[Chem. Pharm. Bull. 22(3) 594—600 (1974)]

UDC 547.269.3'233.09 : 615.31.034.076.9

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

Eiji Owada, Katsuyuki Takahashi,[3] Ryohei Hori[3a] and Takaichi Arita[3]

Faculty of Pharmaceutical Sciences, Hokkaido University[3]

(Received June 18, 1973)

In order to clarify the renal excretion mechanisms of sulfonamides in rabbits the renal clearances of four sulfonamides, which were chosen of widely differing physico-chemical properties, were measured both in control and iodopyracet (IP) infusion periods. In control periods, all of the sulfonamides clearance were lower than inulin clearance, and descending order of these clearances were sulfamethizole (SMZ), sulfanilamide (SA), sulfisoxazole (SI) and sulfamethoxypyridazine (SMP). Sulfamethizole clearance corrected for the protein binding showed net secretion in the tubules, but the other sulfonamide clearances corrected showed net reabsorption. In iodopyracet infusion periods, the clearances of sulfamethizole and sulfisoxazole were significantly depressed below the inulin clearances, suggesting the secretion by the *p*-aminohippurate (PAH) mechanism and the reabsorption concurrently occurred. Further, the transfer rates in each direction into and out of the renal tubules were calculated with the four sulfonamides, and the determinating factors of the urinary excretions were discussed with reference to the physico-chemical properties.

It becomes important to clarify the renal handling of drugs not only for prolonging the drug action but also for prevention of the unexpected adverse reaction with simultaneous administration,[1,4] large[5] and multiple[6] dosing. However, on the studies of the drug excretion the conventional urinary excretion[7] or blood elimination[8] kinetics have not always useful, because these approaches could not reveal quantitatively the functional characteristics of the renal handling. In this connection, the authors previously proposed a possible calculation procedure[9] to estimate each transfer rate in opposite direction(secretion and reabsorption) in the same tubules, using the renal clearance technique.

The present investigation was undertaken to compare with the behavior of four typical sulfonamides, of which physico-chemical properties widely differ, in the rabbit kidney according to the procedure described earlier.[9]

Experimental

Materials——Sulfanilamide, sulfisoxazole and sulfamethizole were J. P. VIII grade. Commercially available sulfamethoxypyridazine was recrystallized from EtOH. mp 182—183°. Inulin for biochemistry

1) Part IX: M. Takada, A. Misawa, K. Fujimoto, R. Hori, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), **22**, 551 (1974).
2) A part of this work was reported at the 89th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1969.
3) Location: *Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo*; *a*) Present address: *Faculty of Pharmaceutical Sciences, Hiroshima University, Kasumi 1-2-3, Hiroshima.*
4) M. Takada, S. Akuzu, A. Misawa, R. Hori, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), **22**, 542 (1974); E. Owada and K. Takahashi, *Yakugaku Zasshi*, **94**, 62 (1974).
5) B.M. Tune, *J. Pharmacol. Exptl. Therap.*, **181**, 250 (1972).
6) I.M. Weiner and J.P. Tinker, *J. Pharmacol. Exptl. Therap.*, **180**, 411 (1972); Y. Suzuki, K. Ina, M. Ito, and I. Yamagami, *Nippon Yakurigaku Kaishi*, **67**, 89 (1971).
7) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 428 (1964).
8) *a*) J. Rieder, *Arzneimittel-Forsch.*, **13**, 81 (1963); *b*) M. Yamazaki, M. Aoki, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **16**, 707 (1968).
9) T. Arita, R. Hori, E. Owada, and K. Takahashi, *Chem. Pharm. Bull.* (Tokyo), **17**, 2526 (1969).

(E. Merck) 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. All other chemicals were reagent grade.

Renal Clearance Experiment——All the experiments were carried out in male and female albino rabbits weighing 2.7—3.5 kg (anesthetized with sodium pentobarbital, 27 mg/kg *i.v.*) by means of the standard renal clearance technique.[10] 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 (to estimate the glomerular filtration rate) was primarily dosed at 120 mg/kg, and was infused at 3 mg/min/body. 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. The detailed procedure was as described in the previous report.[9]

In order to inhibit the proximal tubular secretion of the sulfonamides, iodopyracet was initially given (200 mg/kg *i.v.*) after two or three control clearance periods, and a sustaining infusion of iodopyracet was continued at 15 mg/min/body.

