[Chem. Pharm. Bull. 23(12)3215—3222(1975)]

UDC 547.551.52.09 : 615.31.034

Transformation and Excretion of Drugs in Biological Systems. XI.[1] Renal Excretion Mechanisms of Sulfonamides in Rabbits. (3)[2]

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(Received May 6, 1975)

In order to clarify the renal tubular reabsorption of sulfonamides, the effects of urinary pH and urine flow rate on the renal excretion of four sulfonamides were examined in rabbits. The excretion ratio (ER) of sulfanilamide, sulfamethoxypyridazine and sulfamethizole were little or not affected by modification of the urinary pH, but that of sulfisoxazole was considerably influenced. It was also demonstrated that the ER of these sulfonamides except for sulfamethizole increased with increasing urine flow rate. Only sulfamethizole exhibited as the excretion pattern which was dependent on its plasma level. These different responses to the influencing factors may be described on the basis of their secretion characteristics, reabsorption by non-ionic diffusion and their lipoid solubility.

In the previous paper,[1] the authors reported on the renal clearances of four sulfonamides in rabbits and made comparison against one another for renal handling. The authors were able to confirm that the renal tubular reabsorption of these sulfonamides depended on the lipoid solubility. Although this finding strongly suggests a non-ionic diffusion characteristic in the reabsorption, other investigators have proposed the presence of possible carrier mediated reabsorption in some sulfonamides.[4]

The experiments described below deal with the effect of some physiological factors, such as urinary pH and urine flow rate, on the renal excretion of sulfonamides to clarify the tubular reabsorption mechanism in rabbits.

Experimental

Materials——Sulfanilamide, sulfisoxazole and sulfamethizole used were J.P. VIII grade. Commercially available sulfamethoxypyridazine was recrystallized from EtOH, mp 182—183°. Inulin for biochemistry (E. Merck) and iodopyracet [(3,5-diiodo-4-oxo-1,4-dihydro-1-pyridyl) acetic acid diethanolamine salt], supplied by Daiichi Seiyaku Co., Ltd., mp 154—156° (decomp.) were used in the clearance experiments. All other chemicals used were reagent grade.

Renal Clearance Experiments——All of the experiments were carried out on male and female albino rabbits weighing 2.5—3.5 kg (anesthetized with sodium pentobarbital, 27 mg/kg *i.v.*) by means of the standard renal clearance technique. Priming doses of the sulfonamides were 6 mg/kg for sulfanilamide and 8 mg/kg for the others. The sustaining doses were 0.2 mg/min/body and 0.4 mg/min/body, respectively. Inulin was primarily dosed at 120 mg/kg and infused at 3 mg/min/body to estimate the glomerular filtration rate (GFR). The sulfonamides and inulin were dissolved in saline solution and injected through the auricular vein. The sustaining solution was infused at a rate of 1 ml/min. Details of the procedure to determine the control clearances are as described in the previous report.[1,5]

For the purpose of inhibition of active tubular secretion in only the cases injected with sulfamethizole and sulfisoxazole, the initial iodopyracet dose (200 mg/kg, *i.v.*) was administered after two or three control

1) Part X: E. Owada, K. Takahashi, R. Hori, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), **22**, 594 (1974).
2) A part of this work was reported at the 2nd Symposium on Drug Metabolism and Action, Pharmaceutical Society of Japan, Kyoto, November 1970.
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4) *a*) M.D. Milne, B.H. Scribner, and M.A. Crawford, *Am. J. Med.*, **24**, 709 (1958); *b*) F. Portwich and H. Buttner, *Klin. Wschr.*, **42**, 740 (1964); *c*) M. Sugita, K. Sugita, T. Furukawa, and H. Abe, *Japanese Circulation J.*, **31**, 423 (1967).
5) T. Arita, R. Hori, E. Owada, and K. Takahashi, *Chem. Pharm. Bull.* (Tokyo), **17**, 2526 (1969).

clearance periods, and a sustaining infusion (15 mg/min/body) was continued. In each inhibitory experiment the plasma iodopyracet level was checked. The levels were adequate to reduce the sulfonamide secretion to negligible levels.[1)]

In addition to the experiments described above, another series of experiments were performed. All procedures were the same except that urinary pH or urine flow rate of the rabbits were continuously modified by the following methods.

