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Biological Activities of Drugs. IV.[1] Physicochemical Factors Affecting the Excretion of Sulfonamides in Rats[2]

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Rate constants of acetylation, k_1, and excretion, k_3, for 14 sulfonamides have been studied using pure strain rats. The results were compared with those of rabbits. Values of k_3 in rats were closely related with partition coefficients at pH same as of urine. Excretion of sulfonamides was markedly influenced by urinary pH. The urinary pH of rats was modified to 8.6 ± 0.2 by the administration of $NaHCO_3$. Then, a rate constant for excretion was twice increased. The results suggest that sulfonamides are reabsorbed from the renal tubule in the form of unionized molecules in the filtrate.

Much laborious work has been done in evaluating drug activities on the basis of empirical screening tests employing a trial-and-error approach. It has been increasingly recognized that information of physicochemical properties of drugs should be in cooperated with biological activities of the drugs.

In the previous paper,[1] the effect of physicochemical properties of sulfonamides on the half-life in rabbits was discussed. The importance of lipid solubility of the drug was emphasized. The present report describes a relationship between the lipid solubility of sulfonamides and their rate of excretion in rats. In our previous experiments with rabbits modification of urinary pH was not successful. Presently, we used rats becaues they are omnivorous and their urinary pH is almost equal to that of man, and it can be modified by administering ammonium chloride or sodium bicarbonate. Thus, the effect of urinary pH on the excretion was studied.

Half-Life of Sulfonamides

The blood level of sulfonamides in rats was found to decrease following a first-order reaction rate. A similar decrease was observed in rabbits.[1] Half-lives ($t_{1/2}$) of each drug were calculated from decreasing slopes (Table I). The results of 5 rats were presented in the table.

Table I. Typical Half-life and Percent of Acetylation of Sulfonamides in Rats

Sulfamerazine			Sulfathiazole			Sulfanilamide		
Subjects	$t_{1/2}$ (hr)	Acetylation (%)	Subjects	$t_{1/2}$ (hr)	Acetylation (%)	Subjects	$t_{1/2}$ (hr)	Acetylation (%)
102m	5.68	37.6	145m	2.33	28.2	123m	1.80	79.9
120m	6.10	42.8	140m	2.00	22.8	133m	1.66	86.6
105m	6.45	36.3	126m	2.15	29.8	143m	1.55	81.8
123m	5.67	43.4	130m	2.16	27.2	150m	1.71	86.4
124m	6.30	43.7	144m	2.05	28.5	128m	1.54	86.4
Mean	6.04	40.8	Mean	2.14	27.3	Mean	1.65	84.2

1) Part III: *Chem. Pharm. Bull.* (Tokyo), **16**, 707 (1968).
2) A part of this work was reported at the 24th Annual Meeting of the Pharmaceutical Society of Japan, April 1967.
3) Location: *Toneyama, Toyonaka, Osaka-fu.*

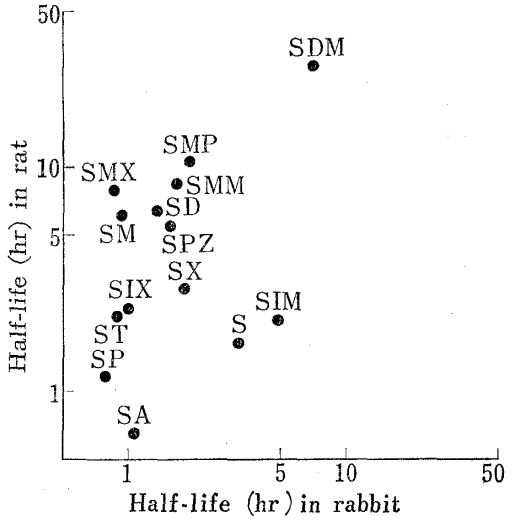

Fig. 1. Relationship of Half-lile of Sulfonamides between Rats and Rabbits

S : Sulfanilamide
SA: Sulfacetamide
SP: Sulfapyridine
ST: Sulfathiazole
SD: Sulfadiazine
SM: Sulfamerazine
SX: N-Sulfanilyl-3,4-xylamide
SIX: Sulfisoxazole
SIM: Sulfisomidine
SPZ: Sulfaphenazole
SMP: Sulfamethoxypyridazine
SDM: Sulfadimethoxine
SMX: Sulfamethoxazole
SMM: Sulfamonomethoxine

It is interesting that inbred rats showed a similar half-life for one drug while hybrid rabbits did not. The average values of half-life and percent of acetylation for each sulfonamide were presented in Table II.

