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Transformation and Excretion of Drugs in Biological Systems. VII.[1] Effect of Biotransformation on Renal Excretion of Sulfonamides

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Sulfanilamide, sulfathiazole, sulfisomezole and their five biotransformed products were applied to renal clearance experiments in dogs and protein binding experiments to dog plasma protein in order to elucidate their renal excretion behaviors.

Clearance ratio of sulfisomezole is considerably low compared with that of other two sulfonamides.

Biotransformation of sulfisomezole to sulfisomezole-N^4-acetate and sulfisomezole-N^1-glucuronide led to remarkable rise of clearance ratio.

N^4-acetylation of sulfathiazole rather reduced clearance ratio compared with the original sulfonamide. On the contrary, N^4-acetylation of sulfanilamide lead to rise of clearance ratio, in spite of high clearance ratio of sulfanilamide.

N^4-acetylated products of the three sulfonamides are considerably secreted through proximal tubule. Proximal tubular secretion of sulfisomezole-N^1-glucuronide is insufficient.

N^4-acetylation of the three sulfonamides increased affinity for dog plasma protein. On the other hand, reduced affinity of sulfisomezole-N^1-glucuronide for dog plasma protein was observed.

Furthermore, correlation between renal excretion and biotransformation of several sulfonamides was extensively discussed.

Many studies concerning biotransformation and renal excretion of various sulfonamides have been undertaken since their introduction into clinical practice. However, elucidation of systematic relationships between biotransformation and renal excretion of sulfonamides, still remains to be solved. Previously we reported the correlation between renal excretion and biotransformation of sulfadimethoxine,[3] sulfisomidine and sulfamethizole.[1] In continuing our program of the investigations, we took up the problem concerning physiological behaviors of sulfanilamide, sulfathiazole, sulfisomezole and their biotransformed products. It is reported[4–6] that the major biotransformed products of sulfanilamide in man is sulfanilamide-N^4-acetate. On biotransformation of sulfathiazole in man, it is reported[7,8] that sulfathiazole-N^4-acetate is the major biotransformed product and little amount of N^4-glucuronide and N^1-glucuronide of sulfathiazole is excreted as minor biotransformed products in man. Furthermore, biotransformation of sulfisomezole in man is well established[9,10] that N^4-acetyla-

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2) Location: *a) Nishi-6-chome, Kita-12-jo, Sapporo; b) Kishi-5-chome, Kita-14-jo, Sapporo.*
3) T. Arita, R. Hori, M. Takada and A. Misawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 930 (1971).
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8) T. Uno and M. Ueda, *Chem. Pharm. Bull.* (Tokyo), **11**, 709 (1963).
9) M. Ueda and K. Kuribayashi, *Yakugaku Zasshi*, **84**, 1104 (1964).
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tion is the major biotransformation pathway of this sulfonamide and sulfisomezole-N^1-glucuronide is also excreted to considerable extent.

In this paper, we describe the intensive research to elucidate the systematic correlation between renal excretion and biotransformation of sulfanilamide, sulfathiazole and sulfisomezole. Furthermore, the relationship between the molecular structural characteristics of several sulfonamides and the susceptibility to renal transport is extensively discussed.

Experimental

Preparation of Materials——Sulfanilamide: Commercially available sulfanilamide was recrystallized from EtOH. mp 165—167°.

Sulfanilamide-N^4-acetate: Sulfanilamide-N^4-acetate was synthesized[11] by acetylation of sulfanilamide. mp 214—216°.

Sulfathiazole: Commercially available sulfathiazole was recrystallized from EtOH. mp 200—204°.

Sulfathiazole-N^4-acetate: Sulfathiazole-N^4-acetate was synthesized[11] by acetylation of sulfathiazole. mp 253—255°.

Sulfathiazole-N^4-glucuronide: Sulfathiazole-N^4-glucuronide was prepared by the method of Ogiya, *et al.*,[12] but it was difficult to purify. So, further attempts to eliminate the contaminated substances were carried out by applying to preparative thin-layer plates (Kiesel gel GF, 1.0 mm in thickness, activated at 110° for 1 hr) and developing with the solvent system of PrOH–H_2O–NH_4OH (6: 2: 1). The plates were dried, areas containing sulfathiazole-N^4-glucuronide were identified by color reactions and ultraviolet ray radiation, and removed from each plate by scraping. The compound was extracted with 1/15M isotonic phosphate buffer solution, and after rapid quantitative determination of the aliquot, the buffer solution was immediately applied to clearance experiments to obviate the rapid hydrolysis. Sulfathiazole-N^4-glucuronide was also identical with previously reported data in paper chromatography.[11]

Sulfisomezole: Commercially available sulfisomezole was recrystallized from EtOH. mp 168—171°.

Sulfisomezole-N^4-acetate: Sulfisomezole-N^4-acetate was synthesized[13] by acetylation of sulfisomezole. mp 223—224°.