Change of Urinary pH——The rabbit urine was gradually acidified by increasing dose of 10% NaH_2PO_4 solution (*i.v.*) at the end of each clearance period.

On the other hand, experiments for alkalization of urine were performed as follows. As rabbits usually excrete alkaline urine (pH 7.5—8.5), each rabbit received either 5—10 ml of 10% NaH_2PO_4 solution (*i.v.* just before the experiment) or 10 ml of 1N HCl (with stomach tube the night before) for acidification of urine. After one or two clearance periods under acidic urine the rabbit urine was alkalized stepwise by increasing doses of 10% $NaHCO_3$ solution (*i.v.*) at the end of each clearance period. One of these clearance experiments is shown in Table I.

Change of Urine Flow Rate——Osmotic diuresis was induced by mannitol injection to increase the urine flow rate. After one or two control clearance periods the rabbit received increasing dose of mannitol (4—13% solution at 1—2 ml/min, *i.v.*) during the experiment. One of these experiments is shown in Table II.

Determination of Relative Lipoid Solubility——Five milliliters of 0.2 mM sulfonamide solution (pH 6—8, isotonic phosphate buffers) was equilibrated with an equal volume of $CHCl_3$ at 37° after which the sulfonamide content in the aqueous phase was determined. The relative lipoid solubility was expressed as $1\text{-}(C_e/C_i)$, where C_i and C_e represent the sulfonamide concentrations in the aqueous phase before and after equilibration.

Analytical Methods——The plasma and urine samples were treated with Somogyi-deproteinizing reagents,[6)] and then analyzed as follows: sulfonamides (not acetylated) by diazotization,[7)] inulin by a modification[8)] of Dische's method,[9)] and iodopyracet by Alpert's method.[10)] The urinary pH was measured with a micro glass electrode (type HG-9005, Toa Electronics Ltd.) within a minute after the sampling.

Results and Discussion

In order to study the effects of urinary pH and urine flow rate on the renal excretion of sulfonamides, four sulfonamides (sulfisoxazole, sulfamethizole, sulfamethoxypyridazine and sulfanilamide) were chosen as typical examples. As previously described,[1)] these sulfonamides possess wide varieties of pK_a and lipoid solubility. It was also evident that only sulfisoxazole and sulfamethizole were secreted into rabbit renal tubules by the *p*-aminohippurate (PAH) mechanism.[1)]

In this report, the renal excretion of sulfonamides is expressed as "Excretion ratio (ER)" and provides information on the transport in the tubules.[1)] ER means the ratio of unbound drug clearance to inulin clearance and is calculated as $U \cdot V/P \cdot f$-GFR, where the abbreviations indicate as follows: U and P, urine and plasma concentrations of sulfonamide; V and GFR, urine flow and glomerular filtration rate; f, fraction of sulfonamide unbound by the plasma protein. Each f value was obtained from the "standard curves" shown in the previous report.[1)]

Relationship between Plasma Level and Renal Excretion of Sulfonamides

All of the control ER values for the sulfonamides were plotted against their plasma levels to examine whether the sulfonamide plasma levels other than urinary pH and urine flow rate affect their own renal excretion or not (Fig. 1).

The ER of three sulfonamides, excluding sulfamethizole, was not a function of the respective plasma levels. Especially, ER of sulfisoxazole ranged widely from net reabsorption (ER$<$1) to net secretion (ER$>$1). This implies that there is a large individual difference in the secretory activity and/or marked effects of physiological factors such as urine pH and flow on the reabsorption process. In the case of sulfamethizole, the ER decreased rapidly while its plasma

6) M. Somogyi, *J. Biol. Chem.*, **86**, 655 (1930).
7) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 428 (1964).
8) T. Arita, R. Hori, M. Takada, S. Akuzu, and A. Misawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 570 (1972).
9) Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **192**, 583 (1951).
10) L.K. Alpert, *Bull. Johns Hopkins Hosp.*, **68**, 522 (1941).

level increased. This pattern is similar to that of PAH,[11] which suggests there is a low transport maximum (Tm) for sulfamethizole secretion.

