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Transformation and Excretion of Drugs in Biological Systems. VI.[1] Correlation between Renal Excretion and Biotransformation of Sulfisomidine and Sulfamethizole

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Sulfisomidine, sulfamethizole and their biotransformed products in man (N^4-acetate, N^4-glucuronide, N^4-sulfonate) were applied to renal clearance experiments in dogs and protein binding experiments to dog plasma protein in order to elucidate their renal excretion mechanisms.

Clearance ratio of N^4-acetate of both sulfonamides were less than that of original sulfonamides. From the results of inhibitory experiments by iodopyracet, it was found that both sulfonamides and their N^4-acetates were actively secreted and N^4-glucuronides exhibited reduced proximal tubular secretion. It was observed that clearance ratio of sulfamethizole-N^4-glucuronide, in spite of the reduced affinity for plasma protein and increased solubility, were lower than that of sulfamethizole.

Sulfisomidine and sulfamethizole are the representative short-acting sulfonamides which are rapidly absorbed and rapidly excreted. They are biotransformed to only a small extent. So their greatest area of clinical usefulness is in the treatment of urinary tract infection. Previously, biotransformation and urinary excretion of sulfisomidine and sulfamethizole in man have been confirmed by many investigators.[3-10] However, systematic studies between biotransformation and renal excretion of these sulfonamides are still remained to be solved. In the present paper, two biotransformed products of sulfisomidine and three biotransformed products of sulfamethizole in man were synthesized chemically and applied to clearance experiment in dog in order to elucidate the relationship between rates and mechanisms of their renal excretion. They were also applied to protein binding experiments. Furthermore, the correlation between structural characteristics and the susceptibility to renal transport was discussed.

Experimental

Preparation of Materials——Sulfisomidine: Commercially available sulfisomidine was recrystallized from EtOH. mp 238°.

Sulfisomidine-N^4-acetate: Sulfisomidine-N^4-acetate was synthesized by acetylation of sulfisomidine.[11] mp 298°.

1) Part V: T. Arita, R. Hori, M. Takada, and A. Misawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 930 (1971).
2) Location: *a) Nishi-6-chome, Kita-12-jo, Sapporo; b) Nishi-5-chome, Kita-14-jo, Sapporo.*
3) T. Uno and Y. Okazaki, *Yakugaku Zasshi*, **80**, 1682 (1960).
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Sulfisomidine-N^4-glucuronide: Sulfisomidine-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 substance were carried out by applying to preparative thin-layer plates (Kieselgel 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 and *Rf* part of sulfisomidine-N^4-glucuronide was checked by illumination of ultraviolet ray. The zones of sulfisomidine-N^4-glucuronide were scraped, eluted with 1/15 M isotonic phosphate buffer solution and the solution was immediately applied to clearance experiments to obviate the rapid hydrolysis.

Sulfamethizole: Commercially available sulfamethizole was recrystallized from EtOH. mp 207—208°.

Sulfamethizole-N^4-acetate: This substance was synthesized by acetylation of sulfamethizole.[11] mp 234—237°.

Sulfamethizole-N^4-glucuronide: Sulfamethizole-N^4-glucuronide was synthesized by the method of Uno, *et al.*[9] Purification of this substance was carried out by preparative thin-layer chromatography as mentioned above.

Sulfamethizole-N^4-sulfonate: Sulfamethizole-N^4-sulfonate was synthesized by the method of Uno, *et al.*[9]

Animal Experiment——Standard methods for renal clearance were employed.[1, 13–15] Male and female dogs weighing 12.0—19.5 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 previous report.[1] 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——The extent of binding of seven substances to dog plasma was determined by the method of equilibrium dialysis as described previously.[1]

Analytical Method——Plasma and urine samples were deproteinized with 10% trichloroacetic acid, and then analyzed as follows: sulfonamides and their biotransformed products by diazotization,[16] inulin by a modification of the method described by Dische, *et al.*,[17] and iodopyracet by the titration method described by Alpert.[18] Hitachi-Horiba model F-4 pH meter with a glass electrode was used to determine pH of urine.

