Transformation and Excretion of Drugs in Biological Systems. I. Renal Excretion Mechanisms of Sulfonamides. (1)[1]

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In order to understand the renal excretion mechanisms of sulfonamides, some renal clearance studies were undertaken with two sulfonamides, sulfanilamide and sulfisoxazole, in rabbits. From the results of inhibitory experiments by iodopyracet, it was found that only sulfisoxazole was actively secreted into the tubule by p-aminohippurate mechanism. Using this method it becomes possible to calculate and separately estimate the net amounts secreted and reabsorbed. The result calculated with sulfisoxazole suggested a significant amount secreted and a higher amount reabsorbed.

It has been increasingly recognized that the elucidation of the renal excretion mechanisms of drugs is important not only for studying pharmacological activity of drugs such as uricosuric agents but also for studying the effectiveness and duration of drug action.
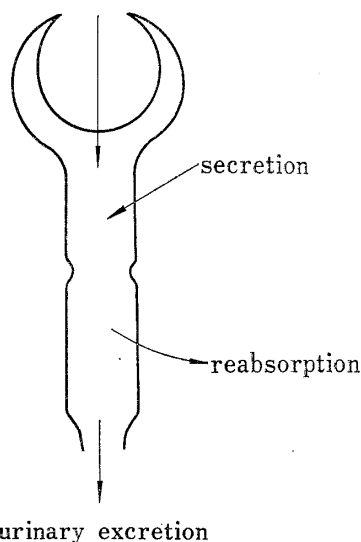
glomerular filtration

urinary excretion

Fig. 1. Schematic Presentation of Renal Tubular Mechanisms of Organic Acid Transport

General excretory mechanisms of organic acids was proposed by Weiner[3] as shown in Fig. 1. In this scheme, a wide variety of organic acids are filtrated, and, in addition, are secreted by the p-aminohippurate (PAH) mechanism. The compounds are then reabsorbed from the tubule by nonionic diffusion.

Although the urinary excretion of sulfonamides has been widely investigated from the view point of prolonging the drug action, the functional characteristics of the renal excretion and the relationship between the excretion rate and some physicochemical properties of sulfonamide are not clear yet. This may be due mainly to a lack of consideration of the quantitative relationship between the three directional transports, glomerular filtration, secretion, and reabsorption, in the nephron.

This work was undertaken to clarify the functional characteristics of the renal transport mechanisms for sulfonamides and the quantitative relationship between these mechanisms, using the renal clearance techniques in rabbits.

1) A part of this work was reported at the 88th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1968.
2) Location: Kita–12–jo, Nishi–6–chome, Sapporo.
3) a) I.M. Weiner and G.H. Mudge, Am. J. Med., 36, 743 (1964); b) I.M. Weiner, Ann. Rev. Pharmacol., 7, 39 (1967).

Experimental

Materials——Sulfanilamide and sulfisoxazole were of J.P. VII grade. Inulin for biochemical purposes and iodopyracet((3,5-diiodo-4-oxo-1,4-dihydro-1-pyridyl)acetic acid diethanolamine salt), mp 154—156° (decomp.), were used in the clearance experiments.

Clearance Method——Standard renal clearance techniques[4] were employed. Male albino rabbits, weighing 2.8—3.3 kg, were anesthetized with sodium pentobarbital (27 mg/kg, *i.v.*). After the midabdominal incision, the ureter was cannulated with a polyethylene catheter for urine collection. Sulfonamides and inulin were infused intravenously at the earlobe, so that the plasma concentration of each drug was relatively constant (below 0.1 mg/ml for sulfonamides and about 0.4 mg/ml for inulin). Inulin was primarily dosed at 300 mg, and was infused at 3 mg/min. The priming dose and sustaining dose of sulfonamides are given in Table I and III. Simultaneously sulfonamide and inulin clearances were determined every 10 minutes. Urine was collected during the period, and blood sample was obtained from the femoral artery at the mid-point of urine collection period.