Relationship between Urinary pH and Renal Excretion of Sulfonamides

To learn the influence of urinary pH, each sulfonamide ER value obtained from individual rabbits in whom the urine flow rate lay within a narrow range (V/GFR: 0.04—0.09), was plotted against the urinary pH as shown in Fig. 2 and 3.

The ER of sulfisoxazole increased with elevation in the urine pH value in both control and inhibitory (secretion blockage) conditions. The same tendency was also observed for sulfamethizole in inhibitory but not in control conditions (Fig. 2 and 3). On the contrary, ER of sulfanilamide and sulfamethoxypyridazine remained relatively constant with change in the urinary pH (Fig. 3).

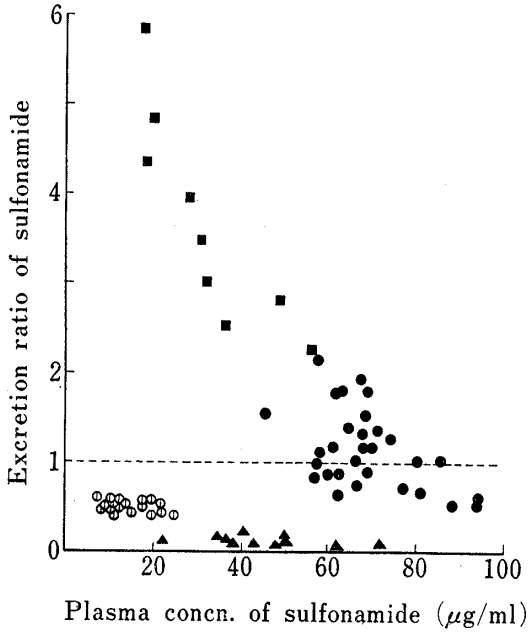

Fig. 1. Relationship between Plasma Levels and Renal Excretion of Sulfamethizole (■), Sulfisoxazole (●), Sulfanilamide (◫) and Sulfamethoxypyridazine (▲)

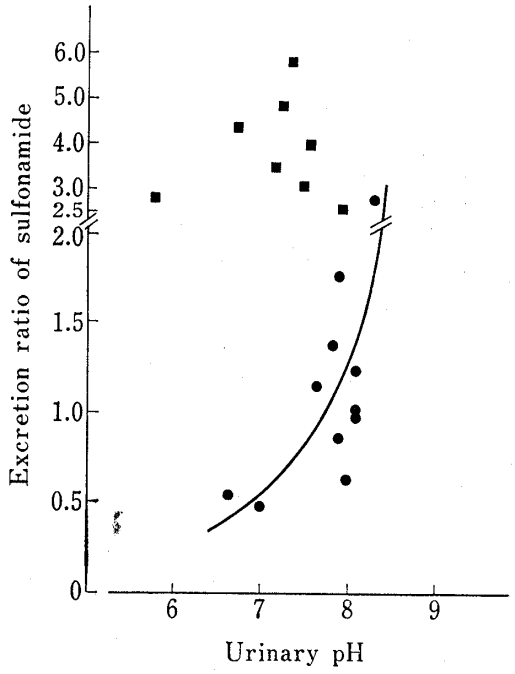

Fig. 2. Relationship between Urinary pH and Renal Excretion of Sulfamethizole (■) and Sulfisoxazole (●)
V/GFR: 0.04—0.09

Effect of Modification of Urinary pH on Renal Excretion of Sulfonamides

For the purpose of further confirmation of the urinary pH effect, the clearances were determined continuously for the same rabbit, in whom urinary pH was gradually modified by the methods described in the experiments. Details of the representative experiment are shown in Table I, and all of the results are summarized in Fig. 4, 5, and 6.

It becomes apparent from Fig. 4 and 5 that the renal excretion of the sulfonamides excluding sulfisoxazole was little affected by the modification of urinary pH. However, in the case of sulfisoxazole, the ER values changed from net reabsorption to net secretion with the alkalization of urine. Although the same tendency was demonstrated for sulfisoxazole in inhibitory condition (Fig. 6), it was difficult to explain the considerable effect of $NaHCO_3$ infusion on the ER beyond unity on the basis of urinary pH alone. The stimulative effect of $NaHCO_3$ on the PAH mechanism might be one of the factors involved. However, except for this point, the results confirmed well the relation mentioned above.