Sulfisomezole-N^1-glucuronide: Three male volunteers each took 4 g of sulfisomezole a day orally, and their urine (10 liters) was collecetd for 48 hr. The urine was adjusted to pH 4 with AcOH and filtered. Two hundred grams of activated charcoal were added to the filtrate. After standing overnight, the charcoal was filtered and washed with H_2O repeatedly. The charcoal was extracted with 1.5 liters of BuOH–MeOH–NH_4OH–H_2O (1: 1: 0.4: 8) at 40° for 25 min and the procedure was repeated three times by the method of Uno, *et al.*,[14] and Ueda, *et al.*,[15] After standing overnight, the extract was filtered, evaporated to dryness under reduced pressure at 37°, and applied to lead salt precipitation procedure to obtain glucuronide gum by the method of Uno, *et al.*[16] The obtained gum was applied to preparative thin-layer chromatography as mentioned previously.[3] Yellow amorphous substance was obtained (0.5 g). mp 167—168°. This substance was also identical with previously reported data in ultraviolet spectrum, infrared spectrum and paper chromatography to be ammonium sulfisomezole-N^1-glucosiduronate.[15,17]

Animal Experiment——Standard laboratory procedures were used for all renal clearance experiments.[3,18-20] Male and female dogs weighing 10.0—17.5 kg were used in these experiments. Each substance was applied to intravenous injection and successive infusion was continued throughout the experiments. The detailed procedure was described in a previous report.[3] 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.

Protein Binding——Binding studies in dog plasma were carried out *in vitro* by equilibrium dialysis with the sulfonamide drugs as described previously.[3] Dog plasma was obtained from the three unanesthetized dogs.

11) T. Uno and M. Kataoka, *Yakugaku Zasshi*, **80**, 1785 (1960).
12) S. Ogiya and H. Kataoka, *Yakugaku Zasshi*, **79**, 949 (1959).
13) H. Kano and K. Ogata, *Ann. Rept. Shionogi Res. Lab.*, **7**, 301 (1957).
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15) M. Ueda and K. Kuribayashi, *Yakugaku Zasshi*, **11**, 1104 (1964).
16) T. Uno, T. Kishima and M. Fujimoto, *Chem. Pharm. Bull.* (Tokyo), **13**, 261 (1965).
17) M. Ueda, Y. Nakagawa and N. Murakami, *Yakugaku Zasshi*, **87**, 451 (1967).
18) M. Sugita, *Nippon Jinzōgaku Kaishi*, **5**, 235 (1963).
19) M. Fujimoto, *Nippon Rinshiyo*, **25**, 1154 (1967).
20) F. Portwich, H. Buttner and K. Engelhardt, *Klin. Wschr.*, **41**, 447 (1963).

Analytical Method——Plasma and urine samples were deproteinized with 10% trichloroacetic acid, and then analyzed as follows: sulfonamides and their biotransformed products by diazotization,[21] inulin by a modification of the method described by Dische, *et al.*[22] (To 1 ml of sample, is added 0.2 ml of 1.5% solution of cysteine hydrochloride. To this 6 ml of 70% sulfuric acid is added and immediately afterwards 0.2 ml of 0.12% alcoholic solution of carbazole. The mixture is shaken and left standing at 40° for 30 min), and iodopyracet by the titration method described by Alpert.[23] Hitachi-Horiba model F-4 pH meter with a glass electrode was used to determine pH of urine.

Result

Renal Excretion of Sulfanilamide and its Biotransformed Products

Four clearance experiments were performed and the results are shown in Fig. 1. The detailed data of each substance are also exemplified in Table I and II, respectively. As shown in Fig. 1, in all studies, sulfanilamide and sulfanilamide-N^4-acetate showed considerable large clearance ratio, which suggests that sulfanilamide as well as sulfanilamide-N^4-acetate are excreted in urine very rapidly. It is very interesting that, in spite of large clearance ratio of sulfanilamide, clearance ratio of sulfanilamide-N^4-acetate which is the major biotransformed product of sulfanilamide, exceeded over clearance ratio of sulfanilamide. Sulfanilamide-N^4-acetate excretion was reduced following administration of iodopyracet at the dosage used in the previously mentioned experiments,[3] and this fact indicates competitive inhibition of proximal tubular secretion of sulfanilamide-N^4-acetate. On the contrary, no significant alteration of clearance ratio of sulfanilamide before and after blockade of proximal tubular secretion was observed.

