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Studies on Absorption of Drugs. VIII.[1] Physicochemical Factors affecting the Absorption of Sulfonamides from the Rat Small Intestine

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The absorption of sulfonamides through the rat intestine was studies with an *in situ* perfusion method employing pH adjusted isotonic saline solutions. The absorption was expressed as the absorbed amounts in one hour and first-order rate constants in terms of the unionized and anionic forms of the drugs. Regression analysis following Hansch, *et al.* were carried out to correlate the intestinal absorption of the drugs with their physicochemical parameters. Physicochemical parameters, such as partition coefficients from chloroform, *n*-octyl alcohol and lecithin contained chloroform, adsorption to the rat blood, adsorption to activated charcoal, molecular weight and dissociation constant, were used. The intestinal absorption of sulfonamides was satisfactorily predicted with the use of equations of the regression analysis.

One of the complicated problems for the scientist engaged in the design of new drugs and new preparations is to predict their biological and therapeutical activities. The therapeutical activity of a drug depends on the drug's concentration in the blood plasma. The concentration is subjected to the pharmacokinetic parameters, *e.g.*, the rate of absorption, distribution, elimination and metabolism. Thus, pharmacokinetic information is important to the design of a drug as well as the planning of dosage regimens.

It has been well established that the biological activity of drugs was subjected to their physicochemical properties.[3] The pharmacokinetic parameters of drugs have been considered to be influenced by physicochemical properties.

Hansch, *et al.*[4,5] have introduced a new method for the analysis of correlation between the biological activity of a series of variously substituted compounds and the substituent constant π, which is a free-energy related parameter used in evaluating the hydrophobic bonding ability of the substituent, and the electronic constant, such as Hammett's sigma factor.

Previously, we have analyzed the inhibitory activity of sulfonamide derivatives for carbonic anhydrase[6] and the bacteriostatic activity of sulfonamides[7] in reference to the physicochemical properties of drugs.

Fujita and Yamazaki[8] have shown that the logarithmic values of the rate of metabolism and renal excretion of bacteriostatic sulfonamides were nicely correlated by linear combina-

1) Part VII: K. Kitao, K. Kubo, T. Morishita, N. Yata and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), in press.
2) Location: *Toneyama, Toyonaka, Osaka.*
3) E.J. Ariëns, "Drug Design," Vol. I, edited by E.J. Ariëns, Academic Press, New York, 1971, pp. 101—162.
4) C. Hansch, "Physico-chemical Aspects of Drug Action," edited by E.J. Ariëns, Pergamon Press, Oxford, 1968, pp. 141—167.
5) C. Hansch, "Drug Design," Vol. I, edited by E.J. Ariëns, Academic Press, New York, 1971, pp. 271—342.
6) N. Kakeya, N. Yata, A. Kamada and M. Aoki, *Chem. Pharm. Bull.* (Tokyo), **17**, 2558 (1969); *idem*, *ibid.*, **18**, 191 (1970).
7) *a*) M. Yamazaki, N. Kakeya, T. Morishita, A. Kamada and M. Aoki, *Chem. Pharm. Bull.* (Tokyo), **18**, 702 (1970); *b*) *Idem*, *ibid.*, **18**, 708 (1970).
8) T. Fujita and M. Yamazaki, "Absorption, Metabolism and Excretion of Drugs," edited by K. Kakemi, Hirokawa Publishing Company, Tokyo, 1971, pp. 283—300.

tion of hydrophobicity constant π and electronegativity parameter ΔpK_a, using the results of an intravenous administration of sulfonamides to rats and rabbits.[9]

Most sulfonamides are usually administered by the oral route with a multiple dosage regimen to secure the plasma level above a minimal effective concentration for a certain period. The pharmacokinetic parameters of sulfonamides for a single oral administration are expediently considered to be available for those of multiple oral administration.[10]

Brodie, *et al.*[11] introduced a pH-partition hypothesis from their studies that most drugs are absorbed from the gastrointestinal tract by a passive diffusion process of unionized molecules across a lipoid barrier and the rate of absorption is related to the partitioning property of molecules between oil and water.

Many workers have studied the mechanism of the gastrointestinal absorption of drugs on the basis of the lipophilic property of their unionized form.

It was reported[12,13] that the amount of drug absorbed (A) through the rat intestine in a certain time from an isotonic perfusion solution with a certain pH was represented by Eq. 1,

$$A = R_m M_0 + R_i I_0 \quad (1)$$

where, M_0 and I_0 are the amount of a drug initially present in the perfusion solution in the unionized and ionized forms, respectively; R_m and R_i are the absorption ratios of unionized and ionized forms, respectively, which are the characteristic values of a drug under the experimental conditions. Eq. 1 was introduced on an assumption that the unionized and ionized forms were passively absorbed independently through the rat intestine. The absorption of sulfonamides in the anionic form was found to be significant and the pH-partition hypothesis should be modified in terms of the absorption of the ionized form.

Presently, the absorption of 15 antibacterial sulfonamides through the rat intestine was measured following an *in situ* perfusion method employing isotonic saline solution adjusted at a certain pH value from 4.0 to 8.0 with HCl or NaOH during the experiments. The rate of absorption in terms of the unionized or anionic form was correlated to the physicochemical parameter of drugs following a regression analysis of Hansch's method. A study was made to confirm a possibility that the pharmacokinetic parameters as well as the therapeutical activity of drugs could be predicted with the physicochemical parameters of drugs. Thus, the intestinal absorption of sulfonamides from perfusing solution of various pH values was estimated employing the results of a structure-activity analysis for the absorption of unionized and anionic forms.

Result and Discussion

Physicochemical Parameters

Physicochemical parameters for 15 sulfonamides were presented in Table I and II as well as drugs' abbreviations. Some of the parameters were already reported in the previous report.[7b] For the sake of verification, the values obtained were presently remeasured.

Partition Coefficient——Partition coefficient of sulfonamides have been reported by some workers employing carbon tetrachloride, benzene,[14] chloroform[14,15] and *n*-octyl alcohol[7b]

9) M. Yamazaki, M. Aoki and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **16**, 707, 721 (1968).
10) E. Krüger-Thiemer, "Physico-chemical Aspects of Drug Action," edited by E.J. Ariëns, Pergamon Press, Oxford, 1968, pp. 63—113.
11) L.S. Schanker, D.J. Tocco, B.B. Brodie and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **123**, 81 (1958); C.A.M. Hogben, D.J. Tocco, B.B. Brodie and L.S. Schanker, *J. Pharmacol. Exptl. Therap.*, **125**, 275 (1959).
12) T. Morishita, N. Yata and A. Kamada, *Yakuzaigaku*, **31**, 187 (1971).
13) T. Morishita, N. Yata, A. Kamada and M. Aoki (the late), *Chem. Pharm. Bull.* (Tokyo), **19**, 1925 (1971).
14) T. Koizumi, T. Arita and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 413 (1964).
15) M. Yamazaki, M. Aoki, A. Kamada and N. Yata, *Yakuzaigaku*, **27**, 37 (1967).