11) R.F. Pitts, "Physiology of the Kidney and Body Fluids," Year Book Medical Publishers, Inc., Chicago, 1968.
12) J.J. Cohen and E.W. Randall, *Am. J. Physiol.*, **206**, 383 (1963).

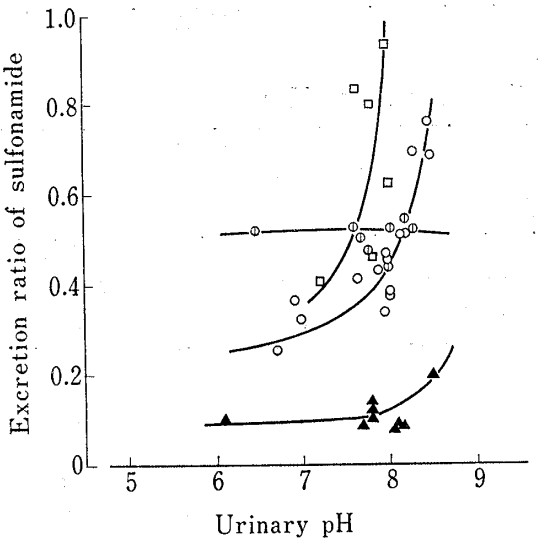

Fig. 3. Relationship between Urinary pH and Renal Excretion of Sulfanilamide (◑), Sulfamethoxypyridazine (▲), Sulfamethizole with Iodopyracet (□), and Sulfisoxazole with Iodopyracet (○)

V/GFR: 0.04—0.09

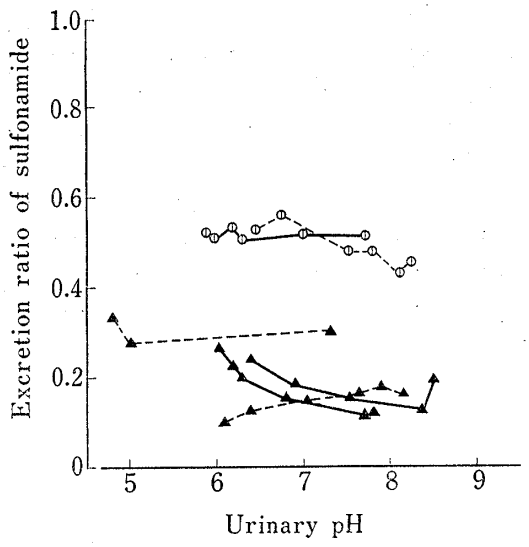

Fig. 4. Effect of Acidification (solid line) and Alkalization (dotted line) of Urine on Renal Excretion of Sulfamethoxypyridazine (▲) and Sulfanilamide (◑)

Table I. Effect of Alkalization of Urine on Renal Excretion of Sulfamethoxypyridazine

10% $NaHCO_3$ soln. *i.v.* injection	Time (min)	V (ml/min)	Blood pH	Urine pH	GFR (ml/min)	Sulfamethoxypyridazine U (μg/ml)	P (μg/ml)	$U \cdot V/P$ (ml/min)	$U \cdot V/P \cdot$GFR	f	ER
0	10— 0	0.732	7.5	6.1	11.2	17.4	50.6	0.252	0.0225	0.22	0.103
2 ml	0										
4 ml	10— 20	0.282	7.5	6.4	14.7	56.8	43.2	0.371	0.0252	0.20	0.126
6 ml	30— 40	0.496	7.5	7.1	12.1	27.0	38.8	0.345	0.0285	0.19	0.150
8 ml	50— 60	0.460	7.6	7.7	12.3	31.4	38.2	0.378	0.0308	0.19	0.163
10 ml	70— 80	0.222	7.6	8.1	8.5	43.8	37.4	0.260	0.0306	0.19	0.164
	90—100	1.140	7.6	7.9	14.2	20.8	44.1	0.536	0.0377	0.20	0.186

Experimental Rabbit No. 5. (3.1 kg) received 10 ml of 1 N HCl (*p.o.*) 18 hr before this experiment. Abbreviation in this and subsequent table: V, urine flow rate; CFR, glomerular filtration rate; U, concn. in urine; P, concn. in plasma; f, fraction nubound to plasma protein; ER, excretion ratio ($U \cdot V/P \cdot f \cdot$GFR).

