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60. Tamotsu Koizumi, Takaichi Arita, and Kiichiro Kakemi : Absorption and Excretion of Drugs. XIX.*1 Some Pharmacokinetic Aspects of Absorption and Excretion of Sulfonamides. (1). Absorption from Rat Stomach.

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Most of the orally administered drugs are absorbed through the wall of gastrointestinal tract to develope their therapeutic activities.

The absorption from the rat stomach of a large number of organic acids and bases was studied by Schanker, Shore, Brodie and Hogben.[1] And the mechanisms of the passage of substances across the biological membrane were classified into five types by Kruhoffer,[2] and the activated diffusion among them was considered to be most important for the absorption of the drugs.

The present report describes the kinetical mechanisms of absorption of sulfonamides through the lipoid barrier at absorption site, and clarifies the relationship of absorption rates and partition coefficients between organic liquid and water.

The drugs need to dissolve in gastrointestinal juice before their absorption, and the relationship between dissolution rate and blood level was previously reported with sulfaethidole.[3]

In aqueous solution, weak acids and bases are composed of dissociated and undissociated molecules, the fraction of either being a function of the pH of the fluid and pKa of the substance. These relationships are expressed in the equations (1, 2, 3, and 4).

$$\text{Fraction of unionized acid} = \frac{[H^+]}{Ka + [H^+]} \qquad (1)$$

$$\text{Fraction of ionized acid} = \frac{Ka}{Ka + [H^+]} \qquad (2)$$

$$\text{Fraction of unionized base} = \frac{Ka}{Ka + [H^+]} \qquad (3)$$

$$\text{Fraction of ionized base} = \frac{[H^+]}{Ka + [H^+]} \qquad (4)$$

In these equations, Ka is the acid dissociation constant of the substance and $[H^+]$ is hydrogen ion concentration of the fluid.

Absorption mechanism of unionized form and that of ionized form being naturally supposed to be different, these mechanisms should be considered separately. Besides sulfonamides have two dissociated forms, positively and negatively charged. The apparent absorption rate consists of partial absorption rates of dissociated and undissociated form, but if proper pH value of the fluid be chosen, absorption rate of undissociated form only can be measured individually. As dissociated molecule is less permeable than undissociated one by passive transport, the absorption mechanism of unionized form is considered as below.

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*1 Part XVIII : Yakugaku Zasshi, **83**, 871 (1963).

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1) L. S. Schanker, P. A. Shore, B. B. Brodie, C. A. M. Hogben : J. Pharmacol. Exp. Therap., **120**, 528 (1957).

2) P. Kruhoffer : J. Pharm. and Pharmacol., **13**, 193 (1961).

3) K. Kakemi, T. Arita, T. Koizumi : Yakugaku Zasshi, **81**, 172 (1961); **82**, 262 (1962).

As far as the lipoid barrier hypothesis is concerned, absorption of unionized molecule by stomach is reduced to a problem of penetration of the molecule through the barrier, and kinetical interpretation is available for passive transport.

When a molecular species in solution penetrate a solid, according to Laidler and Shuler,[4] it can generally be assumed that the rate of diffusion in the liquid is sufficiently rapid so that it need not be taken into consideration. If this is the case, the elementary processes occuring around the surface of the membrane are :

1) Transfer of a drug molecule from the solution to the surface layer of the membrane,

2) Transfer of a drug molecule back into the solution from the membrane, and

3) Diffusion of a drug molecule into the membrane.

At the opposite side of the membrane, the same processes as 1) and 2) are considerable. As far as the begining of absorption is concerned, however, transfer of a molecule from blood to membrane is negligible, amount of drug in plasma being none. And as the rate of transfer from membrane to plasma is fast enough comparing with the rate of diffusion that is rate limiting,*3 a drug molecule diffused though the membrane dissolves into plasma as soon as it reaches the opposite side of the membrane. So that the rate of transfer from membrane to plasma need not be taken into consideration, and the model shown in Fig. 1 is assumed to govern the permeation of sulfonamides through lipoid barrier.