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

	Time	V	Urine	GFR	Sulfaniladmide				Iodopyracet
					U	P	C	CR	P
	(min)	(ml/min)	pH	(ml/min)	(mg/ml)	(mg/ml)	(ml/min)		(mg/ml)
Control	30—20	5.04	6.98	58.4	0.121	0.0172	35.5	0.6079	—
	20—10	5.42	—	54.5	0.120	0.0185	35.2	0.6459	—
	10— 0	6.04	—	55.7	0.116	0.0194	36.2	0.6481	—
Exptl.[a]	15—25	7.74	6.94	54.1	0.100	0.0223	34.7	0.6414	0.5550
	25—35	6.74	—	51.6	0.114	0.0227	33.8	0.6550	0.5090
	35—45	6.30	7.12	48.1	0.122	0.0227	33.9	0.7048	0.5090

dog: ♂ 17.0 kg (dog C in Fig. 1)
a) iodopyracet: 3.54 g *i.v.*, 115.8 mg/min infusion

Protein Binding of Sulfanilamide and its Biotransformed Products

The binding of sulfanilamide and sulfanilamide-N^4-acetate to dog plasma protein was investigated in 0.1 M isotonic phosphate buffer solution at pH 7.4. As shown in Fig. 2, curved lines were obtained by plotting the percentage unbound as a function of the concentration of each compound present in the inner compartment (bound and unbound). The fact that sulfanilamide has extremely low affinity and sulfanilamide-N^4-acetate has a little increased but low affinity to dog plasma protein, was observed.

Renal Excretion of Sulfathiazole and its Biotransformed Products

Six clearance experiments were performed and the results are shown in Fig. 3. The de-

21) T. Koizumi, T. Arita and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 413 (1964).
22) Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **192**, 583 (1951).
23) L.K. Alpert, *Bull. Johns Hopkins Hosp.*, **68**, 522 (1941).

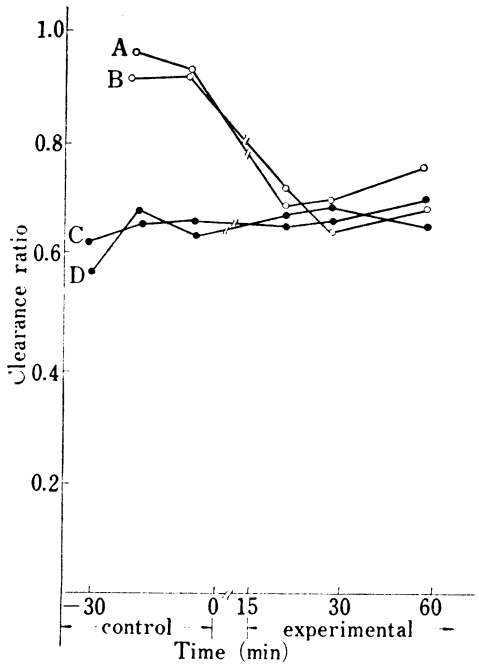

Fig. 1. Clearance Ratio of Sulfanilamide and Sulfanilamide-N⁴-acetate before and after Blockade of Proximal Tubular Secretion

The lines connect the values for each dog.
A: dog ♀ 12.5 kg C: dog ♂ 17.0 kg
B: dog ♂ 15.0 kg D: dog ♂ 13.0 kg
●: sulfanilamide
○: sulfanilamide-N⁴-acetate

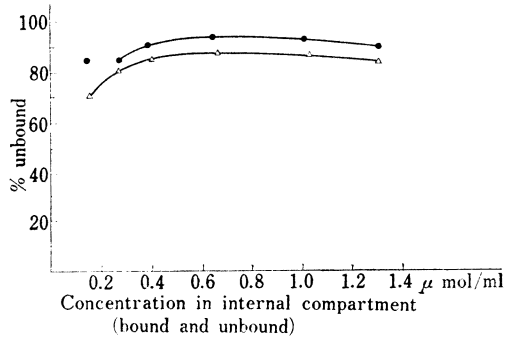

Fig. 2. Comparison of the Binding of Sulfanilamide and Sulfanilamide-N⁴-acetate to Dog Plasma

●: sulfanilamide
△: sulfanilamide-N⁴-acetate

tailed data of each substance are also exemplified in Table III, IV and V, respectively. As shown in Fig. 3, the evident tendency that clearance ratio of sulfathiazole-N⁴-acetate was rather reduced than that of sulfathiazole was observed. Sulfathiazole-N⁴-acetate was considerably excreted through proximal tubule, and sulfathiazole also possessed tolerable affinity for proximal tubular secretory route. Clearance ratio of sulfathiazole-N⁴-glucuronide exceeded slightly over that of sulfathiazole but active secretion of the former compound is quite insufficient.