Determination of Plasma Protein Binding——The extent of binding of the sulfonamides to rabbit plasma protein was determined by the membrane ultrafiltration technique.[11] A large volume of rabbit plasma was obtained by pooling samples from four animals, and then 1 part of sulfonamide solution was added to 9 parts of this plasma, to give the final concentration of 15—200 μg/ml. Two milliliters of the plasma sample was placed in a Centriflo membrane cone (CF-50-A, Amicon Co.), and then centrifuged at 2300 rpm for 16 min under an atmosphare containing 5% CO_2 at room temperature. One half milliliter of the plasma ultrafiltrate was subjected to analysis. To examine the effect of iodopyracet on the binding of the sulfonamides, iodopyracet was added to the plasma at the concentration of 500 μg/ml.

These sulfonamides were little adsorbed by the membrane, hence, the fraction of unbound sulfonamide (f) was calculated as $f=F/P$, where P and F indicated the concentration of sulfonamide in each plasma and corresponding ultrafiltrate, respectively.

Determination of Relative Lipoid Solubility——Five milliliters of the 0.2 mM sulfonamide solution (pH 7.4 isotonic phosphate buffer) was equilibrated with equal volume of $CHCl_3$ at 37°, and then the sulfonamide content in the aqueous phase was determined. The relative lipoid solubilities were expressed as $1-(C_e/C_i)$, where C_i and C_e represent the sulfonamide concentration in aqueous phase before and after the equilibration.

Analytical Methods——The plasma and urine samples were treated with Somogyi-deproteinizing reagents,[12] and then analyzed as follows: the sulfonamides (unacetylated) by the diazotization,[13] inulin by a modification[14] of the method described by Dische, *et al.*,[15] and iodopyracet by the titration method described by Alpart.[16] The urinary pH was measured with a micro glass electrode (type HG-9005, Tōa Electronics Ltd.) within one minute after the sampling.

Result and Discussion

As shown in Table I, the biological half lives in man[17] for the sulfonamides used in this study considerably differ. However, each sulfonamide was rather chosen as a typical one from the physico-chemical viewpoint[13] as follows. Sulfisoxazole as well as sulfaethidole and sulfamethoxazole possesses relatively strong acidity and high lipoid solubility in the sulfonamides. Sulfamethizole such as N^1-acetyl sulfanilamide is also stronger acid but less soluble in lipoid. On the other hand, sulfamethoxypyridazine as well as several long acting sulfonamides is less acidic and highly lipoid soluble. Finally, sulfanilamide is practically neutral, while the lipoid solubility is considerably low.

Effect of Increasing Dose of Iodopyracet on the Sulfonamides Clearances

Preliminarily, the effects of the plasma loading of iodopyracet, which is well known a competitive inhibitor of the *p*-aminohippurate (PAH) mechanism, were observed to examine the secretion of four sulfonamides in the rabbit kidney. As shown in Fig. 1, sulfisoxazole and

10) M. Fujimoto, *Nippon Rinshō*, **25**, 1154 (1967).
11) E. Owada, R. Hori, and T. Arita, *Yakuzaigaku*, **33**, 125 (1973).
12) M. Somogy, *J. Biol. Chem.*, **86**, 655 (1930).
13) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 413 (1964).
14) T. Arita, R. Hori, M. Takada, S. Akuzu, and A. Misawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 570 (1972).
15) Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **192**, 583 (1951).
16) L.K. Alpert, *Bull. Johns Hopkins Hosp.*, **68**, 522 (1941).
17) *a*) J.T. Doluisio and J.V. Swintosky, *Am. J. Pharm.*, **137**, 1179 (1965); *b*) T. Morioka, *Yakuzaigaku*, **23**, 1 (1963).