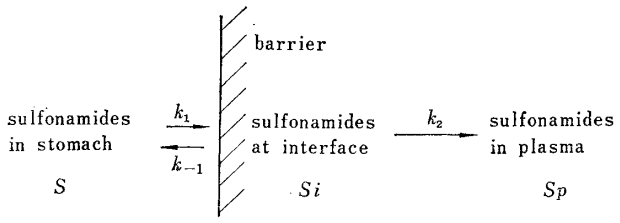

Fig. 1. Schema of Sulfonamide Absorption through Lipoid Barrier

The k's with number subscripts are first order rate constants in reciprocal of hours for the respective processes and hence these are assumed to proceed at a rate directly proportional to amounts of the indicated materials. If at any time (t), S is the amount (mg.) of sulfonamide in the stomach, Si the amount (mg.) of sulfonamide at the surface layer of the membrane, Sp the amount (mg.) of sulfonamide absorbed into plasma, the total amount of sulfonamide being constant, the differentiated equation describes :

$$\frac{dS}{dt}+\frac{dSi}{dt}+\frac{dSp}{dt}=0 \qquad (5)$$

As k_2 is small enough comparing with k_1 and k_{-1}, Si will equilibrate with S at any moment, and equation (6) is described.

$$Si=\frac{k_1}{k_{-1}}S \qquad (6)$$

Differentiating the equation (6), equation (7) is obtained.

$$\frac{dSi}{dt}=\frac{k_1}{k_{-1}}\frac{dS}{dt} \qquad (7)$$

And $\frac{dSp}{dt}$ is given as equation (8).

$$\frac{dSp}{dt}=k_2Si \qquad (8)$$

*3 This is proved to be correct by the experimental fact that the increase of partition coefficient is not accompanied by the increase of absorption rate with the drug of higher partition coefficients, as shown in Tables Ⅲ and Ⅳ.

4) K. J. Laidler, K. E. Shuler : J. Chem. Phys., **17**, 851 (1949).

Substitution of equation (5) with equations (6, 7, and 8) gives equation (9).

$$\frac{dS}{dt}+\frac{k_1}{k_{-1}}\frac{dS}{dt}+k_2\frac{k_1}{k_{-1}}S=0 \qquad (9)$$

Rearrangement of equation (9) gives equation (10).

$$\frac{dS}{dt}=-\frac{k_2\dfrac{k_1}{k_{-1}}}{1+\dfrac{k_1}{k_{-1}}}S \qquad (10)$$

Equation (10) shows that the disappearance of S is first order, and $\dfrac{k_2\frac{k_1}{k_{-1}}}{1+\frac{k_1}{k_{-1}}}$ is then the overall rate constant for removal of unionized drug from the stomach, and is set equal to Ku.

$$Ku=\frac{k_2\dfrac{k_1}{k_{-1}}}{1+\dfrac{k_1}{k_{-1}}} \qquad (11)$$

In the above equation, k_1/k_{-1} represents the partition coefficient of the drug between barrier lipoid and gastric juice, and is set equal to Pl.

k_2, diffusion rate constant of drug through the barrier, is considered after the Fick's law to be proportional to surface area and diffusion constant and equation (12) is obtained,

$$k_2=cAD \qquad (12)$$

where A is the surface area of absorption site, D diffusion constant of sulfonamide in the barrier lipoid, and c constant.

Since chemical and physical characteristics of hypothetical lipoid barrier is not fully studied yet, the direct measurement of Pl is almost impossible. Therefore various kinds of organic solvents (chloroform, ethylene dichloride, isoamyl acetate, benzene, carbon tetrachloride and so on) have been used for the barrier lipoid.

McGowan[5] demonstrated that equation (13) correlate the partition of an uncharged solute between two immiscible liquids.

$$\log P=k_M Pa+E \qquad (13)$$

Pa is the parachor of the solute, P partition coefficient, k_M constant characteristic of the two solvents, and E a correction factor involved in interaction between solute and solvents.