Table I. Abbreviation, Acid Dissociation Constant and Molecular Weight of Sulfonamides

No.	Substance	Abbr.	pK_a	M.W.
1	sulfanilamide	S	10.45	172.21
2	sulfacetamide	SA	5.40	214.24
3	sulfapyridine	SP	8.75	249.29
4	sulfathiazole	ST	7.10	255.32
5	sulfadiazine	SD	6.15	250.28
6	sulfamerazine	SM	6.93	264.30
7	xyloylsulfamine	XS	4.72	304.36
8	sulfisoxazole	SIX	4.62	267.30
9	sulfisomidine	SIM	7.38	278.83
10	sulfamethizole	SMT	5.32	270.33
11	sulfaphenazole	SPZ	5.91	314.35
12	sulfamethoxypyridazine	SMP	7.05	280.32
13	sulfadimethoxine	SDM	6.05	310.33
14	sulfamethoxazole	SMX	5.81	253.29
15	sulfamonomethoxide	SMM	6.03	280.21

Table II. Physicochemical Parameters of Unionized and Ionized Sulfonamides

Substance	log P_c[a]	log P_o[b]	$\log\frac{\beta}{100-\beta}$[c]	$\log\left(\frac{\gamma}{100-\gamma}\right)_m$[d]	$\log\left(\frac{\gamma}{100-\gamma}\right)_i$[e]	log $P_{c\text{-}L}$[f]	log k_{perm}[g]
Sulfanilamide	−1.390	−0.719	−0.935	−0.467	—	—	—
Sulfacetamide	−0.583	−0.959	−0.989	−0.233	−1.003	−2.230	−0.087
Sulfapyridine	0.041	−0.075	−0.665	0.156	—	—	—
Sulfathiazole	−0.730	0.006	−0.320	0.342	−0.011	−1.148	−0.064
Sulfadiazine	0.174	−0.092	−0.720	0.135	−0.854	−1.073	−0.055
Sulfamerazine	0.458	0.154	−0.527	0.323	−0.369	−1.255	−0.095
Xyloylsulfamine	2.114	2.186	−0.019	1.297	−0.043	−0.414	−0.164
Sulfisoxazole	0.738	0.937	−0.203	0.130	−0.724	−1.486	−0.045
Sulfisomidine	−0.542	−0.428	−0.421	0.172	−0.153	−1.751	−0.153
Sulfamethizole	−0.025	0.256	−0.567	0.450	−0.401	−1.466	−0.030
Sulfaphenazole	1.462	1.517	−0.092	0.811	−0.132	−0.285	−0.210
Sulfamethoxypyridazine	0.626	0.315	−0.132	0.411	−0.019	−0.713	−0.233
Sulfadimethoxine	1.688	1.496	0.092	0.973	−0.015	0.056	−0.223
Sulfamethoxazole	0.560	0.890	−0.282	0.339	−0.584	−1.018	−0.045
Sulfamonomethoxine	0.731	0.761	−0.087	0.618	−0.237	−0.879	−0.133

a) Partition coefficient of the unionized form between chloroform and water.
b) Partition coefficient of the unionized form between *n*-octyl alcohol and water.
c) Adsorption to the rat blood at pH 7.4.
d) Adsorption of the unionized form to activated charcoal.
e) Adsorption of the anionic form to activated charcoal.
f) Partition coefficient of the anionic form between lecithin-chloroform and water.
g) Permeation constant of the anionic form through cellophane membrane.

as oil phase. Among these and others, chloroform was recommended by Rieder[16] as a model of lipoid barrier in studying the bacteriostatic activity of sulfonamides. *n*-Octyl alcohol has been frequently used for the structure-activity study of many drugs following a study of Hansch, *et al.*[17] Presently, partition coefficients of sulfonamides were measured at a pH where only the unionized form was present in water phase employing chloroform or *n*-octyl alcohol as oil phase. The oil/water partition coefficient of a drug is subjected to the drug's relative affinity for oil and water phases.

Koizumi, *et al.*[14] found a good correlationship of patrition coefficients of sulfonamides between isoamyl acetate/water and chloroform/water. In general, a better agreement bet-

16) J. Rieder, *Arzneim.-Forsch.*, **13**, 81 (1963).
17) T. Fujita, J. Iwasa and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964).

ween similar solvents is likely to be observed than between dissimilar solvents, which is studied by Hansch[18] for the correlation of partition coefficients from two different solvent systems. The correlation of partition coefficients between chloroform and *n*-octyl alcohol in the present study is expressed by Eq. 2,

$$\log P_o = 0.805 \log P_c + 0.117 \quad (n=15,\ r=0.907) \tag{2}$$

where P_o and P_c are the partition coefficients from *n*-octyl alcohol and chloroform, respectively. n and r are the number of sulfonamides used and the correlation coefficient, respectively.

The partition coefficients were also compared with Koizumi's results[14] of benzene ($r_o=0.895$, $r_c=0.839$), isoamyl acetate ($r_o=0.900$, $r_c=0.932$) and carbon tetrachloride ($r_o=0.695$, $r_c=0.746$); where r_o and r_c are the correlation coefficients for *n*-octyl alcohol and chloroform, respectively. Chloroform and *n*-octyl alcohol are considered to be closely related solvents for the partitioning of sulfonamides as shown by a slope which is approximately unity.

Binding to Protein——In the previous report,[7b] good correlation was obtained for protein binding of sulfonamides in any couple of human plasma, rabbit blood and its plasma; the binding was enhanced with an increase of drug's molecular weight. Presently, a similar analysis was made with rat blood,

$$\beta_{\text{rat blood}} = 0.448\beta_{\text{human plasma}} + 6.106 \quad (n=14,\ r=0.950) \tag{3}$$

$$\beta_{\text{rat blood}} = 0.410\beta_{\text{rabbit plasma}} + 4.420 \quad (n=14,\ r=0.836) \tag{4}$$

$$\beta_{\text{rat blood}} = 0.516\beta_{\text{rabbit blood}} + 2.430 \quad (n=14,\ r=0.864) \tag{5}$$

$$\log \beta/(100-\beta)_{\text{rat blood}} = 0.0079\,\text{M.W.} - 2.481 \quad (n=14,\ r=0.863) \tag{6}$$

where, β is the binding of sulfonamides defined in the previous report[7b] and M.W. is the molecular weight of sulfonamides.