Protein Binding of Sulfathiazole and its Biotransformed Products

Fig. 4 shows the binding of sulfathiazole and its biotransformed products to dog plasma protein in 0.1 M isotonic phosphate buffer solution at pH 7.4. Curved lines were obtained by the method previously mentioned in this paper. Sulfathiazole-N⁴-acetate has considerable high affinity to dog plasma protein as compared with sulfathiazole. Sulfathiazole-N⁴-glucuronide was very unstable under the experimental conditions of dialysis and about 23.3%

Table II. Clearance Ratio of Sulfanilamide-N⁴-acetate before and after Blockade of Proximal Tubular Secretion

	Time	V	Urine	GFR	Sulfanilamide-N⁴-acetate				Iodopyracet
					U	P	C	CR	P
	(min)	(ml/min)	pH	(ml/min)	(mg/ml)	(mg/ml)	(ml/min)		(mg/ml)
Control	30—20	—	—	—	—	—	—	—	—
	20—10	5.20	6.66	46.1	0.270	0.0320	43.9	0.9523	—
	10— 0	6.73	—	56.8	0.223	0.0289	51.9	0.9137	—
Exptl.[a)]	15—25	8.30	6.82	53.5	0.158	0.0341	38.5	0.7196	0.7058
	25—35	7.70	—	57.8	0.169	0.0354	36.8	0.6367	0.7486
	35—45	7.16	7.00	52.6	0.183	0.0362	36.2	0.6882	0.7806

dog: ♀ 12.5 kg (dog A in Fig. 1)
a) iodopyracet: 2.60 g *i.v.*, 85.1 mg/min infusion

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

	Time (min)	V (ml/min)	Urine pH	GFR (ml/min)	Sulfathiazole U (mg/ml)	Sulfathiazole P (mg/ml)	Sulfathiazole C (ml/min)	Sulfathiazole CR	Iodopyracet P (mg/ml)
Control	30—20	2.96	7.46	48.7	0.271	0.0341	23.5	0.4825	—
	20—10	2.92	—	48.9	0.287	0.0327	25.6	0.5235	—
	10— 0	3.10	—	50.4	0.287	0.0337	26.4	0.5238	—
Exptl.[a)]	15—25	3.44	7.22	45.8	0.177	0.0354	17.2	0.3756	0.7868
	25—35	2.96	—	48.8	0.205	0.0357	17.0	0.3484	0.8248
	35—45	3.42	—	46.4	0.186	0.0376	16.9	0.3642	0.9580

dog: ♀, 17.5 m kg (dog F in Fig. 3)
a) iodopyracet: 3.74 g *i.v.*, 122.6 mg/min infusion

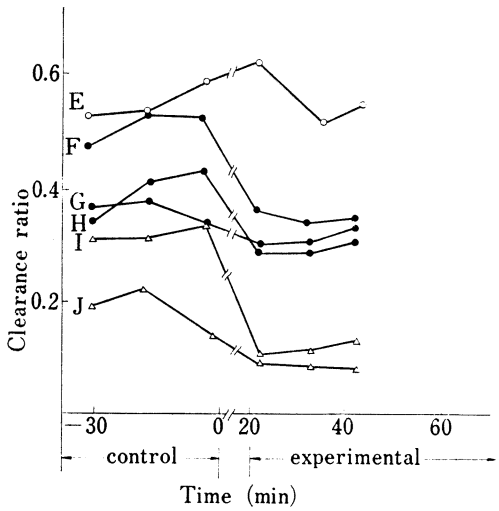

Fig. 3. Clearance Ratio of Sulfathiazole, Sulfathiazole-N⁴-acetate and Sulfathiazole-N⁴-glucuronide before and after Blockade of Proximal Tubular Secretion

The lines connect the values for each dog.
E: dog ♀, 10.0 kg H: dog ♂, 13.2 kg
F: dog ♀, 17.5 kg I: dog ♀, 16.0 kg
G: dog ♀, 10.0 kg J: dog ♂, 16.0 kg
●: sulfathiazole
△: sulfathiazole-N⁴-acetate
○: sulfthiazole-N⁴-glucouronide

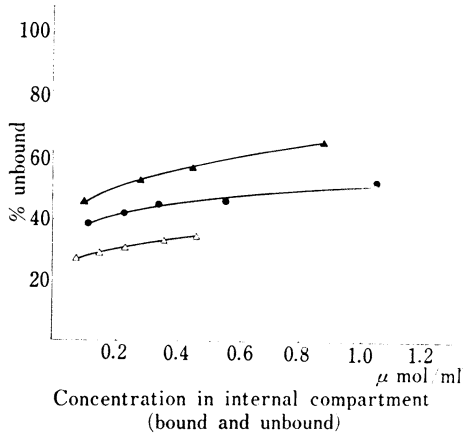

Fig. 4. Comparison of the Binding of Sulfathiazole, Sulfathiazole-N⁴-acetate and Sulfathiazole-N⁴-glucuronide to Dog Plasma

●: sulfathiazole
△: sulfathiazole-N⁴-acetate
▲: sulfathiazole-N⁴-glucuronide

of initial concentrations was hydrolyzed at the end point of incubation time. Curved lines obtained by sulfathiazole-N⁴-glucuronide shown in Fig. 4 indicate the values observed but not revised.

