[Chem. Pharm. Bull. 17(10)2000—2007(1969)]

UDC 615.31.015.11

Biological Activities of Drugs. VII.[1] Structure-Activity Relationship of Sulfonamide Carbonic Anhydrase Inhibitors. (2)[2]

Nobuharu Kakeya, Noboru Yata, Akira Kamada, and Masaru Aoki

Faculty of Pharmaceutical Sciences, Osaka University[3]

(Received December 2, 1968)

Diuretic and natriuretic activities and duration of the diuretic action in rats were studied with 22 sulfonamide derivatives.

In order to clarify the active form of the sulfonamides, the effect of pH of the rat body fluid on the diuretic activity was studied.

1) The diuretic and natriuretic activities of the sulfonamides increased with an increase of their carbonic anhydrase inhibitory activity except for *o*-substituted benzenesulfonamides, disubstituted benzenesulfonamides and 2-amino-1,3,4-thiadiazole-5-sulfonamide.

2) A close correlationship of diuretic and natriuretic activities with the electronic characteristics of the sulfamoyl group, such as Hammett's σ factor, pK_a, NMR chemical shift of the sulfamoyl protons and S=O valence-force constant, was observed for the derivatives with some exceptions.

3) A compound having a large partition coefficient and strong albumin binding ability showed long duration of the diuretic action

4) The diuretic and natriuretic activities of sulfonamides were enhanced with an increase of their dissociation constants. The diuretic activity was enhanced under alkalotic conditions. Thus, it was concluded that active form of the sulfonamide carbonic anhydrase inhibitors was the ionized form.

The diuretic action of carbonic anhydrase inhibitors of sulfonamide derivatives has been well explained on the basis of a depression of the enzymatic reaction, $CO_2+H_2O \rightleftarrows H_2CO_3$, in the renal tubular cells.[4] All sulfonamides which inhibit carbonic anhydrase have the $-SO_2NH_2$ group attached directly to an aromatic nucleus, which is either homocyclic or heterocyclic. Conversion of the free $-SO_2NH_2$ group to $-SO_2NHR$ or $-SO_2NR_2$ results in the abolition of carbonic anhydrase inhibition.[5]

Some studies have been reported on correlations between the diuretic activity of carbonic anhydrase inhibitors *in vivo* and these inhibitors' inhibitory activity for the enzyme *in vitro*.[6,7] But, little consideration has been given to a possible correlationship of the diuretic activity of anticarbonic anhydrase drugs with their physicochemical properties.

We have already reported[1] that the inhibitory activity of sulfonamide derivatives for carbonic anhydrase is closely related with their physicochemical properties, *i.e.*, a derivative which has low electron density at the sulfamoyl group shows a strong inhibitory activity for the enzyme.

Presently, 22 sulfonamide derivatives were studied as to their diuretic and natriuretic activities and duration of the diuretic action in rats. Their activities were discussed on the

1) Part VI: N. Kakeya, M. Aoki, A. Kamada, and N. Yata, *Chem. Pharm. Bull.* (Tokyo), **17**, 1010 (1969).
2) Part of this work was reported at the 18th Annual Meeting of Kinki Branch, Pharmaceutical Society of Japan, November 1968.
3) Location: *Toneyama, Toyonaka, Osaka.*
4) W.B. Schwartz, *New Engl. J. Med.*, **240**, 173 (1949).
5) H.A. Krebs, *Biochem. J.*, **43**, 525 (1948).
6) K.H. Beyer and J.E. Bear, *Pharmacol. Rev.*, **13**, 517 (1961).
7) J.J. Piala, J.W. Poutsiaka, C.I. Smith, J.C. Burke, and B.N. Craver, *J. Pharmacol. Expil. Therap.*, **134**, 273 (1961).

basis of the physicochemical properties, *i.e.*, Hammett's σ factor, pK_a, NMR chemical shift of the sulfamoyl protons, and valence–force constant of the S=O bond. The duration of diuretic action was also discussed in terms of binding to albumin and partitioning between chloroform and water.

