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Plasma Protein Binding, Renal Clearance, and Pharmacokinetics of Diuretics in Rabbits[1a,b]

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The transfer of three commercial diuretics (furosemide, bumetanide and hydroflumethiazide) in the biophase was investigated using rabbits. Pharmacokinetic analysis based on the two-compartment open model was performed. The biological half-lives calculated from the β phase of rabbit plasma level time courses were almost the same and were extremely short (20—40 min), in spite of the large differences among the three diuretics in plasma protein binding. The results of renal clearance experiments using rabbits indicated a large contribution of tubular secretion (approximately 80—90%). Therefore, it is suggested that the plasma protein is involved in the transport of diuretics to the kidney.

Keywords——diuretic; pharmacokinetic parameter; rabbit plasma protein; drug-plasma protein binding; renal clearance experiment; tubular secretion; transport mechanism in elimination

The binding of diuretics to serum protein has been discussed in our previous papers.[1b,3] In the present study, renal clearance of three diuretics (furosemide, bumetanide, and hydroflumethiazide) was determined and the pharmacokinetic parameters based on a two-compartment open model were calculated using rabbits. We also attempted to relate our pharmacokinetic findings, especially the elimination rate constants of diuretics after intravenous administration, to the plasma protein-binding ability and renal clearance of diuretics observed in rabbits.

Experimental

Materials——The diuretics used in this study were furosemide (FM), bumetanide (BM), and hydroflumethiazide (HFT). These drugs were kindly supplied by Hoechst Japan Co. Ltd. and Sankyo Co. Ltd. Phenolsulfonphthalein (PSP) was of J.P. IX grade. All the chemicals and solvents used were of analytical reagent grade. Reagent-grade inulin and probenecid were used for the renal clearance experiment.

Determination of Rabbit Plasma Protein Binding——The equilibrium dialysis method, the general procedure of which was described in our previous report,[4] was used. Rabbit plasma (3 ml) was placed in the chamber of a dialysis apparatus and the diuretic solution, at a concentration of 1 to 10×10^{-5} M before dialysis, was placed in another chamber of the same apparatus. Phosphate buffer (1/15 M, pH 7.4) was used for dilution of the diuretic. Equilibration was done for 6 hr by agitation on a rotary shaker (Toyo Incubator, model 100T) at 30°.

Renal Clearance Experiment——The standard renal clearance technique[5] was used. White male rabbits, weighing 2.6—2.8 kg, were anesthetized by intraperitoneal injection of urethan (1.7 g/kg). A vinyl tube (5 mm × 30 cm) was passed into the rabbit stomach, and 50 ml of warm water (37°) was instilled. The ureter was cannulated with a polyethylene catheter (Atom K.K., NS-510 No. 4, outer diameter; 1.35 mm) for urine collection after mid-abdominal incision. The diuretics, PSP, inulin, and probenecid were infused intravenously through a polyethylene catheter (Atom K.K., NS-510 No. 3, outer diameter; 1.05 mm) cannulated into the

1) *a*) This paper forms Part VI of a series entitled "Interaction between Drugs and Blood Components"; *b*) Part V: M. Nakano, K. Fujii, and S. Goto, *Chem. Pharm. Bull.*, **27**, 101 (1979).
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3) S. Goto, Y. Odawara, M. Nakano, and Y. Araki, *Chem. Pharm. Bull.*, **26**, 2298 (1978).
4) S. Goto, Y. Odawara, M. Nakano, and Y. Araki, *Yakugaku Zasshi*, **97**, 1219 (1977).
5) T. Arita, R. Hori, E. Owada, and K. Takahashi, *Chem. Pharm. Bull.*, **17**, 2526 (1969).