Relationship between Urine Flow Rate and Renal Excretion of Sulfonamides

The influence of urine flow rate was examined next. Each sulfonamide ER value obtained from individual rabbits (urinary pH: 7.5—8.2) was correlated with the urine flow rate (water excretion ratio: V/GFR) as shown in Fig. 7 and 8.

ER of sulfisoxazole increased apparently with increase in the urine flow rate in both control and inhibitory condition. The ER of sulfanilamide also depended on the urine flow rate, but to a lesser extent than that of sulfisoxazole. This correlation was not observed in the cases of sulfamethizole and sulfamethoxypyridazine, however they were investigated in detail by use of increasing urine flow rate as described below (Fig. 9 and 10).

Effect of Increasing Urine Flow Rate on Renal Excretion of Sulfonamides

Another series of experiments were carried out to confirm the effect of urine flow rate. Each rabbit received mannitol infusion and the clearances were continuously determined while gradually increasing diuresis. On of these experiments is exemplified in Table II, and all the results are summarized in Fig. 9 and 10.

The ER of sulfisoxazole increased together with increasing urine flow rate in both control and inhibitory conditions (Fig. 9 and 10). The renal excretion of sulfanilamide and sulfamethoxypyridazine also depended on the urine flow rate, but the degree of dependence was relatively less than that of sulfisoxazole (Fig. 9). Sulfamethizole was excreted in the same

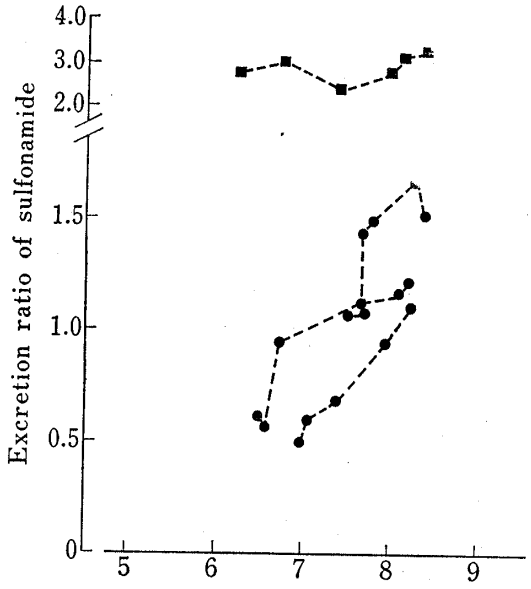

Fig. 5. Effect of Alkalization of Urine on Renal Excretion of Sulfamethizole (■) and Sulfisoxazole (●)

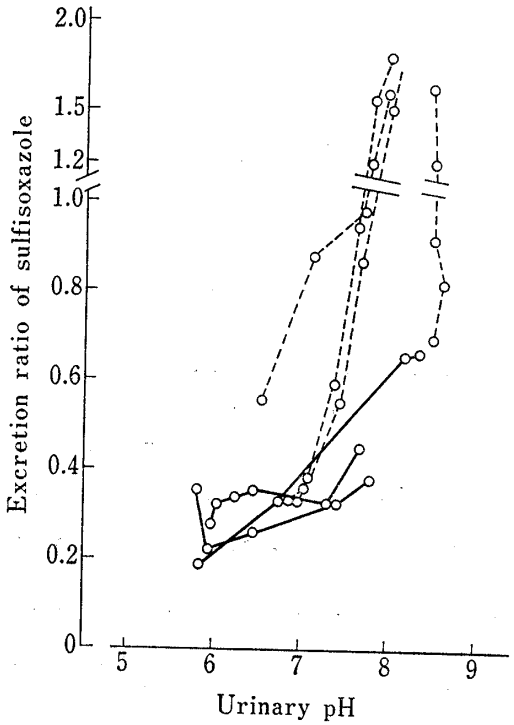

Fig. 6. Effect of Acidification (solid line) and Alkalization (dotted line) of Urine on Renal Excretion of Sulfisoxazole with Iodopyracet

