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

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Blood levels of 14 sulfonamides in rabbits subjected to intravenous administration were found to decrease following a first-order rate. The rate constants for acetylation and excretion were estimated from the first-order half-life in blood and the degree of acetylation in urine. Relationship between physicochemical properties of sulfonamides and rates of excretion was discussed. Binding of sulfonamides with plasma protein was scarcely correlated with the excretion rate. Rate constant of excretion, k_3, was closely correlated with partition coefficients between chloroform and a buffer solution of pH 8.8.

It has been well established that the absorption and excretion of drugs were subjected to the physicochemical properties of drugs. In the previous paper,[1,4] the authors reported physicochemical properties of sulfonamides. In the present paper, physicochemical properties of sulfonamides are discussed in reference to rate of excretion of the drugs in rabbits.

Half-Life of Sulfonamides in Rabbits

Fourteen sulfonamides were employed in studying elimination rates from the blood stream in rabbits after intravenous administration. A logarithm of sulfonamide concentration in blood was plotted against time elapsed after the administration. Results were presented for 4 drugs for a rabbit, subject no. 022f, in Fig. 1. It was found that sulfonamides eliminated from the blood stream followed a first-order rate. But, in some cases as sulfacetamide, N-sulfanilyl-3,4-xylamide, sulfaphenazole, and sulfadimethoxine, a deviation from the first-order elimination was observed in the first 1 hr after administration. The deviation may result from a period of distribution of the drugs into an available volume of the rabbit body.

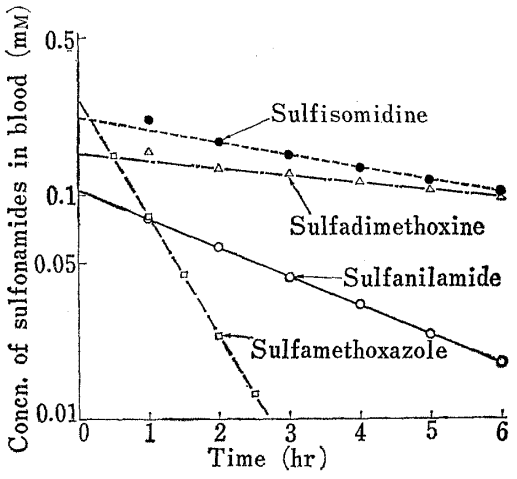

Fig. 1. Blood Concentration of Sulfonamides in Subject 022 f following Intravenous Administration

First-order half-lives of the elimination were obtained from the slopes of Fig. 1. The results were presented in Table I for 6 rabbits using each sulfonamide. Here it must be noticed that the values of half-life for one sulfonamide were distributed over a wide range even in two rabbits of the same venter, but each rabbit showed its constant half-life in experiments. It was also observed that three of 6 rabbits, identification number 015f, 022f, and 023 m in the table, were repeatedly used for the study of excretion

1) Part II: M. Yamazaki, M. Aoki, A. Kamada, and N. Yata, *Yakuzaigaku*, 27, 40 (1967).
2) A part of this work was reported at the 22nd Annual Meeting of the Pharmaceutical Society of Japan, April 1966.
3) Location: *Toneyama, Toyonaka, Osaka-fu.*
4) M. Yamazaki, M. Aoki, A. Kamada, and N. Yata, *Yakuzaigaku*, 27, 37 (1967).