left femoral vein. The infusion rate for sustaining doses was adjusted to 1 ml/min, using a Perista minipump (Mitsumi Kagaku K.K.). Blood samples were obtained from a catheter (Atom K.K., NS-510 No. 3) cannulated into the right femoral artery. Diuretics and inulin clearances were determined every 10 min. Urine was collected during a 10 min period, and a blood sample was taken at the midpoint of the urine collection period. Inulin was initially dosed at 100 mg/kg then infused at a rate of 3 mg/min,[5] and 12 mg/kg of probenecid was given, followed by a 0.3 mg/min sustaining dose. This amount of probenecid was suitable for the complete blockade of diuretic secretion. Urinary pH of the samples collected at each sampling time was checked with a glass microelectrode and was within the range of pH 7—8.5. Renal clearance (C) (in ml/min) was calculated from $C=UV/P$, where U is the urine concentration (mg/ml), P is the plasma concentration (mg/ml), and V is the urine flow rate (ml/min). The clearance ratio (CR) is expressed as $CR=C/GFR$, where GFR is the glomerular filtration rate (in ml/min) calculated as inulin clearance. An outline of the standard renal clearance experiment is shown in Chart 1. The blood pressure was recorded using the method described in the previous paper[6] in order to check the physiological state of rabbits. If this became unusual, all the experiments were stopped immediately.

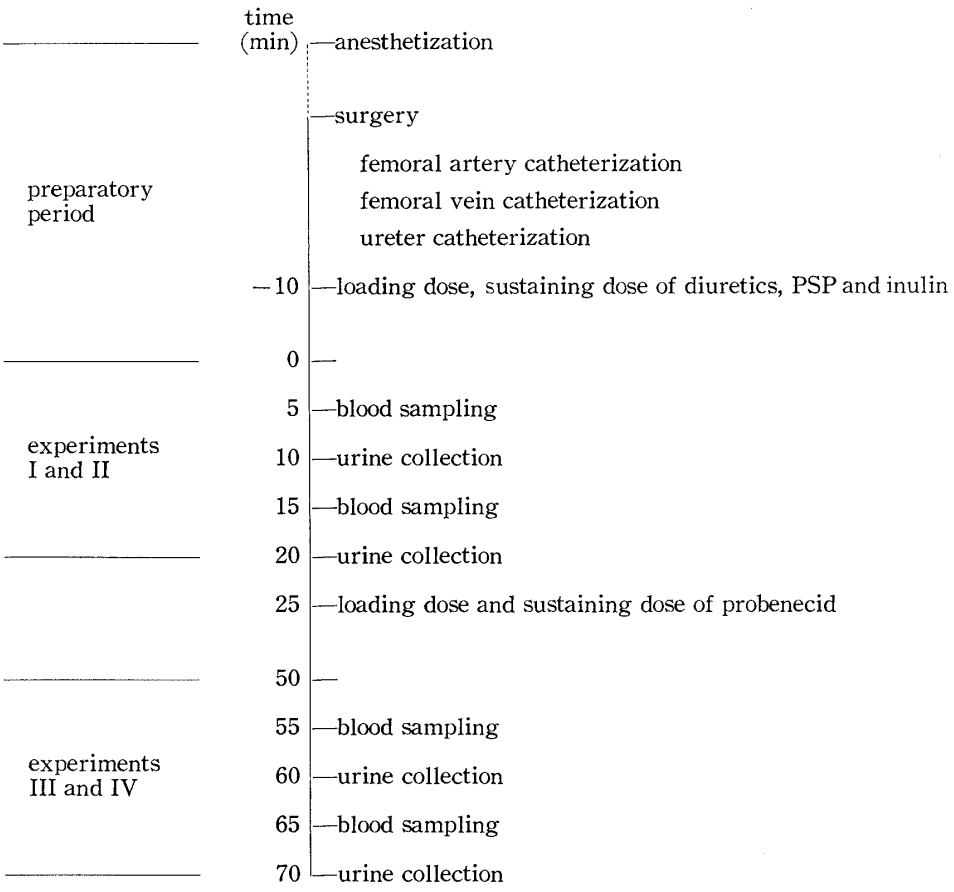

Chart 1. Outline of the Standard Renal Clearance Method

Calculation of Loading and Sustaining Doses——The values of the loading dose (L) and the sustaining rate (S) were calculated using equations (1) and (2).

$$L = [B]_s V_B \quad (1)$$

$$S = k_{13} L \quad (2)$$

where $[B]_s$ is the plasma concentration selected beforehand for each diuretic in renal clearance experiments, V_B is the volume of distribution of the central compartment (B), and k_{13} is the elimination rate constant for the two-compartment open model, as shown in Table XI.