Result

Renal Excretion of Sulfisomidne and Its Biotransformed Products

Seven clearance experiments were performed and the results are shown in Fig. 1. The detailed data of each substance are also exemplified in Table I, II and III respectively. As shown in Fig. 1, sulfisomidine and two biotransformed products showed considerable large clearance ratio, which suggests that sulfisomidine as well as two biotransformed products are excreted in urine very rapidly. The evident tendency that clearance ratio of sulfisomidine-N^4-acetate was rather reduced than sulfisomidine itself was observed. Sulfisomidine and sulfisomidine-N^4-acetate were considerably excreted through proximal tubule, but the reduced proximal tubular secretion of sulfisomidine-N^4-glucuronide was proved.

Protein Binding of Sulfisomidine and its Biotransformed Products

The binding of sulfisomidine and its biotransformed products to dog plasma 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 tendency that sulfisomidine and sulfisomidine-N^4-acetate have high affinities to dog plasma protein was proved. Sulfisomidine-N^4-glucuronide was very unstable under the experimental conditions of dialysis and about 22% of initial concentration was hydrolyzed at the end point of incubation time.

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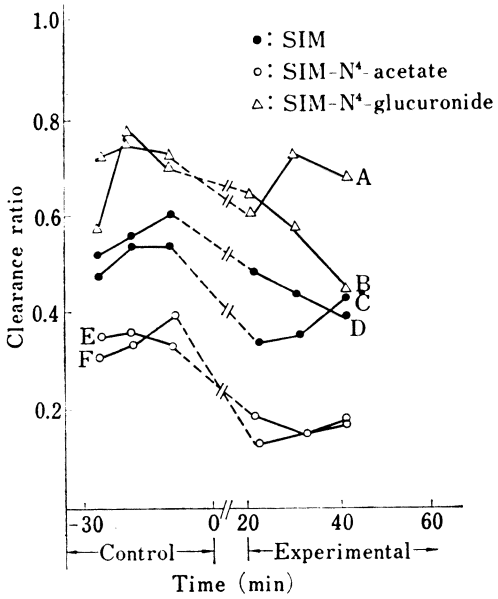

Fig. 1. Clearance Ratio of Sulfisomidine, Sulfisomidine-N⁴-acetate and Sulfisomidine-N⁴-glucuronide before and after Blockade of Proximal Tubular Secretion

The lines connect the values for each dog.
A: dog ♂ 19.5 kg D: dog ♂ 10.0 kg
B: dog ♀ 17.0 kg E: dog ♀ 12.0 kg
C: dog ♂ 13.0 kg F: dog ♂ 15.0 kg

Curved line of sulfisomidine-N⁴-glucuronide shown in Fig. 2 is the observed values and not corrected.

Renal Excretion of Sulfamethizole and Its Biotransformed Products

Ten clearance experiments were performed and the results are shown in Fig. 3 and 4. The detailed data of each substance are also exemplified in Table IV, V, VI,

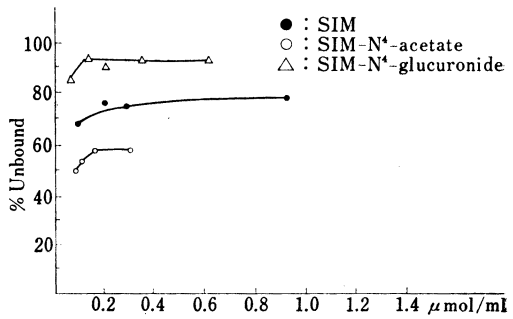

Fig. 2. Comparison of the Binding of Sulfisomidine, Sulfisomidine-N⁴-acetate, and Sulfisomidine-N⁴-glucuronide to Dog Plasma Protein

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

	Time (min)	*V* (ml/min)	Urine pH	*GFR* (ml/min)	Sulfisomidine *U* (mg/ml)	*P* (mg/ml)	*C* (ml/min)	CR
Control	30—20	5.30	7.48	74.4	0.258	0.0390	35.1	0.4718
	20—10	4.52	—	78.2	0.280	0.0304	41.6	0.5320
	10— 0	4.54	—	78.1	0.281	0.0310	41.1	0.5262
Exptl.[a]	15—25	5.40	7.40	75.5	0.154	0.0336	24.8	0.3285
	25—35	5.10	—	66.2	0.159	0.0360	22.5	0.3400
	35—45	5.35	7.62	69.6	0.161	0.0290	29.6	0.4253

dog: ♂ 13.0 kg (dog C in Fig. 1)
a) iodopyracet: 2.34 g *i.v.*, 78.1 mg/min infusion