Table I. Half-life of Sulfonamides in Rabbits

Sulfonamides	Half-life of Sulfonamides in Rabbits (hr) Subject						Mean
	015 f	016 f	018 f	019 f	022 f	023m	
Sulfanilamide	7.30	1.23	4.52	1.73	2.37	2.12	3.21
Sulfacetamide	1.00	0.60	1.65	0.60	1.33	1.26	1.07
Sulfapyridine	1.07	0.72	1.33	0.65	0.38	0.58	0.79
Sulfathiazole	1.12	0.62	1.00	0.57	0.53	1.55	0.90
Sulfadiazine	1.80	1.03	2.00	1.15	0.93	1.23	1.36
Sulfamerazine	1.45	0.48	1.75	0.68	0.61	0.55	0.92
N-Sulfanilyl-3,4-xylamide	2.43	1.33	2.50	1.25	1.65	1.63	1.80
Sulfisoxazole	1.10	1.25	1.08	0.93	0.85	0.85	1.01
Sulfisomidine	6.11	3.13	4.42	4.95	5.66	5.00	4.88
Sulfaphenazole[a]	1.38	1.17	1.52	1.25	1.72	2.12	1.53
Sulfamethoxypyridazine[a]	2.45	1.23	4.35	1.12	1.07	1.28	1.92
Sulfadimethoxine[a]	6.75	2.68	11.75	4.20	8.95	7.45	6.96
Sulfamethoxazole[a]	1.57	0.58	1.12	0.59	0.57	0.78	0.87
Sulfamonomethoxine[a]	2.00	1.28	2.42	1.50	1.23	1.64	1.68

a) Long-acting sulfonamide.

Table II. Percent of Acetylation of Sulfonamides in Rabbits

Sulfonamides	Acetylation of Sulfonamides in Rabbits (%) Subject						Mean
	015 f	016 f	018 f	019 f	022 f	023m	
Sulfanilamide	24.8	75.3	28.6	69.8	66.0	70.4	55.8
Sulfacetamide	7.5	25.6	17.1	18.5	27.3	21.1	19.5
Sulfapyridine	19.5	68.0	28.8	51.8	55.1	51.3	45.8
Sulfathiazole	26.4	60.2	31.1	41.3	50.6	51.4	43.5
Sulfadiazine	49.8	68.1	54.8	64.9	60.2	75.0	62.1
Sulfamerazine	56.5	82.6	60.6	78.6	78.8	76.8	72.3
N-Sulfanilyl-3,4-xylamide	24.6	37.2	30.5	28.7	34.5	32.0	31.3
Sulfisoxazole	20.7	27.2	20.8	19.8	17.2	14.2	20.0
Sulfisomidine	10.1	20.5	8.7	13.7	21.5	17.6	15.4
Sulfaphenazole	31.0	69.8	60.3	62.9	62.6	61.6	58.0
Sulfamethoxypyridazine	59.0	72.8	69.0	65.9	70.4	71.2	68.1
Sulfadimethoxine	57.8	77.6	76.3	68.6	69.3	71.6	70.2
Sulfamethoxazole	62.5	78.1	60.5	78.0	75.9	74.9	71.7
Sulfamonomethoxine	69.5	68.5	56.5	73.1	60.4	76.8	67.5

Table III. The Analysis of Variance of Table I

	Sum of Squares	Degree of Freedom	Variance	Variance Ratio
Variance of Half-lives for 6 Rabbits	31	5	6.2	5.69**
Variance of 14 Sulfonamides	253	13	19.5	17.9 **
Error	71	65	1.09	
Total	355	83		

for more than one year, and they gave the same half-life during the period. In most cases, rabbits were tested at adequate intervals of 2 to 5 weeks. A similar result was reported by Frymoyer[5] for sulfadiazine administered intravenously to 5 rabbits for 3 times at one week interval.

Half-lives of each sulfonamide were studied in 15 rabbits. But the results shown in Table I were of 6 rabbits with 14 sulfonamides. The half-lives of sulfonamides were significantly different (Table III).

It was found that a rabbit with a long half-life for one sulfonamide showed a slow excretion for other sulfonamides.

The half-life of sulfadimethoxine, a typical long-acting sulfonamide, proved to be longest. However, the half-lives for other long-acting sulfonamides, such as sulfaphenazole, sulfamethoxypyridazine, sulfamethoxazole, and sulfamonomethoxine, were rather short compared with those of other non-long-acting sulfonamides, such as sulfanilamide and sulfisomidine.