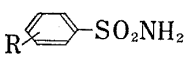
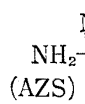
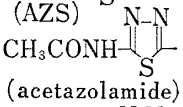
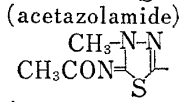
To study the effect of ionization of the drugs on diuretic activity, pH of the rat body fluid was modified by oral administration of $NaHCO_3$.

Results and Discussion

Diuretic and Natriuretic Activities

The diuretic and natriuretic activities of the 22 sulfonamide derivatives in rats are presented in Table I. The activities were expressed as reciprocal of a dose by which the excretion of urine or sodium ion was increased to three times the normal one.

Table I. Diuretic and Natriuretic Activities of Sulfonamide Derivatives

No.	R	Diuretic activity $1/C^{a)}$	Natriuretic activity $1/C^{a)}$
			
1	*p*-CH_3NH	0.42	0.50
2	*p*-NH_2	0.50	0.63
3	*p*-CH_3O	1.54	1.73
4	*p*-CH_3	1.52	1.52
5	*m*-CH_3	1.50	1.50
6	H	1.35	1.43
7	*p*-Cl	1.93	2.00
8	*p*-Br	1.85	1.85
9	*m*-Cl	1.96	2.08
10	*p*-CH_3CO	2.50	2.90
11	*p*-CN	10.00	10.50
12	*m*-NO_2	4.76	5.00
13	*p*-NO_2	6.67	7.00
14	3,4-di-Cl	1.75	1.85
15	3-NO_2-4-Cl	2.10	2.10
16	3-CF_3-4-NO_2	3.80	4.00
17	*o*-CH_3	<0.5	<0.5
18	*o*-Cl	<0.5	<0.5
19	*o*-NO_2	<0.5	<0.5
	$R\text{-}SO_2NH_2$		
20	 (AZS)	4.30	4.50
21	 (acetazolamide)	40.0	40.0
22	 (methazolamide)	33.0	35.0

a) Reciprocal of the dose (mM/kg) which is required to increase the excretion of urine and sodium ion three times higher.

The activities were plotted against Hammett's σ factor (Fig. 1). The correlation coefficients for the diuretic and natriuretic activities were 0.822 and 0.811, respectively. They

increased to 0.909 for the diuretic activity and 0.928 for the natriuretic activity with disubstituted benzenesulfonamides excluded.

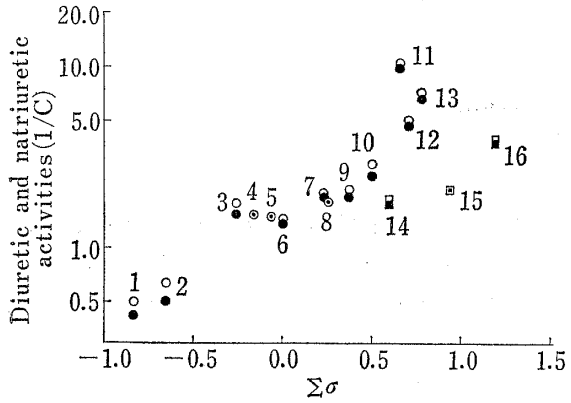


Fig. 1. Relationship between Hammett's σ Factor and Diuretic and Natriuretic Activities

close symbols (●■), diuretic activity
open symbols (○□), natriuretic activity
●○, monosubstituted benzenesulfonamides
■□, disubstituted benzenesulfonamides.
Substituent numbers correspond to those in Table I.