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

	Time (min)	*V* (ml/min)	Urine pH	*GFR* (ml/min)	Sulfisomidine-N⁴-acetate *U* (mg/ml)	*P* (mg/ml)	*C* (ml/min)	CR	Iodopyracet *P* (mg/ml)
Control	30—20	3.54	6.90	54.7	0.0340	0.00645	18.7	0.3419	—
	20—10	2.40	—	48.9	0.0420	0.00613	16.4	0.3354	—
	10— 0	2.06	—	47.0	0.0518	0.00699	15.3	0.3255	—
Exptl.[a]	15—25	4.00	6.90	47.5	0.0178	0.00786	9.1	0.1916	0.5681
	25—35	3.64	—	54.7	0.0189	0.00867	7.9	0.1444	0.5873
	35—45	3.40	—	46.8	0.0205	0.00826	8.4	0.1795	0.6533

dog: ♀ 12.0 kg (dog E in Fig. 1)
a) iodopyracet: 2.50 g *i.v.*, 81.7 mg/min infusion

Table III. Clearance Ratio of Sulfisomidine-N⁴-glucuronide before and after Blockade of Proximal Tubular Secretion

	Time (min)	*V* (ml/min)	Urine pH	*GFR* (ml/min)	Sulfisomidine-N⁴-glucuronide *U* (mg/ml)	*P* (mg/ml)	*C* (ml/min)	CR
Control	30—20	2.12	7.82	62.8	0.478	0.0279	36.3	0.578
	20—10	1.80	—	55.8	0.585	0.0242	43.1	0.772
	10— 0	1.46	—	60.6	0.675	0.0231	42.7	0.705
Exptl.[a)]	20—30	4.72	7.64	74.6	0.259	0.0252	48.3	0.647
	30—40	4.30	—	102.5	0.300	0.0218	59.2	0.578
	40—50	3.60	7.82	93.8	0.275	0.0240	41.3	0.440

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

and VII respectively. It is very interesting that clearance ratio of sulfamethizole approaches approximately to one and considerably exceeds any other biotransformed products in clearance ratio.

Alteration in clearance ratio before and after blockade of proximal tubular secretion by iodopyracet was investigated. The proximal tubular secretion of sulfamethizole, sulfamethizole-N⁴-acetate and sulfamethizole-N⁴-sulfonate were considerably blocked by iodopyracet. On the contrary, only sulfamethizole-N⁴-glucuronide was inert toward iodopyracet load, which fact indicates its insufficient proximal tubular secretion.

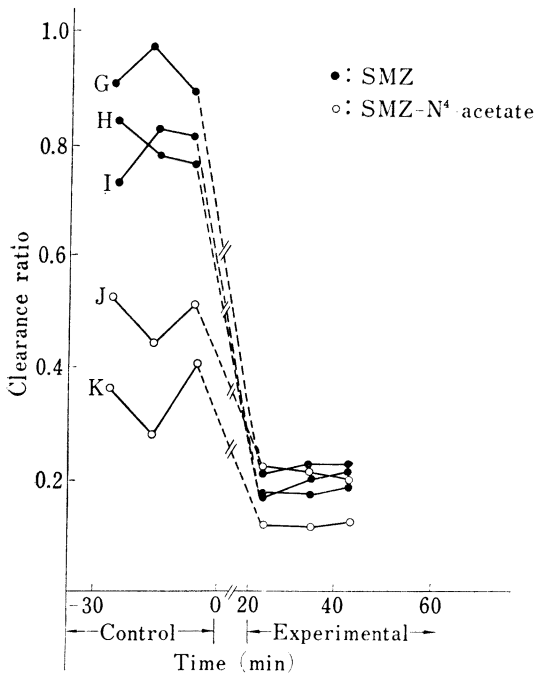

Fig. 3. Clearance Ratio of Sulfamethizole and Sulfamethizole-N⁴-acetate before and after Blockade of Proximal Tubular Secretion