Drug clearance (C) in ml/min is calculated as $C = UV/P$, where U and P, and V indicate urine and plasma concentration of the drug in mg/ml, and urine flow rate in ml/min, respectively. To estimate the renal handling for the drug, clearance ratio(CR) has been conventionally used and is expressed as $CR = C/GFR$, where GFR represents glomerular filtration rate in ml/min calculated as inulin clearance.

When the active tubular secretion of sulfonamides was examined, iodopyracet was infused simultaneously. In general, 580 mg of priming dose and 15 mg/min of sustaining dose of iodopyracet resulted in about 0.5 mg/ml of iodopyracet in plasma, and these doses seem to be adequate for the perfect blockade of the sulfonamides secretion as shown in Fig. 2. In each experiment, plasma concentration of iodopyracet was checked.

Urinary pH was changed by intravenous injection of 10% $NaHCO_3$ solution for alkalization and 10% NaH_2PO_4 solution for acidification, and was determined with a micro glass electrode (type HG–9005, TOA Electronics Ltd.) within a minute.

Urine flow rate was systematically increased by mannitol injection.

Each clearance data given in this paper is the typical one. These data showed results similar to those of other several experiments.

Determination of Plasma Protein Binding——The equilibrium dialysis technique[5] was used. Two milliliters of rabbit plasma containing sulfonamide was placed in a Visking tube, which was then bathed in 4 ml of phosphate buffer (pH 7.4). Equilibration was made for 24 hours at 37°. To examine the effect of iodopyracet on the protein binding of the sulfonamides, iodopyracet was initially added to plasma at the concentration of 1.5 mg/ml.

Analytical Methods——Plasma and urine samples were treated with Somogyi–deproteinizing reagents,[6] and then analyzed as follows: sulfonamides by the diazotization,[7] inulin by a modification of the method described by Dische,[8] and iodopyracet by the titration method described by Alpart.[9]

Results and Discussion

In order to study the renal functional characteristics of sulfonamides excretion, two sulfonamides, sulfanilamide and sulfisoxazole, were chosen as typical examples. The pK_{a2} values are 10.5 and 5.1, respectively. Some of the physicochemical properties such as lipoid solubility and protein binding of these two sulfonamides differ considerably.[10]

Active Tubular Secretion

It has been found that some sulfonamides are actively secreted into renal tubule in dogs,[11] and these secretion are competitively inhibited by iodopyracet[12] which has a remarkable affinity for the PAH mechanism.

4) M. Fujimoto, *Nippon Rinshō*, **25**, 1154 (1967).
5) W. Scholtan, *Arzneimittel–Forsch.*, **11**, 707 (1961).
6) M. Somogyi, *J. Biol. Chem.*, **86**, 655 (1930).
7) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 413 (1964).
8) Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **192**, 583 (1951).
9) L.K. Alpert, *Bull. Johns Hopkins Hosp.*, **68**, 522 (1941).
10) *a*) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 428 (1964); *b*) M. Yamazaki, M. Aoki, and A. Kamada, *ibid.*, **16**, 707 (1968); *c*) Idem, *ibid.*, **16**, 721 (1968).
11) A. Despopoulos and P.X. Callahan, *Am. J. Physiol.*, **203**, 19 (1962).
12) K. Irmscher, D. Gabe, K. Jahnke, and W. Scholtan, *Arzneimittel–Forsch.*, **16**, 1019 (1967).