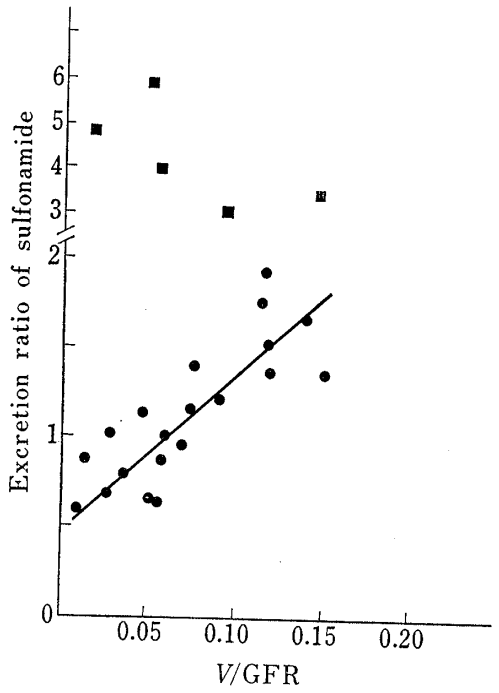

Fig. 7. Relationship between Urine Flow Rate and Renal Excretion of Sulfamethizole(■) and Sulfisoxazole(●)
urinary pH: 7.5—8.2

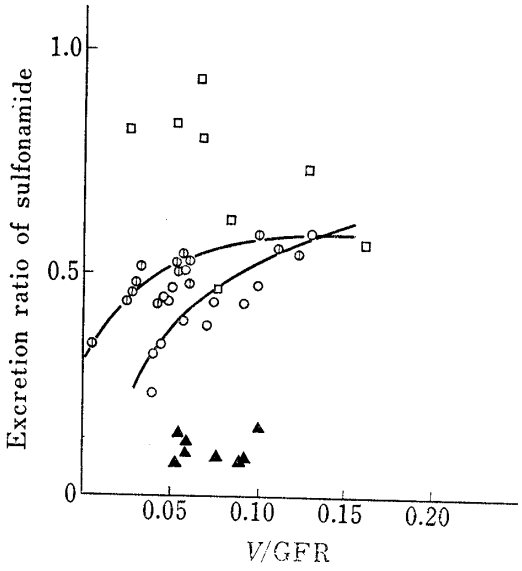

Fig. 8. Relationship between Urine Flow Rate and Renal Excretion of Sulfanilamide(◫), Sulfamethoxypyridazine(▲), Sulfamethizole with Iodopyracet(□) and Sulfisoxazole with Iodopyracet(○)
urinary pH: 7.5—8.2

Table II. Effect of Increased Urine Flow Rate on Renal Excretion of Sulfanilamide

	Time (min)	V (ml/min)	Urine pH	GFR (ml/min)	Sulfanilamide					
					U (μg/ml)	P (μg/ml)	$U \cdot V/P$ (ml/min)	$U \cdot V/P \cdot$ GFR	f	ER
	10— 0	0.720	8.3	10.3	80.2	13.6	4.24	0.412	0.86	0.480
Starting	0									
mannitol	5—15	1.04	8.1	9.39	59.8	14.1	4.42	0.472	0.86	0.580
infusion	20—30	1.41	8.0	9.40	44.2	13.5	4.62	0.494	0.86	0.494
	40—50	1.90	7.9	9.20	33.7	13.2	4.85	0.527	0.86	0.615
	55—65	2.45	7.7	9.11	26.2	12.7	5.07	0.556	0.86	0.650
	75—85	3.12	7.6	8.31	21.8	12.9	5.27	0.635	0.86	0.740

Experimental Rabbit No. 24. (3.0 kg) received mannitol *i.v.* infusion after one control period. Concn. of mannitol in infusate was gradually increased 4—18%. Infusion rate was 1—2 ml/min.

manner in inhibitory condition (Fig. 10). In any case, it is evident from this and the preceding results that renal excretion of the sulfonamides, except for sulfamethizole in the control condition, depended on the urine flow rate.

