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Biological Effects of Furosemide in Relation to Its Disposition in Rabbits[1]

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The purpose of this study was to represent quantitatively the relationship between the time course of pharmacological response and drug disposition data after intravenous administration of furosemide in rabbits. After administration of furosemide, urine flow rate, urinary excretion rate of Na^+ and K^+ were measured as well as excretion rate of intact drug and drug plasma level. From the data observed, a simplified simulation model was constructed and theoretical values were calculated by the model. Calculated values showed reasonable coincidence with the observed values. The urinary excretion rate of furosemide which was used in place of the plasma level, for the practical purposes, was able to correlate quantitatively with diuretic response; urine flow rate and electrolyte excretion rate.

Keywords——correlation between drug disposition and pharmacological response; diuretic action; drug disposition in rabbits; furosemide; simplified renal model

Knowledges and sophisticated techniques on pharmacokinetics having been accumulated, pharmacological significance of pharmacokinetic data should be our next concern. After Dengler's statement;[3] emphasis should be laid on the correlation between pharmacokinetic data and pharmacologically or clinically observed effects, since there seems to be lack of knowledge on this subject. Levy[4] has also stated that the aim of pharmacotherapy is to elicit a desired therapeutic response (rather than a definite "blood level"), and that there must be available adequate information concerning the relationship between drug concentration and pharmacologic effect.

On the other hand, Smolen[5] claims, through his extensive works, that by the use of temporal pharmacological response intensity data, drug-absorption analysis and the description of the biokinetic behavior of a drug is possible. If temporal pharmacological response intensity is used to describe the pharmacokinetic behavior of a drug, the reverse might also be probable. Pharmacological response intensity could be described by pharmacokinetic behavior of a drug.

The aim of the present study is to elucidate correlations between pharmacokinetic behavior and pharmacologic effect of diuretic agents, using furosemide as a model drug.

Theoretical

Brief Description of Renal Physiology and Action of Diuretics[6]

On entrance of the glomerular filtrate into proximal tubule, tubular transport of solute and water begins. As the result of the active transport of Na^+, both Cl^- and HCO_3^- are

1) Presented at the 95th Annual Meeting of Pharmaceutical Society of Japan, Nishinomiya, April, 1975.
2) Location: *3190 Gofuku, Toyama 930, Japan.*
3) H.J. Dengler, "Pharmacological and Clinical Significance of Pharmacokinetics," F.K. Schattauer Verlag, Stuttgart, 1970, p. 1.
4) G. Levy, "Clinical Pharmacokinetics," American Pharmaceutical Association, Academy of Pharmaceutical Sciences, 1974, p. 7.
5) V.F. Smolen and R.D. Schoenwald, *J. Pharm. Sci.*, **60**, 96 (1971); V.F. Smolen, *ibid.*, **60**, 354 (1971); V.F. Smolen, *ibid.*, **60**, 878 (1971); R.D. Schoenwald and V.F. Smolen, *ibid.*, **60**, 1039 (1971); V.F. Smolen, B.D. Turrie, and W.A. Weigand, *ibid.*, **61**, 1941 (1972); V.F. Smolen, *ibid.*, **62**, 77 (1973).
6) G.H. Mudge, "The Pharmacological Basis of Therapeutics," 4th Ed. ed. by L.S. Goodman and A. Gilman, MacMillan, London, 1970, pp. 831—892.

reabsorbed. Almost 90 percent of the filtered Na^+ is reabsorbed by active transport in the proximal tubule. Most of the filtered K^+ is reabsorbed. The proximal tubule is freely permeable to water, and, as solute is reabsorbed, water is reabsorbed at an equivalent rate. The solute concentration in the luminal fluid leaving the proximal tubule is same as that of systemic blood, but the volume flow has been reduced to about 10 percent of the filtration rate.

The tubular fluid then enters the thin segment, which dips into the medulla. In the ascending limb of the loop of Henle, the tubular fluid is diluted and, concomitantly, medullary interstitial fluid is concentrated.