Table I. Physico-chemical Properties[a] and Biological Half Lives[b] of Sulfonamides

Sulfonamide	pK_{a2}	P[c]	$t_{1/2}$[d] (hr)
Sulfamethizole (SMZ)	5.45	0.90	2
Sulfisoxazole (SI)	5.1	4.40	8
Sulfanilamide (SA)	10.43	0.04	11
Sulfamethoxypyridazine (SMP)	7.0	4.14	34

a) ref. 13
b) ref. 17
c) partition coefficient($CHCl_3$/water) determined at unionized pH, 37°
d) biological half life in man

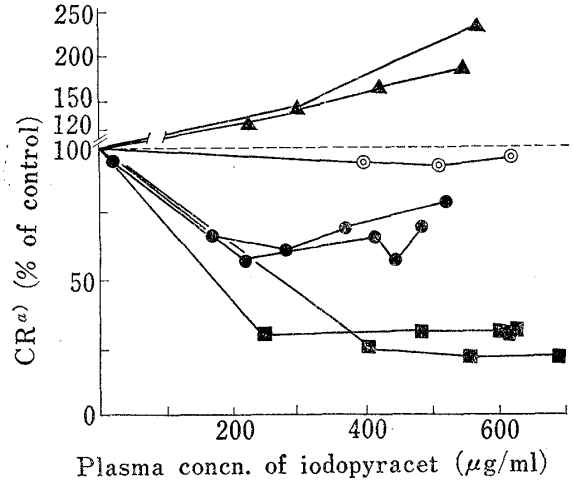

Fig. 1. Effect of Increasing Dose of Iodopyracet (IP) on the Clearance of Sulfonamides; SMZ (■), SI (●), SA (◎), and SMP (▲)

Sulfonamide doses were similar to that described in Experimental. The plasma concn. was less than 80 μg/ml. Iodopyracet dose was increased stepwise after each clearance period.
a) the ratio of sulfonamide clearance to inulin clearance

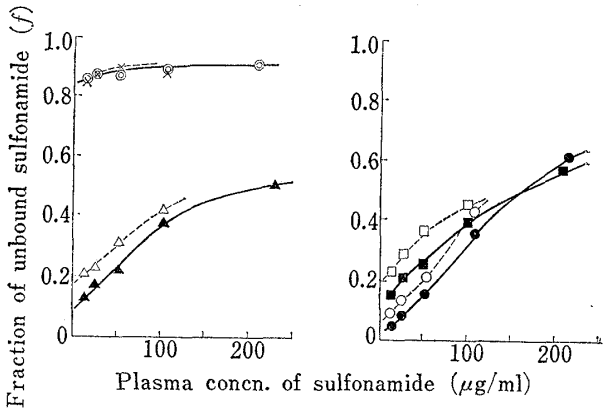

Fig. 2. Plasma Protein Binding of Sulfonamides; SMZ (control ■, with IP □), SI (control ●, with IP ○), SA (control ◎, with IP ×) and SMP (control ▲, with IP △)

Plasma concn. of IP was 500 μg/ml. Each point represents the mean of two experiments.

Table II. Renal Clearance of Sulfamethizole; Effect of Iodopyracet

	Time (min)	V (ml/min)	Urine pH	GFR (ml/min)	Sulfamethizole U (μg/ml)	P (μg/ml)	C (ml/min)	CR	f	ER	Iodopyracet P (μg/ml)
Control	30—20	1.096	7.8	9.92	191	32.6	6.40	0.646	0.21	3.11	
	20—10	0.820	8.0	9.32	224	32.1	5.72	0.614	0.21	2.97	
	10— 0	0.782	8.0	10.21	238	29.8	6.23	0.610	0.20	3.02	
Exptl.[a]	15—25	0.852	8.1	9.25	68	33.3	1.75	0.189	0.30	0.624	619
	25—35	0.740	8.0	9.31	80	33.6	1.77	0.191	0.30	0.628	624
	35—45	0.720	8.0	9.54	88	34.8	1.82	0.191	0.31	0.622	630

a) iodopyracet infusion periods
Exptl. No. 1, rabbit 2.8 kg.
Abbreviations in this and subsequent tables: V, urine flow rate; GFR, glomerular filtration rate; U, concn. in urine; P, concn. in plasma; C, clearance (UV/P); CR, clearance ratio (C/GFR); f, fraction of unbound sulfonamide; ER, excretion ratio (CR/f).

sulfamethizole clearances decreased with the plasma loading of iodopyracet, it suggesting that these sulfonamides were actively secreted and the secretions were blocked to negligible level by the plasma iodopyracet above 400 μg/ml.

