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61. Tamotsu Koizumi, Takaichi Arita, and Kiichiro Kakemi : Absorption and Excretion of Drugs. XX.*1 Some Pharmacokinetic Aspects of Absorption and Excretion of Sulfonamides. (2). Absorption from Rat Small Intestine.

(*Faculty of Pharmaceutical Sciences, Kyoto University*[*2])

In the previous study[1] of this series, the absorption of sulfonamides from rat stomach was demonstrated with a kinetic model, and the absorption rate of unionized form was presented by the following equation :

$$Ku\sqrt{\mathrm{M}}=\frac{abP}{1+aP} \qquad (1)$$

The terms in equation (1) have the following meaning; Ku is absorption rate constant of unionized form, M molecular weight of the drug, P partition coefficient of the drug between organic liquid and water, a a correction factor to P for using organic liquid instead of barrier lipoid, and b a constant about diffusion.

This report describes the results of a similar study performed with rat small intestine for the absorption of sulfonamides.

Experimental

Absorption Experiments——Male rats weighing 130 to 170 g. were fasted for a whole night prior to the experiments but H_2O was allowed freely. The animals were anesthetized with pentobarbital and the small intestine was cannulated for *in situ* recirculation, following the method of Schanker, Tocco, Brodie and Hogben.[2]

Drug solutions were prepared as reported previously on the absorption from rat stomach.[1]

The intestine was first perfused with 50 to 100 ml. of 0.9% NaCl solution, maintained at 37°, and then with 30 ml. of drug solution. The tubing attached to the inflow and outflow cannulae were then transferred to a flask containing 20 ml. of the drug solution. This volume was then continuously circulated through the small intestine for 1 hr. at 37°, using the circulation apparatus shown in Fig. 1.

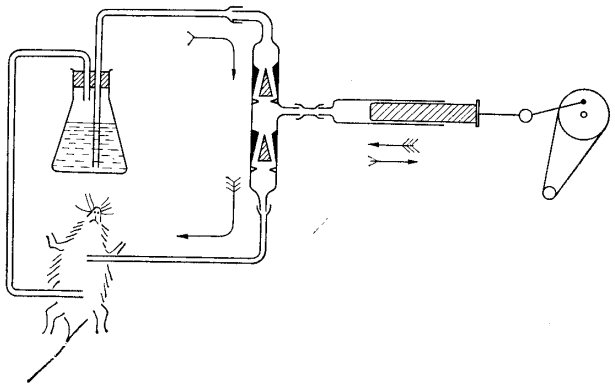

Fig. 1. Apparatus used for Recirculation Experiments of Rat Small Intestine

0.5 ml. of the sample solution was pipetted at 10, 20, 30, 45, and 60 min. after the recirculation was started. The concentrations of the drug and indicator (phenol red) were determined.

Partition Coefficients——Procedure of the measurement and the data obtained were reported previously.[1] Partition coefficients of N^4-acetylsulfonamides obtained are shown in Table I.

Analytical Method——Sulfonamides : reported previously.[1] N^4-acetylsulfonamides : after hydrolysis for 1 hr. in N HCl, concentration was estimated similarly as sulfonamides.

*1 Part XIX : This Bulletin, **12**, 413 (1964).

*2 Yoshidakonoe-cho, Sakyo-ku, Kyoto (小泉 保, 有田隆一, 掛見喜一郎).

1) K. Kakemi, T. Arita, T. Koizumi : This Bulletin, **12**, 413 (1964).

2) L. S. Schanker, D. J. Tocco, B. B. Brodie, C. A. Hogben : J. Pharmacol. Exptl. Therap., **123**, 81 (1958).

Table I. Partition Coefficients and their Reciprocals (chloroform, 37°)

	P	P^{-1}
N^4-Acetylsulfanilamide	0.03	33.3
N^4-Acetylsulfathiazole	0.11	9.09
N^4-Acetylsulfisoxazole	1.65	0.61
N^4-Acetylsulfamethizole	0.38	2.63
N^4-Acetylsulfaethidole	1.66	0.60

Materials——Sulfonamides were J.P. grade, and organic solvents were analytical grade. N^4-acetylsulfonamides were prepared by acetylating the corresponding sulfonamides in regular manner, and recrystallized from EtOH.