A comparison in half-life was made between rats and rabbits[1] revealing no clear relationship (Fig. 1). But except for the experients with sulfacetamide, sulfanilamide and sulfisomedine, rats showed their half-life 2—4 time longer than rabbits.

The same comparison was made in human subjects with Krüger-Thiemer[4] (Fig. 2). The half-life of human subject was longer than that of rats.

Rate constants, K, of eilimination of sulfonamides from the rat blood were found about 2-times larger than excretion rate constants of human subjects[5] (Fig. 3).

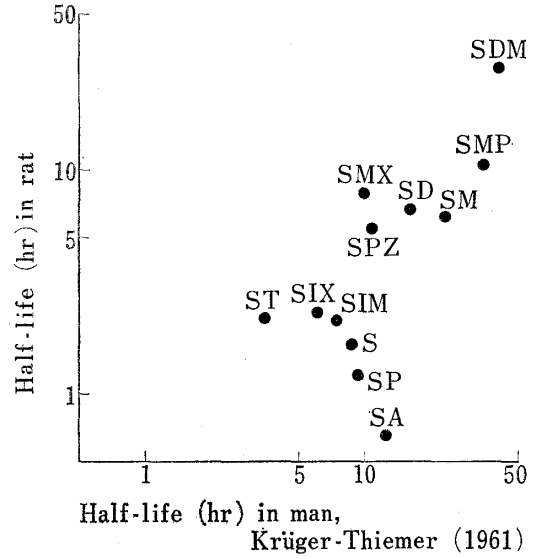

Fig. 2. Relationship of Half-life of Sulfoamides between Rats and Man

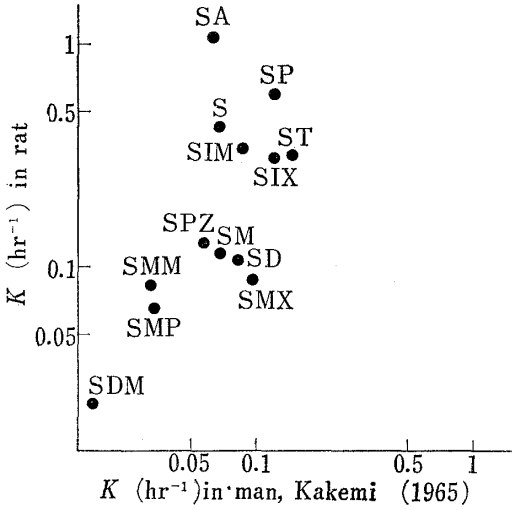

Fig. 3. Relation of K between Rats and Man

Acetylation Rate

Rate constants of acetylation, k_1, and excretion, k_3, for 14 sulfonamides were obtained of rats, being calculated as described previously[1] (Table II). Values of k_1 in rats were compared with those of rabbits (Fig. 4) and human subjects (Fig. 5). There was little correlationship between them. It has been generally accepted that the drug metabolism by human subjects was lower than that of laboratory animals. The same finding was obtained for acetylation of sulfonamides. It must be noticed that rabbits metabolize drugs much more rapidly

4) E. Krüger-Thiemer and F. Bünger, *Arzneimittel-Forsch.*, **11**, 867 (1961).
5) K. Kakemi, T. Arita, and T. Koizumi, *Yakuzaigaku*, **25**, 22 (1965).