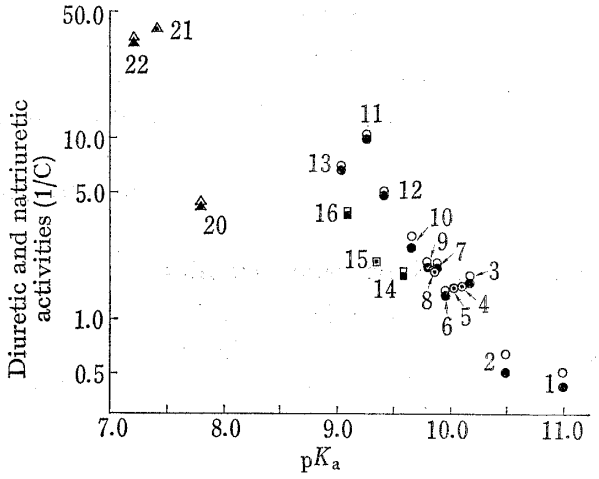


Fig. 2. Relationship between pK_a and Diuretic and Natriuretic Activities

close symbols (●■▲), diuretic activity
open symbols (○□△), natriuretic activity
●○, monosubstituted benzenesulfonamides
■□, disubstituted benzenesulfonamides
▲△, heterocyclic sulfonamides
Substituent numbers correspond to those in Table I.

The diuretic and natriuretic activities increased with a decrease of pK_a (Fig. 2). The correlation coefficients obtained were 0.908 for the diuretic activity and 0.907 for natriuretic activity. They increased to 0.956 for diuresis and 0.957 for natriuresis following exclusion of 2-amino-1,3,4-thiadiazole-5-sulfonamide (AZS).

A linear correlationship was observed between chemical shift of sulfamoyl protons and the biological activities (Fig. 3). The correlation coefficients were 0.909 for the diuretic activity and 0.926 for the natriuretic activity. They increased to 0.956 for the diuretic activity and 0.983 for the natriuretic activity following exclusion of disubstituted compounds.

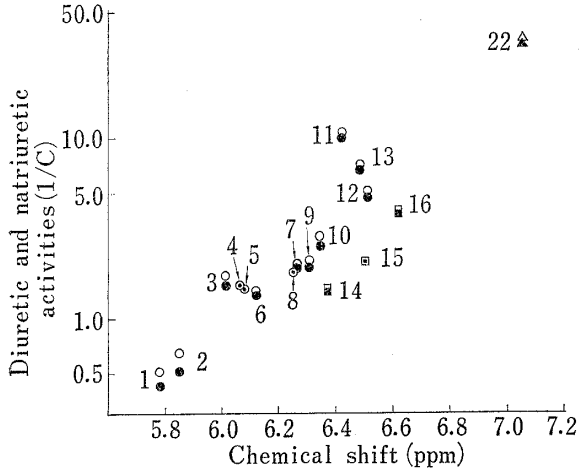


Fig. 3. Relationship between NMR Chemical Shift of Sulfamoyl Protons and Diuretic and Natriuretic Activities

close symbols (●■▲), diuretic activity
open symbols (○□△), natriuretic activity
●○, monosubstituted benzene sulfonamides
■□, disubstituted benzenesulfonamides
▲△, heterocyclic sulfonamide
Substituent numbers correspond to those in Table I.

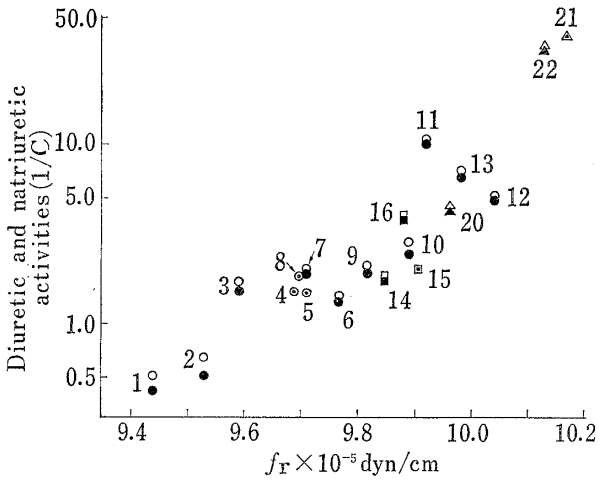


Fig. 4. Relationship between S=O Valence-force Constant and Diuretic and Natriuretic Activities

close symbols (●■▲), diuretic activity
open symbols (○□△), natriuretic activity
●○, monosubstituted benzenesulfonamides
■□, disubstituted benzenesulfonamides
▲△, heterocyclic sulfonamides
Substituent numbers correspond to those in Table I.