Deno[6] reported the k_M values of various organic liquids, that center around 0.013.

Since k_M values are approximately constant, the difference in partition coefficients according to difference of liquids depends mainly on E values, especially for sulfonamides which have many functional groups.

If equation (13) is also available for the partition between barrier lipoid and gastric juice, equation (14) is obtained.

$$\log Pl=k_M Pa+El \qquad (14)$$

5) J.C. McGowan: J. Appl. Chem., **1**, S 120 (1951); **2**, 323, 651 (1952).
6) N.C. Deno, H.E. Berkheimer: J. Chem. Eng. Data, **5**, 1 (1960).

Substracting the both sides of equation (13) from equation (14), equation (15) and equation (16) are obtained which show that the organic liquid, whose E value is in parallel with that of barrier lipoid, gives a partition coefficient directly proportional to Pl.

$$\log Pl - \log P = El - E \tag{15}$$

$$Pl = 10^{(El-E)}P \tag{16}$$

As for diffusion constant D, since it is not known for the drug under studying in lipoid barrier, it is convenient to use equation (17) used by Nelson,[7]

$$DM^{1/2} = \text{const.} \tag{17}$$

where M is molecular weight of the drug.

Substitution of equation (11) with equations (12, 16, and 17) gives equation (18),

$$\sqrt{M}Ku = \frac{abP}{1+aP} \tag{18}$$

where M is molecular weight of sulfonamide, Ku absorption rate of unionized form, P partition coefficient between suitable organic liquid and water, and a and b are constants including A, c, and El-E, respectively.

Reciprocal of both sides of equation (18) gives equation (19) which shows a straight line when $1/\sqrt{M}Ku$ is plotted against $1/P$.

$$\frac{1}{\sqrt{M}Ku} = \frac{1}{b} + \frac{1}{ab}\frac{1}{P} \tag{19}$$

To ascertain the applicability of equation (19), absorption rate from rat stomach was measured and partition coefficients of four organic liquids were determined with each of seventeen sulfonamides.

Experimental

Experimental Procedure for Determination of Absorption Rate——Male rats weighing 130 to 170 g. were fasted for a whole night prior to the experiments. Water was given freely and coprophagy was prevented by using cages with wide-mesh floors. And the method of Schanker, *et al.*[1] was followed.

The drug solution which contained 0.5 mmole/L. of sulfonamide was prepared using the salt solution shown in Table I. Phenol red which was expected to be unabsorbed, was dissolved in the drug solution to indicate any volume change. And the absorption rates were calculated by following equation :

$$K = 2.303 \log \left(\frac{C_{\text{drug initial}} \times C_{\text{phenol red final}}}{C_{\text{drug final}} \times C_{\text{phenol red initial}}} \right) \tag{20}$$

where C is concentration.

Equation (20) was driven by setting the absorption from the stomach to be first order, as given by equation (10).

Materials——Sulfonamides were J.P. grade. Organic solvents used were analytical grade.

Determination of Partition Coefficients——Four ml. of drug solution at theoretically unionized pH was shaken with 4 ml. of organic solvent at 37°, and drug content was determined in H_2O layer after equilibrium was reached. And partition coefficients were calculated by the following equation :

$$P = \frac{\text{Initial concn. of water layer} - \text{Equild concn. of water layer}}{\text{Equild concn. of water layer}} \tag{21}$$

Analytical Method——Sulfonamides were diazotized following regular manner, coupled with 2-diethylaminoethyl-1-naphthylamine and their optical densities were determined at 550 mμ, using Shimazu spectrophotometer type QR-50.

7) E. Nelson : J. Pharmacol. Exp. Therap., **135**, 120 (1962).