In order to examine the active secretion of the sulfonamides in rabbits, the competitive inhibitory experiments were made. As the plasma concentration of iodopyracet was increased, the significant depression of sulfisoxazole excretion was observed in contrast to the constancy of sulfanilamide (Fig. 2). It is obvious from this result that sulfisoxazole is actively secreted, but there is no significant secretion of sulfanilamide. Despopoulos[13] pointed out that one of the substrate specificities of sulfonamides is ionized sulfonamide group. The result of Fig. 2 supported well this assumption.
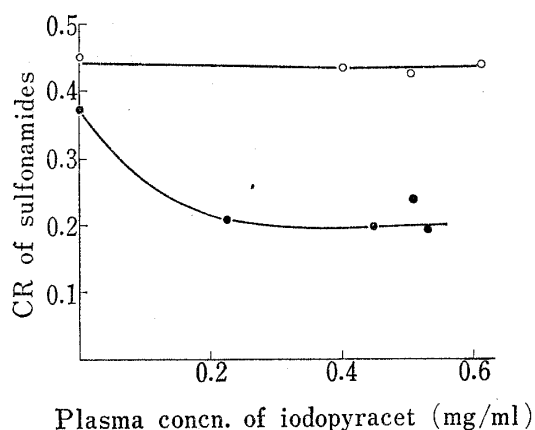
Fig. 2. Effect of Iodopyracet on Renal Excretion of Sulfonamides
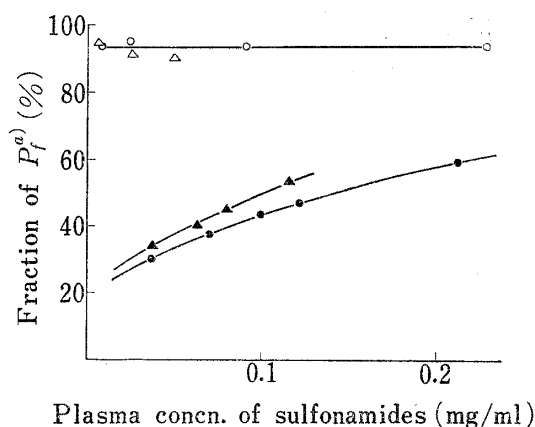
—O—: sulfanilamide
—●—: sulfisoxazole

Fig. 3. Plasma Protein Binding of Sulfonamides

a) P_f represents unbound plasma concn. of sulfonamides.
sulfanilamide
—O—: without iodopyracet   —△—: with iodopyracet
sulfisoxazole
—●—: without iodopyracet   —▲—: with iodopyracet

Effect of Plasma Protein Binding

It is well known that the drugs bound to plasma protein can not be filtrated at glomerulus. To determine the glomerular filterable concentration of the sulfonamides, the rabbit plasma protein binding was measured, and the results are shown in Fig. 3.

The considerable difference was observed between sulfanilamide and sulfisoxazole. From these results, it is assumed that a substantial fraction of sulfanilamide was filtrated, the filtration of sulfisoxazole was only about half that of sulfanilamide. It was also found that the fraction of sulfisoxazole bound to the protein depended on the plasma concentration of

TABLE I. Clearance Ratio of Sulfisoxazole before and after Blockade of Tubular Secretion

Time (min)	V (ml/min)	Urine pH	GFR (ml/min)	Sulfisoxazole				Corrected for binding[a]		
				U (mg/ml)	P (mg/ml)	C (ml/min)	CR	P_f (mg/ml)	C_f (ml/min)	CR_f
—30 to —20	1.65	6.1	10.6	0.0595	0.0593	1.65	0.15	0.0208	4.72	0.45
—20 to —10	1.42	5.8	10.8	0.0673	0.0605	1.58	0.15	0.0211	4.52	0.42
—10 to 0	1.57	5.9	10.9	0.0646	0.0607	1.67	0.15	0.0212	4.78	0.44
Iodopyracet, 580 mg $i.v.$, 15 mg/min infusion										
15—25	1.81	6.6	11.2	0.0287	0.0522	0.99	0.09	0.0191	2.71	0.24
25—35	1.02	6.0	10.0	0.0369	0.0517	0.73	0.07	0.0190	1.99	0.20
35—45	1.60	6.2	12.1	0.0278	0.0485	0.92	0.08	0.0173	2.57	0.21

a) subscript f represents corrected values for plasma protein binding. rabbit: 3.2 kg.
Sulfisoxazole was primarily dosed at 11.2 mg $i.v.$, and was infused at 0.15 mg/min.

