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In Vitro and *in Situ* Models for the Prediction of *in Vivo* Absorption Behavior of Sulfonamides Following Dissolution[1)]

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The purpose of the present study was to assess two recently described models (S-L_w-L_o and S-L_w-*in situ*) for the prediction of drug absorption behavior *in vivo* following dissolution from a compressed tablet. The experiments were carried out by using 11 sulfonamides as test compounds to survey the validity of the models. In the S-L_w-L_o model, the drug appearance in the aqueous phase, C_w, and in the organic phase, C_o, obeyed first order kinetics from 0 to 6 h. In the S-L_w-*in situ* model, the drug disappearance in the reservoir, $(C_w - C_i)^t$, and change in blood levels of the drug, C_b^t, with respect to time were analyzed simultaneously, and the factors affecting the drug absorption are discussed on the basis of the physicochemical and pharmacokinetic properties of the drug. It was found that the C_o^t value was significantly correlated with both $(C_w - C_i)^t$ and C_b^t values. The C_o^t values were also correlated well with the blood levels of the drug *in vivo* following oral administration to rabbits.

The results indicated that the two experimental models are useful to predict the absorption behavior of drugs and their usefulness may be extended to the evaluation of oral bioavailability.

Keywords——sulfonamide; dissolution; interfacial transfer; *in situ* drug perfusion in rabbit intestinal tract; *in vivo* blood level; oral administration to rabbit; multiple regression analysis; partial correlation coefficient; *in vivo* correlation with model

The primary absorption parameters of a drug are known to depend on its solubility and dissolution characteristics, since an orally administered solid drug must dissolve in the gastrointestinal tract prior to absorption across the gastrointestinal mucosa. The development of an efficient and reliable dissolution testing method is necessary to evaluate the bioavailability of solid dosage forms.[2)] We have recently described two model systems[3)] for the prediction of drug absorption following dissolution from compressed tablets. One of the models, S-L_w-L_o, was constructed based on the interfacial transfer of a drug from an aqueous phase (L_w) to an organic phase (L_o) following dissolution of the drug in L_w. The other model, S-L_w-*in situ*, was constructed based on the perfusion of drug solution in rabbit small intestinal tract *in situ* following dissolution of the drug in a reservoir (L_w). Takayama *et al.* recently reported that the S-L_w-L_o model is useful to predict the interfacial transfer behavior of indomethacin following dissolution of its polyvinylpyrrolidone coprecipitate.[4)]

In this paper, the two model systems were evaluated in detail using 11 sulfonamides as test compounds, because their absorption characteristics are well documented.[5)] Furthermore, *in vitro*, *in situ*, and *in vivo* correlations were examined in an attempt to find some reliable bioavailability parameters.

Experimental

Materials——Sulfaguanidine was prepared according to the reported method[6)] and recrystallized from ethanol–water. Other sulfonamides used were the same as those in the previous paper.[7)] All other materials and solvents were of analytical reagent grade, and deionized double-distilled water was used.

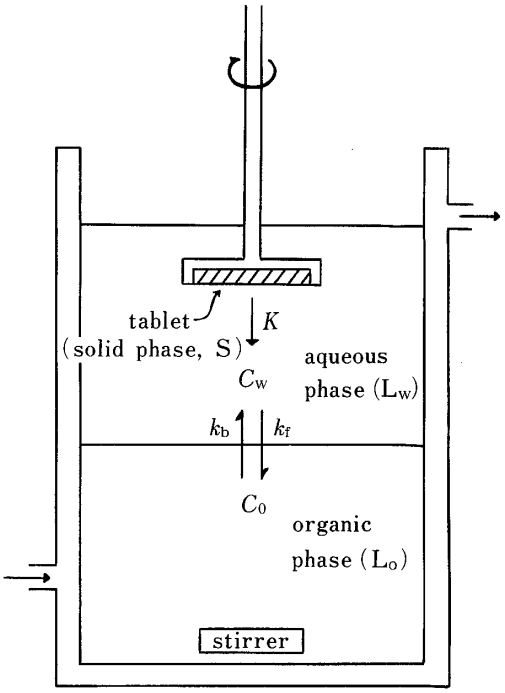

Fig. 1. Schematic Diagram of the S-L_w-L_o Model

K, dissolution rate constant; k_f, rate constant for forward transfer; k_b, rate constant for backward transfer; C_w, concentration in aqueous phase; C_o, concentration in organic phase.