Table I. Salts Components used for Drug Solution

pH	HCl (ml.)	Citric acid (g.)	$Na_2HPO_4 \cdot 12H_2O$ (g.)	KH_2PO_4 (g.)	NaOH (g.)	Sodium borate (g.)	NaCl (g.)	H_2O
1.0	9.2	—	—	—	—	—	6.1	
1.5	7.0	4.2	—	—	1.6	—	4.9	
2.0	6.7	6.3	—	—	2.4	—	4.2	
2.3	6.1	7.7	—	—	2.9	—	3.6	
3.0	5.7	8.4	—	—	3.2	—	3.4	
3.3	4.8	9.5	—	—	3.6	—	3.2	
3.8	4.0	11.6	—	—	4.4	—	2.3	
4.0	3.8	12.6	—	—	4.8	—	2.1	
4.2	2.6	14.7	—	—	5.6	—	1.5	
4.5	1.8	16.8	—	—	6.4	—	0.8	to
4.7	0.9	19.0	—	—	7.2	—	0.1	make
5.0	—	21.0	—	—	8.0	—	—	1000
5.5	—	—	1.2	8.6	—	—	5.0	ml.
5.8	—	—	2.4	8.7	—	—	6.6	
6.4	—	—	7.2	6.4	—	—	5.9	
7.0	—	—	14.3	3.6	—	—	4.3	
7.4	—	—	19.1	1.8	—	—	4.0	
8.0	4.3	—	—	—	—	10.5	3.2	
8.5	2.8	—	—	—	—	13.4	2.5	
9.0	1.4	—	—	—	—	16.2	1.8	
9.3	—	—	—	—	—	19.1	1.0	
10.1	—	—	—	—	1.6	11.5	2.9	
11.6	—	—	—	—	2.0	9.6	3.4	

Results

pH Shift

Influence of gastric juice on sample solution was checked. But difference between pH values of initial and final drug solution was small as shown in Fig. 2.

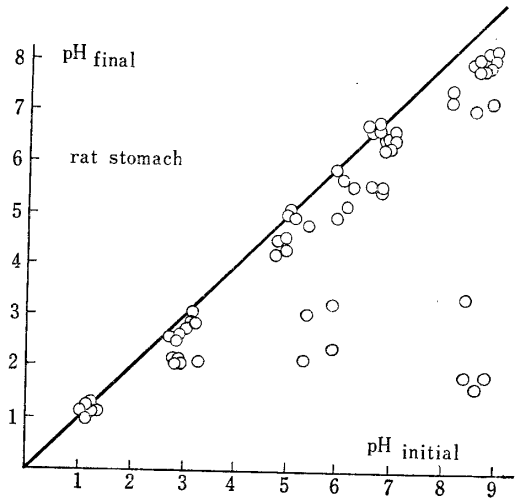

Fig. 2. Plot of Final pH against Initial pH

Absorption Rate *vs.* pH Profile

Absorption rates at various pH values were measured and absorption rate *vs.* pH profile were obtained for sulfanilamide, sulfamerazine, sulfisoxazole, and sulfamethoxypyridazine, and results are shown in Figs. 3 to 6. In every case, absorption rate ascended and descended passing maximum point with increasing pH, showing that the unionized form was 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, calculated from p*Ka* values. The differences were around one pH unit. These discrepancies could be own to the characteristics of gastric juice and absorption site of stomach. This was proved by the fact that the smaller discrepancy of pH was obtained with sulfisoxazole when two times concentrated salt solution, consequently of higher buffer capacity, was used for drug solution, as shown in Fig. 5.

