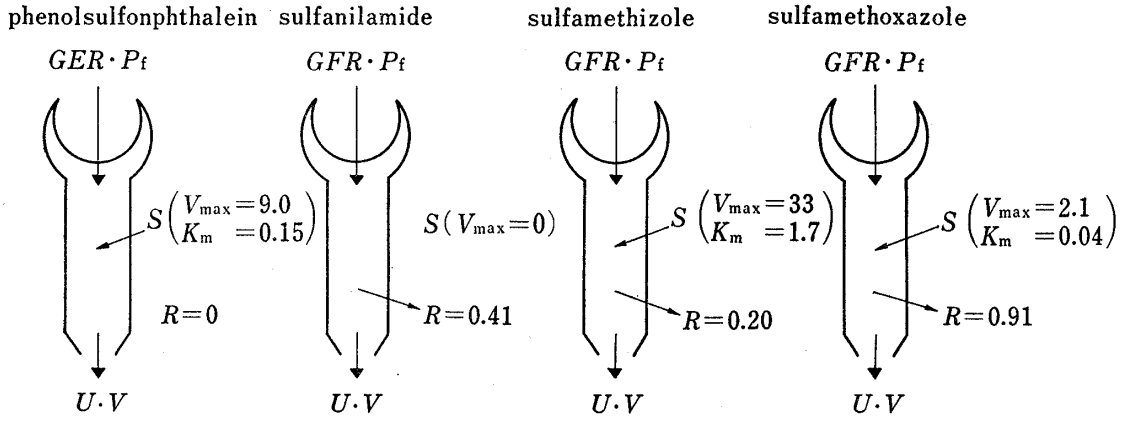

Fig. 5. Schematic Representation of Renal Tubular Transports of Phenolsulfonphthalein and Three Sulfonamides

Abbreviation in this figure: *GFR*, glomerular filtration rate; P_f, unbound drug concentration in plasma; *S*, rate of secretion; V_{max}, maximum velocity of secretion; K_m, Michaelis constant; *R*, reabsorption fraction; $U \cdot V$, urinary excretion rate.
V_{max} and K_m are in μmol/min and mM, respectively.

Table III. Reabsorption Fraction and Apparent Partition Coefficient of Phenolsulfonphthalein and Sulfonamides

	$R^{a)}$	P.C.$^{b)}$	
		Chloroform	Ethyl acetate
Phenolsulfonphthalein	0	<0.001	<0.01
Sulfamethizole	0.20±0.02	0.016	0.07
Sulfanilamide	0.41±0.03	0.032	2.28
Sulfamethoxazole	0.91±0.08	0.087	2.49

a) Reabsorption fraction: the data were obtained from the analysis of Model A by the renal clearance experiments of the single-injection technique. Each value represents the mean ± S.E. from three or four animals.
b) Apparent partition coefficient between chloroform or ethyl acetate and aqueous phosphate buffer, pH 7.4, at 37°.

In conclusion, study was made using this method with drugs which present characteristically different renal handlings. It has demonstrated that the tubular reabsorption of drugs is dependent on nonionic diffusion which can be explained by lipoid solubility of the drug, while tubular secretion can be explained by active transport that conforms to the Michaelis-Menten equation. Further, it is presumed that the tubular secretion of these drugs is dependent upon the unbound drug concentration in plasma. It has been confirmed that the renal handling of drugs can be analyzed under suitable conditions for humans by application of the method proposed.

This method is presently being applied to humans and to dogs with acute renal failure.

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