Results

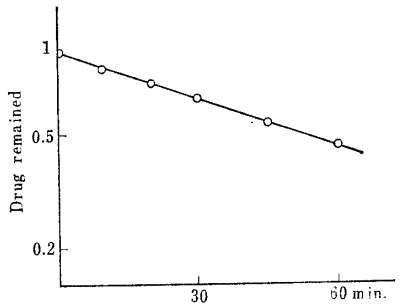

Fig. 2. Logarithmic Plot of Drug Remaining in Recirculating Solution (sulfanilamide pH 7.4)

First Order Absorption of Sulfonamides

The logarithm of remaining sulfonamide, appropriately corrected by the change of concentration of the indicator, was plotted against time. A straight line obtained in Fig. 2 shows that the absorption of sulfonamide from the rat small intestine is first order.

The relative rate of absorption was calculated from the slope of the line multiplied with 2.303.

Influence of Recirculation Speed on Absorption Rate

A stroboscope was used to watch the revolution speed of the wheel of the apparatus which control the flow rate. Recirculation flow rate corresponding the revolution speed was obtained by the repeated examination and shown in Table II.

The absorption rate of sulfamerazine was measured at pH 5.8 with each flow rate of them and results were shown in Table III.

The faster the flow rate, the greater the absorption rate, in the extent of experiments. Higher speed, however, was rejected for the physiological stand point, and the experiments were carried out with the circulation flow rate of 5 to 7 ml./min.

Table II. Revolution Speed of Stroboscope and Corresponding Flow Rate

Revolution speed (r.p.m.)	Flow rate (ml./min.)	Revolution speed (r.p.m.)	Flow rate (ml./min.)
78	10.9	33	5.0
45	6.5	16	2.9

Influence of Volume Change of the Solution on Absorption Rate

If some part of sulfonamide permeates through the water channel or pore on absorption site as well as through lipoid barrier, absorption rate must be affected when a large volume of water is absorbed.

To examine the influence of water absorption, hypotonic solution of sulfanilamide and sulfamerazine was recirculated. The salt components of the hypotonic solution are shown in Table IV, and results of experiments are shown in Table V. In Table V, volume change of circulating solution according to the absorption of water is given as phenol red factor defined as below :

$$\text{Phenol red factor} = \frac{C_{\text{phenol red final}}}{C_{\text{phenol red initial}}} \qquad (2)$$

where C represents concentration.

Absorption rate of sulfamerazine which is larger molecule, was constant and almost was not affected by water absorption, and that of sulfanilamide which is smaller molecule, inclined to increase according with phenol red factor. But increment was small and calculated absorption rate is revealed to give a reasonable value, even if water is absorbed as much as one third of its initial volume.

Table III. Absorption Rate of Unionized Form, *Ku*, at Various Flow Rate

Flow rate (ml./min.)	1	2	3	Mean
11	1.09	1.04	1.08	1.07
7	1.03	0.91	—	0.97
5	0.99	0.97	—	0.98
3	0.91	0.86	—	0.88

sample: sulfamerazine pH 5.8

Table IV. Hypotonic Salt Components used for Determining the Influence of Water Absorption on Absorption Rate of Sulfonamides

	1	2	3
$Na_2HPO_4 \cdot 12H_2O$ (g.)	7.2	7.2	7.2
KH_2PO_4 (g.)	6.4	6.4	6.4
NaCl (g.)	5.9	2.9	0
H_2O	to make 1000 ml.		