Renal Excretion of Sulfisomezole and its Biotransformed Products

Eight renal clearance experiments of sulfisomezole and its biotransformed products were undertaken. The results are shown in Fig. 5. The detailed data of each substance are also exemplified in Table VI—VIII, respectively. As shown in Fig. 5, it is noteworthy that clearance ratio of sulfisomezole is considerably low and acetylation of N⁴-position in sulfisomezole molecule causes great increase of clearance ratio as compared with the original sulfonamide.

Table IV. Clearance Ratio of Sulfathiazole-N⁴-acetate before and after Blockade of Proximal Tubular Secretion

	Time (min)	V (ml/min)	Urine pH	GFR (ml/min)	Sulfathiazole-N⁴-acetate U (mg/ml)	P (mg/ml)	C (ml/min)	CR	Iodopyracet P (mg/ml)
Control	30—20	3.44	6.84	60.3	0.0366	0.00676	18.6	0.3084	—
	20—10	3.66	—	68.6	0.0369	0.00636	21.2	0.3090	—
	10— 0	3.52	—	70.1	0.0405	0.00589	24.2	0.3452	—
Exptl.[a]	15—25	4.60	6.70	74.8	0.0143	0.00851	7.7	0.1029	0.5526
	25—35	4.32	—	74.3	0.0169	0.00890	7.1	0.1104	0.6232
	35—45	4.00	—	70.1	0.0180	0.00707	10.2	0.1455	0.6373

dog: ♀, 16.0 kg (dog I in Fig. 3)
a) iodopyracet: 3.33 g *i.v.*, 109.0 mg/min infusion

Table V. Clearance Ratio of Sulfathiazole-N⁴-glucuronide before and after Blockade of Proximal Tubular Secretion

	Time (min)	V (ml/min)	Urine pH	GFR (ml/min)	Sulfathiazole-N⁴-glucuronide U (mg/ml)	P (mg/ml)	C (ml/min)	CR	Iodopyracet P (mg/ml)
Control	30—20	3.92	7.12	45.0	0.159	0.0264	23.6	0.5244	—
	20—10	4.52	—	46.4	0.135	0.0249	24.5	0.5280	—
	10— 0	4.36	—	38.6	0.130	0.0245	23.2	0.6010	—
Exptl.[a]	15—25	4.10	7.25	36.0	0.147	0.0258	23.3	0.6472	0.9054
	25—35	5.70	—	50.1	0.122	0.0274	25.4	0.5070	1.0432
	35—45	4.62	—	40.3	0.122	0.0254	22.2	0.5509	0.9363

dog: ♀, 10.0 kg (dog E in Fig. 3)
a) iodopyracet: 2.08 g *i.v.*, 68.1 mg/min infusion

Furthermore, sulfisomezole-N¹-glucuronide, which is one of the major biotransformed products of sulfisomezole, also causes remarkable increase of clearance ratio as compared with sulfisomezole. The little but constant decrease of clearance ratio of sulfisomezole after blockade of proximal tubular secretion by iodopyracet was observed, and alteration of clearance ratio of sulfisomezole-N¹-glucuronide under the same experimental condition was not observed. On the contrary, as shown in Fig. 5, sulfisomezole-N⁴-acetate was greatly secreted by proximal tubule.

Table VI. Clearance Ratio of Sulfisomezole before and after Blockade of Proximal Tubular Secretion

	Time (min)	V (ml/min)	Urine pH	GFR (ml/min)	Sulfisomezole U (mg/ml)	P (mg/ml)	C (ml/min)	CR	Iodopyracet P (mg/ml)
Control	30—20	5.06	7.00	70.1	0.198	0.0783	12.8	0.1826	—
	20—10	4.68	—	66.9	0.187	0.0783	11.2	0.1674	—
	10— 0	4.40	—	74.4	0.196	0.0789	10.9	0.1465	—
Expt.[a]	15—25	3.92	6.88	71.6	0.164	0.0783	8.21	0.1147	0.5443
	25—35	3.66	—	68.9	0.165	0.0830	7.20	0.1045	0.5840
	35—45	3.30	—	68.1	0.161	0.0843	6.30	0.0925	0.6517

dog: ♀, 12.0 kg (dog P in Fig. 5)
a) iodopyracet: 2.50 g *i.v.*, 81.7 mg/min infusion

Table VII. Clearance Ratio of Sulfisomezole-N⁴-acetate before and after Blockade of Proximal Tubular Secretion

	Time (min)	V (ml/min)	Urine pH	GFR (ml/min)	Sulfisomezole-N⁴-acetate U (mg/ml)	P (mg/ml)	C (ml/min)	CR	Iodopyracet P (mg/ml)
Control	30—20	6.62	6.84	51.5	0.143	0.0238	39.8	0.7728	—
	20—10	5.54	—	51.5	0.158	0.0254	34.5	0.6699	—
	10— 0	5.00	—	44.1	0.168	0.0268	31.3	0.7098	—
Exptl.[a]	15—25	5.48	6.83	46.0	0.105	0.0309	18.6	0.4043	0.5671
	25—35	5.20	—	53.6	0.116	0.0341	17.7	0.3302	0.6142
	35—45	5.16	—	48.0	0.116	0.0346	17.3	0.3604	0.6865

dog: ♂, 13.0 kg (dog K in Fig. 5)
a) Iodopyract: 2.70 g *i.v.*, 88.4 mg/min infusion