Plasma Concentration Experiment——White male rabbits weighing 2.5—3.5 kg were individually housed in stainless steel cages and fasted for 24 hr before dosing. All doses of drugs were administered from the left aural vein at the level of 10 mg/kg, and a total volume of 2.5—3.5 ml was injected during 1.5—2 min.

6) S. Goto, R. Takamatsu, M. Shibao, and S. Iguchi, *Chem. Pharm. Bull.*, **16**, 332 (1966).

The solutions for injection were prepared as follows. A solution of 100 mg of the drug dissolved in 0.5 ml of 1 N NaOH was diluted to 10 ml with an isotonic phosphate buffer (pH 7.4) in the cases of FM, BM, and PSP. HFT, which is sparingly soluble, was dissolved in a mixture of 0.6 ml of N,N-dimethylformamide and 1.5 ml dioxane, and diluted to 10 ml with isotonic phosphate buffer, pH 7.4. Blood samples (1.5 ml), drawn with a sterile heparinized needle and syringe, were obtained from the right aural vein periodically between 0 and 120 min. The samples were immediately centrifuged to obtain plasma (3000 rpm, 10 min), and the plasma was placed in a fully wrapped test tube and stored in a refrigerator until assay for drugs.

Analytical Methods for Diuretics and PSP in Plasma——FM: Fluorometric measurement[7,8] was used. To 1 ml of plasma, 1 ml of 0.5 N HCl and 10 ml of Et_2O were added, and the mixture was shaken for 5 min then centrifuged (3000 rpm) for 5 min. Five ml of Et_2O phase was collected and 5 ml of 1/15 M phosphate buffer (pH 7.4) was added. After shaking this mixture for 5 min followed by centrifugation (3000 rpm) for 5 min, 2 ml of the aqueous phase was separated. The aqueous sample was mixed thoroughly with 2 ml of 0.5 N HCl and the solution was measured at an excitation wavelength of 347 nm and emission wavelength of 410 nm, using a spectrofluorometer (Hitachi model MPE-4).

BM: The method used was a modification of that of Φstergaard *et al.*[9] Plasma (0.5 ml) was shaken with 4 ml of 1 N HCl and 20 ml of Et_2O for 10 min, then 10 ml of the Et_2O phase was separated and evaporated to dryness. The residue was dissolved in 5 ml of glycine buffer (pH 11.2). After centrifugation, the supernatant was decanted into a fluorometer cell and the fluorescence was read at an excitation wavelength of 327 nm and emission wavelength of 407 nm.

HFT[10]: To 0.5 ml of the plasma solution, 1 ml of pH 4 acetate buffer and then 15 ml of Et_2O were added, and the mixture was agitated for 5 min. The sample was centrifuged (3000 rpm) for 5 min, then 10 ml of the Et_2O layer was transferred to a similar capped centrifuge tube, 3 ml of 0.01 N NaOH was added, and the mixture was shaken for 5 min. After centrifugation (3000 rpm) for 5 min, the Et_2O layer was discarded. Residual Et_2O in the aqueous phase was evaporated off under a stream of nitrogen, 1 ml of 1 N HCl was added, and the fluorescence was measured at an excitation wavelength of 333 nm and emission wavelength of 388 nm.

PSP: To 1 ml of the plasma, 7 ml of H_2O, 1 ml of 10% $ZnSO_4$, and 1 ml of 0.5 N NaOH were added for deproteinization. The mixture was centrifuged (3000 rpm) for 5 min, then the clear supernatant (3 ml) was collected, and basified by the addition of 1 ml of 0.5 N NaOH. Colorimetric measurement at 555 nm was done with a spectrophotometer (Hitachi model 181).