Table II. Kinetic Parameters of Sulfonamides in Rats

Sulfonamides	$t_{1/2}$ (hr)	Acetylation (%)	K (hr^{-1})	k_1 (hr^{-1})	k_3 (hr^{-1})
Sulfanilamide	1.65	84.2	0.421	0.355	0.066
Sulfacetamide	0.65	40.2	1.085	0.434	0.651
Sulfapyridine	1.18	53.4	0.587	0.315	0.272
Sulfathiazole	2.14	27.3	0.325	0.088	0.237
Sulfadiazine	6.48	32.3	0.107	0.034	0.073
Sulfamerazine	6.04	40.8	0.115	0.047	0.068
N–Sulfanilyl–3,4–xylamide	2.85	52.6	0.245	0.129	0.116
Sulfisoxazole	2.30	20.6	0.305	0.062	0.243
Sulfisomidine	2.05	32.3	0.339	0.110	0.229
Sulfaphenazole[a)]	5.46	77.7	0.128	0.100	0.028
Sulfamethoxypyridazine[a)]	10.5	56.8	0.066	0.038	0.028
Sulfadimethoxine[a)]	28.5	49.2	0.024	0.012	0,012
Sulfamethoxazole[a)]	7.87	47.8	0.088	0.042	0.046
Sulfamonomethoxine[a)]	8.37	53.2	0.083	0.044	0.039

a) Long-acting sulfonamide.
Five animals at minimum were used for each experiment.

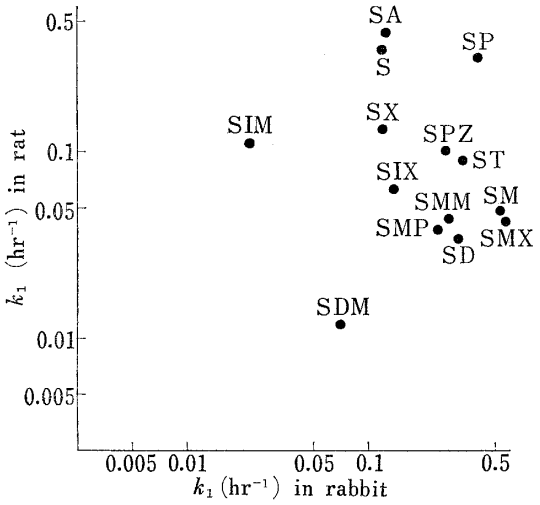

Fig. 4. Relationship of k_1 between Rats and Rabbits

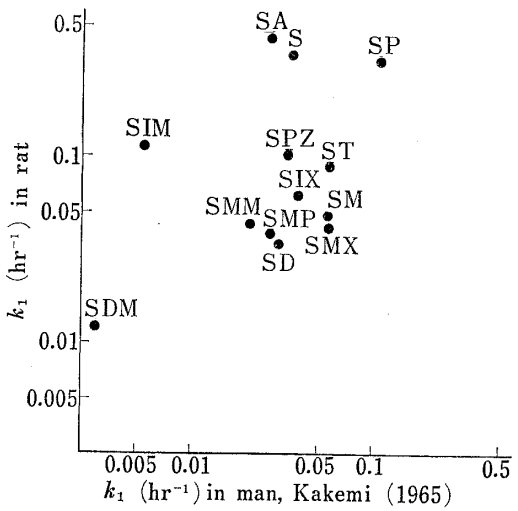

Fig. 5. Relationship of k_1 between Rats and Man

than human subjects. Therefore rabbits' data only may not be sufficient on screening of new drugs.

It was difficult to establish a clear relationship between k_1 and pK_a, superdelocalizability of π-electrons, or Michaelis' constants. But, in contrast with the results of rabbits, the acetylation rate in rats was observed to decrease with an increase of binding of sulfonamides to human plasma protein (Fig. 6).

Rate of Excretion

Rates of excretion of sulfonamides were studied after an intravenous administration in the rat tail. The result was compared with that of rabbits on the basis of k_3 (Fig. 7). The excretion rate of rats proved to be smaller than that of rabbits with a linear relationship (correlation coefficient 0.793). But there was little correlationship between the present k_3 of rats and k_3 of man determined by Koizumi[6] (Fig. 8). In general, the sulfonamides studied were excreted from man slower than rats and rabbits.

6) Y. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 428 (1964).