A close correlation was obtained between S=O valence–force constant and the biological activities (Fig. 4). The correlation coefficients were 0.907 for the diuretic activity and 0.902 for the natriuretic activity.

A comparison was made between the inhibitory activity for carbonic anhydrase *in vitro* and diuretic and natriuretic activities *in vivo*. The correlation coefficients between the carbonic anhydrase inhibitory constants at 0.2° and the diuretic and natriuretic activities were 0.810 and 0.801, respectively (Fig. 5A). They increased to 0.955 for the diuretic activity and 0.953 for the natriuretic activity except for disubstituted benzenesulfonamides and AZS. The correlation coefficients of the inhibitory activity at 15° with the diuretic and natriuretic activities were 0.813 and 0.802, respectively (Fig. 5B). They increased to 0.933 for the diuretic activity and 0.928 for the natriuretic one by exclusion of disubstituted compounds.

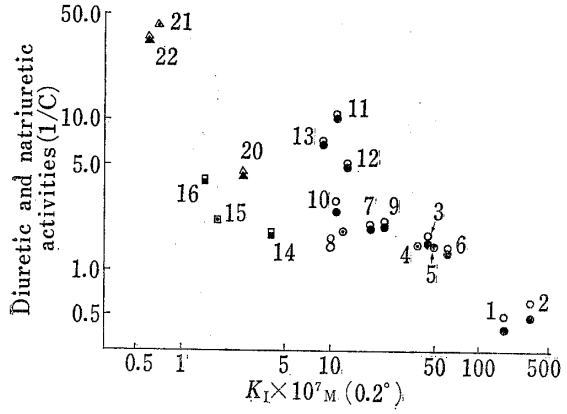


Fig. 5A. Relationship between Carbonic Anhydrase Inhibitory Constant at 0.2° and Diuretic and Natriuretic Activities

close symbols (●■▲), diuretic activity
open symbols (○□△), natriuretic activity
●○, monosubstituted benzenesulfonamides
■□, disubstituted benzenesulfonamides
▲△, heterocyclic sulfonamides.
Substituent numbers correspond to those in Table I.

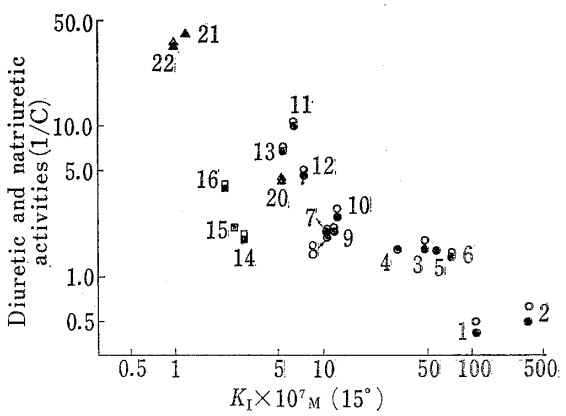


Fig. 5B. Relationship between Carbonic Anhydrase Inhibitory Constant at 15° and Diuretic and Natriuretic Activities

close symbols (●■▲), diuretic activity
open symbols (○□△), natriuretic activity
●○, monosubstituted benzenesulfonamides
■□, disubstituted benzenesulfonamides
▲△, heterocyclic sulfonamides
Substituent numbers correspond to those in Table I.