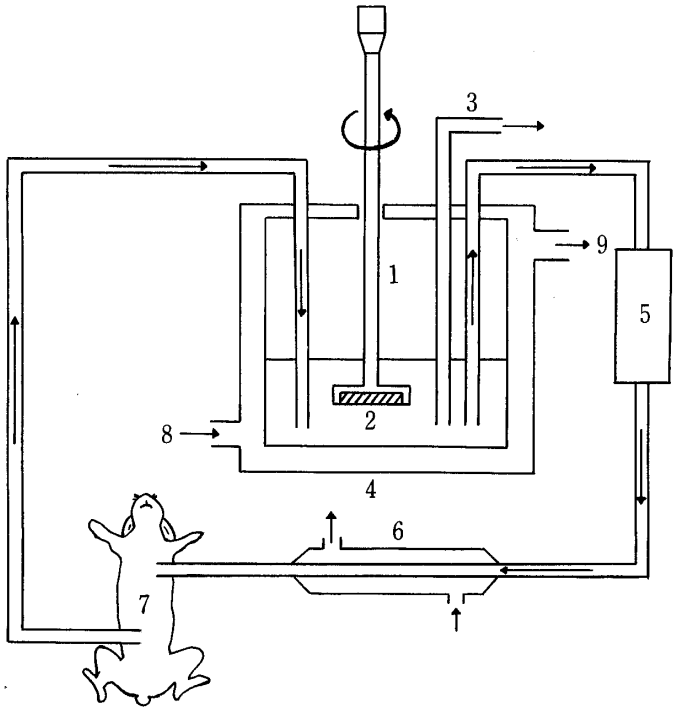

Fig. 2. Schematic Diagram of the S-L_w-*in Situ* Model

1, stainless disk holder; 2, tablet; 3, sampling tube; 4, jacket beaker; 5, circulation pump, 6, thermostat; 7, rabbit small intestine; 8, circulating water inlet; 9, circulating water outlet.

S-L_w-L_o Experiment——This model basically consisted of a rotating disk dissolution apparatus[8] with an organic phase (Fig. 1). The aqueous phase (L_w) and organic phase (L_o) consisted of 75 ml of isotonic phosphate buffer (pH 7.4) and 75 ml of 1,2-dichloroethane–*n*-octanol (6:4), respectively. By considering the sink condition, the composition of L_o was suitably chosen to constitute the lower phase and to give as large a partition coefficient of the drug as possible. These solutions were mutually saturated prior to experiments. The disks (solid phase, S) of 2 cm diameter were prepared by compressing drug powder under a pressure of 400 kg/cm^2 in a hydraulic press (Riken Seiki Co., Ltd., model P-16B). The rotation speed of the disks and stirring rate of the organic phase were both 90 rpm to minimize the disturbance of the interface. The entire system was maintained at 37 °C by means of a circulating water-bath. At predetermined intervals, 0.5 ml samples were withdrawn simultaneously from L_w and L_o through stainless steel tubes. The drug concentrations were determined spectrophotometrically. The dissolution rates and interfacial transfer parameters (see Fig. 1) were calculated[9] by the method described previously.[3]

S-L_w-*in Situ* Experiment——This perfusion system basically consisted of a 60 cm loop of rabbit small intestine (male albino rabbits, 2.5—3.0 kg) *in situ*, a reciprocating pump, a constant-temperature solution reservoir, and a rotating disk apparatus (Fig. 2), since the primary absorption site of sulfonamides is known to be the small intestine.[10] The rabbit was anesthetized with ethyl carbamate (1.2 g/kg), and connected to the perfusion system after the surgical operation. All the sections were securely connected with medical grade polyethylene tubes and stainless steel materials to provide a closed system. The entire system was maintained at 37 °C by using a circulating water-bath. A perfusing solution (135 ml) of isotonic phosphate buffer (pH 7.4) was circulated at a flow rate of 30 ml/min through the system. The lag time from the reservoir to the intestinal tract was about 3 min. The rotating speed of the disks (solid phase, S) of 2 cm diameter was 90 rpm. At appropriate intervals, 0.5 ml samples of the perfusing solution were taken from the reservoir through a stainless steel tube. The drug concentration was determined spectrophotometrically. The blood levels of the drug were simultaneously determined by collecting blood samples from ear veins of the rabbits, during the S-L_w- *in situ* experiments. The assays of drugs in blood were performed after hydrolysis in hot 0.1 N hydrochloric acid solution by the Bratton–Marshall method.[11]

***In Vivo* Absorption Experiment**——Male albino rabbits (2.5—3.0 kg) were employed in the absorption study. Intervals of at least one week were adopted to minimize the residual or cumulative effect of the preceding dose. A 100 mesh drug powder (100 mg/kg of body weight) was administered orally along with 80 ml of water by means of a stomach catheter. At predetermined times blood samples (0.6 ml) were collected from the ear veins by using a 1.0 ml syringe containing 0.1 ml of 3.8% sodium citrate. The drug concentrations of the blood samples were determined in

the same way as in the S-L_w-*in situ* experiments.