Inulin[11,12]: After deproteinization and centrifugation as described for the determination of PSP, 1 ml of the supernatant was collected, and 6 ml of 75% H_2SO_4, 0.2 ml of 1.5% cysteine-HCl, and 0.2 ml of 0.2% carbazole–EtOH solution were added. The mixture was incubated at 40° for 20 min. After cooling, the absorbance of the solution was measured at 560 nm within 15 min.

Analytical Methods for Diuretics, PSP, and Inulin in Urine——FM: To 1 ml of urine, 1 ml of 0.5 N HCl and 10 ml of Et_2O were added, and the mixture was shaken for 5 min then centrifuged (3000 rpm) for 5 min. Five ml of the Et_2O phase was collected and evaporated down under a stream of nitrogen. The residue was hydrolyzed for 40 min at 70° in 5 ml of 1 N HCl solution, then 0.5 ml of 0.2% $NaNO_2$ was added, and the mixture was cooled in an ice bath. After shaking for 3 min, 0.5 ml of 0.5% H_2NSO_3H was added and the mixture was shaken for 5 min. Next, 2 ml of EtOH and 0.5 ml of 0.2% Tsuda reagent were added, and the mixture was allowed to stand for 30 min. The color developed was measured at 535 nm.

BM[13,14]: A mixture of 1 ml of urine sample, 1 ml of 1 N HCl, and 7 ml of Et_2O was agitated for 15 min, centrifuged (3000 rpm) for 5 min, and then 5 ml of the Et_2O layer was evaporated down in a stream of nitrogen. The residue was dissolved in 0.2 ml of MeOH. The MeOH solution (0.05 ml) was spotted on a silica gel plate (20 × 20 cm) (Merck Co.), together with BM standard solution (0.05 ml), and the plate was developed with cyclohexane–$CHCl_3$–glacial AcOH (2: 2: 1, v/v). The band corresponding to BM was visualized under a UV lamp, marked by comparison with a standard, and the appropriate area was scraped off the TLC plate. To this sample, 3 ml of 0.1 M glycine buffer (pH 3.1) was added and the mixture was shaken for 15 min then centrifuged (3000 rpm) for 5 min. The supernatant was collected and the solution was measured by a fluorometric method (excitation wavelength of 340 nm and emission wavelength of 435 nm).

7) P. Hajdú and A. Haüssler, *Arzneim.-Forsch.*, **14**, 709 (1964).
8) A. Haüssler and P. Hajdú, *Arzneim.-Forsch.*, **14**, 710 (1964).
9) E.H. Φstergaard, M.P. E.H. Magnussen, C.K. Nielsen, E. Eilertsen, and H.H. Frey, *Arzneim.-Forsch.*, **22**, 66 (1972).
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13) D.L. Davies, A.F. Lant, N.R. Millard, A.J. Smith, J.W. Ward, and G.M. Wilson, *Clin. Pharmacol. Ther.*, **15**, 141 (1973).
14) M.R. Cohen, E. Hinsch, R. Vergona, J. Ryan, S.J. Kolis, and M.A. Schwartz, *J. Pharm. Exp. Ther.*, **197**, 697 (1976).

HFT and PSP: The same method as above was used.

Inulin: After diluting the sample solution (supernatant) to ten volumes, the same method as above was applied.

Results

Rabbit Plasma Protein Binding

The variation in the binding percentages of HFT with undiluted rabbit plasma as a function of plasma concentration of HFT is shown in Fig. 1. The binding percentages of the three diuretics and PSP at extremely low concentrations were obtained by extrapolation of the curve to the ordinate, where the concentration of HFT is zero. The obtained values are summarized in Table I. It was found that there is a considerable difference among the three diuretics.