Adsorption to Activated Charcoal——Nogami, *et al.*[19] reported relation between the adsorption of sulfonamides to carbon black and their partition coefficients, binding to bovine serum albumin and intestinal absorption rate. Kakemi, *et al.*[20–22] found that the absorption rates of drugs from the rat small intestine are closely related to their adsorption to the intestinal mucosa. Thus, it may be considered that the adsorbing ability of a drug is important to their gastrointestinal absorption as well as their partitioning ability to the lipoid moiety of the intestinal tract. To confirm the possibility as a parameter for the gastrointestinal absorption study, the adsorption of sulfonamides to activated charcoal was measured for unionized and ionized froms. Good correlation was obtained for the partition coefficient with the adsorption of the unionized form but not with that of anionic form,

$$\log \gamma/(100-\gamma)_m = 0.454 \log P_o + 0.175 \quad (n=15,\ r=0.899) \tag{7}$$

$$\log \gamma/(100-\gamma)_m = 0.413 \log P_c + 0.217 \quad (n=15,\ r=0.886) \tag{8}$$

18) C. Hansch, "Drug Design," Vol. I, edited by E.J. Ariëns, Academic Press, New York, 1971, p. 279.
19) H. Nogami, T. Nagai and S. Wada, *Chem. Pharm. Bull.* (Tokyo), **18**, 342 (1970).
20) K. Kakemi, T. Arita, R. Hori and R. Konishi, *Chem. Pharm. Bull.* (Tokyo), **15**, 1883 (1967).
21) K. Kakemi, T. Arita, R. Hori, R. Konishi and K. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **17**, 248 (1969).
22) K. Kakemi, T. Arita, R. Hori, R. Konishi, K. Nishimura, H. Matsui and T. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **17**, 255 (1969).

$$\log \gamma/(100-\gamma)_i = 0.190 \log P_o - 0.454 \quad (9)$$
$$(n=13,\ r=0.480)$$
$$\log \gamma/(100-\gamma)_i = 0.146 \log P_c - 0.424 \quad (10)$$
$$(n=13,\ r=0.373)$$

where, γ is the adsorption of sulfonamides to activated charcoal and m and i denote the unionized and ionized forms, respectively. The present finding suggests that the adsorption of unionized molecules is subjected to their hydrophobic property.

Partition of Anionic Sulfonamides——Furusawa, *et al.*[23] reported that a migration of anionic drug molecules from water to chloroform is enhanced by the addition of lecithin to chloroform, while cations and unionized molecules remained uninfluenced. We have already reported that four sulfonamide anions were absorbed through the rat intestine, with the mechanism left unclarified.[13] Aoki, *et al.*[24] reported the absorption of the ionized form of drugs and Kakemi, *et al.*[22] also reported the similar redults. To study the structure-activity relationship in the absorption of anionic sulfonamides, their chloroform–water distribution in the presence of egg lecithin in oil phase was measured. The results were compared with those without lecithin,

$$\log P_{c\text{-lecithin}} = 0.782 P_c - 0.038 \quad (11)$$
$$(n=5,\ r=0.993)$$

where, $P_{c\text{-lecithin}}$ is the partition coefficient of the unionized sulfonamides between lecithin-chloroform and water.

Dissociation constants of sulfonamides and their solubility to chloroform and to water reported by Yamazaki, *et al.*[15] were presently adopted.

Absorption through the Rat Intestine

Previously,[13] it was reported that rat intestinal absorption of four sulfonamides from perfusing saline solutions, which were adjusted at a certain pH beteeen 4.0—8.0 with the addition of 0.1N HCl or 0.1N NaOH during absorption experiments was expressed by Eq. 1. R_m and R_i for 15 sulfonamides were calculated employing Eq. 1 and pK_a's (Table I). The present results of (A) at pH 6.5 were found to correlate with Kimura's results[25] at pH 6.5 with a correlation coefficient of 0.969.

Sulfonamides in perfusing solution are absorbed through the rat intestine following a passive transfer process. Thus, R_m and R_i are expressed by Eq. 12 and 13,

$$k_m = -\ln (1-R_m) \quad (12)$$
$$k_i = -\ln (1-R_i) \quad (13)$$

where k_m and k_i are the first-order absorption rate constants of unionized and anionic forms, respectively. Here, R_m and R_i are the values in an hour experiment, thus, the unit of k_m and k_i is hr^{-1} (Table III). It is to be noted that the absorption of ionized form can not be ignored. Thus, a pH-partition hypothesis developed by Brodie, *et al.*[11] should be reconsidered since they asserted that a lipoid membrane of intestine allows the passage of drugs in their undissociated form. Kakemi, *et al.*[22] found some discrepancies from the pH-partition theory in their experiment with many drugs and interpreted the intestinal absorption of ionized form on the basis of the binding process in absorption.

23) S. Furusawa, K. Okumura and H. Sezaki, *J. Pharm. Pharmacol.*, **24**, 272 (1972).
24) M. Aoki, A. Kamada, N. Yata, K. Kishimoto, C. Mugino, H. Sakaguchi, K. Tanabe and I. Mimura, *Chem. Pharm. Bull.* (Tokyo), **17**, 1109 (1969).
25) T. Kimura, K. Inui and H. Sezaki, *Yakuzaigaku*, **31**, 167 (1971).

Table III. Absorption Ratio and Absorption Rate Constant of Unionized and Ionized Sulfonamides

Substance	R_m	R_i	k_m	k_i
Sulfanilamide	0.306	—[a]	0.365 hr^{-1}	— hr^{-1}
Sulfacetamide	0.111	0.103	0.118	0.109
Sulfapyridine	0.428	—[a]	0.559	—
Sulfathiazole	0.178	0.180	0.196	0.198
Sulfadiazine	0.385	0.252	0.486	0.290
Sulfamerazine	0.402	0.260	0.514	0.301
Xyloylsulfamine	0.654	0.474	1.061	0.642
Sulfisoxazole	0.508	0.367	0.709	0.457
Sulfisomidine	0.164	0.137	0.179	0.147
Sulfamethizole	0.235	0.155	0.268	0.168
Sulfaphenazole	0.741	0.466	1.351	0.627
Sulfamethoxypyridazine	0.495	0.393	0.683	0.499
Sulfadimethoxine	0.715	0.573	1.255	0.851
Sulfamethoxazole	0.709	0.533	1.234	0.761
Sulfamonomethoxine	0.683	0.523	1.149	0.740

a) These values were not obtained because of their large pK_a.