Determination of Partition Coefficients and Solubilities——The partition coefficients (*PC*) were determined after shaking 10 ml of isotonic phosphate buffer solution (pH 7.4) containing a drug (5×10^{-4} M) and 10 ml of 1,2-dichloroethane–*n*-octanol (6:4) solution for 2 h at 37 °C. The *PC* was defined as the ratio of the equilibrium concentration in the organic phase to that in the aqueous phase. For the solubility (S_s) determination, an excess amount of drug was shaken with isotonic phosphate buffer (pH 7.4) at 37 °C for 24 h. After filtration, a sample was diluted with the same buffer solution and analyzed spectrophotometrically.

Results and Discussion

Analysis of S-L_w-L_o Data

In the interfacial transfer of a drug following dissolution from a compressed tablet (Fig. 1), the rates of change in the drug concentrations in L_w and L_o can be expressed by Eqs. (1) and (2), respectively.

$$\frac{dC_w}{dt}=K\cdot(S_s-C_w)-k_f\cdot C_w+k_b\cdot C_o \quad (1)$$

$$\frac{dC_o}{dt}=k_f\cdot C_w-k_b\cdot C_o \quad (2)$$

In these equations, C_w and C_o represent the concentrations of drugs in L_w and L_o, k_f and k_b are the first-order rate constants for foward- and backward-interfacial transfer,[12] respectively, K is the dissolution rate constant,[13] and S_s is the saturated concentration of drug in L_w.

Figure 3 shows a typical example of interfacial transfer behavior in the case of sulfapyridine following dissolution. Under these experimental conditions, the tablets maintained a constant surface area, and the drug appearances in L_w and in L_o were found to obey first-order kinetics. The solid lines indicate the theoretical C_w, C_o, and C_w+C_o values estimated by the Runge–Kutta–Gill method[14] from Eqs. (1) and (2). The parameters (K, S_s, k_f, and k_b) used for the computation are listed in Table I; they were determined in the same way as previously reported.[3] As shown in Fig. 3, a good fit was obtained between the experimental values and theoretical ones. Similar results were obtained for other sulfonamides studied. Therefore, even though the model is a simple one, the analysis of S-L_w-L_o data may afford valuable information[5] regarding the interfacial transfer of the drugs following

Table I. Physical Parameters for Sulfonamides at pH 7.4 and 37 °C

Compound	S_s[a] ($\times10^3$ M)	K[b] ($\times10\,h^{-1}$)	k_f[b] ($\times10\,h^{-1}$)	k_b[b] ($\times10\,h^{-1}$)	PC[a]	pK_a[c]
Sulfanilamide	71.7	3.70	0.633	1.73	0.367	10.5
Sulfisomezole	19.7	3.56	2.34	2.32	1.01	5.72
Sulfaphenazole	4.78	1.78	3.90	0.582	6.70	5.87
Sulfisoxazole	27.5	3.37	0.885	4.19	0.211	4.79
Sulfathiazole	4.34	3.11	2.40	0.900	2.67	7.23
Sulfisomidine	7.47	3.12	1.49	1.69	0.882	7.47
Sulfadimethoxine	2.53	3.11	2.86	0.188	15.2	5.98
Sulfamethizole	32.6	3.08	0.342	3.19	0.107	5.22
Sulfapyridine	1.87	2.68	3.38	0.826	4.06	8.56
Sulfadiadine	3.04	2.69	0.629	1.25	0.502	6.37
Sulfaguanidine	9.23	2.95	0.156	1.61	0.097	12.1

a) Experimental values (see the text).
b) Calculated values from Eqs. (1) and (2).
c) For dissociation of amide proton; cited in ref. 6.