In the distal tubule and collecting duct, cation exchange predominates. Luminal Na^+ is reabsorbed in exchange for both H^+ and K^+. The distal tubule is permeable to water in the presence of the antidiuretic hormone (ADH.) In the absence of ADH, water reabsorption does not occur, and the volume of fluid flowing out of the distal tubule is approximately same as the volume flowing into it.

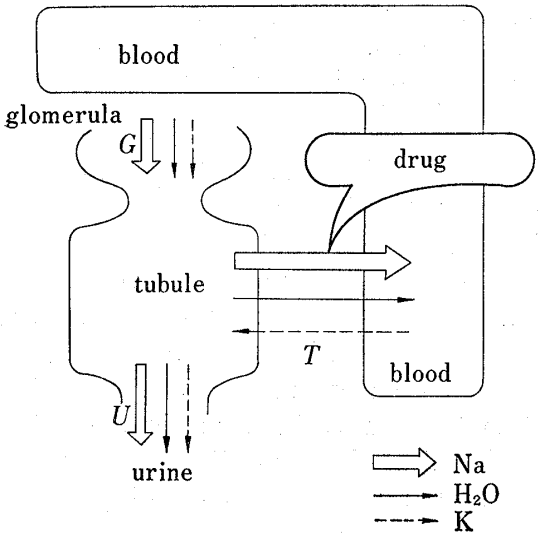

Fig. 1. Simplified Renal Model

Clinically useful diuretic agents decrease Na^+ reabsorption and thus lead to an increase in the rate of Na^+ excretion. This increase in the rate of Na^+ excretion is the primary and desired effect, and the increase in urine flow is an automatic consequence. Furosemide clearly inhibits Na^+ reabsorption in the ascending loop of Henle.[7] However, proximal sites of action may also be involved.[8] Partial distal inhibition of Na^+ reabsorption is also probable.[9]

General Description of the Simplified Renal Model

On the basis of renal physiology and diuretic action briefly described above, a simplified model is defined as follows (Fig. 1).

G, T and U represents, respectively, glomerular filtration rate, tubular reabsorption rate and urinary excretion rate of the species indicated by the subscripts. At steady state, material balance gives Eqs. 1 through 4.

$$G^\circ_{H_2O} = T^\circ_{H_2O} + U^\circ_{H_2O} \quad \text{Eq. 1}$$

$$G^\circ_{K^+} = T^\circ_{K^+} \quad \text{Eq. 2}$$

$$G^\circ_{Na^+} = T^\circ_{Na^+} + U^\circ_{Na^+} + U^\circ_{K^+} \quad \text{Eq. 3}$$

$$T^\circ_{H_2O} = fT^\circ_{Na^+} \quad \text{Eq. 4}$$

Eqs. 2 and 3 state that most of the filtered K^+ is reabsorbed and that in the distal tubule and collecting duct cation exchange predominates, luminal Na^+ being reabsorbed in exchange for both K^+ and H^+. Concentration of H^+, however, is negligibly low compared with that of K^+ and/or Na^+, and Eq. 3 does not include $U^\circ_{H^+}$ term. Eq. 4 expresses that water reabsorption occurs at tubule depending on the active reabsorption of Na^+, where f is assumed a constant.

Administration of furosemide gives no effects on the glomerular filtration but inhibits tubular reabsorption of Na^+. Consequently, following equations hold.

$$T_{Na^+} = T^\circ_{Na^+}(1.0-X) \quad \text{Eq. 5}$$

7) W. Suki, F.C. Rector, Jr., and D.W. Seldin, *J. Clin. Invest.*, **44**, 1458 (1965); P.A.F. Morrin, *Can. J. Physiol. Pharmacol.*, **44**, 129 (1966).
8) B.M. Brenner, R.I. Keimowitz, F.S. Wright, and R.W. Berliner, *J. Clin. Invest.*, **48**, 290 (1969).
9) A.G. Fraser, J.F. Cowie, A.T. Lambie, and J.S. Robson, *J. Pharmacol. Exp. Ther.*, **158**, 475 (1967).

$$T_{H_2O} + U_{H_2O} = T^{\circ}_{H_2O} + U^{\circ}_{H_2O} \quad \text{Eq. 6}$$

$$T_{Na^+} + U_{Na^+} + U_{K^+} = T^{\circ}_{Na^+} + U^{\circ}_{Na^+} + U^{\circ}_{K^+} \quad \text{Eq. 7}$$

$$T_{H_2O} = fT_{Na^+} \quad \text{Eq. 8}$$

Where X is inhibition fraction of Na^+ transport and a function of furosemide plasma level.