13) A. Despopoulos, *J. Theoret. Biol.*, **8**, 163 (1965).

itself, and was affected a little by the addition of iodopyracet.   However, these effects could be eliminated by the correction for the protein binding.

One of the inhibitory experimental data were corrected by the protein binding on the basis of the data of Fig. 3.   It was obvious that these effects were not an influencing factor to the depression of sulfisoxazole excretion through the blockade of tubular secretion (Table I).

Separate Estimation of Amounts Secreted and Reabsorbed

Generally speaking, clearance ratio indicates only the overall renal excretion of the drug under experimental conditions, because some drugs are transported in the opposite direction by more than two mechanisms in the same tubule.   Several atempts have been made to elucidate these mechanisms mainly by using the stop flow technique[14] and the modified clearance techniques.[12,15]   But the quantitative relationship between secretion and reabsorption has remained obscure in many drugs.

A possible calculation of amounts secreted and reabsorbed in the tubule is described as below.

If the drug is filtrated, secreted, and then reabsorbed in the nephrons, the relation of the net amounts transported in each direction is represented as Fig. 4a.   In this scheme, the net amount of the drug filtrated at the glomeruli is represented as $GFR \cdot P_f$, and the net amount of the drug excreted in the urine is calculated as UV.
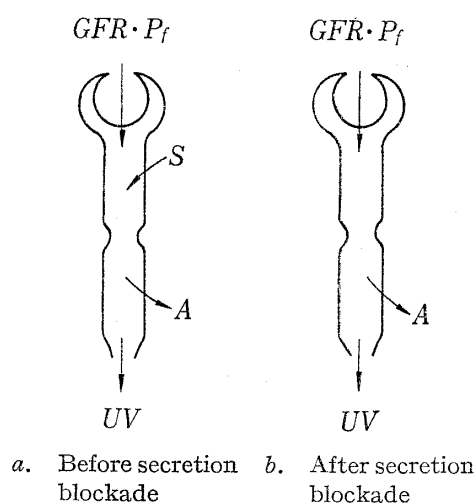

a. Before secretion   b. After secretion
    blockade   blockade

Fig. 4.   Relation of Net Amounts Transported for Each Direction in Tubule
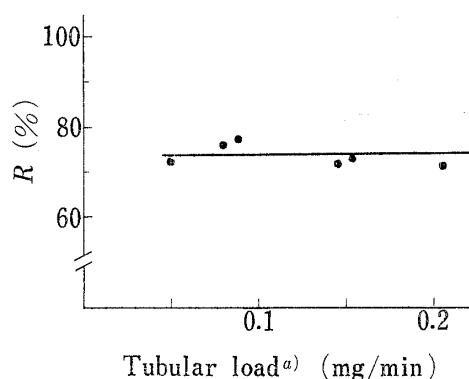

Tubular load[a] (mg/min)

Fig. 5.   Effect of Tubular Load of Sulfisoxazole on Its Tubular Reabsorption

Tubular secretion blockade was made the same condition as described in Experimental.
a) Plasma concentration of sulfisoxazole was decreased from 0.05 to 0.025 mg/ml.

Since the tubular load of the drug is the sum of $GFR \cdot P_f$ and S, which indicates the net amount of the drug secreted into the tubules, the net amount of the drug reabsorbed from the tubules (A) is theoretically represented as

$$A = (GFR \cdot P_f + S) - UV \tag{1}$$

and fraction of the tubular load reabsorbed (R) is

$$R = \frac{(GFR \cdot P_f + S) - UV}{GFR \cdot P_f + S} \tag{2}$$

From Eq. (2), the net amount secreted is calculated as

$$S = \frac{UV}{1 - R} - GFR \cdot P_f \tag{3}$$

where only R can not be determined experimentally by the usual clearance method.