TABLE III. Renal Clearance of Sulfamethoxypyridazine; Effect of Iodopyracet

	Time (min)	V (ml/min)	Urine pH	GFR (ml/min)	Sulfamethoxypyridazine U (μg/ml)	P (μg/ml)	C (ml/min)	CR	f	ER	Iodopyracet P (μg/ml)
Control	30—20	0.820	7.9	12.6	13.5	42.3	0.262	0.0208	0.20	0.105	
	20—10	0.660	7.8	15.1	19.1	43.0	0.293	0.0194	0.20	0.097	
	10— 0	0.800	7.7	13.0	14.4	44.8	0.257	0.0198	0.20	0.097	
Exptl.[a]	15—25	0.718	7.8	11.9	13.9	26.9	0.371	0.0313	0.24	0.132	429
	25—35	0.854	7.7	10.3	11.4	24.9	0.392	0.0379	0.23	0.163	386
	35—45	0.508	7.8	9.3	16.0	23.4	0.347	0.0373	0.23	0.163	352

a) iodopyracet infusion periods
Exptl. No. 15, rabbit 3.0 kg

TABLE IV. Renal Clearance[a] of Sulfonamides; Effect of Iodopyracet

Exptl. No.	Sulfonamide	Condition[b]	IP P (μg/ml)	V/GFR	Urine pH	Sulfonamide P (μg/ml)	CR	ER
1	SMZ	C		0.092	8.0	31.5	0.623	3.03
		I	624	0.082	8.0	33.9	0.190	0.625
2	SMZ	C		0.146	7.6	31.4	0.709	3.45
		I	839	0.127	7.7	38.3	0.232	0.729
3	SMZ	C		0.025	7.2	18.1	0.767	4.35
		I	497	0.024	8.0	24.7	0.227	0.821
4	SMZ	C		0.054	8.1	27.5	0.784	3.98
		I	808	0.066	7.9	27.9	0.267	0.932
5	SMZ	C		0.048	7.8	18.4	1.041	5.88
		I	624	0.066	7.8	23.4	0.219	0.803
6	SA	C		0.036	4.6	17.6	0.354	0.411
		I	783	0.077	5.7	18.6	0.456	0.530
7	SA	C		0.099	8.0	19.8	0.506	0.587
		I	540	0.107	8.0	18.4	0.561	0.652
8	SA	C		0.096	7.3	9.2	0.420	0.491
		I	469	0.103	7.8	8.6	0.466	0.545
9	SA	C		0.029	7.7	10.3	0.407	0.475
		I	550	0.057	7.8	11.1	0.451	0.526
10	SI	C		0.143	5.9	60.2	0.151	0.846
		I	—	0.132	6.3	50.8	0.079	0.374
11	SI	C		0.057	8.0	62.5	0.117	0.622
		I	—	0.046	8.0	43.9	0.083	0.450
12	SI	C		0.036	8.1	57.1	0.141	0.832
		I	—	0.050	8.0	57.8	0.109	0.465
13	SI	C		0.053	8.0	81.1	0.164	0.651
		I	557	0.057	8.0	66.3	0.103	0.388
14	SMP	C		0.053	8.1	48.5	0.017	0.079
		I	453	0.032	7.8	32.7	0.032	0.126
15	SMP	C		0.057	7.8	43.4	0.020	0.100
		I	389	0.066	7.8	25.1	0.036	0.152
16	SMP	C		0.054	7.8	37.9	0.027	0.141
		I	918	0.059	7.3	21.8	0.054	0.238

a) Each value is the mean of data from two or three clearance periods.
b) C: control, I: iodopyracet infusion

Plasma Protein Binding of the Sulfonamides

In order to estimate the glomerular filterable concentration of the sulfonamides, the extent of binding to rabbit plasma protein was determined and the results are shown in Fig. 2. Three sulfonamides except for sulfanilamide bound highly to the plasma protein and the extent depended markedly on the concentration of the sulfonamides. Some displacing activities of iodopyracet to bound sulfonamides were also demonstrated. Hence, in the clearance experiments described below the fraction of sulfonamide unbound (f) was determined from the plasma concentration using these "standard curves" with or without iodopyracet.