Table V. Correlation of Phenol Red Factor and Absorption Rate of Sulfanilamide and Sulfamerazine

	Sulfanilamide		Sulfamerazine	
	Phenol red factor	K (hr^{-1})	Phenol red factor	K (hr^{-1})
Buffer (1)	1.00	0.67	1.01	0.94
isotonic	1.00	0.67	1.03	1.23
Buffer (2)	1.15	0.70	1.18	1.18
2/3 tonic	1.27	0.80	1.29	1.18
Buffer (3)	1.40	0.72	1.39	0.83
1/3 tonic	1.44	0.74	1.45	0.95
	1.47	0.78	1.48	1.16

pH Shift

pH of the drug solution was changed significantly during the recirculation, as reported by Hogben, Tocco, Brodie, and Schanker.[3]

The final pH was plotted against the initial pH and spots were almost straighten out in a line as shown in Fig. 3. Extent of pH shift is of course considered to depend on buffer capacity of the solution, and experiment with a half dilute solution afforded the dotted line in the same figure. The two lines cross each other at pH about 6.5.

3) C. A. Hogben, D. J. Tocco, B. B. Brodie, L. S. Schanker: J. Pharmacol. Exptl. Therap., **125**, 275 (1959).

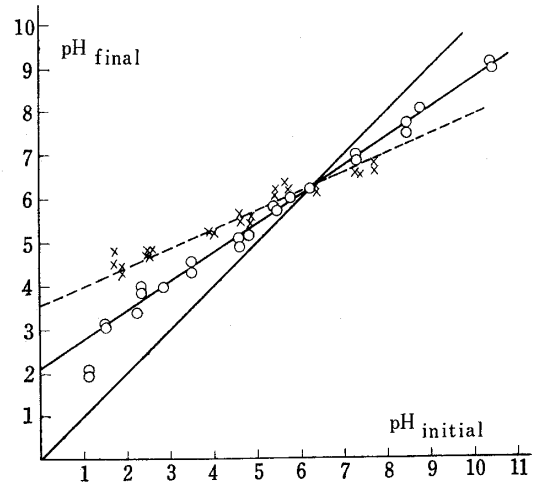

Fig. 3. Plot of Final pH against Initial pH at the Recirculation Experiments

solid line: solution of components of Table I
dotted line: solution of half dilute concentration

This means that pH of the solution inclines to approach the value of 6.5 during circulation, and that seems to be a pH value of the digestive juice.

Absorption Rate *vs.* pH Profile

Absorption rates at various pH were measured and pH profile was obtained with sulfonamides and results are shown in Figs. 4 to 7. In every case absorption rate ascended and descended passing maximum point with increasing pH, showing that the unionized form is absorbed predominantly.

But the pH value of the solution, from which the drug was absorbed most rapidly, did not coincide with the pH value where unionized form fraction is maximum, even if pH shift during circulation is taken into account.

pH of absorption rate maximum and pH of maximum unionized form is shown in Table Ⅵ.

Table Ⅵ. pH of Unionized Form Maximum and that of Absorption Rate Maximum

	pH			pH	
	Unionized	K_{max}		Unionized	K_{max}
Sulfapyridine	5.5	5.8	Sulfisoxazole	3.3	4.4
Sulfamethoxazole	3.8	5.0	Sulfisomidine	4.9	5.7
Sulfamerazine	4.7	5.6	*p*-Aminobenzoic acid	3.5	4.5