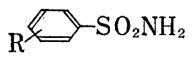
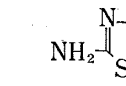
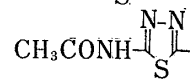
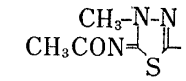
It was interesting that the biological activity of disubstituted benzenesulfonamides and AZS *in vivo* was weaker than that expected from the inhibitory activity for carbonic anhydrase *in vitro* and the electronic characteristics of the sulfamoyl group of monosubstituted benzenesulfonamides.

Presently, the presence of some physicochemical factors was suggested in transfering molecules in question from the gastrointestinal tract to a particular site where diuresis is performed. The lipid–water partitioning and protein binding of drugs should be taken into consideration for the process.

Determinations were made of association constants for bovine serum albumin at pH 7.4 and chloroform–water partition coefficients of unionized form of sulfonamides (Table II). Here it was considered that most studies of the partition coefficient had advocated chloroform as a model of lipid barriers in the absorption process. Drugs permeate the lipid barrier mostly in the form of unionized molecule. The diuretic compounds studied had pK_a of 7—11 and the pH at the absorbing site of gastrointestinal tract was considered to be around 6 or below. Thus, the compounds existed mainly as unionized molecules ih the tract. The partition coefficient of unionized molecules was sufficient for the present study.

Binding of drugs to tissues, especially to plasma protein, was also important for the distribution to drugs to particular sites of biological activity. Many studies have revealed that drugs bound to plasma protein cannot penetrate through the body membrane. For example,

Table II. Association Constant with Albumin and Chloroform–Water Partition Coefficient of Sulfonamide Derivatives

No.	R	Association constant[a] $K \times 10^{-3}$M^{-1}	Partition coefficient[b]
			
1	*p*-CH_3NH	0.22	0.31
2	*p*-NH_2	0.05	0.025
3	*p*-CH_3O	0.39	1.72
4	*p*-CH_3	0.51	2.61
5	*m*-CH_3	0.26	2.55
6	H	0.17	0.70
7	*p*-Cl	1.08	1.69
8	*p*-Br	1.10	2.98
9	*m*-Cl	0.65	2.19
10	*p*-CH_3CO	0.18	0.53
11	*p*-CN	0.25	0.30
12	*m*-NO_2	0.30	0.53
13	*p*-NO_2	0.28	0.31
14	3,4-di-Cl	3.48	4.07
15	3-NO_2-4-Cl	1.57	1.30
16	3-CF_3-4-NO_2	2.08	1.90
17	*o*-CH_3	0.23	3.53
18	*o*-Cl	0.26	3.53
19	*o*-NO_2	0.26	1.69
	R-SO_2NH_2		
20		0.10	0.0002
21		1.00	0.005
22		2.60	0.05

a) pH 7.4, 30° *b*) partition coefficient of unionized form, 30°

it is only the unbound fraction of drug in plasma which penetrates the blood–brain and blood–cerebrospinal fluids,[8–10] and the renal tubular epithelium[11,12] and the gastrointestinal epithelium.[13–15] It has been generally shown that a transference of drugs to active sites increases with an increase of partition coefficient.[16–18] But, when drug have too high a

8) B.B. Brodie, H. Kurz, and L.S. Shanker, *J. Pharmacol. Exptl. Therap.*, **118**, 17 (1956).
9) D.P. Rall, J.R. Stabenau, and C.G. Zubrod, *J. Pharmacol. Exptl. Therap.*, **125**, 185 (1959).
10) S. Mayer, R.P. Maickel, and B.B. Brodie, *J. Pharmacol. Exptl. Therap.*, **127**, 205 (1959).
11) I. Sperber, *Pharmacol. Rev.*, **11**, 109 (1959).
12) L. Peters, *Pharmacol. Rev.*, **12**, 1 (1960).
13) P.A. Shore, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **119**, 361 (1957).
14) C.A.M. Hogben, D.J. Tocco, B.B. Brodie, and L.S. Shanker, *J. Pharmacol. Exptl. Therap.*, **125**, 275 (1959).
15) L.S. Shanker, *J. Pharmacol. Exptl. Therap.*, **126**, 283 (1959).
16) L.S. Shanker, P.A. Shore, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **120**, 528 (1957).
17) L.S. Shanker, D.J. Tocco, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **123**, 81 (1958).
18) L.S. Shanker and P.A. Nafpliotis, *Federation Proc.*, **19**, 136 (1960).