14) a) R.L. Malvin, W.S. Wilde, and L.P. Sullivan, *Am. J. Physiol.*, **194**, 135 (1958); b) M. Sugita, *Nippon Jinzōgaku Kaishi*, **5**, 235 (1963).

Here, if the tubular secretion of the drug is perfectly blocked as shown Fig. 4*b*, Eq. (1) and (2) are simplified as shown in Eq. (4) and (5) respectively.

$$A = GFR \cdot P_f - UV \qquad (4)$$

$$R = \frac{GFR \cdot P_f - UV}{GFR \cdot P_f} \qquad (5)$$

In this case, R can be easily calculated since the drug is only reabsorbed as well as the case of sulfanilamide.

Now, it is assumed that the fraction of the tubular load reabsorbed does not change before and after the blockade of the tubular secretion. From this assumption, the net amounts secreted and reabsorbed can be calculated from Eq. (1) and (3) using the R value and the clearance data before the secretion blockade. The R value is obtained from Eq. (5) using the data after the secretion blockade.

The assumption described above would be supported by some experimental results. The clearance ratio of sulfanilamide, which was not actively secreted, was changed little by the addition of iodopyracet as shown in Fig. 2. The similar results were obtained with sulfadimethoxine in dogs.[16] This seems to suggest that the tubular reabsorption process of sulfonamides is affected little by the iodopyracet loaded into tubular lumen. It was also examined whether or not the sulfonamide tubular load decreased by the blockade of secretion changes its reabsorption fraction (R). From the result of Fig. 5, the change of the sulfisoxazole load at this range did not affect its fraction.

The separate estimation of the amounts secreted and reabsorbed was carried out with sulfisoxazole using the data of Table I, in which the renal functions were relatively constant before and after the tubular secretion blockade. This caution may be necessary to exclude the other influencing factors, such as GFR, urine flow rate, urinary pH and plasma concentration of the drug. The results are listed in Table II, and summarized in Fig. 6.

TABLE II. Net Amounts of Sulfisoxazole Transported for Each Direction in Tubules

Time (min)	mg/min				R
	$GFR \cdot P_f$	UV	S	A	
−30 to −20	0.22	0.10	0.23	0.35	
−20 to −10	0.23	0.10	0.21	0.34	0.78[a]
−10 to 0	0.23	0.10	0.23	0.36	
Tubular secretion blockade					
15—25	0.21	0.05		0.16	0.76
25—35	0.19	0.04		0.15	0.80
35—45	0.21	0.04		0.16	0.79

a) the average of the values after secretion blockade

Similar calculation was made for sulfanilamide (Table III) using Eq. (4) and (5), and the result are compared with that of sulfisoxazole (Fig. 7). In comparison with sulfanilamide, the renal handling of sulfisoxazole would be more complicated, and is characterized by the considerable secretion rate and higher reabsorption rate.

15) *a*) I.M. Weiner, J.A. Washington, Jr., and G.H. Mudge, *Bull. Johns Hopkins Hosp.*, **106**, 333 (1960);
    b) I.M. Weiner, K.C. Blanchard, and G.H. Mudge, *Am. J. Physiol.*, **207**, 953 (1964).
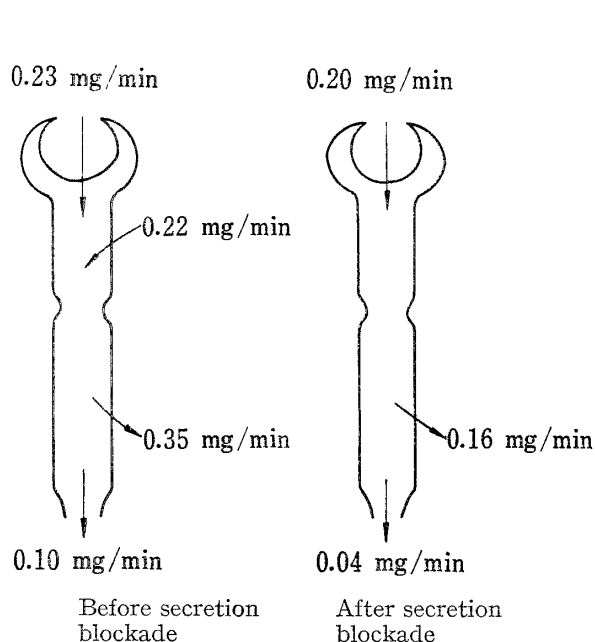16) T. Arita, R. Hori, and M. Takada, unpublished data.