Renal Excretion and Tubular Reabsorption of Sulfonamides

It has been recognized that non-ionic diffusion is one of the important processes of renal tubular reabsorption of drugs.[4a] In general, modification of urinary pH changes the fraction of diffusible (unionized) species of acidic drugs, such as sulfonamides, in urine. This causes alteration in tubular reabsorption, and consequently a change in renal excretion of drugs. However, this effect may be attributed to both pK_a and lipoid solubility of acidic drugs.[13]

Fig. 11 shows the effect of pH on the relative lipoid solubility of the sulfonamides as a combined index of pK_a and lipoid solubility. If non-ionic diffusion played an important role

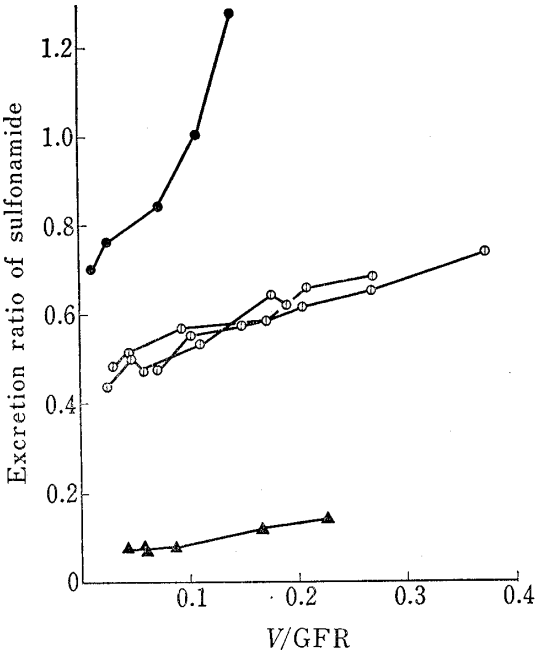

Fig. 9. Effect of Increased Urine Flow Rate on Renal Excretion of Sulfisoxazole (●), Sulfanilamide(◑) and Sulfamethoxypyridazine(▲)

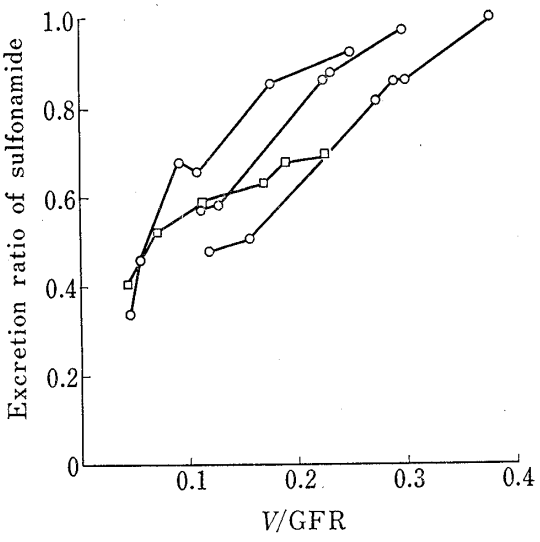

Fig. 10. Effect of Increased Urine Flow Rate on Renal Excretion of Sulfisoxazole with Iodopyracet(○) and Sulfamethizole with Iodopyracet(□)

13) I.M. Weiner and G.H. Mudge, *Am. J. Med.*, **36**, 743 (1964).

in the reabsorption of sulfonamides, it might be expected that change in urinary pH considerably affected the renal excretion of sulfisoxazole and sulfamethizole, but to a lesser degree or not at all sulfamethoxypyridazine and sulfanilamide.

As mentioned above in this report, the experimental results were in accord with the expectation for three sulfonamides but not for sulfamethizole. Considerably larger secretion than reabsorption of sulfamethizole,[1] as well as the effect of its plasma levels (Fig. 1) may have masked the urinary pH effect on the reabsorption. It is noteworthy that a well inverted relation was demonstrated between the patterns shown in Fig. 11 and 3, in which each sulfonamide secretion was regarded to be negligible. This suggests that there was large contribution of non-ionic diffusion to the reabsorption of sulfonamides.