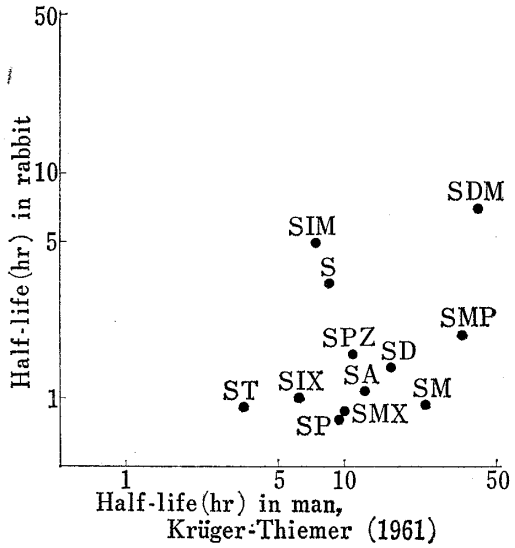

Fig 2. Relationship of Half-life of Sulfonamides between Rabbits and Man

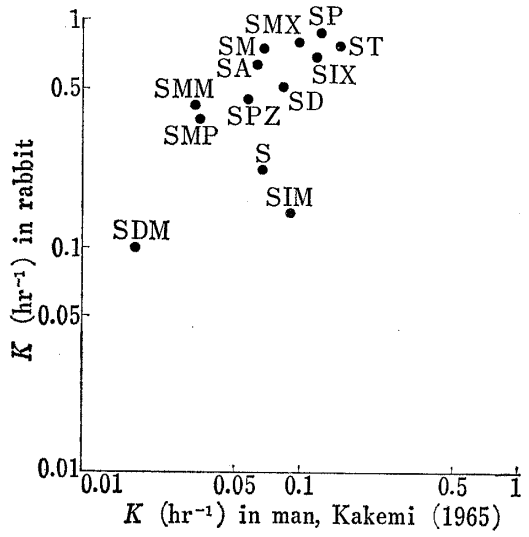

Fig. 3. Relationship of K between Rabbits and Man

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

The present results showed five to ten times shorter half-lives than that for man (Krüger-Thiemer[6]) (Fig. 2). A decreasing rate constant of sulfonamides in the blood of rabbits was compared with an excretion rate constant[7] in the urine of man (Fig. 3). It was found that no good correlation between rabbits and man was present in Fig. 2 and 3.

Acetylation of Sulfonamides in Rabbits

Acetylation (A) of a sulfonamide in rabbits was calculated by assaying total and unacetylated amount of sulfonamide in the urine (Table II).

$$A=\frac{\left(\begin{array}{l}\text{cumulative excretion}\\ \text{of total sulfonamide}\end{array}\right)-\left(\begin{array}{l}\text{cumulative excretion of}\\ \text{unacetylated sulfonamide}\end{array}\right)}{(\text{cumulative excretion of total sulfonamide})}$$

The acetylation was considerably varying individually in rabbits and sulfonamides.

5) J.W. Frymoyer and R.F. Jacox, *J. Lab. Clin. Med.*, **62**, 891 (1963).
6) E. Krüger-Thiemer and F. Bünger, *Arzneimittel-Forsch.*, **11**, 867 (1961).
7) K. Kakemi, T. Arita, and T. Koizumi, *Yakuzaigaku*, **25**, 22 (1965).

The degree of acetylation in the urine was closely correlated with the half-life in the blood (Table I and II).