Renal Excretion of the Sulfonamides and the Effect of Iodopyracet

Sixteen clearance experiments were performed with four sulfonamides both in control and iodopyracet infused periods. The details of each representative experiment with sulfamethizole and sulfamethoxypyridazine are presented in Table II and III, respectively.

Table IV shows the results of all the experiments identical to those described above. It is noteworthy that in most of the experiments the renal functions such as water excretion ratio (V/GFR) and urinary pH, which also affect on the excretion of the drugs,[9] changed insignificantly before and after the iodopyracet infusion.

The ratios (CR) of the sulfonamides clearance to inulin clearance, as the conventional measure of the overall renal excretion, are compared with each other (Fig. 3). In control periods, all the sulfonamides exhibited apparently net reabsorption (CR$<$1), but the difference of the CR values between the maximum and the minimum was approximately forty times. The observed differences between control and inhibitory CR were statistically significant with sulfamethizole and sulfisoxazole, but insignificant both with sulfanilamide and sulfamethoxypyridazine.

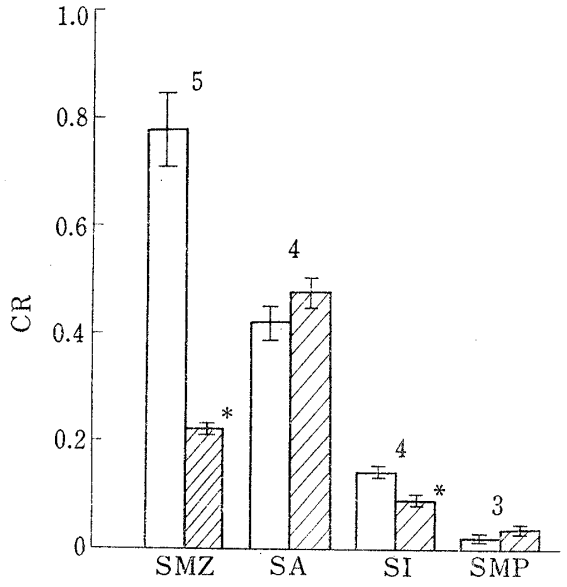

Fig. 3. Clearance Ratio of Sulfonamides in Rabbits.

□: control, ▨: IP infusion
Values are given as the mean ± S.E.; number of animals is given in parentheses. The asterisks indicate significant difference (SMZ, $p<0.001$; SI, $p<0.01$; SA and SMP, $p>0.1$; t test).

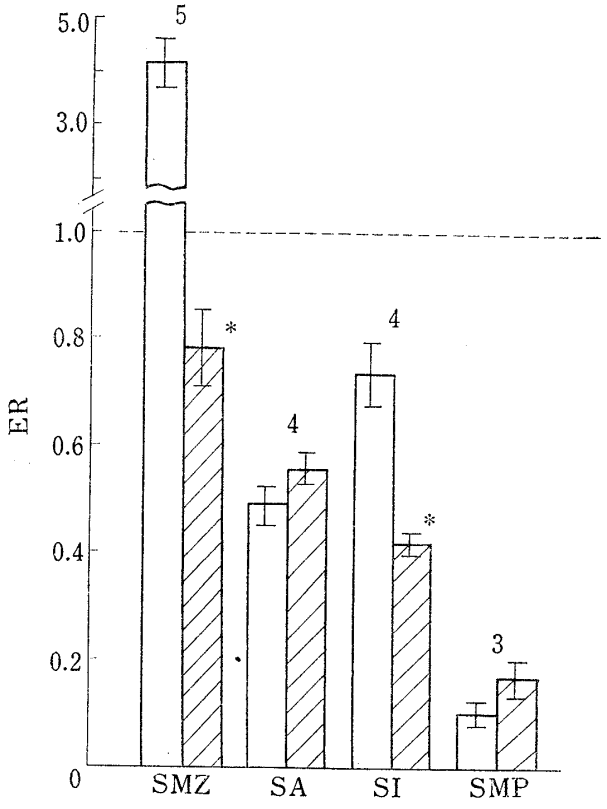

Fig. 4. Excretion Ratio of Sulfonamides in Rabbits

□: control, ▨: IP infusion
Values are given as the mean ± S.E.; number of animals is given in parentheses. The asterisks indicate significant difference (SMZ and SI, $p<0.001$; SA and SMP, $p>0.1$).