The lines connect the values for each dog.
G: dog ♂ 12.5 kg
H: dog ♂ 13.5 kg
I: dog ♂ 14.5 kg
J: dog ♂ 15.0 kg
K: dog ♂ 16.5 kg

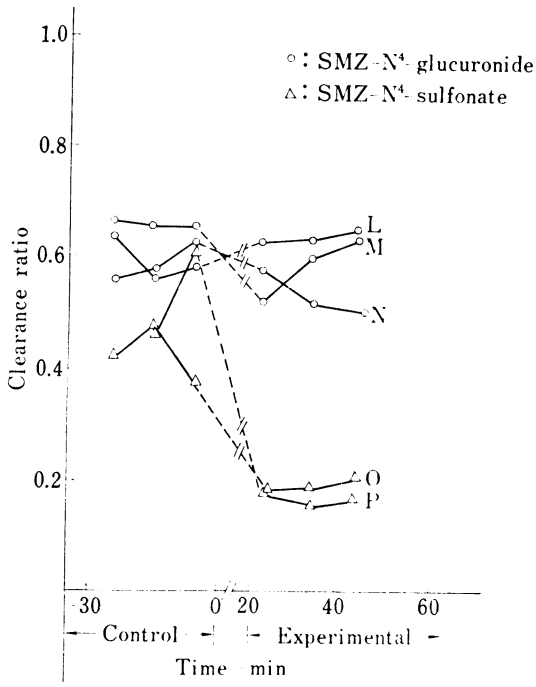

Fig. 4. Clearance Ratio of Sulfamethizole-N⁴-glucuronide and Sulfamethizole-N⁴-sulfonate before and after Blockade of Proximal Tubular Secretion

The lines connect the values for each dog.
L: dog ♂ 16.5 kg
M: dog ♂ 18.0 kg
N: dog ♀ 12.0 kg
O: dog ♂ 12.5 kg
P: dog ♂ 12.0 kg

TABLE IV. Clearance Ratio of Sulfamethizole before and after Blockade of Proximal Tubular Secretion

	Time (min)	V (ml/min)	Urine pH	GFR (ml/min)	Sulfamethizole U (mg/ml)	Sulfamethizole P (mg/ml)	Sulfamethizole C (ml/min)	Sulfamethizole CR	Iodopyracet P (mg/ml)
Control	30—20	3.30	6.98	82.4	0.556	0.0303	60.6	0.7354	—
	20—10	3.30	—	78.4	0.554	0.0281	65.1	0.8304	—
	10— 0	3.40	—	81.2	0.506	0.0260	66.2	0.8153	—
Exptl.[a]	15—25	4.56	7.08	93.6	0.129	0.0349	16.8	0.1795	0.5692
	25—35	4.18	—	93.3	0.143	0.0363	16.4	0.1758	0.5954
	35—45	4.09	—	83.5	0.156	0.0389	16.4	0.1964	0.6740

dog: ♂ 14.5 kg (dog I in Fig. 3)
a) iodopyracet: 3.00 g *i.v.*, 98.8 mg/min infusion

TABLE V. Clearance Ratio of Sulfamethizole-N⁴-acetate before and after Blockade of Proximal Tubular Secretion

	Time (min)	V (ml/min)	Urine pH	GFR (ml/min)	Sulfamethizole-N⁴-acetate U (mg/ml)	Sulfamethizole-N⁴-acetate P (mg/ml)	Sulfamethizole-N⁴-acetate C (ml/min)	Sulfamethizole-N⁴-acetate CR	Iodopyracet P (mg/ml)
Control	30—20	3.26	7.38	48.8	0.478	0.0613	25.4	0.5205	—
	20—10	2.98	—	53.5	0.521	0.0670	23.2	0.4336	—
	10— 0	3.12	—	48.2	0.497	0.0630	24.6	0.5104	—
Exptl.[a]	15—25	2.78	7.14	43.0	0.238	0.0672	9.85	0.2291	0.8048
	25—35	2.98	—	45.1	0.232	0.0690	10.0	0.2217	1.1383
	35—45	3.16	—	44.2	0.233	0.0780	9.44	0.2136	1.1919

dog: ♂ 15.0 kg (dog J in Fig. 3)
a) iodopyracet: 3.12 g *i.v.*, 102 mg/min infusion