Kinetics of Excretion of Sulfonamides

As stated above, the disappearance of sulfonamide from the rabbit blood follows a rate of first-order reaction. It is expressed as follows[8]:

$$\log C = -\left(\frac{K}{2.303}\right)t + \log C_0 \qquad (1)$$

where C=sulfonamide concentration in blood; K=rate constant for elimination from the blood stream; t=time after intravenous administration; and $\log C_0$=an intercept of the concentration axis at time zero when $\log C$ is plotted against time. The first-order half-life of a sulfonamide may be presented as follows:

$$t_{1/2} = \frac{0.693}{K} \qquad (2)$$

Here it may be assumed that K is a rate constant for the disappearance of sulfonamide from blood stream of the rabbit. Here, it must be admitted that the rate constant for excretion in the urine should be used for the following calculation of the excretion parameters of the sulfonamides. But, in the present study, the rate constant of elimination from the blood stream (K) was used assuming that the eliminated fraction of the drugs from the blood stream was effectively excreted into the urine. The sulfonamides introduced intravenously are considered to distribute rapidly over a definite distribution volume of the rabbits. The distribution volume may be maintained constant during the experimental period of elimination of the drugs from the blood stream. Thus, K may be quite similar with the rate constant of excretion in the urine. The present assumption is well supported by similar results for the authors' study[9] of elimination of the sulfonamides from the rat blood stream and the study of excretion into the rat urine by Nogami.[10] K is assumed a sum of rate constants for excretion from the kidney and for acetylation in the rabbit.[11] The fraction of glucuronides is neglected for the calculation of excretion because of the small amount in the rabbit urine.[12]

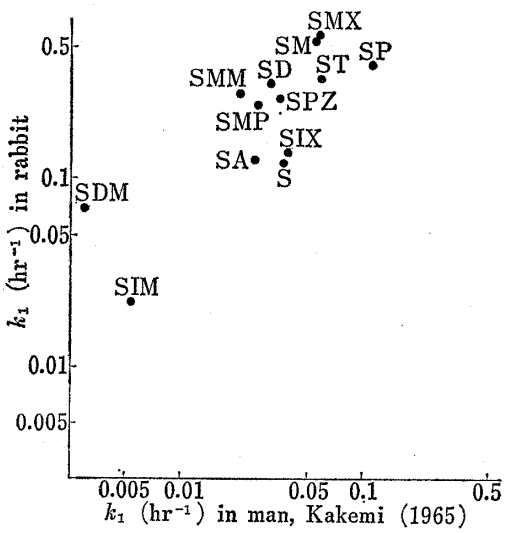

Fig. 4. Relationship of k_1 between Rabbits and Man

$$K = k_1 + k_3 \qquad (3)$$

$$f = \frac{k_3}{k_1 + k_3} \qquad (4)$$

where k_1 is a rate constant of acetylation (hr^{-1}), k_3 a rate constant of excretion (hr^{-1}), and f a fraction of unacetylated sulfonamide excreted in the urine against the total sulfonamide excreted. From Eq. 5 f is determined.

$$f = \frac{100 - A}{100} \qquad (5)$$

8) J.V. Swintosky, *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 395 (1956).
9) M. Yamazaki, M. Aoki, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **16** 721(1968).
10) H. Nogami, J. Hasegawa, M. Hanano, and K. Imaoka, the 24th Annual Meeting of the Pharmaceutical Society of Japan, April 1967.
11) E. Nelson and I. O'Reilly, *J. Pharmacol. Exptl. Therap.*, **129**, 368 (1960).
12) J.W. Bridges, M. R. Kibby, and R. T. Williams, *Biochem. J.*, **91**, 12 p (1964).

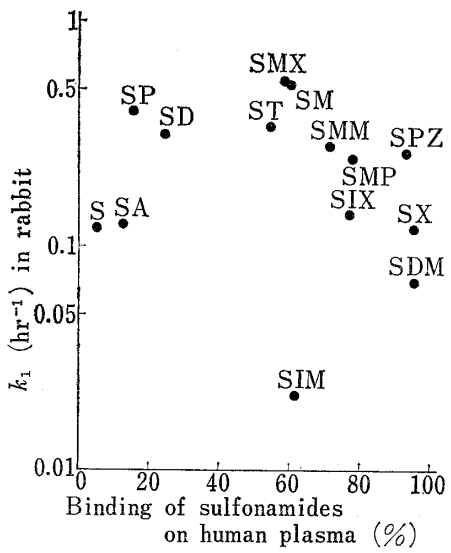

Fig. 5. Relationship between Binding of Sulfonamides on Human Plasma and k_1 in Rabbits