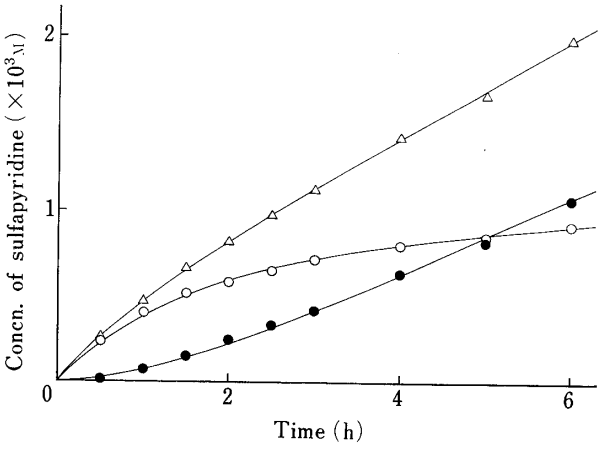

Fig. 3. Interfacial Transfer of Sulfapyridine following Dissolution at 37 °C in the S-L_w-L_o Model

○, C_w; ●, C_o; △, C_w+C_o.
The solid line indicates the theoretical curve obtained from Eqs. (1) and (2). Each point represents the mean of 4 experiments.

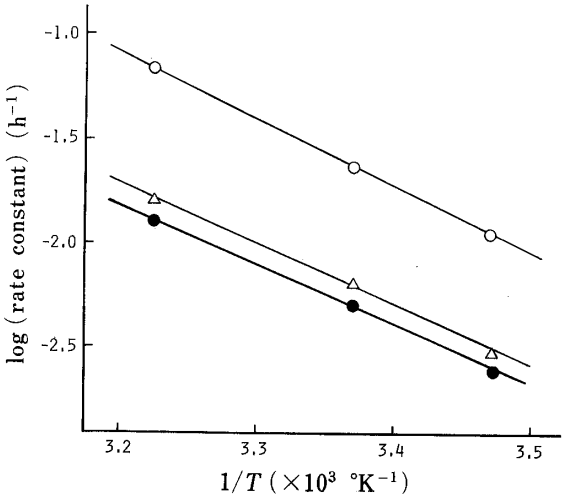

Fig. 4. Arrhenius Plots for Various Rate Constants of Sulfisomidine in the S-L_w-L_o Model

○, K; ●, k_f; △, k_b.
Each point represents the mean of 4 experiments.

dissolution. In particular, C_o value may be a useful parameter to predict drug levels in L_o with respect to time. As a typical example, multiple regression analysis for S-L_w-L_o data of 11 sulfonamides gave the following result:

$$\log C_o^{t=2} = 0.738 \log K + 0.942 \log S_s + 0.775 \log k_f - 0.290 \log k_b - 0.620 \quad (3)$$

$(n=11,\ r=0.098,\ s=0.115,\ F=36.5)$

where $C_o^{t=2}$ is the drug appearance in L_o at time = 2 h after the start of dissolution. The partial correlation coefficients for $\log S_s$, $\log K$, $\log k_f$, and $\log k_b$ were found to be 0.957, 0.512, 0.958, and −0.616, respectively. This indicates that S_s and k_f make relatively large contributions to C_0 value.

Figure 4 shows the Arrhenius plots of rate constants (K, k_f, and k_b) for sulfisomidine, as an example. The activation energies (E_a) calculated from the slopes of the linear plots gave values of 6.38, 5.83, and 6.15 kcal/mol for K, k_f, and k_b, respectively. Similar E_a values were obtained for other drugs such as sulfisomezole and sulfapyridine. This indicates that the dissolution and interfacial transfer processes of sulfonamides may be mainly diffusion-controlled,[13,15] as described previously.[3] Since the sulfonamides employed in this study are known to be absorbed mainly by passive diffusion,[4] the S-L_w-L_o model may be suitable to simulate the absorption behavior of such a drug *in vivo*.

Analysis of S-L_w-*in Situ* Data

In the S-L_w-*in situ* model (Fig. 2), the rate of change in drug concentration (C_i) in L_w following dissolution from a compressed tablet, and that in blood level (C_b) can be expressed by Eqs. (4) and (5), respectively.