Substitution and rearrangement give Eqs. 9 and 10.

$$U_{Na^+} + U_{K^+} = U^{\circ}_{Na^+} + U^{\circ}_{K^+} + T^{\circ}_{Na^+}X \quad \text{Eq. 9}$$

$$U_{H_2O} = [U^{\circ}_{H_2O} - f(U^{\circ}_{Na^+} + U^{\circ}_{K^+})] + f(U_{Na^+} + U_{K^+}) \quad \text{Eq. 10}$$

Experimental

Animal——Unanesthetized male rabbits weighing 2—3 kg were fixed on the back. Nelaton's catheter was inserted to the bladdar to take urine samples. In order to block ADH, according to LeZotte, *et al.*,[10] 5.0 w/v% glucose solution was infused intravenously (1.0 ml/min) throughout the experiment. Five hours after the start of glucose infusion, steady urine flow, Na^+ and K^+ excretion was attained, and the drug solution was administered intravenously. Blood samples were taken from the merginal vein of the ear other than that glucose was being infused.

Drug——Furosemide used was J.P. grade which was kindly supplied by TOYO PHARMAR Co. Ltd. Drug solution contained 20 mg of furosemide and 7 mg of sodium carbonate in 2 ml. Drug was administered at 1.25, 2.5, 5.0 and 10.0 mg/kg body weight dose level.

Assay——Furosemide concentrations in plasma were measured fluorometrically.[11] To 1.0 ml of plasma, five ml of ether and one drop of conc. HCl were added and the mixture was shaken for 3 minutes. Four ml of the ether phase was withdrawn and the organic solvent was evaporated off. Five ml of 0.01 N HCl was added and mixed thoroughly and was subjected to fluorometry (activation maximum 340 nm, fluorescence maximum 410 nm).

Furosemide concentration in urine was determined colorimetrically.[11] Diluted urine and one drop of conc. HCl were shaken with 5 ml of ether for 3 minutes in order to separate basic metabolites. Four ml of ether phase was withdrawn and the organic solvent was evaporated off. Five ml of 1 N HCl was added to the residue and hydrolyzed for 45 minutes at 70°. After the mixture was cooled, diazotization was performed with 0.5 ml of 0.2% $NaNO_2$ and 0.5 ml of 1% ammonium sulfamate and coupled with 2-dimethylaminoethyl-1-naphthalene for color development. Absorbance of the resulting dye was determined at 535 nm.

Sodium and potassium in plasma and in urine were measured by flamephotometry.

Inulin concentration in plasma and in urine were determined by the method of Dische.[12]

Results and Discussion

Pharmacokinetics of Furosemide

Plasma concentrations of furosemide after intravenous administration to rabbits with various dose level were normalized by dividing by the corresponding dose, semi-logarithmic plot of which *vs.* time is contained in Fig. 2(a). Similarly, semi-logarithmic plot of average excretion rate normalized by dividing by the corresponding dose against the mid point of the time interval is contained in Fig. 2(b). Only to avoid confusion, representative data were shown in Figs. 2 through 5. Even though the values scatter at low concentration region, it is evident that dose dependency is not likely, and that the plots follow two-compartment open model.