Absorption of Unionized Form

Previously,[13] it was reported that the intestinal absorption of sulfonamide is markedly influenced by buffer components and pH adjusted isotonic saline solution is recommended for the absorption study. Koizumi, *et al.*[26] studied the rat intestinal absorption of unionized sulfonamides with an *in situ* perfusion method employing buffer solutions. Good correlation was obtained between the present results employing pH adjusted isotonic saline solution and Koizumi's results with a correlation coefficient of 0.918 (Fig. 1).

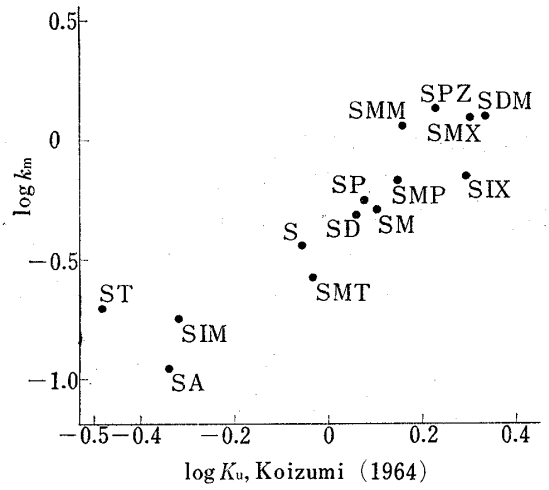

Fig. 1. Relationship between Absorption Rate Constants of the Unionized Form and Those of Koizumi (1964)

It has been well established that the partition coefficient of a drug is one of the important parameters for the permeation of the drug through the live membrane.[27,28] The absorption of the unionized form increased with an increase of the partition coefficients from chlorofoem and *n*-octyl alcohol (Fig. 2 and 3). Poor correlation was observed between the absorption and the partition coefficient[14] from carbon tetrachloride (r=0.508). But good correlation was reported between the intestinal absorption of barbiturates and their partition coefficient of carbon tetrachloride/isotonic buffer.[20] Thus, it may be considered that an adequate organic solvent is important for the study of partition coefficient.

Nogami, *et al.*[19] have studied a relation between the adsorption of sulfonamides to carbon black and their intestinal absorption with a conclusion that the hydrophobic interaction of the drugs with the intestinal mucosa is one of the important parameters for absorption.

26) T. Koizumi, T. Arita and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 421 (1964).
27) R. Collander, *Trans. Faraday Soc.*, 33, 985 (1937).
28) G.A.J. van Os, "Molecular Pharmacology," edited by E.J. Ariëns, Academic Press, New York, 1964, p. 8.

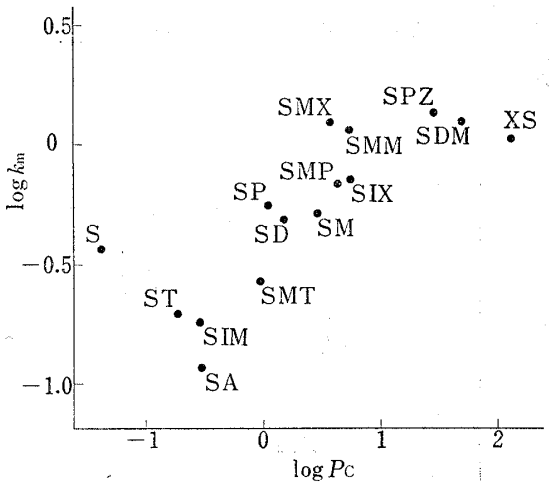

Fig. 2. Relationship between Absorption of the Unionized Form and Partition Coefficient (Chloroform/Acetate Buffer)

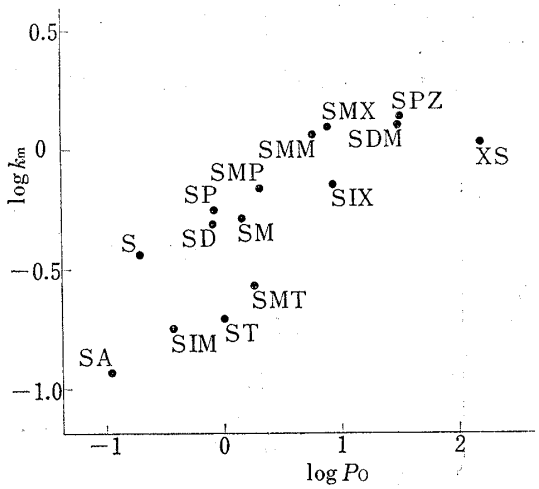

Fig. 3. Relationship between Absorption of the Unionized Form and Partition Coefficient (*n*-Octyl Alcohol/Acetate Buffer)

In the present study, correlation of the absorption of the unionized form with the adsorption to activated charcoal was not clear, but absorption was likely to increase with an increase of adsorption. It seems that the absorption of unionized sulfonamides can not be explained on the basis of their hydrophobic interaction alone.

Absorption of Anionic Form

Brodie, *et al.*[11] introduced a pH-partition hypothesis for the gastrointestinal absorption of drugs on the basis of an experimental result that only unionized molecules were permeated through the mucosal membrane. Recently, many workers have reported the contribution of ionized molecules to the absorption.[22,24] The absorption of ionized molecules through a passive diffusion process has been suggested on the basis of ion-pair absorption by Higuchi,[29] and supported on the basis of adsorption to the intestine, enhanced migration to chloroform in the presence of lecithin.[23]

Presently, the intestinal absorption of anionic sulfonamides was studied in reference to their physicochemical properties. Water-soluble substances of small molecular size are considered to be absorbed by a simple diffusion process through water filled pores of the intestine. Thus, the ion size of sulfonamides and their electric charge are important for a simple diffusion. No marked correlations were found between the absorption of the anionic form and the molecular weight of sulfonamides and the permeation of the anionic form through cellophane membrane.

Recently, Furusawa, *et al.*[23] reported that the anionic form of many drugs easily migrated from aqueous phase to chloroform with the addition of lecithin to chloroform.

A major constituent of the live membrane is lipid in which phospholipids are contained 60—90%.[30] Lipid in the membrane of the rat liver mitochondria contains 42% lecithin.[31] Lecithin is contained in the lipids of natural origins and possibly in the intestinal membrane.

29) T. Higuchi, Thermodynamics, Kinetics and Mechanisms of Interface Transport of Organic Ammonium Species, seminar presented at The Upjohn Company, July 12, 1967 from J.G. Wagner, "Biopharmaceutics and Relevant Pharmacokinetics," Drug Intelligence Publications, Illinois, 1971, p. 28.
30) R. Sato, "Seitaimaku no Seikagaku," eds. by T. Oda, R. Sato and M. Nokano, Asakura, Tokyo, 1969, p. 16.
31) M. Kates, *Adv. Lipid Res.*, 2, 17 (1964).