TABLE VI. Clearance Ratio of Sulfamethizole-N⁴-glucuronide before and after Blockade of Proximal Tubular Secretion

	Time (min)	V (ml/min)	Urine pH	GFR (ml/min)	Sulfamethizole-N⁴-glucuronide U (mg/ml)	Sulfamethizole-N⁴-glucuronide P (mg/ml)	Sulfamethizole-N⁴-glucuronide C (ml/min)	Sulfamethizole-N⁴-glucuronide CR	Iodopyracet P (mg/ml)
Control	30—20	2.74	7.20	64.3	0.279	0.0187	40.9	0.6361	—
	20—10	3.20	—	67.0	0.256	0.0211	38.8	0.5791	—
	10— 0	3.36	—	68.8	0.258	0.0214	40.5	0.5887	—
Exptl.[a]	15—25	2.00	7.02	36.6	0.280	0.0240	23.3	0.6367	1.0010
	25—35	3.20	—	53.4	0.252	0.0237	34.0	0.6367	1.0242
	35—45	3.44	—	51.4	0.222	0.0225	34.0	0.6615	0.8040

dog: ♂ 16.5 kg (dog L in Fig. 4)
a) iodopyracet: 3.43 g *i.v.*, 112.3 mg/min infusion

TABLE VII. Clearance Ratio of Sulfamethizole-N⁴-sulfonate before and after Blockade of Proximal Tubular Secretion

	Time (min)	V (ml/min)	Urine pH	GFR (ml/min)	Sulfamethizole-N⁴-sulfonate U (mg/ml)	Sulfamethizole-N⁴-sulfonate P (mg/ml)	Sulfamethizole-N⁴-sulfonate C (ml/min)	Sulfamethizole-N⁴-sulfonate CR	Iodopyracet P (mg/ml)
Control	30—20	3.20	—	55.1	0.393	0.0534	23.5	0.4265	—
	20—10	3.52	—	56.9	0.386	0.0493	27.5	0.4833	—
	10— 0	3.67	6.80	67.7	0.369	0.0536	25.2	0.3722	—
Exptl.[a]	15—25	3.96	—	61.0	0.147	0.0539	10.8	0.1770	0.6114
	25—35	3.80	—	62.4	0.159	0.0518	11.6	0.1859	0.6660
	35—45	3.44	6.92	57.4	0.198	0.0572	11.9	0.2073	0.6400

dog: ♂ 12.5 kg (dog O in Fig. 4)
a) iodopyracet: 2.60 g *i.v.*, 85.1 mg/min infusion

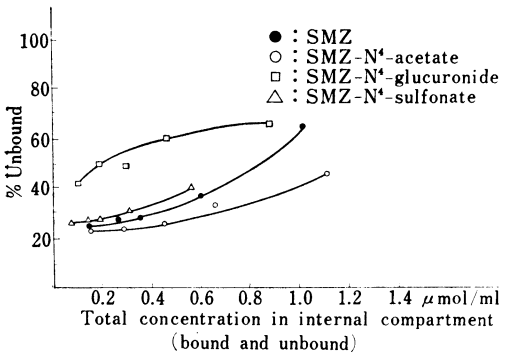

Fig. 5. Comparison of the Binding of Sulfamethizole, Sulfamethizole-N⁴-acetate, Sulfamethizole-N⁴-glucuronide, and Sulfamethizole -N⁴-sulfonate to Dog Plasma Protein

Protein Binding of Sulfamethizole and Its Biotransformed Products

Fig. 5 shows the binding of sulfamethizole and its biotransformed products to dog plasma in 0.1 M isotonic phosphate buffer solution at pH 7.4. Curved lines were obtained by the method previously mentioned in this paper. Sulfamethizole-N⁴-acetate and sulfamethizole exhibited high affinities to dog plasma protein, in spite of their high renal excretion rate. Sulfamethizole-N⁴-glucuronide was very unstable under the experimental condition of dialysis and about 33% of initial concentrations were hydrolyzed at the end point of incubation time. Curved line of sulfamethizole-N⁴-glucuronide shown in Fig. 5 is the observed values and not corrected.