Plasma concentration, P, and urinary excretion rate, E, are expressed by the following two-exponential equations.

$$P = A \exp(-\alpha t) + B \exp(-\beta t) \quad \text{Eq. 11}$$

$$E = A' \exp(-\alpha' t) + B' \exp(-\beta' t) \quad \text{Eq. 12}$$

10) L.A. LeZotte, K.M. MacGaffey, E.W. Moore, and H. Jick, *Clin. Sci.*, **31**, 371 (1966).
11) P. Hajdu and A. Haussler, *Arznei.-Forsch.*, **14**, 709 (1964); A. Haussler and P. Hajdu, *ibid.*, **14**, 710 (1964).
12) Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **192**, 583 (1951).

Where A, B, α, β, A', B', α' and β' are constants. Pharmacokinetic parameters obtained for each rabbit with the least square method are shown in Table I.

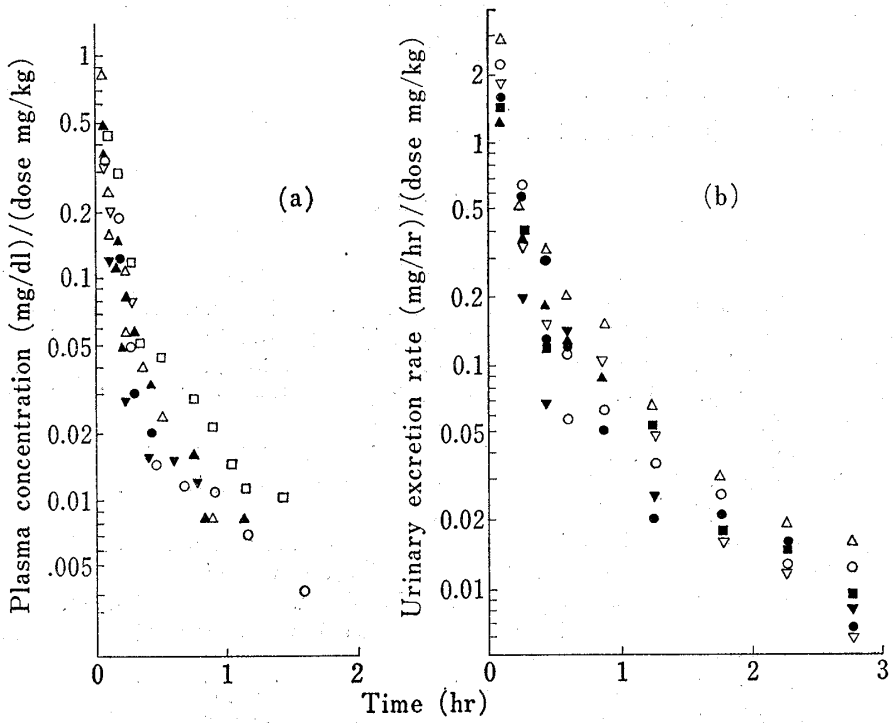

Fig. 2. Drug Disposition of Furosemide

Table I. Pharmacokinetic Parameters of Furosemide Disposition in Rabbits

Rabbit (kg)	Dose mg/kg	A mg/dl	B mg/dl	α h^{-1}	β h^{-1}	A′ mg/h	B′ mg/h	α' h^{-1}	β' h^{-1}
S-3(2.2)	10.0	6.52	0.137	0.168	0.0116	20.1	0.532	0.121	0.0109
T-3(2.3)	10.0	—	—	—	—	42.2	1.57	0.133	0.0172
U-3(2.0)	10.0	3.48	0.075	0.137	0.0107	28.8	0.629	0.124	0.0101
N-3(2.7)	5.0	3.39	0.266	0.160	0.0196	—	—	—	—
W-3(2.5)	5.0	2.02	0.093	0.529	0.0329	15.9	0.308	0.150	0.0114
Y-3(2.4)	2.5	—	—	—	—	8.4	0.164	0.215	0.0128
V-3(2.6)	2.5	1.10	0.068	0.151	0.0224	13.3	0.771	0.243	0.0251
Z-2(2.3)	1.25	4.69	0.329	0.904	0.0835	2.6	0.266	0.152	0.0169
O-3(3.2)	1.25	—	—	—	—	18.5	1.03	0.243	0.0180
R-3(2.6)	1.25	0.80	0.047	0.248	0.0305	11.2	0.776	0.277	0.0278

Comparison of Fig. 2(a) with 2(b), and examination of Table I suggest that urinary excretion rate of furosemide is directly proportional to its concentration in plasma.