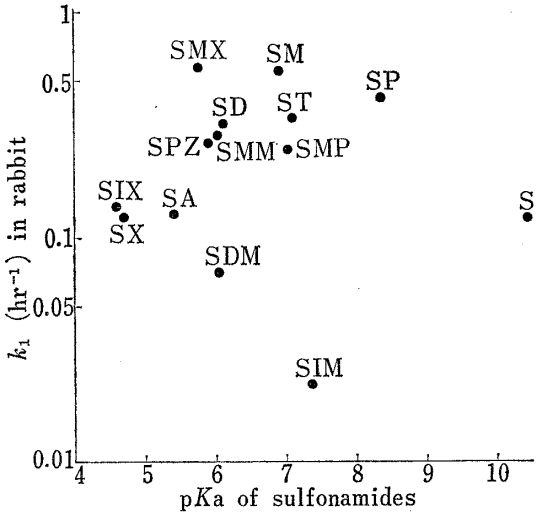

Fig. 6. Relationship between pK_a of Sulfonamides and k_1 in Rabbits

Table IV. Kinetic Parameters of Sulfonamides in Rabbits

Sulfonamides	$t_{1/2}$ (hr)	Acetylation (%)	K (hr^{-1})	k_1 (hr^{-1})	k_3 (hr^{-1})
Sulfanilamide	3.21	55.8	0.216	0.121	0.095
Sulfacetamide	1.07	19.5	0.648	0.126	0.522
Sulfapyridine	0.79	45.8	0.877	0.402	0.475
Sulfathiazole	0.90	43.5	0.770	0.335	0.435
Sulfadiazine	1.36	62.1	0.510	0.317	0.193
Sulfamerazine	0.92	72.3	0.753	0.544	0.209
N–Sulfanilyl–3,4–xylamide	1.80	31.3	0.385	0.121	0.264
Sulfisoxazole	1.01	20.0	0.686	0.137	0.549
Sulfisomidine	4.88	15.4	0.142	0.022	0.120
Sulfaphanazole	1.53	58.0	0.453	0.263	0.190
Sulfamethoxypyridazine	1.92	68.1	0.361	0.246	0.115
Sulfadimethoxine	6.96	70.2	0.100	0.070	0.030
Sulfamethoxazole	0.87	71.7	0.797	0.571	0.226
Sulfamonomethoxine	1.68	67.5	0.413	0.279	0.134

Table IV shows the mean values of half–life, acetylation, K, k_1, and k_3 in rabbits, respectively.

Acetylation Rate

A rate of acetylation in rabbits was compared with that of man determined by Kakemi[7] (Fig. 4). k_1 of rabbits was about ten–times larger than that of man with a significant correlationship. It has been considered that the acetylating ability of herbivorous animals for drugs is much stronger than that of omnivorous animals. A similar consideration was made by Bridges.[12,13]

Newbould[14] studied the effect of protein–binding on acetylation of sulfonamide employing a perfusion technique of the rabbit liver. He observed that the acetylation of sulfaphenazole, a highly bound sulfonamide to protein, was reduced in ten–times in the presence of plasma

13) J.W. Bridges and R.T. Williams, *J. Pharm. Pharmacol.*, **15**, 565 (1963).
14) B.B. Newbould and R. Kilpatrick, *Lancet*, **1960**, I, 887.

protein. In the present study, there was found no linear relationship between k_1 of rabbits and degree of binding to human plasma (Fig. 5).

k_1 showed little relationship to pK_a (Fig. 6), Michaelis' constant measured by Kakemi[7] on a pigion liver extract, and π-electron superdelocalizability of sulfonamides. The acetylation of sulfonamides in a living body seems difficult to explain only on the basis of physicochemical properties of sulfonamides.