Discussion

Sulfisomidine and sulfamethizole possess the unique characteristics compared with other many sulfonamides. First, sulfisomidine and sulfamethizole are excreted very rapidly in urine, so these sulfonamides are classified into one group belonging to short-acting sulfonamides. Second, both sulfonamides are biotransformed in very small extent, and excreted mainly in unchanged form in human. These characteristics led to apply them in clinical treatment of urinary tract infection. Concerning biotransformation of sulfisomidine, it has been reported that in man small amounts of sulfisomidine-N⁴-acetate and N⁴-glucuronide are excreted, on the other hand, large amount of unchanged sulfisomidine is excreted in urine.[7,8,10] As shown in Fig. 1, clearance ratio of sulfisomiidne-N⁴-acetate was appreciably decreased as compared with clearance ratio of sulfisomidine. On the contrary, clearance ratio of sulfisomidine-N⁴-glucuronide was higher than that of sulfisomidine. It has also been reported that in man sulfamethizole is excreted mainly in unchanged form, and small amounts of its N⁴-acetate, N⁴-glucuronide and N⁴-sulfonate are excreted in urine.[11] It is worthy to note that three biotransformed products of sulfamethizole exhibited rather reduced clearance ratio than their original substances as shown in Fig. 3 and 4. It has been the general impression that conjugated products containing glucuronic acid or sulfuric acid are more soluble at physiological pH and have much lower affinities to plasma protein (Fig. 2, 5) than their original substances, and accordingly they might be excreted more rapidly than their original substances. However, from the results of this paper, it is satisfactory to consider that the renal excretion rate of biotransformed products of certain sulfonamides are not always accelerated comparing with the original sulfonamides. The above mentioned facts and the results in the preceding report[1] will be used to find some relationship between extent of biotransformation and alteration of clearance ratio for several sulfonamides. Namely, as to short-acting sulfonamides which are excreted rapidly and slightly biotransformed in man, clearance ratio of their biotransformed products is not always increased compared with the original sulfonamides and the alteration in clearance ratio is restricted within comparatively narrow extent. On the contrary, as to long-acting sulfonamides which are excreted very slowly and considerably biotransformed in man such as sulfadimethoxine, biotransformation causes a marked enhance in clearance ratio compared with the original sulfonamides. Proxima ltubular secretion of drugs is also one of the important factors controlling their renal excretion as well as glomerular filtration.

The excretion of sulfisomidine, sulfamethizole and their biotransformed products were studied under the conditions in which their proximal tubular secretion is blocked by iodopyracet to elucidate their renal excretion behaviors. As shown in Fig. 1, the clearance ratio of sulfisomidine and its biotransformed products were decreased after blockade of proximal tubular secretion by iodopyracet, which shows that they are actively secreted through proximal tubule. Although sulfisomidine-N^4-acetate as well as sulfisomidine was shown to be excreted considerably through proximal tubule, sulfisomidine-N^4-glucuronide showed the reduced affinity for proximal tubular secretion.

As shown in Fig. 3 and 4, considerable proximal tubular secretion of sulfamethizole, its biotransformed products, sulfamethizole-N^4-acetate and sulfamethizole-N^4-sulfonate were observed, but sulfamethizole-N^4-glucuronide which is another biotransformed product of sulfamethizole was only insufficiently excreted through proximal tubular secretion.

Protein bindings of the two sulfonamides and their biotransformed products were also studied. It is well known that excretion rate of the drug which is eliminated by glomerular filtration is greatly influenced by protein binding, because the drug immediately available for renal excretion through glomerular filtration is the portion presented in plasma as free form unbound to plasma protein. As shown in Fig. 2 and 5, sulfisomidine and sulfamethizole are highly bound to dog plasma protein in spite of their rapid excretion rates. The similar tendencies were observed in protein binding of sulfisomidine-N^4-acetate, sulfamethizole-N^4-acetate and N^4-sulfonate. It is quite possible to consider that their major renal excretory route is not only glomerular filtration but also proximal tubular secretion to which protein binding is seemed to display a different influence compared with the influence to glomerular filtration. From these experimental results, protein binding of drugs as well as biotransformation of drugs seems to play an important role for controlling renal excretion rates of drugs. On this subject, more detailed investigations will be necessary.