Clearance of Inulin

Three hundred mg of inulin was administered intravenously and its concentration in plasma and the amount excreted in urine were measured. Inulin clearance, a measure of glomerular filtration rate, was calculated and is shown in Table II.

Table II. Inulin Clearance in Rabbits

Rabbit	Body weight kg	Inulin clearance dl/h
R-3	2.6	8.8
Y-3	2.4	13.1
A-2	2.9	9.4
V-3	2.6	9.7

Urinary Excretion and Plasma Concentration of Na^+ and K^+ at the Absence of Furosemide

Urine flow rate and urinary excretion rate of Na^+ and K^+ were determined after the glucose infusion was started. Results are shown in Table III. It is clear from the Table that the steady state is attained in 5 hours. Steady state plasma concentrations of Na^+ and K^+ are also contained in Table III.

Table III. Attainment of Steady State and Effect of Furosemide on Urine Flow during Glucose Infusion[a)]

Time min		E mg/h	U_{H_2O} ml/h	U_{Na^+} mEq/h	U_{K^+} mEq/h	P_{Na^+} mEq/l	P_{K^+} mEq/l
Glucose infusion 5.0 w/v% glucose 1.0 ml/min							
−300	−180	0.0	12.5	0.463	0.364	—	—
−180	−150	0.0	30.4	0.084	0.192	—	—
−150	−120	0.0	51.6	0.096	0.282	—	—
−120	−90	0.0	50.2	0.093	0.258	—	—
−90	−60	0.0	61.0	0.123	0.317	136	21.1
−60	−30	0.0	58.0	0.110	0.282	—	—
−30	0	0.0	51.0	0.103	0.261	—	—
Furosemide administration 2.5 mg/kg *i.v.*							
0	10	4.62	180.0	16.8	2.07	136	20.9
10	20	0.903	121.0	11.3	1.50	136	21.3
20	30	0.381	51.6	3.75	0.714	133	20.1
30	45	0.336	33.6	1.67	0.493	—	—
45	60	0.255	29.2	0.911	0.378	134	19.7
60	90	0.120	48.8	0.262	0.483	—	—
90	120	0.042	48.2	0.129	0.391	133	20.1
120	150	—	52.2	0.118	0.350	—	—
150	180	—	49.0	0.108	0.296	134	19.5

a) U's and P's with subscripts are urinary excretion rate and plasma concentration, respectively, of the species indicated. E is urinary excretion rate of furosemide.

Urinary Excretion of Na^+ and K^+ in Relation to Furosemide Disposition

After 5 hours of glucose infusion, steady state having been attained, furosemide was administered intravenously. Alteration of urine flow rate, Na^+ and K^+ excretion rate due to furosemide are shown in Table III with furosemide excretion rate which is the measure of drug plasma level.

Since the change in electrolyte excretion is based on the inhibition of active reabsorption of Na^+ at renal tubule, it is reasonable to test the relationship between plasma level of furosemide and electrolyte excretion rate. The sum of the excretion rate of Na^+ and K^+ (mEq/hr) was plotted against logarithm of furosemide excretion rate, E, (instead of plasma concentration, P, because these values are parallel and that measurement of urinary concentration is more accurate than that in plasma), which is shown in Fig. 3.