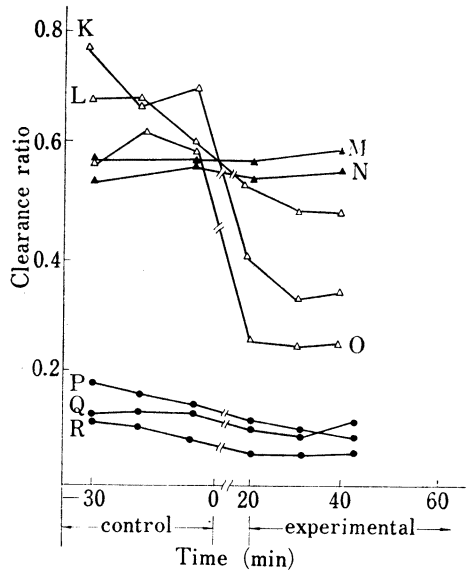

Fig. 5. Clearance Ratio of Sulfisomezole, Sulfisomezole-N⁴-acetate and Sulfisomezole-N¹-glucuronide before and after Blockade of Proximal Tubular Secretion
The lines connect the values for each dog.
K: dog ♂ 13.0 kg L: dog ♀ 14.0 kg
M: dog ♂ 11.5 kg N: dog ♂ 13.0 kg
O: dog ♂ 14.0 kg P: dog ♀ 12.0 kg
Q: dog ♀ 15.0 kg R: dog ♂ 13.5 kg
●: sulfisomezole
△: sulfisomezole-N⁴-acetate
▲: sulfisomezole-N¹-glucuronide

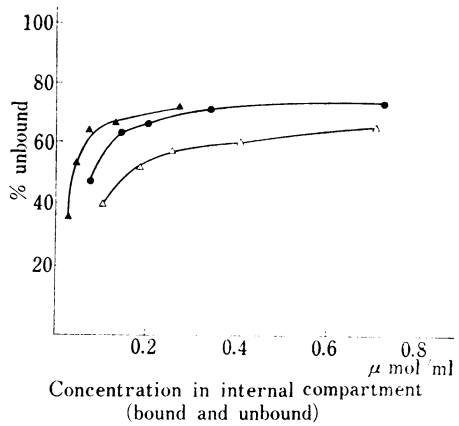

Fig. 6. Comparison of the Binding of Sulfisomezole, Sulfisomezole-N⁴-acetate and Sulfisomezole-N¹-glucuronide to Dog Plasma
●: sulfisomezole
△: sulfisomezole-N⁴-acetate
▲: sulfisomezole-N¹-glucuronide

Protein Binding of Sulfisomezole and its Biotransformed Products

Fig. 6 shows the binding of sulfisomezole and its biotransformed products to dog plasma protein in 0.1 M isotonic phosphate buffer solution at pH 7.4. Curved lines were obtained by the method previously mentioned in this paper. Of the three compounds, sulfisomezole-N⁴-aceate possesses the highest affinity for dog plasma protein as compared with the other compounds. The reduced protein binding of sulfisomezole-N¹-glucuronide was observed.

TABLE VIII. Clearance Ratio of Sulfisomezole-N^1-glucuronide before and after Blockade of Proximal Tubular Secretion

	Time	V	Urine	GFR	Sulfisomezole-N^1-glucuronide				Iodopyracet
					U	P	C	CR	P
	(min)	(ml/min)	pH	(ml/min)	(mg/ml)	(mg/ml)	(ml/min)		(mg/ml)
Control	20—10	2.92	6.43	42.5	0.0892	0.0109	23.9	0.5624	—
	10— 0	3.10	—	39.6	0.0818	0.0113	22.4	0.5657	—
Exptl.[a]	15—25	2.96	6.72	41.8	0.0858	0.0111	22.9	0.5478	0.5165
	25—35	3.18	—	41.6	0.0924	0.0121	24.7	0.5938	0.5794

dog: ♂, 11.5 kg (dog M in Fig. 5)
a) iodopyracet: 2.39 g *i.v.*, 78.3 mg/min infusion

Discussion

Up to the present, numerous derivatives of sulfanilamide have been synthesized and numbers of the less toxic ones have been subjected to extensive clinical trial. Particularly, the recent remarkable development of sulfonamides is characterized by the discoveries of so-called long-acting sulfonamides, which are excreted very slowly in urine and display the prolonged pharmacological effects such as sulfadimethoxine.