0.23 mg/min   0.20 mg/min

0.22 mg/min

0.35 mg/min   0.16 mg/min

0.10 mg/min   0.04 mg/min

Before secretion   After secretion
blockade   blockade

Fig. 6.   Relation of Net Amounts of Sulfisoxazole
Transported for Each Direction in Tubules

100   100

94   6   33   67

32

43   51

51   14

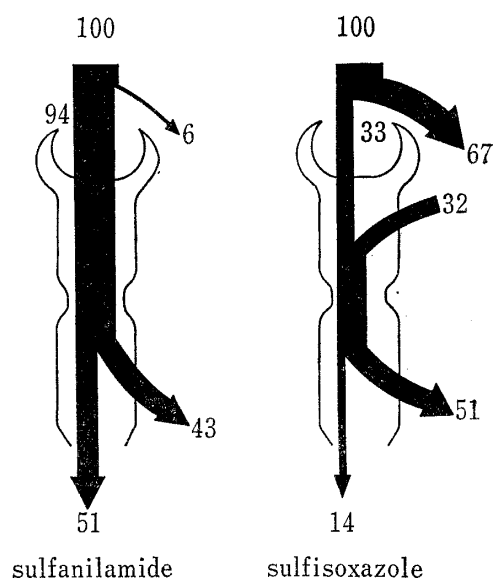sulfanilamide   sulfisoxazole

Fig. 7.   Quantitative Comparison of
Renal Excretion of Sulfonamides

The figures in this scheme represent percent of
net transported amounts to $GFR \cdot P$.

Table III.   Clearance Ratio of Sulfanilamide before and after Blockade of Tubular Secretion

Time (min)	V (ml/min)	Urine pH	GFR (ml/min)	Sulfanilamide				Corrected for binding		
				U (mg/ml)	P (mg/ml)	C (ml/min)	CR	P_f (mg/ml)	C_f (ml/min)	CR_f
-20 to -10	1.14	8.0	9.6	0.0913	0.0208	5.00	0.52	0.0196	5.31	0.55
-10 to 0	0.81	8.0	10.1	0.1144	0.0187	4.94	0.49	0.0176	5.25	0.52
			iodopyracet, 580 mg $i.v.$, 15 mg/min infusion							
15—25	1.18	8.0	11.7	0.0967	0.0187	6.10	0.52	0.0176	6.48	0.55
25—35	1.31	7.9	11.7	0.0937	0.0186	6.60	0.56	0.0175	7.01	0.60

rabbit: 3.3 kg
Sulfanilamide was primarily dosed at 45 mg $i.v.$, and was infused at 0.3 mg/min.

Tubular Reabsorption

Though several studies concerning the tubular reabsorption process of sulfonamides have been made, the functional characteristic is controversial.   Some of the urinary excretion[10a] and plasma elimination[17] or blood elimination[10b] kinetic studies suggested nonionic diffusion process.   On the other hand, other possible mechanisms such as ionic diffusion or active transport[14b,18,19] were assumed from the results of some sulfonamides renal clearance studies.

In order to clarify this point, some influencing factors on the tubular reabsorption process were examined with two sulfonamides.