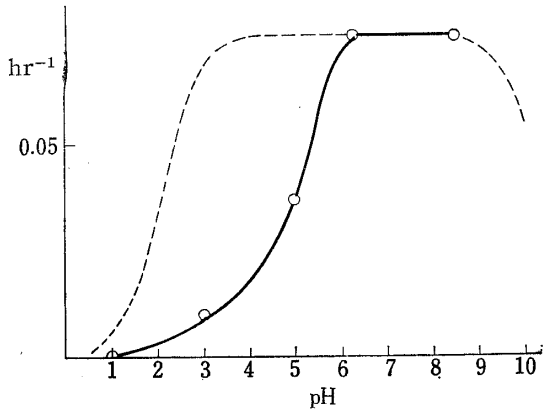

Fig. 3. Absorption Rate *vs.* pH Profile of Sulfanilamide

—○— Represents obtained data
– – – Theoretical fraction of unioinized form

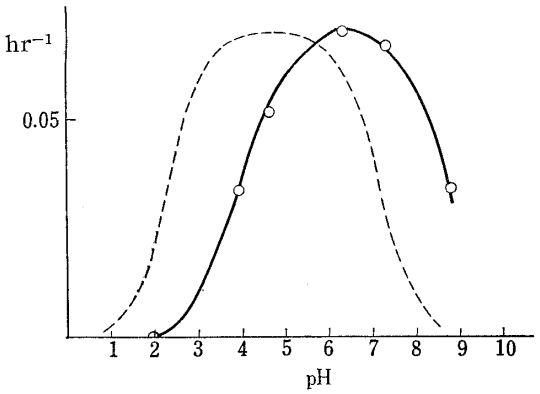

Fig. 4. Absorption Rate *vs.* pH Profile of Sulfamerazine

—○— Represents data obtained from experiments
– – – Theoretical fraction of unionized form

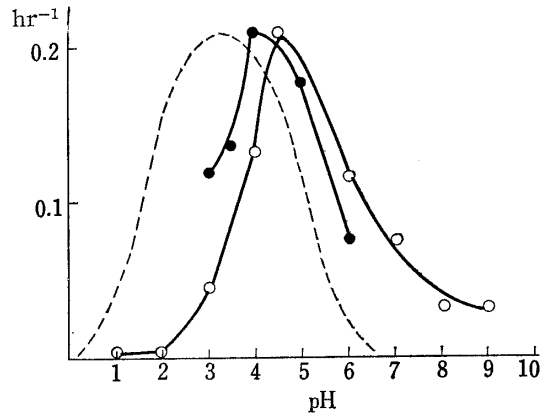

Fig. 5. Absorption Rate *vs.* pH Profile of Sulfisoxazole

—○— Represents data obtained from experiments with regular concentration
—●— With two times concentrated salt solution
– – – Theoretical fraction of unionized form

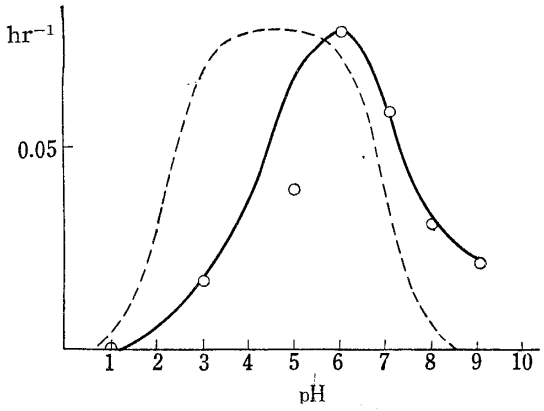

Fig. 6. Absorption Rate *vs.* pH Profile of Sulfamethoxypyridazine

—○— Represents data obtained from experiments
– – – Theoretical fraction of unionized form

TABLE II. p*Ka*'s, Intermediate pH, and pH Values of Drug Solutions used for Absorption Rate Measurement