In the view of clinical importance and pharmacological interest, many investigations concerning biotransformation, renal excretion and protein binding of numerous sulfonamides have been undertaken. However, the physicochemical properties of the individual sulfonamide are so various and the experimental data concerning their physiological behaviors are so complicated that, the systematic correlation between molecular structural characteristics following biotransformation of sulfonamides, and their physiological behaviors have still remained to be elucidated.

As mentioned previously, clearance ratio of sulfisomezole is very low compared with other short-acting sulfonamides such as sulfathiazole and sulfanilamide, thus indicating that effective plasma levels of sulfisomezole are maintained for a long time after an oral dose, possibly by virtue of preferential distal tubular reabsorption. On the contrary, considerable high clearance ratio of sulfathiazole and sulfanilamide was proved. As for N^4-acetylation products of the three sulfonamides, it would be noticeable that N^4-acetylation of sulfisomezole induces a great rise of clearance ratio compared with sulfisomezole. On the other hand, sulfathiazole-N^4-acetate exhibits rather reduced clearance ratio than sulfathiazole. N^1-glucuronidation of sulfisomezole is one of major biotransformations and sulfisomezole-N^1-glucuronide causes great increase of clearance ratio compared with sulfisomezole. Sulfisomezole well agrees in the great rise of clearance ratio of N^1-glucuronide with sulfadimethoxine.[3] Sulfathiazole-N^4-glucuronide which is one of minor biotransformed products of sulfathiazole, also caused slight increases of clearance ratio compared with that of sulfathiazole. Sulfathiazole-N^4-glucuronide also agrees in instability with N^4-glucuronides of other sulfonamides.[1,3]

One of major purposes of our researches was to demonstrate whether or not a given compound was subject to proximal tubular secretion. Clearance experiments were carried out under conditions which would inhibit proximal tubular secretion by iodopyracet infusion. Slight but distinct proximal tubular secretion of sulfisomezole, and considerable proximal tubular secretion of sulfathiazole were observed. On the other hand, the secretion of sulfanilamide was quite insufficient. Every N^4-acetylation product of the three sulfonamides was remarkably secreted through proximal tubule. Particularly, extent of secretion of sulfisomezole-N^4-acetate seemed to exceed over those of N^4-acetylation products of other two sulfonamides. Proximal tubular secretion of sulfisomezole-N^1-glucuronide was not observed.

As one of important factors for determining renal excretion rate of drugs, metabolic

biotransformation would play an essential role *in vivo* as well as other intensive factors. In general, the main metabolic biotransformation of sulfonamides in man is N^4-acetate formation, which is classified into synthetic reactions *in vivo*. N^4-Acetylation products of sulfonamides usually result in great decrease of solubility in body fluid. Some of sulfonamides are also extensively biotransformed to N^1-glucuronide, which markedly increase solubility in body fluid. Adding to previously mentioned two synthetic processes, biosynthesis of N^4-glucuronide and N^4-sulfonate of certain sulfonamides has been proved as minor biotransformed products. As the result of metabolic biotransformation of sulfonamides in various ways described above, physicochemical characteristics of each biotransformed products would be considerably altered compared with those of original unchanged sulfonamides. Thus, the physiological behaviors of each biotransformed compound would alter markedly, reflecting diversity in their physicochemical characteristics. As for correlation between molecular features of sulfonamides and the renal excretory patterns, several reports and reviews have been published.[24-27] However, the diversity of molecular structural changes after metabolic biotransformation of the drugs has been the major deterrent to organization and unification of experimental data. Nevertheless, such an organization and unification would be essential to an understanding of detailed physiological behaviors of the drug substantially. Concerning relationships between renal excretion and biotransformation of sulfonamides, the following conclusions can be drawn from the results of present investigation and our preceding report.[1,3]

Table IX. Physiochemical Properties and Renal Excretory Behaviors of Sulfonamides

Compounds	p*Ka*	Protein binding (%)	Clearance ratio in Dog	Proximal tubular secretion
Sulfanilamide	10.08	10.2	0.5662—0.8881(4)	—
Sulfathiazole	7.25	55.5	0.3450—0.5238(3)	+
Sulfisomidine	7.57	23.2	0.4720—0.6020(3)	+ +
Sulfamethizole	5.45	69.8	0.7354—0.9756(3)	+ + +
Sulfisomezole	6.05	25.3	0.0765—0.1826(3)	+
Sulfadimethoxine	6.32	72.0	0.0270—0.1030(3)	—
Sulfanilamide-N^4-acetate	5.78	17.1	0.9049—1.333 (2)	+ +
Sulfathiazole-N^4-acetate	6.80	67.8	0.1400—0.3452(2)	+ +
Sulfisomidine-N^4-acetate	7.08	42.0	0.3255—0.5960(3)	+ +
Sulfamethizole-N^4-acetate		76.7	0.2843—0.5205(2)	+ +
Sulfisomezole-N^4-acetate	5.54	36.7	0.5532—0.7098(3)	+ +
Sulfadimethoxine-N^4-acetate	6.01	61.8	0.1320—0.2900(3)	+ +
Sulfathiazole-N^4-glucuronide			0.5244—0.6010(1)	
Sulfisomidine-N^4-glucuronide			0.5780—1.224 (3)	
Sulfamethizole-N^4-glucuronide			0.5625—0.6648(3)	
Sulfadimethoxine-N^4-glucuronide			0.2000—0.4191(4)	
Sulfisomezole-N^1-glucuronide		22.0	0.4925—0.7112(3)	—
Sulfadimethoxine-N^1-glucuronide		18.2	0.5280—0.8370(3)	—
Sulfamethizole-N^4-sulfonate		70.0	0.3722—0.4833(2)	+ +