Excretion Rate

Rate constants of excretion of sulfonamides from rabbits were calculated in the same way as acetylation. When k_3 was compared in rabbits and man reported by Koizumi, [15]it was found that the excretion rate for rabbits was 5 to 20-times larger than that of man (Fig. 7). pH of the rabbit urine is more alkaline than that of man, and sulfonamides enhanced an ionization in an alkaline urine. The ionized molecules of sulfonamides is difficult to be reabsorbed through the kidney tubule because of its low lipid-solubility, so that the rate of excretion is more enhanced in rabbits than in man.

Binding of sulfonamides to plasma protein has been studied on the basis of drug activity. Interactions of sulfonamides with plasma protein, mainly with albumin, are reversible.[1] Sulfonamides bound to plasma protein do not permeate the glomerular membrane. Thus, a highly bound sulfonamide may give a small value of k_3. There was little relationship between k_3 of rabbit and binding of sulfonamides to human plasma (Fig. 8). Ciceri[16] also found no correlationship between a blood sulfonamide retention and a protein binding.

Anton[17] observed that the affinity of sulfonamides for plasma protein differed markedly depending upon the species of animals. It is considered that a different affinity may be responsible for rates of metabolism and excretion of a drug in different species of animals. This consideration is likely to be admitted in the present experiment, in which a dried human plasma was used for the study of protein binding in a non-physiological medium while rabbits were employed for a study of excretion.

Rieder[18] found a possible relation between persistence of sulfonamides in the human body and their partition between lipid solvents and water. In a pathway of absorption, drugs are to penetrate lipophilic barriers. Thus, drugs need a high lipid solubility to facilitate penetration. The same consideration may be acceptable for the reabsorption of sulfonamides through the kidney tubules.

Values of k_3 of sulfonamides were plotted against partition coefficients between chloroform and a phosphate buffer of pH 7.4 (a physiological pH of blood) (Fig. 9). A linear relationship was found for the sulfonamides studied except for sulfanilamide and sulfapyridine. The exception of the two drugs may be considered as follow:

Many drugs are weak organic electrolytes and they exist as ionized and unionized molecules in the body. Only unionized molecules are lipid soluble and ionized ones can hardly penetrate the lipophilic membrane. Thus, the penetration through the membrane may be subjected to two physicochemical factors, the degree of ionization, and the solubility of unionized molecules to lipid.

Here, the penetration of a drug through the tubular membrane should be taken into consideration. A schematic illustration of the kidney of man is presented (Fig. 11). The kidney tubule is covered with closely packed epithelial cells forming a continuous membrane. The pH values of the plasma and filtrate at the upper part of the glomerule are assumed to be 7.4. Now, a sulfonamide, having pK_a (sulfonamide group) of 7.4, is administered to man. The sulfonamide dissociates in an equal amount of ionized and unionized molecules in the plasma

15) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 428 (1964).
16) C. Ciceri, T. Chieli, and C. Bertazzoli, *Farmaco*, **20**, 259 (1965).
17) A.H. Anton, *J. Pharmacol. Exptl. Therap.*, **129**, 282 (1960).
18) J. Rieder, *Arzneimittel-Forsch.*, **13**, 81 (1963).

and the glomerular filtrate as illustrated in Fig. 11. The filtrate flows down to the lower part of the tubule, where pH is reported to decrease 5—6 from 7.4. Here the pH is assumed to be 5.4. The sulfonamide remains unionized in 99% at pH 5.4. If unionized molecules have a high lipid solubility, they can be easily reabsorbed into the plasma through the lower part of the tubule.[19] Thus, it is essential to assure pH at the site of reabsorption.