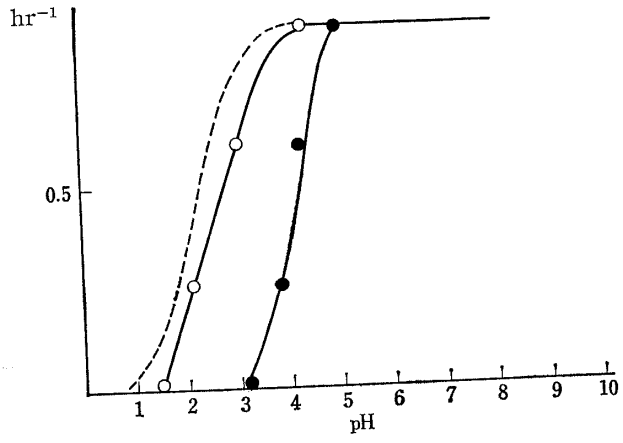

Fig. 4. Absorption Rate *vs.* pH Profile of Sulfanilamide

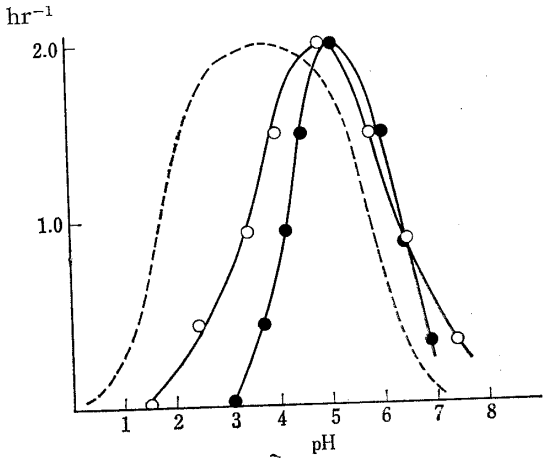

Fig. 5. Absorption Rate *vs.* pH Profile of Sulfamethoxazole

—○— Represents initial pH
—●— Final pH
– – – Theoretical fraction of unionized form

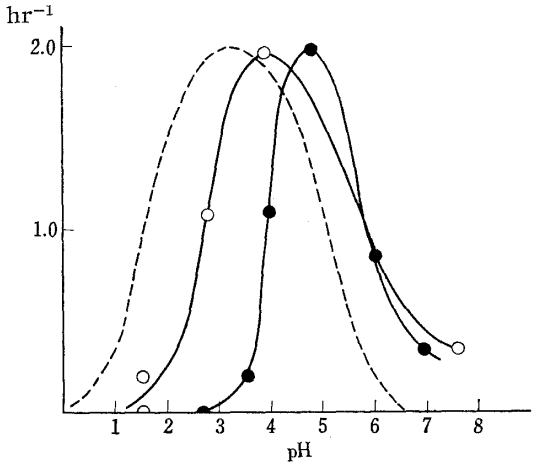

Fig. 6. Absorption Rate *vs.* pH Profile of Sulfisoxazole

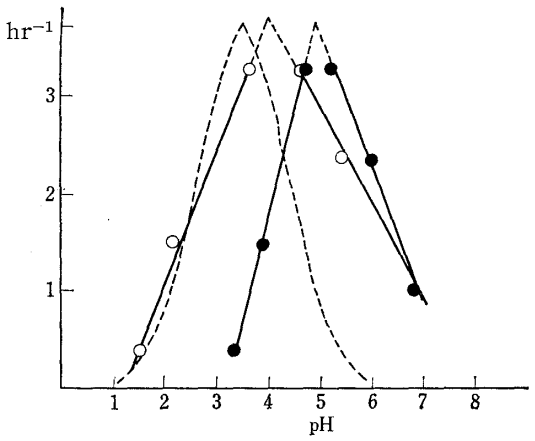

Fig. 7. Absorption Rate *vs.* pH Profile of *p*-Aminobenzoic Acid

—○— Represents initial pH
—●— Final pH
– – – Theoretical fraction of unionized form

Steady State Experiments

As the cause of this pH discrepancy, following can be considered : 1) dependancy of barrier permeability on pH, 2) dependancy of barrier peameability on direction of transport, 3) influence of potential gradient between plasma and digestive juice,[4] and 4) influence of zeta potential at the surface of barrier.

But at the steady state, there is no net passage of drug from intestine to plasma or from plasma to intestine, and influences of 1) and 2) are excluded.

Sulfisomidine solution of 50 mg. per 10 ml. was injected intravenously and simultaneously the solution of various concentration of the drug was recirculated through the small intestine at pH 9.3 and the steady state was attained after trial and error procedure.