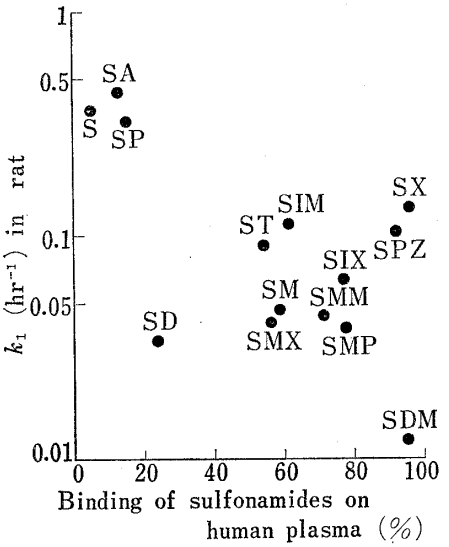

Fig. 6. Relationship between Binding of Sulfonamides on Human Plasma and k_1 in Rats

It has been generally accepted that a drug is effectively excreted from the kidney in the form of molecules unbound to plasma. Thus, it is necessary to explain the excretion of sulfonamides from the kidney on the basis of binding of drugs to the plasma as well as influence of pH changes of the glomerular filtrate.[1] The influence of binding of sulfonamides to plasma protein on their excretion was presented in Fig. 9. The correlation coefficient was 0.518. It was observed that a highly bound sulfonamide was excreted slowly from rats.

The excretion rate of sulfonamides in rats was plotted against partition coefficients between chloroform and a phosphate buffer of pH 6.4 (the same pH as in the rat urine) (Fig. 10). The excretion rate decreased with an increase of the partition coefficient. Here, sulfanilamide showed an exceptional behaviour which has been left unexplain.

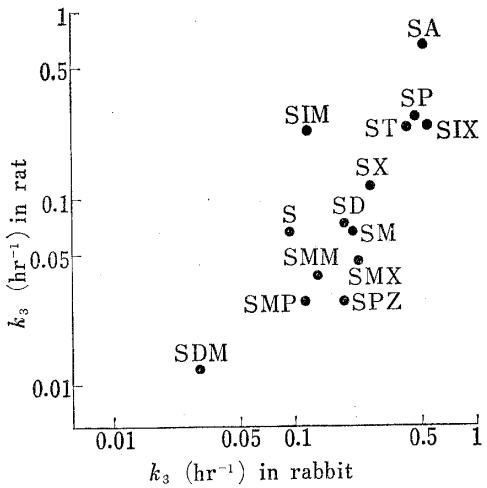

Fig. 7. Relationship of k_3 between Rats and Rabbits

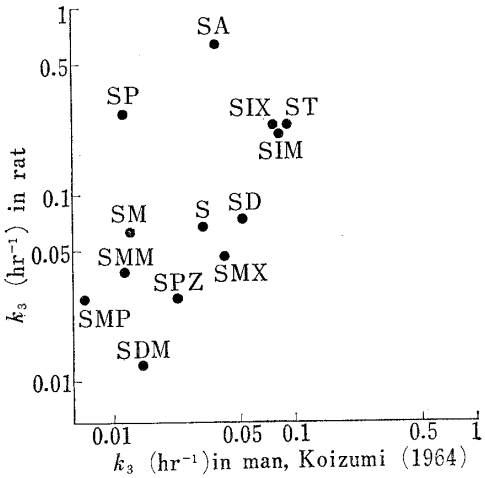

Fig. 8. Relationship of k_3 between Rats and Man

The correlation coefficient was 0.874 by exclusion of sulfanilamide. It may be concluded that a sulfonamide highly soluble to lipid permeates easily through the wall membrance of kidney tubules of rats with low k_3 in the rate of excretion from the kidney.

Effect of Change of Urinary pH on Excretion Rate

It has been well known that the excretion rates of drugs were influenced by urinary pH. Beckett[7] observed that the renal excretion of amphetamine, a basic drug, was enhanced when pH of urine was following oral administration of ammonium chloride in man. Kostenbauder[8] also demonstrated that the half-life of sulfaethidole, considered as acidic in this case, was 11.4 hr when urinary pH was 5.0, and the half-life decreased to 4.2 hr when urinary pH was increased to 8.0. A similar observation was presented by Dayton[9] for probenecid.

7) A.H. Beckett and M. Rowland, *J. Pharm. Pharmacol.*, **17**, 628 (1965).
8) H.B. Kostenbauder, J.B. Portnoff, and J.V. Swintosky, *J. Pharm. Sci.*, **51**, 1084 (1962).
9) P.G. Dayton, T.F. Yu, W. Chen, L. Berger, L.A. West, and A.B. Gutman, *J. Pharmacol. Exptl. Therap.*, **140**, 278 (1963).