In each animal and in each amount administered, combined electrolyte excretion rate is proportional to log excretion rate of the drug until the drug excretion rate is reduced to a certain value, E_{min}, when combined elecrtolyte excretion rate is as low as the steady state value. When drug excretion rate is below E_{min}, pharmacological response is nil. Values of the critical drug excretion rate E_{min} and the proportionality constant, S, for each animal are listed in Table IV. E_{min} value varies from 0.08 to 0.31 mg/hr. With the dose levels employed in this work, maximal electolyte excretion rate could not be attained, therefore, inhibition percent of Na^+ active transport was not able to calculate. Consequently, Eq. 13 was used in place of Eq. 9 for simulation purpose below.

$$U_{Na^+} + U_{K^+} = U^{\circ}_{Na^+} + U^{\circ}_{K^+} + S(\log E - \log E_{min}) \qquad \text{Eq. 13}$$

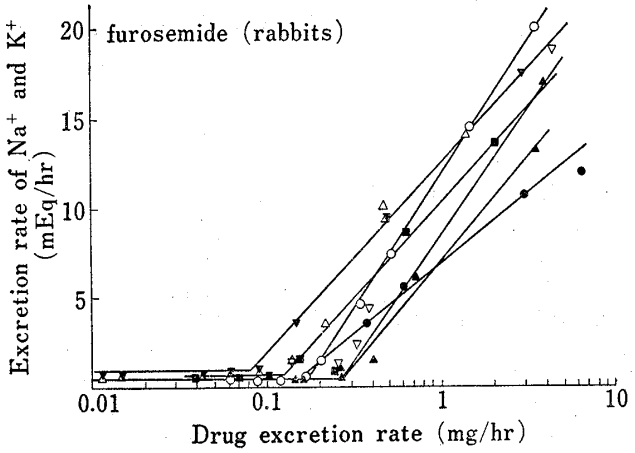

Fig. 3. Excretion Rate of Na^+ and K^+ *vs.* Drug Excretion Rate

	Rabbit	Dose (mg/kg)
○,	S-3	10.0.
●,	T-3	10.0.
■,	W-3	5.0.
▽,	V-3	2.5.
▼,	Y-3	2.5.
△,	Z-2	1.25.
▲,	O-3	1.25.

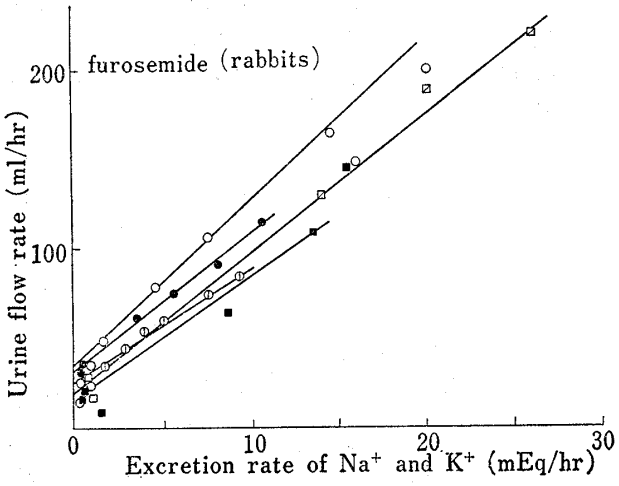

Fig. 4. Urine Flow Rate *vs* Excretion Rate of Na^+ and K^+

	Rabbit	Dose (mg/kg)
○,	S-3	10.0.
●,	T-3	10.0.
◐,	U-3	10.0.
▨,	N-3	5.0.
■,	W-3	5.0.

TABLE IV. Parameters of Diuretic Response (1)

Rabbit (kg)	Dose mg/kg	$U^{\circ}_{H_2O}$ ml/h	$U^{\circ}_{Na^+}$ mEq/h	S mEq/h	E_{min} mg/h	f ml/mEq	*b* ml/h
S-3(2.2)	10.0	36.0	0.13	15.1	0.17	8.7	40.0
T-3(2.3)	10.0	47.0	0.24	7.57	0.12	7.9	30.0
U-3(2.0)	10.0	38.0	0.37	4.38	0.14	7.1	21.0
N-3(2.7)	5.0	52.0	0.31	—	—	7.2	34.0
W-3(2.5)	5.0	27.0	0.24	11.0	0.12	7.3	27.0
Y-3(2.4)	2.5	36.0	0.22	10.5	0.08	8.6	11.0
V-3(2.6)	2.5	55.0	0.44	14.2	0.23	8.1	16.0
Z-2(2.3)	1.25	37.0	0.26	12.9	0.13	7.9	23.0
O-3(3.2)	1.25	38.0	0.25	15.1	0.31	10.1	15.0
R-3(2.6)	1.25	32.0	0.17	11.3	0.27	9.4	30.0