Protein binding were done with the sulfonamodes at the concentration of 0.3 mM in the presence of dog plasma.
The p*Ka* values were obtained from the literatures.[28,29]
Number in parenthesis indicates number of dog applied to renal clearance experiments.

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(1) As shown in Table IX, clearance ratio of long-acting sulfonamides is extremely low to certify slow urinary excretion due to predominant distal tubular reabsorption. On the contrary, clearance ratio of short-acting sulfonamides is generally high due to reduced distal tubular reabsorption.

(2) Clearance ratio of N^4-acetylated sulfonamides is considerably high, but the alteration in clearance ratio is restricted within comparatively limited extent. Clearance ratio of N^4-acetylated products of long-acting sulfonamides rises remarkably compared with the original sulfonamides. Renal excretion rate of N^4-acetylated products of certain short-acting sulfonamides are not always accelerated, but retarded compared with the original sulfonamides.

(3) N^4-Acetylated products of sulfonamides are all characterized by their remarkable proximal tubular secretion. It is noteworthy that the common characteristics of N^4-acetylated products of sulfonamides for proximal tubular secretion would be without regard to existence of proximal tubular secretion in original sulfonamides.

(4) N^1-Glucuronidation of long-acting sulfonamides results in great rise of clearance ratio compared with original sulfonamides, although only two long-acting sulfonamides were applied to renal clearance experiments. Proximal tubular secretion of this biotransformed product was not observed. It is also demonstrated that N^1-glucuronidation of sulfonamides causes in increased solubility in physiological fluid and results in decreased affinity for dog plasma protein compared with original sulfonamides. Possibly, these alterations of physicochemical characteristics following N^1-glucuronidation of sulfonamides, would increase clearance ratio and accelerate the renal excretory rate of the biotransformed products.

(5) Clearance ratio of N^4-glucuronide of sulfonamides is considerably high. However, this biotransformed product is very unstable even under physiological conditions and decomposed easily. For this reason, recognition pertaining to the existence of proximal tubular secretion of this compound is obscure.

(6) N^4-Sulfonate of sulfonamide, although only sulfamethizole-N^4-sulfonate was applied to clearance experiments, is remarkably secreted through proximal tubule as Despopoulos proposed.[25]

As mentioned above, correlation between renal excretion behaviors and biotransformation of sulfonamides was partially elucidated, but many unsolved problems still remain to be solved. On these subjects, more profound investigations will be necessary.

Another important factor to be considered in controlling renal excretion rate is the extent and strength of binding of sulfonamides to plasma protein. Actually, renal excretion of drugs would be affected by binding to plasma protein, because the drug immediately available for renal excretion through glomerular filtration is the portion present in plasma as free form unbound to plasma protein. Furthermore, it has been reported[30] that the biotransformation of drugs apparently is modified by plasma protein binding. Concerning protein binding of sulfonamides, several reports[31-35] have been published. However, relationship between renal excretion and protein binding still remains to be solved.

From our preceding reports[1,3] and this paper, several approaches to role of protein binding on renal excretion of several sulfonamides, are described below.

(1) N^4-Acetylation generally increases affinities for plasma protein compared with original sulfonamides. In spite of the increased protein binding, every N^4-acetate is secreted through proximal tubule remarkably.

(2) N^1-Glucuronidation of sulfonamides (although only two compounds were applied

30) B.B. Brodie and C.A.M. Hogben, *J. Pharm. Pharmacol.*, **9**, 345 (1957).
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to the experiments) reduces affinity for plasma protein remarkably. Such reduced protein binding of N^1-glucuronidation products seems to be one of the important factors determining renal excretion behaviors of the compounds.

(3) N^4-Glucuronidation of sulfonamides seems to decrease affinity for plasma protein, but this biotransformed product is very unstable in physiological condition and decomposed easily. This evidence shows that it is impossible to obtain accurate information concerning protein binding.

The binding of sulfonamides and the biotransformed products by plasma protein continues to be an interesting subject for active research. However, caution is necessary to prevent overemphasizing the general importance of binding phenomenon in the renal excretion behavior of the drugs in the body.

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