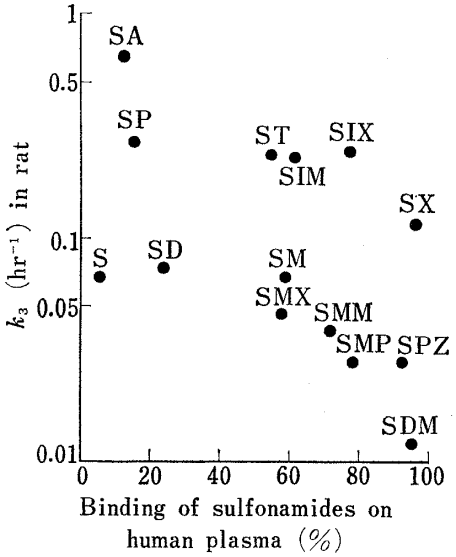

Fig. 9. Relationship between Binding of Sulfonamides on Human Plasma and k_3 in Rats

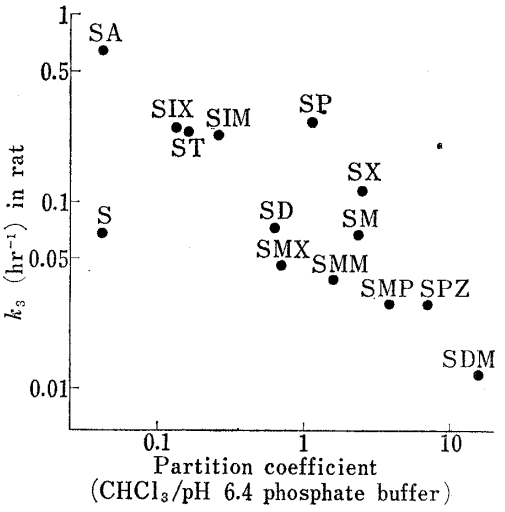

Fig. 10. Relationship between k_3 in Rats and Partition Coefficient ($CHCl_3$/pH 6.4 Phosphate Buffer)

The change of half-life of drugs with change of urinary pH may be attributed to the degree of pH when the drugs are transferred from renal tubules. Many substances in the blood including drugs are filtered passively through the glomeruli of renal tubules, and reabsorbed into the plasma through the tubules. Many drugs seem to be reabsorbed passively.[10] They are mostly weak electrolytes. Thus, change of pH of the renal filtrate will cause a considerable change in an ionized and unionized molecules ratio of a drug in the filtrate. The unionized molecules diffuses easily the lipid wall which separates plasma from the tubular filtrate. It is difficult to measure the pH of the filtrate in tubules between glomerulus and the bladder . But the pH of the filtrate in the glomerulus and the bladder may be consistent with the pH of plasma and urine.

A normal pH of rat urine was 6.4±0.2. Oral administration of sodium bicarbonate increased pH to 8.6±0.2. The alkaline urine may affect the half-life of sulfonamides. For example, a mean half-life of sulfamerazine for 5 rats, having a normal urinary pH, was 6.04 hr (Table III). But, the half-life decreased to 3.84 hr when rats were administered sodium bicarbonate. It is also observed that the acetylation of sulfamerazine decreased from 40.8% to 28.3% following administration of sodium bicarbonate. It has been commonly accepted that the acetylation of sulfonamides was decreased by a simultaneous administration of sodium bicarbonate and possibly resulting in prevention of untowards effects of sulfonamides, such as urinary calculus and hematuria. The rate constants of acetylation and excretion for sulfamerazine were calculated by usual procedures. It was very interesting that k_3 of alkaline urine was two-times that of a normal urine, but k_1 showed little change. It may be considered that the administration of sodium bicarbonate did not affect acetylation in rats, but the apparent degree of acetylation in urine was decreased as a result of an enhanced excretion (k_3) of the drug. The increase of k_3 may be mainly ascribed to a decrease of unionized molecules in alkaline urine at the site of reabsorption in the kidney.