$$\frac{dC_i}{dt} = K \cdot (S_s - C_i) - k_i \cdot C_i \quad (4)$$

$$\frac{dC_b}{dt} = k_i \cdot C_i \quad (5)$$

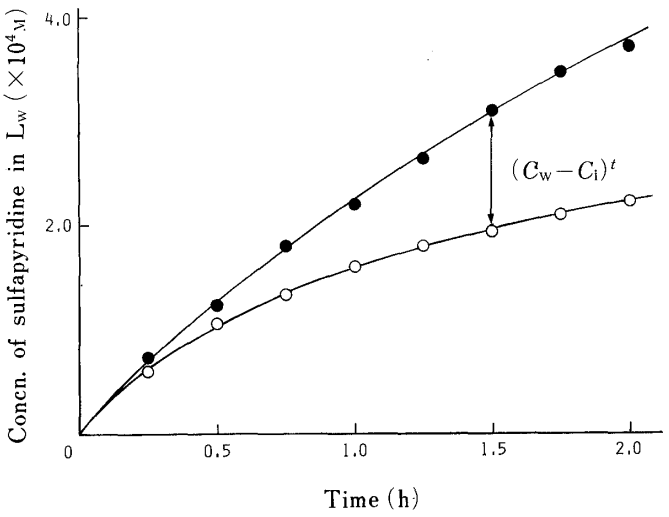

Fig. 5. Estimation of the Amount of Drug Absorbed with Respect to Time in the S-L_w-*in Situ* Model

○, C_i value in S-L_w-*in situ* experiment; ●, C_w value in S-L_w experiment.
The plot shows the value every 15 min after the start of dissolution. Each point represents the mean of 4 experiments.

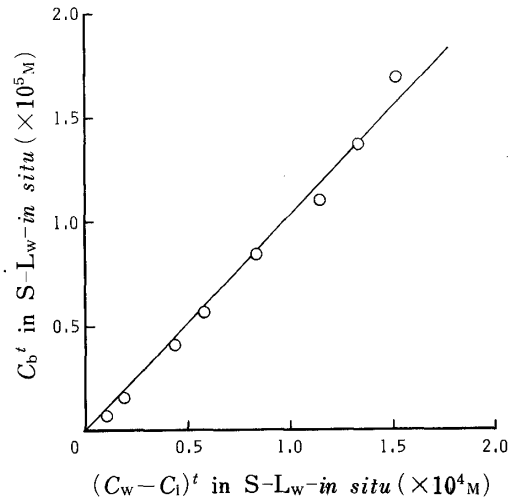

Fig. 6. Relationship between $(C_w - C_i)^t$ and C_b^t in the S-L_w-*in Situ* Model for Sulfapyridine

The plot shows the value every 15 min after the start of dissolution. Each point represents the mean of 4 experiments.

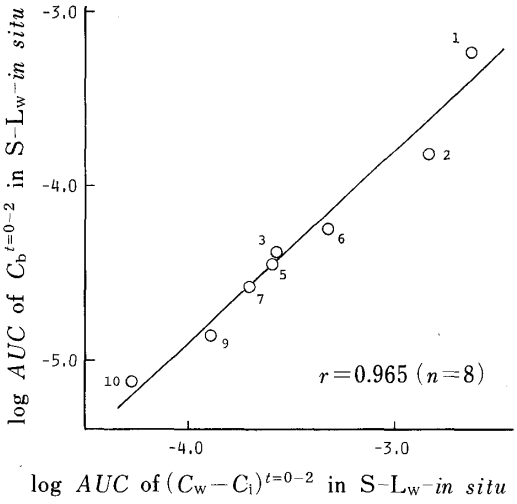

Fig. 7. Relationship between log*AUC* of $(C_w - C_i)^{t=0-2}$ and log*AUC* of $C_b^{t=0-2}$ in the S-L_w-*in Situ* Model

The numbers refer to the compounds in Table I. Each *AUC* value is the mean of 4 experiments.

where k_i is apparent absorption rate constant of the drug. In this experiment C_i and C_b values were simultaneously measured, and it was found that the changes in both values with respect to time were first-order. For the prediction of the amount of the drug absorbed in S-L_w-*in situ* experiments, the amount of drug dissolved (C_w) in L_w was separately measured by means of a dissolution study (S-L_w model) using the same volume of polyethylene tubing instead of the rabbit intestinal tract, and then the C_i value was subtracted from the C_w value. As shown in Fig. 5, the $(C_w - C_i)^t$ value was defined as the amount of drug absorbed with respect to time in the S-L_w-*in situ* model.