partition coefficient, they show less absorption to the active site. Thus, an optimal partition coefficient is required for a maximal absorption.[19]

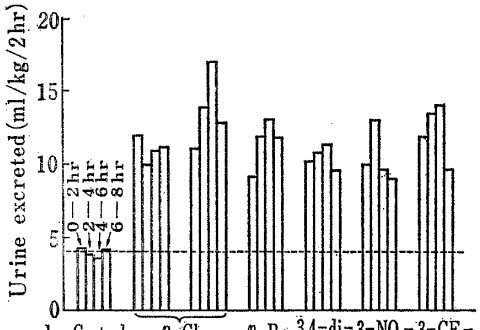
The deviation of diuretic and natriuretic activities of AZS from the monosubstituted compounds may be attributed to its low partition coefficient. The deviation of the activity of disubstituted derivatives from the monosubstituted compounds may be also attributed to their large binding ability to albumin. Despite a remarkable binding to albumin and low partition coefficient, it was found that acetazolamide and methazolamide had a strong diuretic activity. This finding was left unexplained here.

Little activity of the *o*-substituted compounds was also unexplained from the results of carbonic anhydrase inhibition constant,[1] electronic characteristics,[1] association constant to albumin or partition coefficient studied (Table I, II). Generally speaking diuretic and natriuretic activities were subjected to an inhibitory activity to carbonic anhydrase and electronic characteristics of the sulfamoyl group as well as to physicochemical properties, *e.g.*, binding to albumin and partition coefficient.

Duration of Biological Activity

It has been well known that drugs having a strong binding ability to protein and a marked lipid solubility show a slow decrease in their plasma concentration and sustain their biological activity.[20,21]

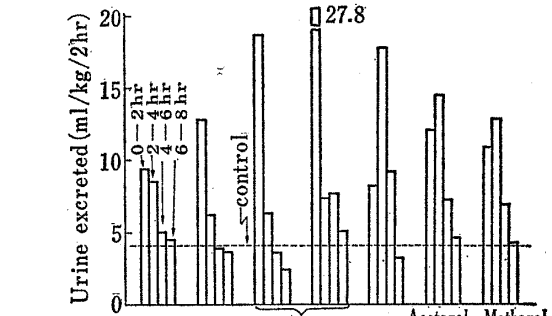
The diuretic action of sulfonamide derivatives in rats was compared with binding to the bovine serum albumin at pH 7.4 or chloroform–water partition coefficient of unionized molecules. Sulfonamides which had partition coefficients larger than 1.3 and association constants larger than $1.08\times10^{3}\text{M}^{-1}$ sustaineed a marked diuretic action 8 hours after the administration (Fig. 6A). But sulfonamides which had small values in one of the two physicochemical constants showed a strong diuretic activity during 0—4 hours following a rapid decrease to the normal excretion at 6—8 hours (Fig. 6B).



Compound	Control	*p*-Cl		*p*-Br	3,4-di-Cl	3-NO_2-4-Cl	3-CF_3-4-NO_2
Dose (mM/kg)	—	0.5	0.75	0.5	0.75	0.5	0.5
Partition coefficient	—	1.69		2.98	4.07	1.30	1.90
Association constant $K\times10^{-3}\text{M}^{-1}$	—	1.08		1.10	3.48	1.57	2.08

Fig. 6A. Relationship of Duration of Diuretic Action with Chloroform–Water Partition Coefficient and Association Constant with Albumin

The excreted amount of urine is the mean value of 10 rats.