To study the influence of an acidic urine on excretion of sulfonamides, ammonium chloride was administered orally to rats. The pH of the urine decreased to 5.4±0.2. A mean half-life of sulfamerazine was 6.90 hr for the acidic urine. pK_a (sulfonamide group) of sulfamerazine determined spectrophotometrically was 6.93.[11] Thus, the percent fractions

10) B.B. Brodie, and C.A.M. Hogben, *J. Pharm. Pharmacol.*, **9**, 345 (1957).
11) M. Yamazaki, M. Aoki, A. Kamada, and Yata, *Yakuzaigaku*, **27**, 37 (1967).

of unionized sulfamerazine in plasma, in normal–, acidic–, and alkaline–urine were as follows: in plasma, pH 7.4, 25%; in urine, pH 5.4, 97%; pH 6.4, 77%; pH 8.6, 2%. Although the pH of tubular filtrate at the site reabsorption of sulfamerazine was not determined, the tubular filtrate is likely to be alkaline when urine was maintained at pH 8.6 and acidic at pH 5.6.

The same experiments were carried out for three sulfonamides (Table III).

Sulfanilamide, having pK_a (sulfonamide group) of 10.45, mainly exists as an unionized form at a pH range of 5.4—8.8. And there was no significant difference in partition coefficients at pH 5.4, 6.4, and 8.8 (Table IV). Thus, k_3 of sulfanilamide was not influenced by a change of urinary pH from alkaline to acidic. The k_3 of a sulfonamide, which has a large pK_a value, was little influenced by change of urinary pH so long as the partition coefficient was not influenced by change of urinary pH.

It is interesting that k_3 of rats at alkaline urine was almost equal to that in rabbits. Here it must be noticed that the urinary pH of rabbits was 8.8.

Thus, the importance of partition coefficient at urinary pH was to be noted for the study of excretion of sulfonamides as well as their absorption at gastrointestinal pH.

Table III. The Mean Half-life and Percent of Acetylation of Sulfonamides as Function of Urinary pH

Urine	$t_{1/2}$ (hr)	Acetylation (%)	K (hr^{-1})	k_1 (hr^{-1})	k_3 (hr^{-1})
		Sulfamerazine			
Acid	6.90	40.4	0.101	0.041	0.060
Normal	6.04	40.8	0.115	0.047	0.068
Alkaline	3.84	28.3	0.180	0.051	0.129
		Sulfathiazole			
Acid	2.31	34.0	0.305	0.103	0.202
Normal	2.14	27.3	0.325	0.088	0.237
Alakline	1.17	17.5	0.595	0.103	0.492
		Sulfisoxazole			
Acid	3.98	28.7	0.175	0.050	0.125
Normal	2.30	20.6	0.305	0.062	0.243
Alakline	1.41	13.9	0.494	0.068	0.426
		Sulfanilamide			
Acid	1.67	82.6	0.418	0.346	0.072
Normal	1.65	84.2	0.421	0.355	0.066
Alkaline	1.57	84.2	0.441	0.371	0.069

Five animals at minimum were used for each experiment.

Table IV. Effect of Urinary pH on Rate Constants for Excretion of Sulfonamidse in Rats

Sulfonamides	pK_a	Partition Coefficient			Rate Constant for Excretion of Sulfonamides, k_3 (hr^{-1})			
					Rat			
		pH 5.4	pH 6.4	pH 8.8	Acid Urine	Normal Urine	Alkaline Urine	Rabbit [a]
Sulfanilamide	10.45	0.0398	0.0407	0.0358	0.072	0.066	0.069	0.095
Sulfathiazole	7.10	0.171	0.159	0.0056	0.202	0.237	0.492	0.435
Sulfamerazine	6.93	2.77	2.37	0.0515	0.060	0.068	0.129	0.209
Sulfisoxazole	4.62	0.806	0.146	0.0021	0.125	0.243	0.426	0.549

a) From the previous paper.[1]
Five animals at minimum were used for each experiment.

Experimental

Method——Male rats, Sprague–Dawley strain, weighing from 300 to 400 g, were supplied by the Inbred Animal Laboratory, Osaka Univertisy. Sulfonamides were administered intravenously into a tail vein at a dose of 0.5 ml/100 g body weight (=0.2 mmole/kg). Samples of blood were chronologically collected by cardiac punctures. Urine was collected 24 and 48 hr after intravenous administration of the drug while the animal was kept in a metabolic cage. Procedures for preparation of sulfonamide soltuions and assay of the drugs were described in the previous paper.[1]

Control of Urinary pH——Five-tenth ml of 8% NH_4Cl or $NaHCO_3$ solution per 100 g of body weight (=0.4 g/kg) was given orally, under a condition with a desired urinary pH.