Urine Flow Rate and Combined Electrolyte Excretion Rate

Since water reabsorption is subjective to active Na^+ reabsorption, close relationship is expected between urine flow rate and combined electrolyte excretion rate. Fig. 4 contains the plot of urine flow rate against combined electrolyte excretion rate. Throughout the electrolyte excretion range caused by furosemide administration, straight line with an intercept

on y axis was obtained. This fact verifies Eq. 10. Values of the intercept on y axis, b, and the slope of the straight line, f, obtained for each animal from the graph are shown in Table IV.

Eq. 10 indicates that the theoretical value of the intercept on y axis of the straight line of Fig. 4 is given by $U^{\circ}_{H_2O}$-f($U^{\circ}_{Na^+}+U^{\circ}_{K^+}$). In most of the rabbits, observed value of intercept, b, was little smaller than the theoretical prediction.

K+ Fraction

Plot of K^+ fraction, $U_{K^+}/(U_{Na^+}+U_{K^+})$, against log combined electrolyte concentration in urine gives straight line (Fig. 5), suggesting that K^+ fraction is dependent on combined electrolyte concentration. The urine samples collected after hydrochlorothiazide administration, however, gave a quite different pattern, indicating that K^+ fraction is not necessarily dependent on combined electrolyte concentation but on the diuretic agent used.

Fig. 6 contains the plot of K^+ fraction *vs.* furosemide excretion rate. Where drug excretion rate is above a certain value, E_2, and below another certain value, E_1, K^+ fraction stays fairly constant, at K_{f2} and K_{f1}, respectively. Between these levels ,the plot is straight line, suggesting that the drug concentration effects on K^+ fraction.

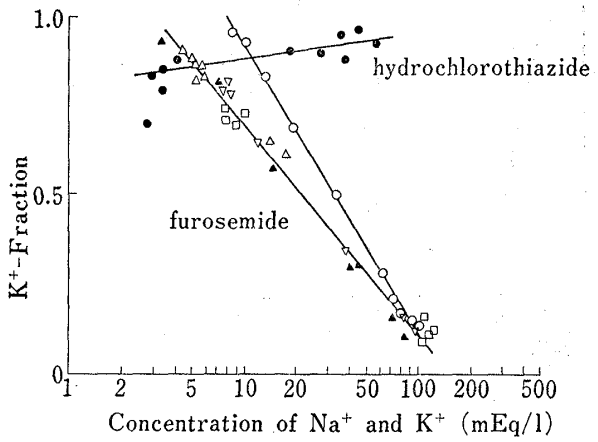

Fig. 5. K^+-Fraction *vs.* Concentration of Na^+ and K^+ in Urine

Furosemide	Dose (mg/kg)
○,	10.0.
□,	5.0.
▽,	2.5.
△, ▲,	1.25.
Hydrochlorothiazide	
●,	5.0.

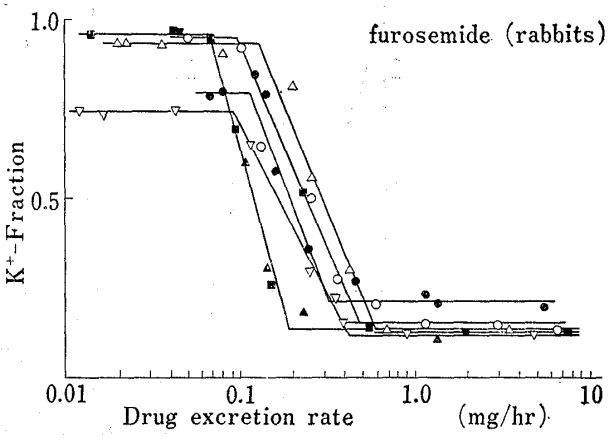

Fig. 6. K^+- Fraction *vs.* Drug Excretion Rate

	Rabbit	Dose (mg/kg)
○,	T-3	10.0.
●,	U-3	10.0.
■,	W-3	5.0.
▽,	V-3	2.5.
△,	R-3	1.25.
▲,	Z-2	1.25.