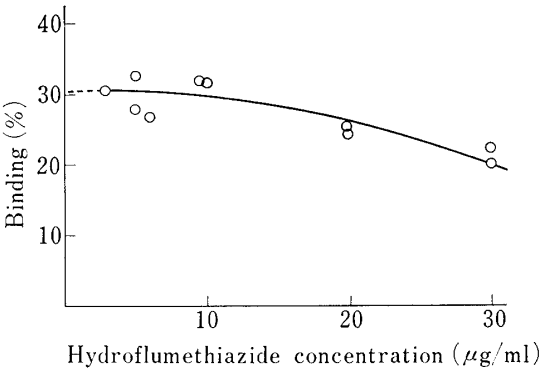


Fig. 1. Relationship between Percentage of Plasma Protein Binding and Concentration of Hydroflumethiazide in Rabbit Plasma

Table I. Rabbit Plasma Protein Binding of the Three Diuretics and Phenolsulfonphthalein (PSP)

Drugs	Binding (%)[a]
Furosemide (FM)	90.0
Bumetanide (BM)	81.4
Hydroflumethiazide (HFT)	30.3
Phenolsulfonphthalein (PSP)	92.0

a) By extrapolation of the experimental curve to the ordinate, where the concentration of drugs is zero, as shown in Fig. 1.

The addition of probenecid affected the protein binding of diuretics and PSP slightly, but these differences disappeared at extremely low concentrations of the drugs.

Some Experimental Trials related to the Renal Clearance Method

Renal Clearance Values of Left and Right Kidneys: The difference in renal functions between left and right kidneys in the same rabbit was checked. The same operative treatment was carried out on left and right ureters, and urine samples were collected at 10, 20, 30, and 40 min after the start of the experiment. FM was used as a model compound. The experimental results are summarized in Table II. It is clear that the renal functions of both kidneys are almost the same as in a healthy rabbit.

Table II. Renal Clearance of the Left and Right Kidneys in the Rabbit

Time (min)	Urine flow (ml/min)		Inulin clearance (ml/min)		Furosemide conc. (μg/ml)			Furosemide clearance (ml/min)	
					Plasma	Urine			
	Left	Right	Left	Right		Left	Right	Left	Right
0—10	0.62	0.78	8.2	8.6	4.2	34.9	30.3	5.1	5.6
10—20	0.42	0.57	7.0	8.5	4.4	38.7	52.7	3.7	6.9
20—30	0.59	0.56	11.0	8.4	4.5	61.8	46.7	8.2	5.8
30—40	0.59	0.58	12.0	9.2	4.6	59.7	44.9	7.5	5.6
Mean	0.56	0.62	9.6	8.7	4.4	48.6	43.7	6.7	6.0

Effect of Probenecid on the Renal Secretion of Diuretics: Competitive inhibition experiments were carried out using probenecid. Increasing plasma concentrations of probenecid sharply reduced the renal clearance of diuretics and PSP. A 12 mg/kg loading dose followed by a 0.3 mg/min sustaining rate for the administration of probenecid was used in the secretion blockage experiments, except for BM. In the inhibitory experiment for BM, 40 mg/kg of probenecid as the loading dose and 1 mg/min as the sustaining rate were selected. Complete secretion blockage could be achieved under the above administration conditions.

Table III. Renal Clearance Data for Furosemide using the Rabbit

Experiment No.	Time (min)	Urine flow (ml/min)	Inulin clearance (ml/min)	FM concentration (μg/ml)		FM clearance (ml/min)	Clearance ratio (CR)
				Plasma	Urine		
Rabbit, male, 2.7 kg: FM 500 μg/kg loading followed by 41 μg/min sustaining in 0.9% NaCl[a]							
I	0—10	0.56	8.3	3.7	58.2	8.8	1.1
II	10—20	0.40	7.9	3.7	72.2	7.8	1.0
Probenecid, 12 mg/kg loading followed by 0.3 mg/min sustaining in 0.9% NaCl							
III	50—60	0.40	17.8	7.3	34.0	1.9	0.1
IV	60—70	0.40	12.3	7.7	23.5	1.2	0.1

a) This administration schedule was selected to obtained 3.0 μg/ml plasma concentration of FM.