Compound	*p*-CH_3	H	*m*-NO_2	*p*-CN	Acetazolamide	Methazolamide
Dose (mM/kg)	0.75	0.75	0.25	0.5	0.25	0.05	0.05
Partition coefficient	2.61	0.70	0.53	0.30	0.005	0.05
Association constant $K\times10^{-3}\text{M}^{-1}$	0.51	0.17	0.30	0.25	1.00	2.60

Fig. 6B. Relationship of Duration of Diuretic Action with Chloroform–Water Partition Coefficient and Association Constant with Albumin

The excreted amout of urine is the mean value of 10 rats.

Here, it was recognized that chloroform–water partition coefficient and albumin binding were important factors for the duration of diuretic action of sulfonamide derivatives.

19) C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, **86**, 1616 (1964).
20) B.B. Brodie and C.A.M. Hogben, *J. Pharm. Pharmacol.*, **9**, 345 (1957).
21) R.K. Richard and J.D. Taylor, *Anesthesiology*, **17**, 414 (1956).

Effect of pH of Body Fluid on the Activity

Ford[22] found that carbonic anhydrase inhibitors showed poor diuretic activity under acidotic conditions, while a strong diuretic activity in normal and alkalotic conditions. Maren[23] reported that a poor diuretic activity of sulfonamide carbonic anhydrase inhibitors in acidosis was ascribed to an inhibited enzymatic activity due to increase of hydrogen ion concentration.

To elucidate an active form of sulfonamides for diuretic and natriuretic activities, pH of rat's body fluid was modified. The diuretic activity of sulfonamide derivatives in normal and alkalotic conditions was presented in Fig. 7. The maximum excretion rate of urine after the administration of diuretic compounds was 35 ml/kg/4 hr in the two conditions. It suggests that the physiological conditions for the maximum excretion rate is not affected by the alkalotic conditions in rats, but the excretion rate without the drugs differed considerably between the two conditions. Benzenesulfonamide showed no diuretic action with a dose of 0.125 mM/kg to rats in normal conditions, but it showed the maximum excretion rate with the same dose in the alkalotic conditions. Similar enhancement of the diuretic action in the alkalotic conditions was observed for *p*-Cl and *p*-NO_2 derivatives. Such enhancement of the activity was attributable to the increased concentration of ionized molecules under alkalotic conditions. Thus, the present observations, as well as the results of pK_a-activity correlationship described previously, will lead to a conclusion that the diuretic action of sulfonamide derivatives is subjected to the concentration of ionized molecules.

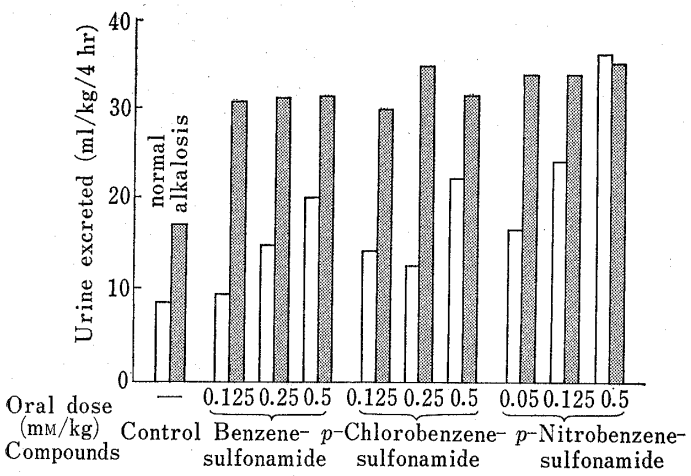


Fig. 7. Effect of Alkalosis on the Diuretic Activity

The excreted amout of urine is the mean value of 10 rats

Experimental

Diuretic and Natriuretic Activities——Sulfonamide derivatives were dissolved in an aqueous solution of 1% Tween 60 and 0.85% NaCl. Tween 60 was used to enhance solubility of the derivatives.