Thus, a study was made on the partitioning of sulfonamides between water and lecithin-chloroform. A close linear correlation was found in 13 sulfonamides excluding sulfanilamide and sulfapyridine owing to their large pK_a (Fig. 4).

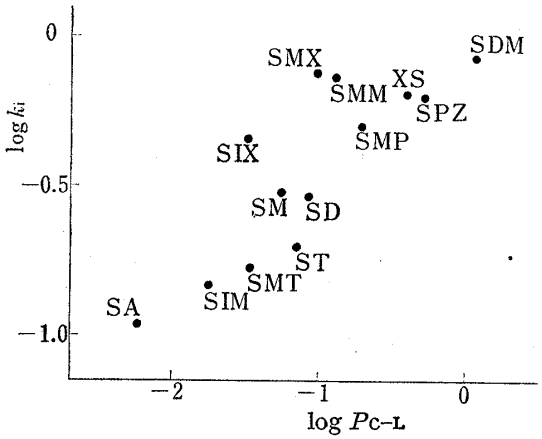

Fig. 4. Relationship between Absorption of the Anionic Form and Partition Coefficient (Lecithin-chloroform/Ammonium Buffer)

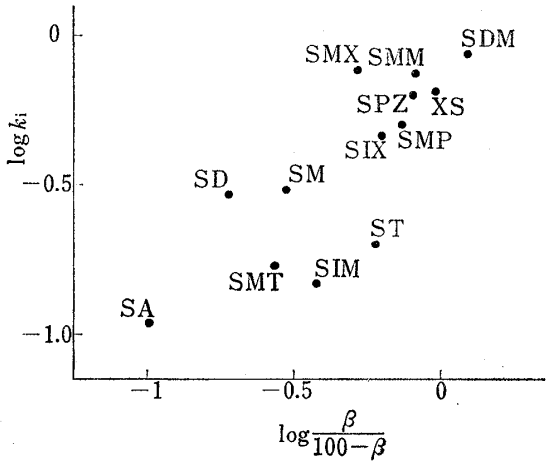

Fig. 5. Relationship between Absorption of the Anionic Form and Adsorption of the Rat Blood at pH 7.4

It was suggested that the absorption of ionized drugs was closely correlated with their adsorption to the intestinal membrane.[22] Thus, the absorption of the anionic form was compared with the adsorption to the rat blood at pH 7.4 (Fig. 5). A similar comparison was made with the adsorption of the anionic form to activated charcoal resulting in insufficient correlation coefficients.

Relationship between Physicochemical Parameters and Intestinal Absorption

Hansch, *et al.*[4,5] have introduced a method to correlate physicochemical parameters of drugs to their biological activity. Previously,[6,7] it was reported that the carbonic anhydrase inhibitory and bacteriostatic activities of sulfonamide derivatives were subjected to the drugs' physicochemical properties. Lien[32,33] reported that absorption of acidic and basic drugs through the rat intestine, buccal membrane and skin was markedly influenced by the partition coefficient and dissociation constant.

Presently, the absorption of unionized and anionic sulfonamides was correlated with the drugs' physicochemical parameters on the basis of Hansch's method for the analysis of a structure-activity relationships.

Absorption of Unionized Form——A linear correlationship of 15 sulfonamides with one of their physicochemical parameters was presented by Eq. 14—18 (Table IV).

A remarked correlation was obtained with partition coefficients from *n*-octyl alcohol and chloroform.

A two-parameter analysis employing a square term of the partition coefficients revealed that the addition of square term did not influence significantly on the results (Eq. 19 and 20).

Yamazaki, *et al.*[7b] reported that the partition coefficient of sulfonamides from chloroform was calculated with their solubilities to chloroform and water. A comparison of Eq. 21 with Eq. 14 revealed that the solubilities to chloroform and water was sufficient for the present analysis.

32) E.J. Lien, *Drug Intel. Clin. Pharm.*, 4, 7 (1970).
33) E.J. Lien, R.T. Koda and G.L. Tong, *Drug Intel. Clin. Pharm.*, 5, 38 (1971).

Table IV. Linear and Nonlinear Relationships between Absorption Rate Constant of the Unionized Form and Physicochemical Parameters[a]
$\log k_m = a + bX + cY + dZ$

a	b	X	c	Y	d	Z	r	s	Eq.
−0.380	0.288	$\log P_c$					0.803	0.211	(14)
−0.410	0.319	$\log P_o$					0.823	0.201	(15)
0.026	0.841	$\log \frac{\beta}{100-\beta}$					0.740	0.238	(16)
−0.455	0.489	$\log \left(\frac{\gamma}{100-\gamma}\right)_m$					0.637	0.273	(17)
−7.254	2.890	log MW					0.577	0.289	(18)
−0.376	0.292	$\log P_c$	−0.005	$(\log P_c)^2$			0.803	0.219	(19)
−0.372	0.426	$\log P_o$	−0.092	$(\log P_o)^2$			0.854	0.191	(20)
−0.046	0.368	$\log S_c$**[b]	0.230	$\log 1/S_w$**			0.829	0.206	(21)
−0.305	−0.394	$\log \left(\frac{\gamma}{100-\gamma}\right)_m$	0.598	$\log P_o$	−0.087	$(\log P_o)^2$	0.883	0.180	(22)
2.785	−1.317	log MW	0.530	$\log P_o$	−0.115	$(\log P_o)^2$	0.869	0.190	(23)
−0.018	0.366	$\log P_c$*	0.582	$\log \frac{\beta}{100-\beta}$ **	−0.494	$\log \left(\frac{\gamma}{100-\gamma}\right)_m$ ***	0.888	0.177	(24)
8.096	−3.357	log MW**	0.594	$\log S_c$*	0.408	$\log 1/S_w$*	0.888	0.177	(25)

a) Fifteen sulfonamides are used for the analysis.
b) One asterisk indicates significance at the 1% level, two asterisks at the 5% level and three asterisks at the 10% level.