Table IV. Renal Clearance Data for Bumetanide using the Rabbit

Experiment No.	Time (min)	Urine flow (ml/min)	Inulin clearance (ml/min)	BM concentration (μg/ml)		BM clearance (ml/min)	Clearance ratio (CR)
				Plasma	Urine		
Rabbit, male, 2.7 kg: BM 750 μg/kg loading followed by 52 μg/min sustaining in 0.9% NaCl[a]							
I	0—10	1.20	17.2	9.9	150	18.2	1.1
II	10—20	0.96	17.3	8.3	172	19.9	1.2
Probenecid, 40 mg/kg loading followed by 1 mg/min sustaining in 0.9% NaCl							
III	50—60	1.36	18.4	19.3	48.6	3.4	0.2
IV	60—70	1.14	17.4	20.1	56.7	3.2	0.2

a) This administration schedule was selected to obtain 5.5 μg/ml plasma concentration of BM.

Table V. Renal Clearance Data for Hydroflumethiazide using the Rabbit

Experiment No.	Time (min)	Urine flow (ml/min)	Inulin clearance (ml/min)	HFT concentration (μg/ml)		HFT clearance (ml/min)	Clearance ratio (CR)
				Plasma	Urine		
Rabbit, male, 2.7 kg: HFT 550 μg/kg loading followed by 14 μg/min sustaining in 0.9% NaCl[a]							
I	0—10	0.46	5.3	1.6	44.4	12.8	2.4
II	10—20	0.40	5.4	1.5	38.9	10.4	1.9
Probenecid, 12 mg/kg loading followed by 0.3 mg/min sustaining in 0.9% NaCl							
III	50—60	0.40	8.9	1.9	13.7	2.9	0.3
IV	60—70	0.42	11.1	2.3	13.7	2.5	0.2

a) This administration schedule was selected to obtain 1.7 μg/ml plasma concentration of HFT.

Table VI. Renal Clearance Data for Phenolsulfonphthalein using the Rabbit

Experiment No.	Time (min)	Urine flow (ml/min)	Inulin clearance (ml/min)	PSP concentration (μg/ml)		PSP clearance (ml/min)	Clearance ratio (CR)
				Plasma	Urine		
Rabbit, male, 2.5 kg: PSP 500 μg/kg loading followed by 32 μg/min sustaining in 0.9% NaCl[a]							
I	0—10	0.18	8.5	13.9	414	5.4	0.6
II	10—20	0.20	8.6	12.5	405	6.5	0.8
Probenecid, 12 mg/kg loading followed by 0.3 mg/min sustaining in 0.9% NaCl							
III	50—60	0.23	10.6	18.9	72.9	0.9	0.1
IV	60—70	0.12	8.8	20.0	116	0.7	0.1

a) This administration schedule was selected to obtain 9.8 μg/ml plasma concentration of PSP.

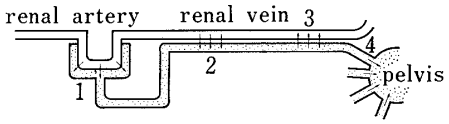
Renal Clearance Experiments in Rabbits

In an attempt to define the renal excretion mechanisms of the three diuretics, a standard renal clearance experiment[5] was carried out, and the results for the three diuretics and PSP are shown in Tables III—VI. Inhibitory experiments with probenecid were also carried out, and the results are illustrated in Tables III—VI as experiments III and IV. The estimated values of filtration, secretion, reabsorption and urine excretion for the three diuretics and PSP are shown in Tables VII—X. These values were calculated based on the assumption that the renal functions were maintained constant before and after the tubular secretion blockage.[5]

Table VII. Transfer Rate[a,b] of Furosemide in Each Direction in Renal Tubules

Experiment	Time (min)	Transfer rate (μg/min)			
		Filtration	Secretion	Reabsorption	Excretion
I	0—10	3.1	29.8	0.3	32.6
II	10—20	2.9	26.2	0.2	28.9
III	50—60	13.0	—	—	13.6
IV	60—70	9.5	—	0.1	9.4

a) Transfer directions in renal tubule of diuretics are as follows:
1-filtration, 2-secretion, 3-reabsorption, 4-excretion.



b) These values were calculated using the equations in reference (5) from the data in Tables III—VI.