Since net transport of the drug is zero at the steady state, equation (3) is presented for acid and equation (4) for base, and rearrangement gives equations (5) and (6) respectively.

$$\frac{C_p([H^+]_pKu+KaKi)}{Ka+[H^+]_p}=\frac{C_s([H^+]_sKu+KaKi)}{Ka+[H^+]_s} \tag{3}$$

$$\frac{C_p([H^+]_pKi+KaKu)}{Ka+[H^+]_p}=\frac{C_s([H^+]_sKi+KaKu)}{Ka+[H^+]_s} \tag{4}$$

(acid) $$[H^+]_s=\frac{Ka(r[H^+]_p+Ka)-KaR(Ka+[H^+]_p)}{Rr(Ka+[H^+]_p)-(r[H^+]_p+Ka)} \tag{5}$$

$$r=Ku/Ki \qquad R=C_s/C_p$$

(base) $$[H^+]_s=R(Ka+[H^+]_p)-Ka \quad \text{as} \quad r=\infty \tag{6}$$

where C is concentration, Ku and Ki are absorption rates of unionized form and ionized form respectively, Ka is acid dissociation constant, R is steady state distribution ratio—— the concentration of the drug in small intestine divided by the concentration of drug in plasma which is not bound to protein, r is absorption rate ratio of unionized form to ionized form, and subscripts p and s represent plasma and absorption site respectively.

Experiments with sulfisomidine and sulfanilamide afforded the data shown in Table Ⅶ.

4) J. Weatherby : Arch. intrn. Pharmacodynamie, **135**, 127 (1962).

Table VII. Data for the Calculation of Virtual pH

	pKa	pH_{soln}	R	pH virtual	
				$r=\infty$	$r=10$
Sulfisomidine (as acid)	7.5	9.1	2.06	8.06	8.22
Sulfanilamide (as base)	2.4	4.3	1.38	2.78	—

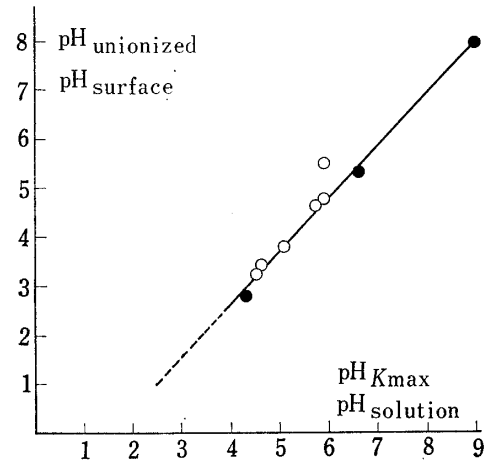

Fig. 8. Plot of Unionized-fraction-maximum pH against Absorption-rate-maximum pH

Black circle were obtained from absorption rate *vs.* pH profile, and solid circle from steady state experiments.

Difference between virtual pH at absorption site and pH of drug solution was the same magnitude as pH descrepancy as shown in Fig. 8.

According to Albert,[5] pH at surface is described as below :

$$pH_{surface} = pH_{solution} + \text{zeta potential(v.)}/0.06 \quad (7)$$

So it is reasonable to consider that the virtual pH at the absorption site does not coincide with pH of circulating solution measured, by the influence of potential gradient and zeta potential.

Absorption Rate of Unionized Sulfonamides

Considering the results above, virtual pH and pH shift should be taken into account to decide the initial pH that will give absorption