Figure 6 shows the relationship between $(C_w - C_i)^t$ value and C_b^t value of sulfapyridine, as a typical example, a good correlation was obtained. The correlation coefficient and the regression line are as follows,

$$C_b^t = 0.108\ (C_w - C_i)^t + 7.67 \times 10^{-7} \quad (r = 0.997,\ p < 0.001) \tag{6}$$

The intercept is nearly equal to zero and the slope is 0.1. This indicates that the $(C_w - C_i)^t$

value may be an extracted parameter that reflects the amount of the drug absorbed *in situ*. It should also be noted in Eq. (6) that the S-L_w-*in situ* method is much more sensitive and convenient for predicting the blood levels of the drug following dissolution compared with the direct measurement of blood samples. Moreover, the areas under the curves of blood concentrations (AUC of $C_b^{t=0-2}=\int_0^2 C_b dt$) and those of the amount of drug absorbed in the S-L_w-*in situ* model (AUC of $(C_w-C_i)^{t=0-2}=\int_0^2 C_w dt-\int_0^2 C_i dt$) at time $=0-2$ h were determined for several sulfonamides according to the trapezoidal rule.[16] It is noteworthy in Fig. 7 that these AUC values give a correlation coefficient of $r=0.965$ for all the drugs studied. These results indicate that the $(C_w-C_i)^t$ value is useful to predict the absorption behavior of the drug *in situ* following dissolution.

In Vivo Correlations with *in Vitro* and *in Situ* Models

The correlation of *in vitro* or *in situ* absorption parameters with *in vivo* bioavailability parameters has received widespread attention in recent years because *in vivo* studies, particularly human testing, are very costly and time-consuming. The importance of an efficient and reliable experimental model that can mimic the drug behavior *in vivo* is thus clear. Based on the above considerations, the fit of the S-L_w-L_o data to S-L_w-*in situ* data was evaluated prior to making *in vivo* correlations.

Figure 8 shows the relationship between C_o^t and $(C_w-C_i)^t$ of sulfapyridine, as an example, where the measurements were taken every 15 min up to 2 h after the start of dissolution. An excellent correlation between *in vitro* and *in situ* absorption parameters was observed. The correlation coefficient and the regression line are as follows,

$$(C_w-C_i)^t=0.631\ C_o^t+1.10\times10^{-5} \quad (r=0.995,\ p<0.001) \tag{7}$$

The intercept is nearly equal to zero and the slope is 0.6. This suggests that the *in situ* situation is well reflected by the interfacial transfer process *in vitro*. As shown in Fig. 9, the AUC values of $C_o^{t=0-2}$ were also well correlated with those of $(C_w-C_i)^{t=0-2}$ for all the drugs studied ($r=0.963$).

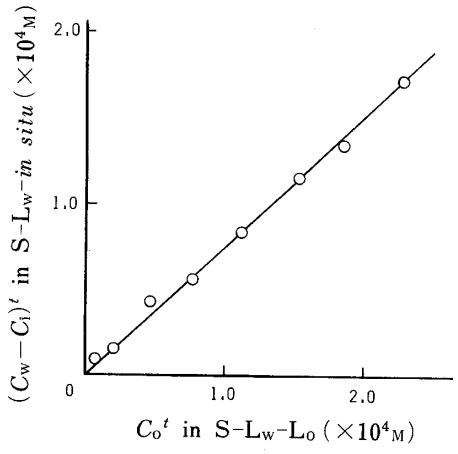

Fig. 8. Relationship between C_o^t in S-L_w-L_o and $(C_w-C_i)^t$ in the S-L_w-*in Situ* Model for Sulfapyridine

The plot shows the value every 15 min after the start of dissolution. Each point represents the mean of 4 experiments.

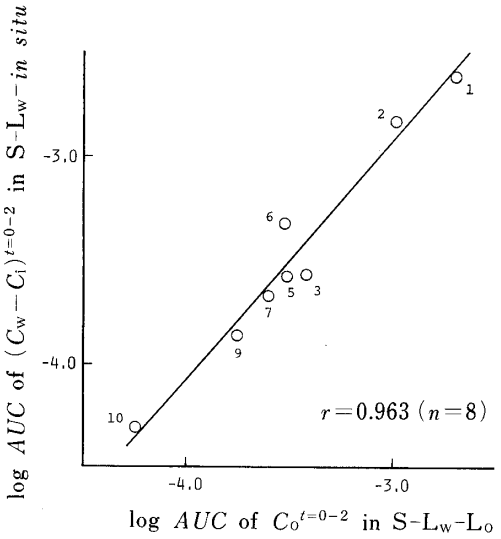

Fig. 9. Relationship between log AUC of $C_o^{t=0-2}$ in the S-L_w-L_o Model and log AUC of $(C_w-C_i)^{t=0-2}$ in the S-L_w-*in Situ* Model

The numbers refer to the compounds in Table I. Each AUC value is the mean of 4 experiments.