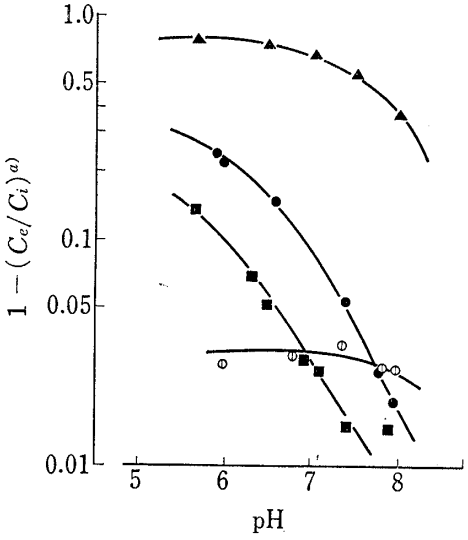

Fig. 11. Effect of pH on Relative Lipoid Solubility of Sulfamethoxypyridazine (▲), Sulfisoxazole (●), Sulfanilamide(ⓘ) and Sulfamethizole (■)

a) Relative Lipoid solubility; see Text

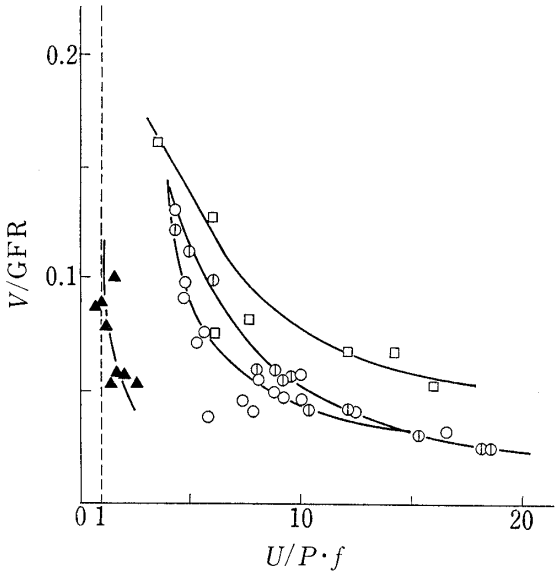

Fig. 12. Relationship between Water Excretion Ratio and Urine/Plasma Water Concentration Ratio of Sulfamethoxypyridazine(▲), Sulfisoxazole with Iodopyracet (○), Sulfanilamide(ⓘ) and Sulfamethizole with Iodopyracet(□)

urinary pH: 7.5—8.2

It is well known that increased urine flow rate reduces the concentration ratio of drugs in urine and plasma, and consequently reduces the reabsorption by non-ionic diffusion.[4a] As already mentioned above, renal excretion of these sulfonamides except for sulfamethizole were dependent on the urine flow rate. The urine flow rate independence of sulfamethizole could be explained on the basis of the same reason for the urinary pH independence.

Figure 12 shows the relationship between the concentration ratio of sulfonamides in urine and plasma water ($U/P\cdot f$) and water excretion ratio (V/GFR) using the same data shown in Fig. 8 (under the condition in which the tubular secretion was negligible). It is evident that all $U/P\cdot f$ ratios were larger than unity and in ascending order, the ratios among the sulfonamides were sulfamethoxypyridazine, sulfisoxazole, sulfanilamide and sulfamethizole in all levels of water excretion ratio. This order corresponded well to the order of relative lipoid solubility (at pH 7.4).[1] These results suggest that the active up-hill transport is insignificant for the sulfonamide reabsorption process and the permeability of the sulfonamides through the tubular wall is dependent on the lipoid solubility.

In conclusion, different effects of urinary pH, as well as urine flow rate on the renal excretion were observed among the sulfonamides and on the condition with or without secretion

blockage. However, the experimental results presented in this report were almost explicable on the basis of the tubular reabsorption by non-ionic diffusion and lipoid solubility of the sulfonamides. It is necessary to give consideration to the fact that despite the high lipoid solubility of sulfamethoxypyridazine, the renal excretion of this sulfonamide was relatively little affected by the change in urine flow rate. In this connection, further study will be undertaken.

Acknowledgement The authors thank Miss Mariko Ikeda for her technical assistance, and Daiichi Seiyaku Co., Ltd. for supplying the iodopyracet pure powder.