TABLE V. Parameters of Diuretic Response (2)

Rabbit (kg)	Dose mg/kg	K_{f1}	E_1 mg/h	K_{f2}	E_2 mg/h
S-3(2.2)	10.0	0.80	0.13	0.13	0.42
T-3(2.3)	10.0	0.94	0.10	0.15	0.48
U-3(2.0)	10.0	0.80	0.13	0.20	0.33
N-3(2.7)	5.0	—	—	—	—
W-3(2.5)	5.0	0.96	0.068	0.11	0.19
Y-3(2.4)	2.5	0.79	0.044	0.11	0.18
V-3(2.6)	2.5	0.75	0.096	0.11	0.42
Z-2(2.3)	1.25	0.86	0.070	0.10	0.25
O-3(3.2)	1.25	0.85	0.17	0.13	0.49
R-3(2.6)	1.25	0.93	0.16	0.11	0.54

This feature is expressed by Eq. 14.

$$\text{K}^+ \text{ fraction} = \frac{U_{K^+}}{U_{Na^+}+U_{K^+}} \begin{cases} = K_{f_1} & (E<E_1) \\ = K_{f_1}-(K_{f_1}-K_{f_2}) \log (E/E_1)/\log (E_2/E_1) & (E_1 \leq E \leq E_2) \\ = K_{f_2} & (E_2<E) \end{cases} \qquad \text{Eq. 14}$$

Parameters, E_1, E_2, K_{f1} and K_{f2}, are shown in Table V.

On the basis of the observations reported above, disposition of furosemide and diuretic response could be correlated with Eqs. 10 through 14. Simulated values for rabbit S–3 obtained by a computer using the parameters listed in Tables I, IV and V are compared with experimental values in Fig. 7.

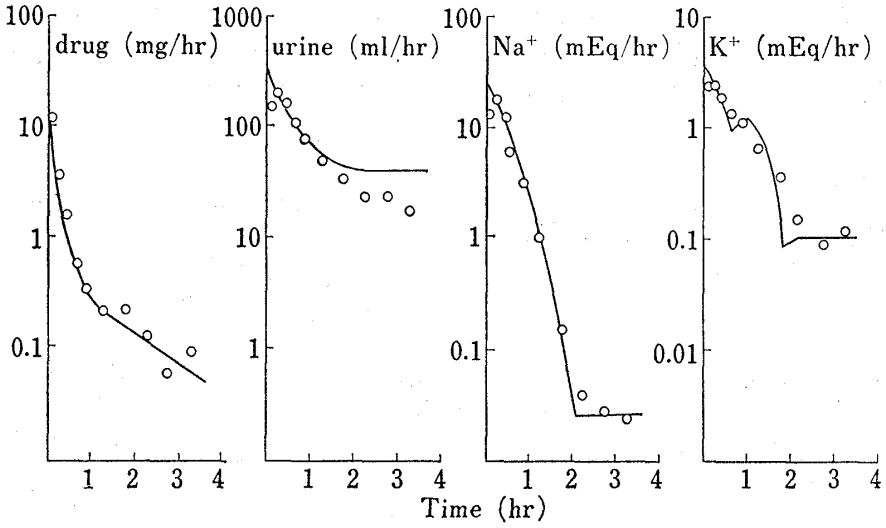

Fig. 7. Comparison of Theoretical Values and Observed Data

Furosemide 10 mg/kg *i.v.* rabbit (S-3).

In spite of the simplicity of the model employed, predicted values show reasonable coincidence with the observed values, which verifies this sort of approach. In this particular instance, time course of plasma furosemide (though urinary excretion rate of the intact drug was used in place of plasma level, in practice) was able to correlate quantitatively with diuretic response; urine flow rate and electrolyte excretion rate. We trust this kind of relationship will be of great help in better dosage schedule.