	Sulfonamides	pKa_1	pKa_2	p*Hi*	pH exp.
1	Sulfanilamide	2.36	10.43	6.4	6.3
2	Sulfanilacetamide	1.78	5.38	3.6	4.5
3	Sulfaguanidine	2.75	12.05	7.4	6.3
4	Sulfapyridine	2.58	8.43	5.5	6.3
5	Sulfadiazine	2.00	6.48	4.2	6.3
6	Sulfamethoxazole	1.76	5.8	3.8	4.5
7	Sulfathiazole	2.36	7.12	4.7	6.3
8	Sulfamerazine	2.26	7.06	4.7	6.3
9	Sulfisoxazole	1.55	5.1	3.3	4.5
10	Sulfamethizole	2.00	5.45	3.7	4.5
11	Sulfisomidine	2.36	7.5	4.9	6.3
12	Sulfamethazine	2.36	7.38	4.9	6.3
13	Sulfamethoxypyridazine	2.06	7.0	4.5	6.3
14	Sulfamonomethoxine	2.0	5.9	4.0	4.5
15	Sulfaethidole	1.93	5.6	3.8	4.5
16	Sulfadimethoxine	2.02	6.7	4.4	6.3
17	Sulfaphenazole	1.9	6.5	4.2	4.5

Determination of Absorption Rate of Unionized Form (*Ku*)

Since difference of pH exists between drug solution and absorption site, drug solution of pH value one unit higher than the pH where fraction of unionized form was calculated to be maximum, was used for determination of *Ku*.

pH values of drug solution, experimentally used to determine *Ku*, were listed in Table II. *Ku*'s obtained are shown in Table III with $Ku\sqrt{M}$ and their reciprocals.

Table III. Molecular Weights and Absorption Rates of Unionized Sulfonamides

	Sulfonamides	M	$\sqrt{M}$	*Ku* (hr^{-1})	$Ku\sqrt{M}$	$(Ku\sqrt{M})^{-1}$
1	Sulfanilamide	172.21	13.1	0.075	0.983	1.0
2	Sulfanilacetamide	214.24	14.6	0.068	0.993	1.0
3	Sulfaguanidine	232.26	15.2	0.010	0.152	6.6
4	Sulfapyridine	249.29	15.7	0.087	1.37	0.73
5	Sulfadiazine	250.28	15.8	0.090	1.42	0.70
6	Sulfamethoxazole	253.30	15.9	0.20	3.18	0.31
7	Sulfathiazole	255.32	16.0	0.061	0.977	1.0
8	Sulfamerazine	264.30	16.2	0.070	1.14	0.88
9	Sulfisoxazole	267.30	16.3	0.21	3.42	0.29
10	Sulfamethizole	270.33	16.4	0.094	1.54	0.65
11	Sulfisomidine	278.34	16.7	0.027	0.45	2.2
12	Sulfamethazine	278.34	16.7	0.14	2.32	0.43
13	Sulfamethoxypyridazine	280.31	16.7	0.079	1.32	0.76
14	Sulfamonomethoxine	280.31	16.7	0.20	3.38	0.30
15	Sulfaethidole	284.36	16.9	0.18	3.04	0.33
16	Sulfadimethoxine	310.34	17.6	0.19	3.34	0.30
17	Sulfaphenazole	314.35	17.7	0.20	3.45	0.29

Partition Coefficients

To select a suitable organic liquid, partition coefficients were determined with four organic solvents, carbon tetrachloride, benzene, chloroform, and isoamyl acetate, according to the classification of Sandell.[8] Partition coefficients obtained are shown in Table IV.