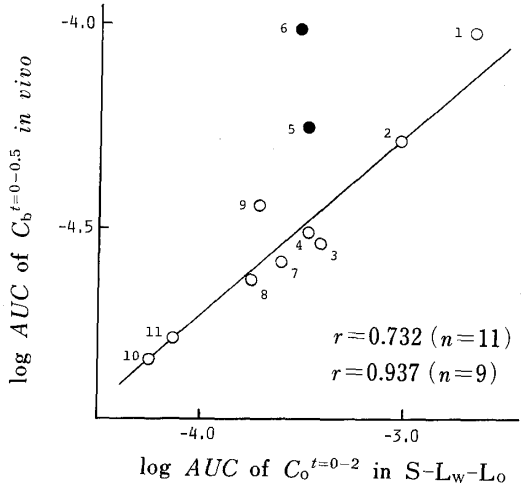

Fig. 10. Relationship between log*AUC* of $C_o^{t=0-2}$ in the S-L_w-L_o Model and log*AUC* of $C_b^{t=0-0.5}$ *in Vivo*

The numbers refer to the compounds in Table I. Each *AUC* value is the mean of 4 experiments.

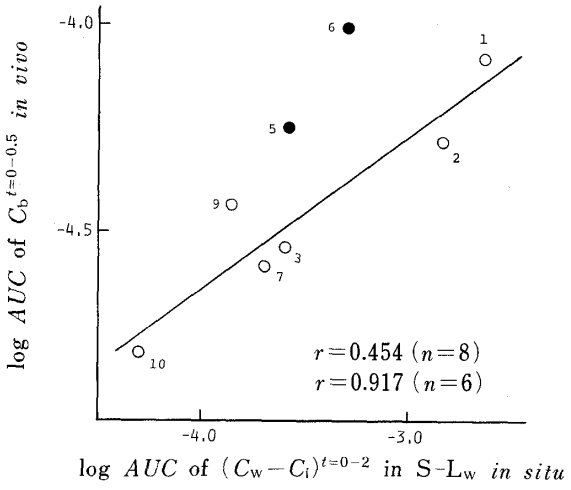

Fig. 11. Relationship between log*AUC* of $(C_w-C_i)^{t=0-2}$ in S-L_w-*in Situ* and log*AUC* of $C_b^{t=0-0.5}$ *in Vivo*

The numbers refer to the compounds in Table I. Each *AUC* value is the mean of 4 experiments.

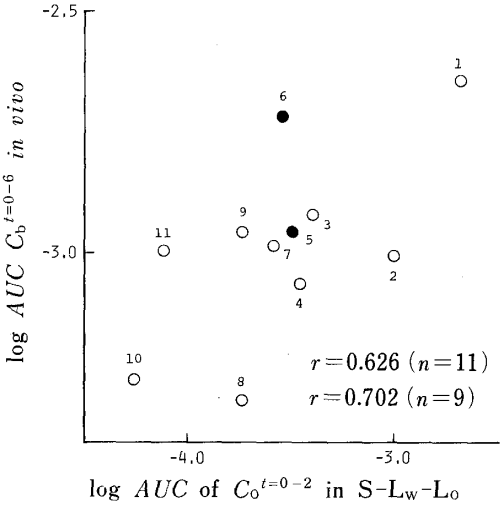

Fig. 12. Relationship between log*AUC* of $C_o^{t=0-2}$ in S-L_w-L_o and log*AUC* of $C_b^{t=0-6}$ *in Vivo*

The numbers refer to the compounds in Table I. Each *AUC* value is the mean of 4 experiments.