Male albino rats, Sprauge–Dawley strain, weighing from 200 to 300 g, were kept fasting for 17 hours before experiment. The drug solution was administered orally in a load of 25 ml/kg. In collecting the urine over a 4-hour period, rats were placed separately in metabolism cages. The sodium ion in urine samples was determined with a Kotaki flame photometer (model FP-3).

The excreted amounts of the urine and sodium ion during 4 hours after administration were plotted against dosage in a logarithmic scale (Fig. 8). The diuretic and natriuretic activities were expressed as reciprocals of the dose (C, mM/kg) which was required to increase the excretion of urine and sodium ion three times higher.

Duration of the Diuretic Activity——One to 2.5 time doses of C were employed for the present study. A drug was dissolved in an aqueous solution of 1% Tween 60. The animals were fed *ad lib.* but without water for 17 hours before experiment. A drug solution, 50 ml/kg, was administered orally. The excreted urine was collected at 2-hour intervals for 8 hours. Water was given freely after administration of the drug solution.

Alkalosis——In order to study the influences of alkalosis on diuretic activity, $NaHCO_3$ (1 g/kg) was administered orally for consecutive 3 days before experiment.

22) R.V. Ford, *J. Lab. Clin. Med.*, **53**, 53 (1959).
23) T.H. Maren, *Bull. Johns. Hopkins Hosp.*, **98**, 159 (1956).

Partition Coefficient——Chloroform–water partition coefficients were measured spectrophotometrically of 15 ml of 2 mM aqueous solution of compound and 5 ml of chloroform after shaking for 2 hours at 30°. The pH of aqueous phase was adjusted so that the compound might exist mainly in unionized molecules: pH 5.5 phosphate buffer for *p*-aminobenzenesulfonamide, *p*-methylaminobenzenesulfonamide and 2-amino-1,3,4-thiadiazole-5-sulfonamide; 0.002 N H_2SO_4 for the other 19 compounds.

Binding to Albumin——Five % of bovine serum albumin (Armour Pharmaceutical Co.) was dissolved in a pH 7.4 phosphate buffer. The diuretic compounds were dissolved in a pH 7.4 phosphate buffer at the concentration of 0.1—10 mM. The binding was determined with an equilibrium dialysis method employing a cellophane membrane. Five ml of the protein solution was taken into a dialyzing bag which was placed in a glass tube containing 10 ml of the compound solution. After shaking at 30° for 7 hr, the unbound drug was measured spectrophotometrically. The association constants of sulfonamides to albumin were calculated following the Scatchard equation.[24]

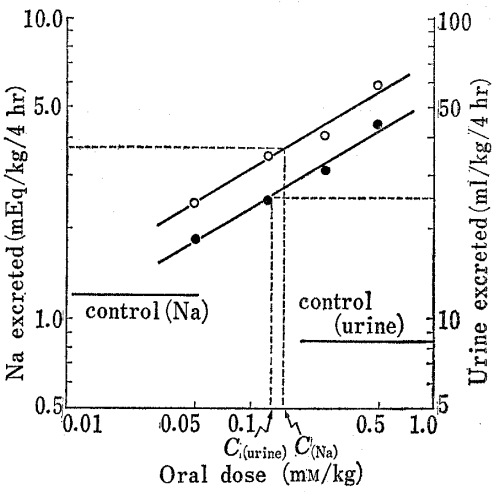


Fig. 8. A Logarithm Plot of Dose–Response for *p*-Nitrobenzenesulfonamide

$C_{(urine)}$ and $C_{(Na)}$ are the dose (mM/kg) which is required to increase the excretion of urine and sodium ion three times higher, respectively
—○—: Na —●—: urine
Each plot in the figure is a mean value of 10 rats.

24) G. Scatchard, *Ann. N.Y. Acad. Sci.*, **51**, 660 (1949).