It was found that the urinary pH of a rabbit, a herbivorous animal, was around 8.8. Partition coefficients between chloroform and a tris aminomethane buffer (pH 8.8.) were compared with k_3 values (Fig. 10). The correlation coefficient was 0.512, and increased to 0.829 by exclusion of sulfapyridine. The deviation of sulfapyridine has been left an explain. But it may be respon-

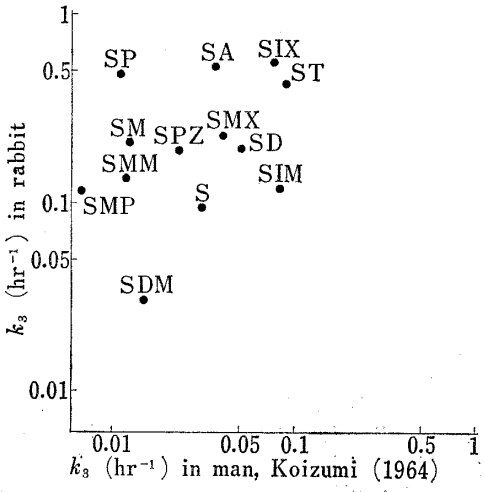

Fig. 7. Relationship of k_3 between Rabibts and Man

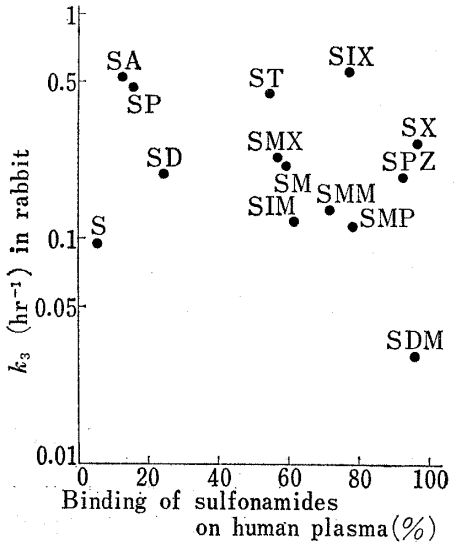

Fig. 8. Relationship between Binding of Sulfonamides of Human Plasma and k_3 in Rabbits

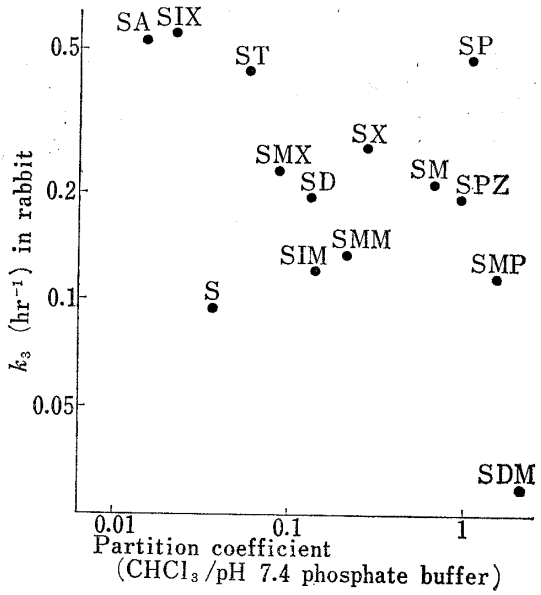

Fig. 9. Relationship between k_3 in Rabbits and Partition Coefficient ($CHCl_3$/pH 7.4 Phosphate Buffer)

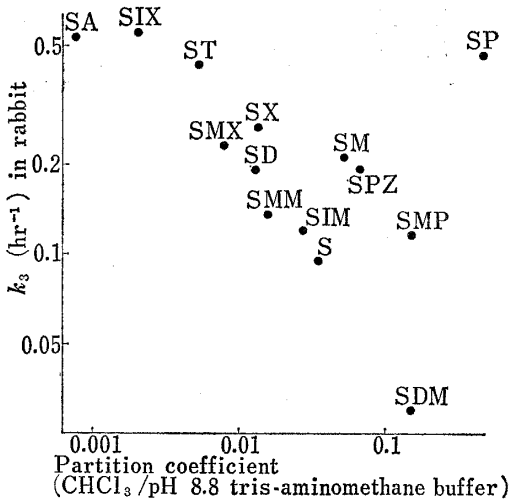

Fig. 10. Relationship between k_3 in Rabbits and Partition Coefficient ($CHCl_3$/pH 8.8 Tris-aminomethane Buffer)