"Excretion ratio" (ER) means the ratio of unbound drug clearance to inulin clearance and is calculated as CR/f.[18] ER yields information on net direction and extent of the transport in the renal tubules. As shown in Fig. 4, in control periods sulfamethizole gave considerably high ER than unity, it being obvious net secretion. On the other hand, net reabsorption of three other sulfonamides were demonstrated. The inhibitory effect of iodopyracet was also manifested with sulfamethizole and sulfisoxazole, therefore the bidirectional transport in the tubules is evident especially for sulfisoxazole. No significant effect of iodopyracet for ER of sulfanilamide and sulfamethoxypyridazine suggested little or no tubular secretion process for these sulfonamides as well as the competitive reabsorption process.

Quantitative Comparison of the Renal Handling of the Sulfonamides

According to the procedure described earlier,[9] the rates of the sulfonamides into and out of the tubules were calculated from the original clearance data, and are given in Table V. Further, the percentage of each transfer rate to "the maximum filtration rate" ($GFR \cdot P$, *i.e.* the hypothetic filtration rate if the bound fraction is zero), calculated from the data in Table V, is summarized in Fig. 5.

Table V. Rates of Glomerular Filtration ($GFR \cdot P \cdot f$), Tubular Secretion (S), Tubular Reabsorption (A) and Urinary Excretion (UV) of Sulfonamides in the Rabbit Kidney

Exptl. No.	Sulfonamide	μg/min[a]					R[c]
		$GFR \cdot P$	$GFR \cdot P \cdot f$	UV	S[b]	A	
1	SMZ	309	64	193	246	117	0.38
2	SMZ	291	60	206	222	76	0.27
3	SMZ	243	43	187	185	41	0.18
4	SMZ	286	56	225	185	17	0.07
5	SMZ	216	38	224	241	55	0.20
6	SA	209	180	75		106	0.59
7	SA	195	168	98		70	0.41
8	SA	81	69	35		35	0.51
9	SA	157	135	64		71	0.53
10	SI	651	116	98	143	161	0.63
11	SI	990	186	116	72	142	0.55
12	SI	600	102	84	79	97	0.54
13	SI	1008	253	164	169	258	0.61
14	SMP	707	151	12		139	0.92
15	SMP	588	118	12		107	0.90
16	SMP	309	58	8		50	0.86

a) Each value is the mean of data calculated from two or three control clearance periods.
b) Secretion of SA and SMP were regarded as negligible level.
c) Fraction of tubular load reabsorbed; the mean of data calculated from IP infusion periods (SMZ, SI), or control periods (SA, SMP).

These schematic diagrams revealed the determinating factors of the urinary excretion, *i.e.* the renal clearance of each sulfonamide. For example, a profound secretion and a little reabsorption are responsible for the marked excretion of sulfamethizole in the urine. Sulfisoxazole is also handled by the kidney in a similar manner as above, but relatively faster rate of reabsorption than that of secretion is characteristic. Comparatively rapid excretion of sulfanilamide mainly due to its large rate of filtration. On the contrary, in addition to a small rate of filtration, a considerable high fractional reabsorption of sulfamethoxypyridazine results in the extremely low urinary excretion.

18) H. Büttner, F. Portwich, E. Manzke, and N. Staudt, *Klin. Wschr.*, **42**, 103 (1964).