Table VIII. Transfer Rate[a,b] of Bumetanide in Each Direction in Renal Tubules

Experiment	Time (min)	Transfer rate (μg/min)			
		Filtration	Secretion	Reabsorption	Excretion
I	0—10	31.8	149	0.8	180
II	10—20	26.7	139	0.7	165
III	50—60	66.0	—	—	66.0
IV	60—70	65.1	—	0.3	64.8

a), *b*) Refer to Table VII.

Pharmacokinetic Analysis of the Disappearance of Diuretics and Phenolsulfonphthalein in Rabbits

Plasma concentrations of the three diuretics and PSP against time after intravenous administration to rabbits were plotted on semi-logarithmic paper. Pharmacokinetic analysis

Table IX. Transfer Rate[a,b] of Hydroflumethiazide in Each Direction in Renal Tubules

Experiment	Time (min)	Transfer rate (μg/min)			
		Filtration	Secretion	Reabsorption	Excretion
I	0—10	5.8	46.5	31.9	20.4
II	10—20	5.9	34.1	24.4	15.6
III	50—60	11.6	—	6.1	5.5
IV	60—70	17.8	—	12.0	5.8

a), b) Refer to Table VII.

Table X. Transfer Rate[a,b] of Phenolsulfonphthalein in Each Direction in Renal Tubules

Experiment	Time (min)	Transfer rate (μg/min)			
		Filtration	Secretion	Reabsorption	Excretion
I	0—10	9.4	65.6	0.5	74.5
II	10—20	8.6	73.0	0.6	81.0
III	50—60	15.9	—	—	16.8
IV	60—70	14.0	—	0.1	13.9

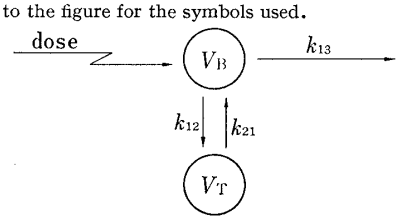
a), b) Refer to Table VII.

Table XI. Summary of Pharmacokinetic Parameters[a,b] for Diuretics and Phenolsulfonphthalein

Parameter	Furosemide (FM)	Bumetanide (BM)	Hydroflumethiazide (HFT)	Phenolsulfonphthalein (PSP)
P (μg/ml)	57.5 ±2.0	74.0 ±6.6	35.2 ±1.9	180.0 ±5.7
Q (μg/ml)	3.1 ±0.7	4.7 ±1.6	19.5 ±0.7	32.7 ±1.8
α (min^{-1})	0.103±0.013	0.086±0.014	0.121±0.028	0.082±0.008
β (min^{-1})	0.107±0.004	0.017±0.003	0.021±0.003	0.030±0.003
$t_{0.5}$ (min)	42.2 ±9.8	41.7 ±7.4	33.4 ±4.8	23.3 ±2.1
k_{12} (min^{-1})	0.017±0.004	0.013±0.004	0.041±0.012	0.007±0.004
k_{21} (min^{-1})	0.021±0.005	0.021±0.005	0.057±0.011	0.038±0.003
k_{13} (min^{-1})	0.093±0.010	0.069±0.008	0.045±0.008	0.065±0.006
V_B (ml/kg)	165.2 ±7.4	139.0 ±15.1	183.0 ±7.7	50.0 ±1.8
V_T (ml/kg)	130.5 ±19.7	81.1 ±7.8	131.0 ±8.8	9.5 ±3.6

a) Values are averages (±S.D.) of three experiments, and were calculated using the "stripping" technique (J.G. Wagner, "Fundamentals of Clinical Pharmacokinetics," First ed., Drug Intelligence Publication, Inc., Hamilton, Illinois, 1975, pp. 59—62). Not corrected; in order to obtain the intrinsic parameters related to plasma concentration curve after single *i.v.* bolus or rapid injection, the method of Loo and Riegelman (J.C.K. Loo and S. Riegelman, *J. Pharm. Sci.*, **59**, 53 (1970)) must be applied.

b) Refer to the figure for the symbols used.