19) B.B. Brodie and C. A. M. Hogben, *J. Pharm. Pharmacol.*, 9, 345 (1957).

sible for its low water-solubility.[4]

To make sure the importance of drug dissociation at a reabsorption site, it was attempted to modify the urinary pH of rabbits by administering ammonium chloride, hydrochloric acid lemonade, or animal protein without success.

It may be concluded that a lipophilic property, more precisely a partition coefficient between chloroform and water of urinary pH, is one of the most important factors which influence the excretion rate of sulfonamides from the kidney. Here it must be admitted that the present assumption to regard the biologic membrane as an organic solvent may lead to some faulty conclusion. But the present pH-partition hypothesis at the reabsorption site may be well advocated by the present study. Thus, the rate of excretion from the kidney, one of the biopharmaceutic properties of sulfonamide was well explained on the basis of partition.

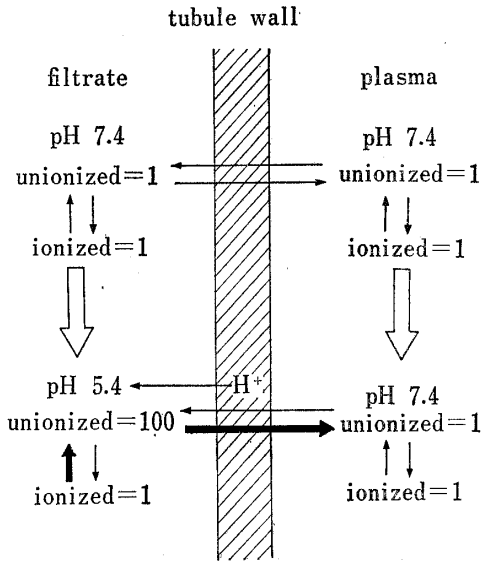

Fig. 11. Influence of pH of Urine on Excretion of Sulfonamide ($pK_a=7.4$)

Experimental

Material——Fourteen sulfonamides, J.P. VII grade, were listed in Table I. They were routinly purified by recrystallization. The melting points were as follows: sulfanilamide, 165°; sulfacetamide, 182°; sulfapyridine, 190°; sulfathiazole, 201°; sulfadiazine, 251°; sulfamerazine, 234°; N-sulfanilyl-3,4-xylamide, 210°; sulfisoxazole, 195°; sulfisomidine, 243°; sulfaphenazole, 182°; sulfamethoxypyridazine, 181°; sulfadimethoxine, 198°; sulfamethoxazole, 169°; sulfamonomethoxine, 204°. Solutions for injection were prepared by dissolving 0.5 M sulfonamides in water following J.P. VII.

Physico-chemical Properties——Partition coefficient, pK_a and binding of sulfonamides to human plasma were measured as reported previously.[1,4]

Animals——Male and female albino rabbits, weighing 3—4 kg, were supplied by market. Their strains were not identified. They were given compressed food for rabbits (Oriental Yeast Co.) and water *ad libitum*.

Method——The animals were kept fasting for 12 hr before experiment. Sulfonamides, 0.4 ml per kg (=0.2 mmole/kg) were administered intravenously at the earlobe in a period of 2 minutes. Five-tenth ml samples of blood were collected from the opposite marginal ear vein. Urine was collected 24 and 48 hr after administration. The sulfonamides were recovered from the urine at 75—90 per cent. A modified Bratton and Marshall method[4] was employed for assaying unacetylated and total sulfonamides.