It is well known that urine flow rate is one of the influencing factors on nonionic diffusion process, because this change affects the concentration ratio of the drug in urine and plasma (U/P).[20]   The effect of the urine flow rate on the tubular reabsorption of the sulfonamides

17) J. Rieder, *Arzneimittel–Forsch.*, 13, 81 (1963).
18) F. Portwich and H. Büttner, *Klin. Wschr.*, 42, 740 (1964).
19) D.P. Earle, Jr., *J. Clin. Invest.*, 23, 914 (1944).
20) M.D. Milne, B.H. Scribner, and M.A. Crawford, *Am. J. Med.*, 24, 709 (1958).
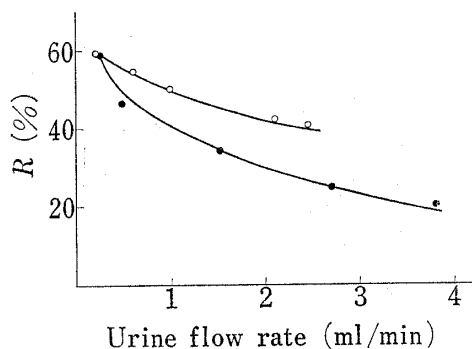
Fig. 8.   Influence of Urine Flow Rate
on Tubular Reabsorption of Sulfon-
amides

—O—: sulfanilamide
—●—: sulfisoxazole (under the condition of
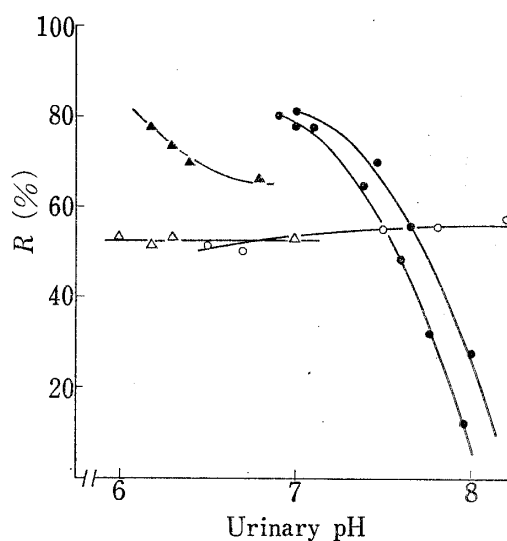   secretion blockade)

Fig. 9.   Influence of Urinary pH on
Tubular Reabsorption of Sulfonamides

sulfanilamide
   —△—: acidification   —O—: alkalization
sulfisoxazole (under the condition of secretion
blockade)
   —▲—: acidification   —●—: alkalization

are shown in Fig. 8.   As the urine flow rate increased, the gradual decrease of the reabsorp-
tion rate was observed for both sulfonamides.   This may suggest that the reabsorption process
of the sulfonamides is concerned with U/P of the drugs.

Urinary pH is another factor affecting the nonionic diffusion process.[20]   The considerably
different responses to urinary pH change were observed between sulfanilamide and sulfisoxazole
as shown in Fig. 9.

According to Yamazaki,[10c] one possible interpretation of these behaviours might be
based on the nonionic diffusion process.   From its pKa_2 value of 10.5, sulfanilamide exists
almost in molecular form in experimental urine pH, and its lipoid solubility remaines constant.
While the ionized form of sulfisoxazole increases considerably in alkaline urine, accompanied
by a marked change of lipoid solubility, because of its lower pKa_2 value of 5.1.   If diffusable
sulfonamides are predominantly molecular form, similar patterns should be found in the
reabsorption behaviours.

These results suggested the nonionic diffusion process, but this may be regarded as appro-
priate only for sulfanilamide.   Because some other possible mechanisms such as ionic diffusion
or other transport systems related in the acid-base balance[20] would not be neglected, futher
study will be undertaken to understand this point.