Analysis was made employing the partition coefficient from *n*-octyl alcohol and the adsorption to activated charcoal or the drugs' molecular weight resulting in an improvement on the correlation coefficient (Eq. 22 and 23). But the terms of adsorption and molecular weight in the respective equations were justified at less than the 0.90 level of significance ($F_{1,11}$= 2.55 and 1.16, $F_{1,11,0.1}$=3.23). A three-parameter analysis employing $\log P_c$ and the adsorptions to activated charcoal and rat blood resulted in a marked imporvement on the correlation coefficient (Eq. 24) with good significance levels ($\log P_c$, $F_{1,11}$=11.5, $F_{1,11,0.01}$=9.65; $\log \beta/100-\beta$, $F_{1,11}$=6.10, $F_{1,11,0.05}$=4.84; $\log (\gamma/100-\gamma)_m$, $F_{1,11}$=3.95, $F_{1,11,0.10}$=3.23). It was interesting that the correlation coefficients in any couple of the three parameters were around 0.8. Thus, it is considered that the intestinal absorption of unionized sulfonamides is subjected

Table V. Linear and Nonlinear Relationships between Absorption Rate Constant of the Unionized Form and Physicochemical Parameters[a]
$\log k_m = a + bX + cY + dZ$

a	b	X	c	Y	d	Z	r	s	Eq.
−0.437	0.356	$\log P_c$					0.863	0.184	(26)
−0.438	0.346	$\log P_o$					0.839	0.199	(27)
0.029	0.835	$\log \frac{\beta}{100-\beta}$					0.737	0.247	(28)
−0.519	0.598	$\log \left(\frac{\gamma}{100-\gamma}\right)_m$					0.671	0.270	(29)
−12.672	5.112	log MW					0.707	0.258	(30)
−0.389	0.568	$\log P_c$	−0.167	$(\log P_c)^2$*[b]			0.936	0.134	(31)
−0.400	0.511	$\log P_o$	−0.130	$(\log P_o)^2$**			0.893	0.172	(32)
−0.209	0.421	$\log S_c$*	0.317	$\log 1/S_w$*			0.907	0.161	(33)
−0.243	0.299	$\log \frac{\beta}{100-\beta}$ ***	0.496	$\log P_c$	−0.174	$(\log P_c)^2$*	0.952	0.122	(34)
−0.393	0.233	$\log P_o$***	0.382	$\log P_c$	−0.191	$(\log P_c)^2$*	0.956	0.117	(35)
−0.396	0.383	$\log P_c$*	0.204	$\log P_o$	−0.170	$(\log P_o)^2$*	0.949	0.126	(36)

a) Fourteen sulfonamides are used for the analysis excluding sulfanilamide.
b) One asterisk indicates significance at the 1% level, two asterisks at the 5% level and three asterisks at the 10% level.

to the partitioning property as well as the adsorbing ability to the intestinal membrane with complex mechanisms.

It was revealed that sulfanilamide, a parent substance of sulfonamide derivatives, had a large absorption rate unexplainable from its partition coefficient (Fig. 2). A similar unusually rapid urinary excretion was reported by Yamazaki, *et al.*[9] A small molecular weight may be considered to be one of the reasons reflecting a small hydrophobicity and a rapid diffusivity in the intestine.

Presently, a correlation analysis was made excluding sulfanilamide (Table V).

It is noteworthy that the coefficients in Eq. 26 and 31 of chloroform are similar to those of *n*-octyl alcohol in the respective Eq. 27 and 32.

Eq. 35 and 36 were obtained employing the partition coefficients from chloroform and *n*-octyl alcohol with marked improvement on the correlation coefficients.

It may be suggested that better results can be obtained with the use of partition coefficients from several organic solvents or mixed solvents for a regression analysis.

The calculated values of log k_m are presented in Table VI as well as the experimental ones.

Table VI. Observed and Calculated Absorption Rate Constants of Unionized Sulfonamides

Compd. No.	log k_m obsd.	log k_m Calcd.						
		Eq. 15	Eq. 21	Eq. 24	Eq. 25	Eq. 31	Eq. 34	Eq. 35
1	−0.437	−0.640	−0.817	−0.571	−0.611	—	—	—
2	−0.929	−0.716	−0.609	−0.681	−0.587	−0.755	−0.867	−0.886
3	−0.253	−0.434	−0.320	−0.467	−0.298	−0.366	−0.421	−0.395
4	−0.708	−0.408	−0.610	−0.640	−0.616	−0.892	−0.793	−0.772
5	−0.313	−0.439	−0.374	−0.440	−0.354	−0.295	−0.377	−0.354
6	−0.289	−0.361	−0.227	−0.317	−0.215	−0.164	−0.209	−0.223
7	0.026	0.288	0.171	0.103	0.268	0.067	0.024	0.068
8	−0.149	−0.111	−0.227	0.069	−0.227	−0.061	−0.032	0.003
9	−0.747	−0.547	−0.521	−0.546	−0.795	−0.746	−0.689	−0.756
10	−0.572	−0.328	−0.377	−0.579	−0.091	−0.403	−0.425	−0.343
11	0.131	0.075	0.004	0.062	−0.109	0.085	0.084	0.110
12	−0.165	−0.309	−0.097	−0.069	0.149	−0.099	−0.040	−0.155
13	0.099	0.068	0.124	0.172	−0.116	0.095	0.128	0.055
14	0.091	−0.126	−0.194	−0.145	−0.043	−0.123	−0.104	−0.032
15	0.060	−0.167	−0.082	−0.107	−0.509	−0.063	0.001	−0.039

Absorption of Anionic Form——Sulfonamides ionize in the cationic and anionic forms being subjected to their pK_a values and the pH values of solutions. Generally, sulfonamides ionize in the cationic form in gastric juice and in the anionic in intestinal fluid. Thus, to consider the contribution of ionization to the intestinal absorption, the anionic form is sufficient for the present study.

The absorption rate constants of the anionic form of 13 sulfonamides were correlated with physicochemical parameters employing a single-parameter analysis (Table VII).

The absorption rate constants were found to correlate with partitioning ability of the anionic form between lecithin-chloroform and water (Eq. 37) and binding of drugs to the rat blood at pH 7.4 (Eq. 38), but not with adsorption of the anionic form on activated charcoal, molecular weight of the drugs and the rate of permeation of the anionic form through the cellophane membrane.

Comparing Eq. 38 with Eq. 16, it was found that binding to the rat blood was closely correlated with the absorption of the anionic form but not with that of the unionized form.