Table IV. Partition Coefficients and their Reciprocals

	Sulfonamide	CCl_4		Benzene		$CHCl_3$		Isoamyl acetate	
		P	P^{-1}	*P*	P^{-1}	*P*	P^{-1}	*P*	P^{-1}
1	Sulfanilamide	0.003	333	0.009	111	0.04	25	0.36	2.78
2	Sulfanilacetamide	0.017	59	0.029	34.5	0.22	4.6	0.87	1.15
3	Sulfaguanidine	0.001	1000	0.034	29.4	0.01	100	0.03	33.3
4	Sulfapyridine	0.006	167	0.176	5.7	1.06	0.94	2.24	0.45
5	Sulfadiazine	0.006	167	0.128	7.8	1.16	0.86	1.54	0.65
6	Sulfamethoxazole	0.032	31	0.641	1.6	3.58	0.28	22.0	0.05
7	Sulfathiazole	0.027	37	0.110	9.1	0.15	6.67	0.52	1.92
8	Sulfamerazine	0.022	46	0.202	5.0	2.40	0.42	2.1	0.48
9	Sulfisoxazole	0.033	30	0.848	1.2	4.40	0.23	22.4	0.05
10	Sulfamethizole	0.015	67	0.017	58.8	0.90	1.11	2.2	0.45
11	Sulfisomidine	0.013	77	0.062	16.1	0.28	3.57	0.40	2.50
12	Sulfamethazine	0.045	22	0.375	2.7	5.40	0.19	3.61	0.28
13	Sulfamethoxypyridazine	0.003	333	0.266	3.8	4.14	0.24	1.31	0.76
14	Sulfamonomethoxine	0.198	5	0.793	1.3	4.26	0.23	14.7	0.07
15	Sulfaethidole	0.054	19	0.220	4.6	3.12	0.32	8.0	0.13
16	Sulfadimethoxine	0.234	4	5.03	0.2	30.6	0.03	77.8	0.01
17	Sulfaphenazole	0.148	7	4.31	0.2	25.4	0.03	87.9	0.01

8) K.B. Sandell: Monat. Chem., **89**, 36 (1958).

Discussion

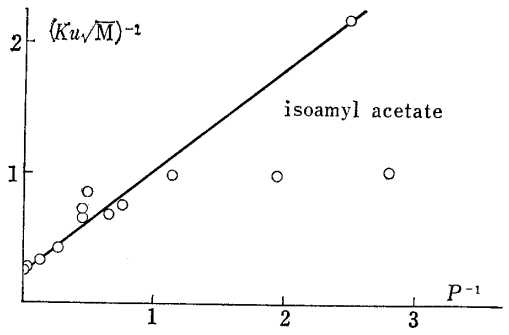

Fig. 7. Plot of $(Ku\sqrt{M})^{-1}$ against Reciprocal of Partition Coefficient between Isoamyl Acetate and Water

Reciprocal of $\sqrt{M}Ku$ were plotted against reciprocal of partition coefficients between isoamyl acetate and water, and Fig. 7 was obtained.

An examination of Fig. 7 shows that good linearity exists among the spots, though some of the spots are scattered in higher $1/P$ region.

This indicates that the model proposed accounts for the mechanism that has the significance on the partition to and diffusion through the barrier, of sulfonamides. Of sulfanilamide, sulfaguanidine, and sulfathiazole which have smaller molecular weight and lower partition coefficient, spots drop far from the line of the figure, showing the possibility of another mechanism of transport: simple diffusion through water channel, for example.

a and *b* values calculated from the results are shown in Table V. *a* represents the difference of *E* values between barrier lipoid and the liquid used, and that is the correction factor for using partition coefficient between organic liquid and water instead of partition coefficient between barrier lipoid and gastric juice. *b*, which is the maximum rate of absorption of sulfonamide multiplied by the square root of the molecular weight of the drug, is around 4. Diffusion is the rate limitting step, and absorption rate of unionized sulfonamides never exceeds the value of $4/\sqrt{\text{mol. weight}}$ $\text{hr},^{-1}$ as far as the mechanism proposed is available.

Table V. *a* and *b* Values calculated from the Data obtained

	a	*b*		*a*	*b*
Isoamyl acetate	0.30	4.3	Benzene	2.0	4.3
Chloroform	0.46	4.0	Carbon tetrachloride	*a*)	*a*)

a) Spots are scattered around.

Summary

Kinetical mechanism of absorption of sulfonamides through the lipoid barrier is described. Assuming permeation of the drug through the barrier to be governed by three processes, namely, 1) transfer of a drug from the solution to the membrane surface, 2) transfer back to the solution from the surface, and 3) diffusion into the barrier, relationship of absorption rate and partition coefficient are obtained. And that was supported by the experimental data obtained.

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