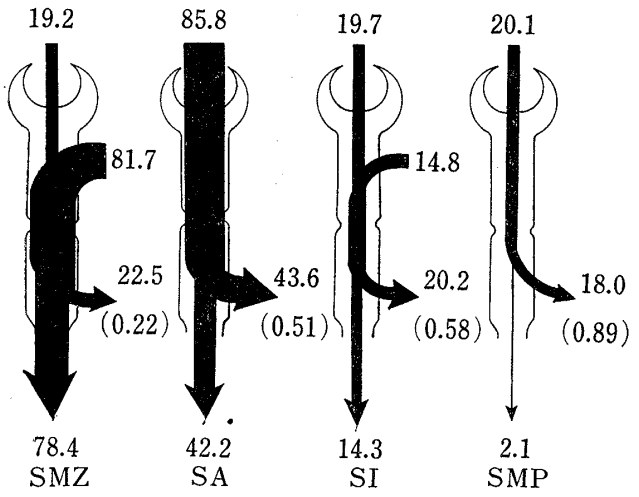

Fig. 5. Quantitative Comparison of Renal Excretion of Sulfonamides in Rabbits

The figures in this scheme represent the rates of transfer (% of GFR·P). Fraction of tubular load reabsorbed are given in parentheses.

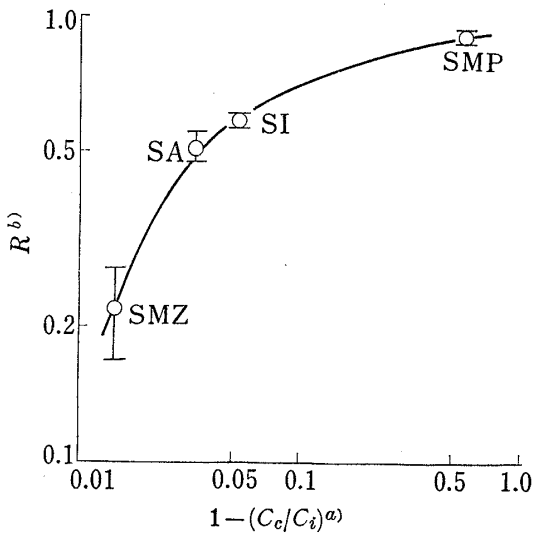

Fig. 6. Relationship between Relative Lipoid Solubility and Tubular Reabsorption of Sulfonamides

a) relative lipoid solubility; see Experimental.
b) Fraction of tubular load reabsorbed; mean ± S.E. (3—5 animals)

On the secretory component, Despopoulos[19] pointed out that the ionization of N^1 position of sulfonamides is essential for the interaction with the PAH mechanism. As shown in Table I, sulfamethizole and sulfisoxazole are relatively stronger acids than sulfanilamide and sulfamethoxypyridazine. Therefore the formers could be readily secreted than the latters. The protein binding seems to be insignificant role for the secretion of the sulfonamides, because these sulfonamides except for sulfanilamide bound to the plasma protein to same extent.

Not the rate of reabsorption but the fraction of the tubular load reabsorbed (R) also widely varied among the sulfonamides. However, these values roughly proportionated to the relative lipoid solubilities (pH 7.4) as shown in Fig. 6. The similar but somewhat less correlations were observed between the lipoid solubility and the $t_{1/2}$ in man,[8a)] and the urinary excretion rate constant (k_3) in rabbits[8b)] with many sulfonamides. Although some other investigators have proposed the possible carrier mediated reabsorption for some sulfonamides,[20] the result in Fig. 6 might re-emphasized the role of non-ionic diffusion for the renal tubular reabsorption of the sulfonamides.

In conclusion, except for the not explicable instances such as the proximal tubular reabsorption of N^4-acetyl sulfamerazine,[21] the sulfonamides seem to be generally handled by the kidney according to the manner, "pump-and-leak system" proposed by Mudge and Weiner.[22]

Acknowledgement The authors are indebted to Mr. T. Noguchi for his technical assistance.

19) A. Despopoulos, *J. Theoret. Biol.*, **8**, 163 (1965).
20) M.D. Milne, B.H. Scribner, and M.A. Crawford, *Am. J. Med.*, **24**, 709 (1958); F. Portwich and H. Büttner, *Klin. Wschr.*, **42**, 740 (1964); M. Sugita, K. Sugita, T. Furukawa, and H. Abe, *Japanese Circulation J.*, **31**. 423(1967).
21) H. Sonnenberg, H. Oelert, and K. Baumann, *Arch. Ges. Physiol.*, **286**, 171 (1965).
22) G.H. Mudge and I.M. Weiner, "Proceeding of the First International Pharmacological Meeting," Vol. 4, ed. by C.A.M. Hogben, Pergamon Press, New York, 1963, pp. 157—164.