TABLE VII. Linear and Nonlinear Relationships between Absorption Rate Constant of the Anionic Form and Physicochemical Parameters[a]

$$\log k_i = a + bX + cY + dZ$$

a	b	X	c	Y	d	Z	r	s	Eq.
−0.008	0.833	$\log P_{c\text{-}L}$					0.833	0.178	(37)
−0.171	0.821	$\log\frac{\beta}{100-\beta}$					0.823	0.182	(38)
−0.326	0.325	$\log\left(\frac{\gamma}{100-\gamma}\right)_i$					0.362	0.299	(39)
−10.320	4.067	log MW					0.657	0.242	(40)
−0.659	−1.864	$\log k_{perm}$					0.432	0.289	(41)
−0.050	0.238	$\log P_{c\text{-}L}$	0.426	$\log\frac{\beta}{100-\beta}$			0.868	0.167	(42)
−0.001	0.508	$\log P_{c\text{-}L}$*[b]	−0.272	$\log\left(\frac{\gamma}{100-\gamma}\right)_i$			0.865	0.169	(43)
−0.210	1.263	$\log\frac{\beta}{100-\beta}$*	−0.529	$\log\left(\frac{\gamma}{100-\gamma}\right)_i$**			0.910	0.139	(44)
−0.078	0.266	$\log P_{c\text{-}L}$*	0.849	$\log\frac{\beta}{100-\beta}$*	−0.561	$\log\left(\frac{\gamma}{100-\gamma}\right)_i$*	0.961	0.099	(45)

a) Thirteen sulfonamides are used for the analysis excluding sulfanilamide and sulfapyridine.
b) One asterisks indicates significance at the 1% level, two asterisk at the 5% level.

The results of a multi-parameter analysis are presented by Eq. 42—45 in Table VII.

Eq. 42 and 43 revealed that the addition of the adsorption parameter from rat blood or activated charcoal to the lecithin-chloroform partitioning parameter increased the correlation coefficient of the equations. A comparison of Eq. 44 with Eq. 38 and 39 revealed that Eq. 44 accounts for 83% (r^2=0.828) of the variance of the data, whereas Eq. 38 accounts for 68% (r^2=0.677) and Eq. 39 accounts for only 13% (r^2=0.131) of the variance.

The addition of the lecithin-chloroform partitioning parameter to Eq. 43 yields Eq. 45 resulting a marked increase in the correlation coefficient.

The calculated values of log k_i are presented in Table VIII as well as the experimental ones.

TABLE VIII. Observed and Calculated Absorption Rate Constant of Anionic Sulfonamides

Compd. No.	log k_i				
	Obsd.	Calcd.			
		Eq. 37	Eq. 38	Eq. 44	Eq. 45
1	—	—	—	—	—
2	−0.964	−0.924	−0.982	−0.929	−0.948
3	—	—	—	—	—
4	−0.702	−0.480	−0.433	−0.608	−0.649
5	−0.532	−0.449	−0.762	−0.668	−0.495
6	−0.521	−0.524	−0.603	−0.681	−0.652
7	−0.192	−0.178	−0.186	−0.211	−0.180
8	−0.340	−0.619	−0.337	−0.084	−0.239
9	−0.863	−0.727	−0.516	−0.661	−0.815
10	−0.774	−0.610	−0.636	−0.714	−0.724
11	−0.202	−0.125	−0.246	−0.256	−0.158
12	−0.302	−0.301	−0.279	−0.367	−0.369
13	−0.070	0.015	−0.095	−0.086	0.024
14	−0.118	−0.426	−0.402	−0.257	−0.260
15	−0.131	−0.369	−0.242	−0.195	−0.252

Prediction of Intestinal Absorption with Physicochemical Parameters

Structure-activity relationships of drugs have been studied extensively for many years to clarify the biological activity of drugs in reference to their physicochemical properties. The relationship between the physicochemical properties of drugs and their pharmacokinetic parameters as well as their dosage schedule is also considered to be one of the important purposes in studying the structure-activity.

Recently, Nogami, *et al.*[34] have introduced the substituent constant for the intestinal absorption of benzene derivatives employing Danielli's activated diffusion model.[35] They found that the constants of substituent groups were experimentally defined from the absorption of various compounds having the substituent groups and that the absorption rate coefficient of a compound was predicted from the addition rule of the constants of substituent groups.

In the previous study,[13] it was established that the intestinal absorption of sulfonamides was influenced by the pH value of perfusion solution. The amount of a sulfonamide absorbed from perfusion solution at a certain pH value through the rat intestine was calculated with the rates of absorption of the unionized and anionic forms and pK_a value of the drug.

The rate of absorption of the unionized and anionic forms is expressed in terms of physicochemical parameters,

$$\log k_m = f(x_i) \text{ or } k_m = 10^{f(x_i)}$$
$$\log k_i = g(x_i') \text{ or } k_i = 10^{g(x_i')}$$

where, x_i and x_i' are the physicochemical parameters used for the regression analysis. Thus, R_m and R_i are expressed by Eq. 46 and 47.

$$R_m = (1-e^{-k_m}) \text{ or } R_m = [1-\exp\{-10^{f(x_i)}\}] \quad (46)$$
$$R_i = (1-e^{-k_i}) \text{ or } R_i = [1-\exp\{-10^{g(x_i')}\}] \quad (47)$$

TABLE IX. Observed and Calculated Absorption Ratios of Sulfonamides

Compd. No.	pH 4.0			pH 5.8			pH 6.5			pH 7.6		
	Obsd.	Calcd.		Obsd.	Calcd.		Obsd.	Calcd.		Obsd.	Calcd.	
		Eq. 25—45	Eq. 36—45		Eq. 25—45	Eq. 36—45		Eq. 25—45	Eq. 36—45		Eq. 25—45	Eq. 36—45
1	0.306	0.217	—	0.293	0.217	—	0.316	0.217	—	0.308	0.217	—
2	0.115	0.223	0.121	0.110	0.141	0.111	0.100	0.116	0.108	0.105	0.107	0.107
3	0.437	0.369	0.332	0.410	0.396	0.332	0.432	0.396	0.332	0.413	0.396	0.332
4	0.171	0.215	0.155	0.180	0.214	0.158	0.184	0.212	0.165	0.172	0.205	0.190
5	0.398	0.357	0.357	0.353	0.332	0.332	0.288	0.300	0.300	0.251	0.277	0.277
6	0.395	0.456	0.451	0.387	0.438	0.434	0.385	0.389	0.383	0.278	0.245	0.244
7	0.638	0.786	0.657	0.487	0.511	0.499	0.468	0.489	0.487	0.488	0.484	0.484
8	0.471	0.446	0.597	0.367	0.439	0.451	0.373	0.438	0.441	0.358	0.438	0.439
9	0.167	0.148	0.161	0.160	0.148	0.160	0.172	0.147	0.159	0.140	0.144	0.149
10	0.242	0.262	0.356	0.163	0.195	0.220	0.173	0.178	0.184	0.152	0.173	0.173
11	0.725	0.555	0.721	0.598	0.532	0.627	0.556	0.512	0.547	0.457	0.502	0.506
12	0.503	0.540	0.503	0.476	0.530	0.495	0.470	0.498	0.479	0.401	0.390	0.382
13	0.730	0.755	0.678	0.658	0.718	0.669	0.638	0.679	0.659	0.565	0.655	0.653
14	0.715	0.533	0.603	0.644	0.479	0.515	0.558	0.442	0.454	0.532	0.424	0.426
15	0.675	0.594	0.598	0.605	0.534	0.536	0.586	0.471	0.472	0.514	0.433	0.433
r[a]		0.904	0.947		0.935	0.954		0.942	0.953		0.931	0.933

a) correlation coefficient between observed and calculated values

34) H. Nogami, M. Hanano and H. Yamada, *Chem. Pharm. Bull.* (Tokyo), **16**, 389, 580, 586 (1968).
35) H. Davson and J.F. Danielli, "The Permeability of Natural Membranes," Cambridge, 1952, p. 324.