The semilogarithmic plot of plasma concentration of diuretics and PSP ($[B]_t$) against time (t) after intravenous administration is biphasic ($[B]_t = Pe^{-\alpha t} + Qe^{-\beta t}$).

B: central compartment, T: peripheral compartment.

based on the two-compartment open model was done using the feathering method. The values obtained (P, Q, α, β) were used to calculate the apparent volume of the central compartment (V_B) and the rate constant of elimination from the central compartment (k_{13}). The half-life period ($t_{0.5}$) of the late portion of each biphasic curve was determined graphically. These constants are summarized in Table XI.

In these rabbits, the average elimination half-lives of the three diuretics were extremely small and approximately similar (20—40 min).

Discussion

The elimination half-life ($t_{0.5}$) can be calculated by means of equation (3), using the drug elimination constant (β) at the late portion of the biexponential curve.

$$t_{0.5} = \frac{0.693}{\beta} \tag{3}$$

On the other hand, the excretion half-life ($t'_{0.5}$) can be calculated as follows,

$$t'_{0.5} = \frac{0.693 \cdot V_B \cdot \text{body weight}}{C} \tag{4}$$

where C is the renal clearance of diuretics.

It has been accepted that the plasma protein binding of drugs usually affects their distribution in the body and their elimination from the body. There was a considerable difference among diuretics as regards plasma protein binding, as shown in Table I. However, regardless of the extents of plasma protein binding, β values for the three diuretics and PSP were almost the same, and their half-lives ($t_{0.5}$) were extremely small (20—40 min). It is clear that the extent of plasma protein binding of diuretics and PSP is not the only factor affecting the elimination rate of these drugs.

The half-life values ($t'_{0.5}$) calculated using eq. (4) from the renal clearance as shown in Tables III—VI are 36 min for FM, 15 min for BM, 27 min for HFT, and 17 min for PSP. Compared with $t_{0.5}$ calculated from β, as shown in Table XI, these values are similar. It may be considered from the above results that the main elimination route for these diuretics is glomerular excretion.

The renal clearance ratio for the three diuretics and PSP was relatively higher in the control period (experiments I and II). Probenecid caused a decrease in the renal clearances and clearance ratios of diuretics and PSP (experiments III and IV). This shows that the diuretics and PSP are secreted in the renal tubules and that the contribution of tubular secretion in the renal excretion process in quite large (Tables VII—X).

Gillette[15] stated that the effect of drug-plasma protein binding on transfer in the biophase is complex and it may either hasten (transport mechanism) or retard (storage mechanism) the elimination of drugs, depending on the mechanism of elimination.

It may be concluded from this study that binding to plasma protein may serve for the transport of the diuretics and PSP to the kidney. The diuretic-plasma protein complex may dissociate rapidly in the renal system and then the free diuretics are rapidly secreted through the proximal convoluted and loop Henle segments. Because the secretion rates of drugs are quite fast, further dissociation of the drug-plasma protein complex will proceed continuously. It seems probable that the rate of dissociation of the drug-plasma protein complex seldom becomes a rate-limiting step in the excretion of diuretics.

The high clearance of HFT may indicate a high degree of tubular secretion in renal excretion, and the data from the tubular secretion blockage experiment also suggest clearly that HFT is reabsorbed by the renal tubules.

15) J.R. Gillette, *Ann. N.Y. Acad. Sci.*, **226**, 6 (1973).