Thus, it is interesting to examine the *in vivo* correlations with S-L_w-L_o data and S-L_w-*in situ* data. The *AUC* values of blood concentrations after oral administration of the rabbits *in vivo* (*AUC* of $C_b^{t=0-0.5}$) were calculated up to 30 min after the start of experiments as a tentative measure of the absorption process. These *AUC* of $C_b^{t=0-0.5}$ values were plotted against the corresponding *AUC* values of S-L_w-L_o and those of S-L_w-*in situ* data, as shown in Figs. 10 and 11, respectively. When two data points of sulfathiazole and sulfisomidine were excluded, good correlations of *AUC* of $C_b^{t=0-0.5}$ *in vivo* with both *AUC* of $C_o^{t=0-2}$ and *AUC* of $(C_w-C_i)^{t=0-2}$ were observed with correlation coefficients $r>0.9$.[17] The anomalous behavior of the two drugs may be due to the pK_a values (see Table I) being close to the pH value of the dissolution medium, which may exert a subtle effect on the rate of dissolution and/or absorption of such weak organic electrolytes. It is interesting to note that the *in vivo* data appeared to be rather well correlated with the S-L_w-L_o data (Fig. 10) compared with S-L_w-*in situ* data (Fig. 11), although the numbers of drugs examined were not identical. This may indicate that the S-L_w-L_o model is somewhat superior to the S-L_w-*in situ* model for the prediction of drug absorption behavior *in vivo*. The greatest advantage of the S-L_w-L_o model is that the C_o^t value can be easily obtained by simple procedure, and the reproducibility and

accuracy ($\pm 3\%$) were not less than for the S-L_w-*in situ* data. It should be noted in Fig. 12, however, that the correlation coefficient between *in vivo* data and S-L_w-L_o data decreased significantly, when *AUC* values were taken up to 6 h. During such a long period, the blood levels of the drugs must be markedly changed by the distribution and elimination processes *in vivo*.[18]

From the above results, it is concluded that the S-L_w-L_o model is particularly useful to predict the initial absorption behavior of a drug following dissolution *in vivo*. On the other hand, the S-L_w-*in situ* model may provide more realistic experiment conditions to mimic the *in vivo* physiological process,[19] although various factors affecting the drug absorption such as interactions of the drug with biological substances should be taken into account. Since passive diffusion is the most common mechanism of drug absorption, the two models described here may be extended to evaluate the oral bioavailability of such drugs. For application to practical dosage forms, however, some improvements to the models may be necessary, including appropriate adjustment of experimental conditions and of the overall size of the system; this problem is being investigated.

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References and Notes

1) A part of this study was presented at the 14th Symposium on Drug Metabolism and Action, Fukuoka, November 1982.
2) K. G. Nelson and K. W. Miller, "Principles and Perspectives in Drug Bioavailability," ed. by J. Blanchard, R. J. Sawchuk, and B. B. Brodie, S. Kargel Inc., Basel, 1979, pp. 20—50.
3) K. Uekama, J. Fujisaki, and F. Hirayama, *Yakugaku Zasshi*, **100**, 1087 (1980).
4) K. Takayama, N. Nambu, and T. Nagai, *Chem. Pharm. Bull.*, **29**, 2718 (1981).
5) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.*, **12**, 421 (1964); K. Kakemi, T. Arita, and S. Muranishi, *ibid.*, **13**, 861 (1965).
6) K. Ohto, Y. Yamanouchi, and K. Sugino, *Nippon Kagaku Kaishi*, **63**, 1670 (1942).
7) K. Uekama, F. Hirayama, M. Otagiri, Y. Otagiri, and K. Ikeda, *Chem. Pharm. Bull.*, **26**, 1162 (1978).
8) H. Nogami, T. Nagai, and A. Suzuki, *Chem. Pharm. Bull.*, **14**, 329 (1966).
9) The calculation was carried out on a FACOM M-140 with a program written by the authors.
10) Y. Imamura and H. Ichibagase, *Chem. Pharm. Bull.*, **21**, 668 (1973).
11) A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).
12) A. Noyes and W. Whitney, *J. Am. Chem. Soc.*, **19**, 930 (1897).
13) G. E. Schumacher and J. B. Nagwaker, *J. Pharm. Sci.*, **63**, 240 (1974).
14) "Instruction Manual of FACOM FORTRAN SSL," Fujitsu Co., 1976.
15) L. L. Bircumshaw and A. C. Riddiford, *Quart. Rev.* (London), **6**, 157 (1952).
16) K. Takada and S. Asada, "Essential of Pharmacokinetics," Hirokawa Publishing Co., Tokyo, 1978, pp. 27—29.
17) A good correlation between *AUC* of $C_b^{t=0-0.5}$ *in vivo* and *AUC* of $C_b^{t=0-2}$ in S-L_w-*in situ* was also obtained, with a correlation coefficient of $r=0.898$.
18) M. Yamazaki, M. Aoki, and A. Kamada, *Chem. Pharm. Bull.*, **16**, 707 (1968).
19) T. Yotsuyanagi, A. Molokhia, S. Hwang, N. F. H. Ho, G. L. Flynn, and W. I. Higuchi, *J. Pharm. Sci.*, **64**, 71 (1975).