The degree of ionization of acid and its unionized fraction at a certain pH are expressed by Eq. 48 and 49.

$$M_0/(M_0+I_0) = 1/\{1+10^{(pH-pK_a)}\} \quad (48)$$

$$I_0/(M_0+I_0) = 10^{(pH-pK_a)}/\{1+10^{(pH-pK_a)}\} \quad (49)$$

Substituting Eq. 46, 47, 48 and 50 into Eq. 1, one obtains Eq. 50.

$$\text{Fraction absorbed} = 1 - \frac{\exp\{-10^{f(x_i)}\}+10^{(pH-pK_a)}\times\exp\{-10^{g(x_i')}\}}{1+10^{(pH-pK_a)}} \quad (50)$$

The absorbed fraction at various pHs' in a certain period is calculated employing Eq. 50 and the equations which are obtained from a regression analysis of the unionized and anionic form.

The relationships between the absorbed values and the calculated ones at pH values of 4.0, 5.8, 6.5 and 7.6 of perfusion solution are presented in Table IX and Fig. 6.

Presently, it was revealed that the amounts of absorption of sulfonamide through the rat intestine enabled one to predict from their physicochemical parameters with accuracy. The present study is still in the early stage. In order to complete the approach described above, it is obviously necessary to study accurate quantitative measurements of physicochemical parameters as well as pharmacokinetic ones. After a regression analysis has been carried out employing these parameters, we may be able to obtain helpful information in the study of a better understanding of the drug action.

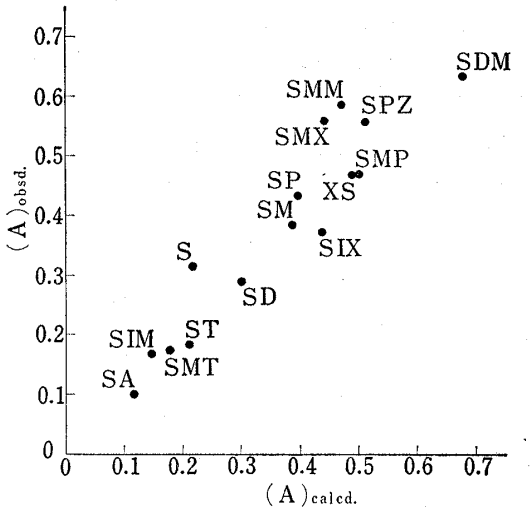

Fig. 6. Relationship between Observed Absorption Ratios of Sulfonamides at pH 6.5 and Those Calculated with Equations 25 and 45

Experimental

Intestinal Absorption——Procedures were generally similar to those in the previous paper.[24] A sulfonamide was dissolved in an isotonic saline solution at a concentration 1 mM, whose pH was adjusted at a certain value with 0.1N HCl or 0.1N NaOH before and during the perfusion experiments. With preliminary experiments, decrease in absorption was observed at pH below 3.8 and higher than 8.5 due to a possible modification of membrane to the absorption. Thus, pH-profile of absorption was studied at pH 4.0—8.0. The disappearance of sulfonamides from the perfusing solution was found to follow a first-order reaction kinetics for more than one hour. The absorbed amount in one hour was employed for the calculation of R_m, R_i, k_m and k_i.

Partition Coefficient of Unionized Form——Partition coefficient of sulfonamides between an acetate buffer of pH 3.0—3.5 with an ionic strength 0.1 and chloroform and *n*-octyl alcohol was measured at 30°. A possible ionization of amino and heterocyclic groups was neglected and the present value was assumed as the partition coefficient of the unionized form.

Adsorption to Rat Blood——An equilibrium dialysis method following a previous report[7b] was employed. Twenty ml of blood was taken from each male rat, SD strain with 400—450 g body weight, through a cardiopuncture. Blood from each rat was mixed and added an aliquot amount of 3.8% sodium citrate to prevent from coagulation. A cellophane bag (Visking Company) with 2 ml blood was placed into 5 ml of a pH 7.4 phosphate buffer containing 0.07 mM sulfonamide. After being shaked at 37° for 7 hr, a concentration in the buffer was measured spectrophotometrically. To obtain a concentration of control, similar experiment was performed with 2 ml of the phosphate buffer instead of blood. Per cent of adsorption β was calculated with following equation.

$$\beta = \frac{\text{concn. of control} - \text{concn. with blood}}{\text{concn. of control}} \times 100$$

Adsorption to Activated Charcoal——Ten mg of activated charcoal (Takeda Chemical Industries) was taken into a 200 ml glass flask with a glass stopper. One hundred ml of 0.1 mM sulfonamide buffer solution was placed into the flask. The flask was shaked at 37° for 15 hr. Following an equiliblium was established, a concentration of the sulfonamide was measured spectrophotometrically. Per cent of adsorption γ was calculated with following equation. An acetate buffer of pH 3.0—3.5 was used to study the adsorption of

$$\gamma = \frac{\text{initial concn.} - \text{concn. after adsorption}}{\text{initial concn.}} \times 100$$

the unionized form, and an ammonium buffer of pH 8.7 for the ionized form.

Partitioning of Anionic Form to Lecithin-containing Chloroform——Ovo-lecithin dissolved in chloroform at 10 mg/ml was used as oil phase and an ammonium buffer of pH 7.77 with an ionic strength 0.1 as water phase. Chloroform and the buffer were saturated each other before use. Five ml of chloroform containing lecithin was taken into a test tube with a glass stopper. Equal volume of the buffer solution containing 0.1 mM of a sulfonamide was placed on the oil phase without disturbing the interface. The test tube was placed horizontally in a water-bath at 30° to result in the maximum interfacial area. To accelerate the partitioning, the tube was rocked several times every 15 minutes interval for 2 hr without disturbing the interface. A concentration of the drug in the buffer was measured spectrophotometrically, following the buffer was centrifuged to remove a possible existence of chloroform. Partition coefficient of the anionic form was calculated with a correction of the unionized form present in the buffer at the pH value.

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