Table VIII. Absorption Rates of Sulfonamides, and N^4-Acetylsulfonamides

	Sulfonamide	pH		Ku	$Ku\sqrt{M}$	$(Ku\sqrt{M})^{-1}$
		initial	final			
1	Sulfanilamide	7.4	6.8	0.88	11.5	0.087
2	Sulfanilacetamide	4.5	5.1	0.46	6.7	0.149
3	Sulfaguanidine	7.4	6.8	0.04	0.6	1.67
4	Sulfapyridine	6.3	6.3	1.19	18.7	0.053
5	Sulfadiazine	4.9	5.2	1.14	18.0	0.055
6	Sulfamethoxazole	4.8	5.2	2.00	31.8	0.031
7	Sulfathiazole	6.3	6.3	0.33	5.3	0.189
8	Sulfamerazine	6.3	6.3	1.27	20.6	0.049
9	Sulfisoxazole	3.9	4.9	1.96	32.0	0.031
10	Sulfamethizole	4.5	5.1	0.93	15.3	0.065
11	Sulfisomidine	6.3	6.3	0.48	8.0	0.125
12	Sulfamethazine	6.3	6.2	1.25	20.9	0.048
13	Sulfamethoxypyridazine	6.3	6.3	1.41	23.5	0.043
14	Sulfamonomethoxine	4.8	5.2	1.44	24.0	0.042
15	Sulfaethidole	4.5	5.0	1.33	22.5	0.044
16	Sulfadimethoxine	6.3	6.3	2.16	38.0	0.026
17	Sulfaphenazole	2.6	4.4	1.67	29.6	0.034
18	N^4-Acetylsulfanilamide	2.7	4.4	0.18	2.63	0.38
19	N^4-Acetylsulfathiazole	2.7	4.3	0.09	1.55	0.65
20	N^4-Acetylsulfisoxazole	2.9	4.2	0.47	8.28	0.12
21	N^4-Acetylsulfamethizole	2.5	4.3	0.30	5.3	0.19
22	N^4-Acetylsulfaethidole	3.0	4.6	0.50	9.1	0.11

5) A. Albert : Pharmacol. Revs., 4, 136 (1952).

rate of unionized form individually. Authors managed as below : sulfanilacetamide, for example, has *pKa*'s 1.78 and 5.38, and therefore unionized pH of sulfanilacetamide is 3.58. Consulting Fig. 8, solution pH 4.9 is obtained which corresponds to surface pH 3.58. Since circulating solution changes its pH during operation, the initial pH is decided as 4.5 from Fig. 3 in order to give mean value of circulation pH 4.9. The recirculation experiment with sulfanilacetamide was thus started with initial pH 4.5.

The analogus treatments were taken to calculate the initial pH's with other sulfonamides, and absorption rates of unionized forms were determined. Results are shown in Table Ⅷ.

Discussion

$1/Ku\sqrt{M}$ was plotted against reciprocal of partition coefficient between chloroform and water, and Fig. 9 was obtained, which shows that the good linearity exists among the spots. This indicates that the model proposed for the absorption from stomach, also accounts for sulfonamides absorption from rat small intestine.

Absorption rate from stomach showed best linearity when it was plotted against reciprocal of partition coefficient between isopentyl acetate and water, but absorption from small intestine afforded the best linearity with chloroform. The difference of organic solvent which gave suitable P in these experiments (from stomach and from small intestine) is considered to due to difference in physicochemical characteristics of organic solvents used, from barrier lipoids.

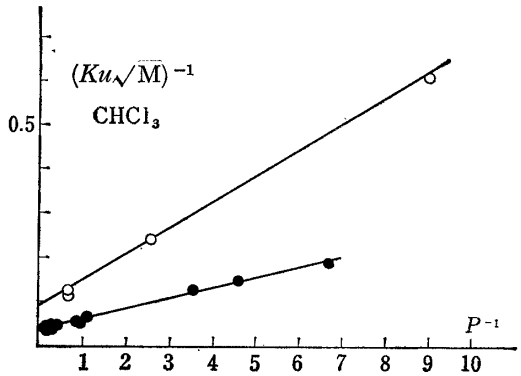

Fig. 9. Plot of $(Ku\sqrt{M})^{-1}$ against Reciprocal of Partition Coefficisnt between Chloroform and Water

—●— Sulfonamide
—○— N^4-Acetylsulfonamide

Summary

Rat small intestine was recirculated *in situ* with seventeen kinds of sulfonamide and five N^4-acetyl derivatives, and absorption rate of the unionized drug was determined.

The kinetical model proposed for the absorption of the drug from rat stomach also accounts for the absorption from rat small intestine, though the organic solvents that give suitable P are different.

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