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162. Hideo Yamada and Ryuichi Yamamoto : Biopharmaceutical Studies on Factors Affecting Rate of Absorption of Drugs. I. Absorption of Salicylamide in Micellar Solution.

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Factors affecting the rate of absorption of drugs are very interesting subjects from the pharmaceutical point of view. Especially addition of the surfactant is an important factor. Kinetic studies on the effects of the non-ionic surfactant on drug absorption were recently investigated by Kakemi, *et al.*[1,2] using the rectum and by Levy, *et al.*[3] using the stomach. Nogami, *et al.*[4] studied the absorption of the ionic surfactant from the rat small intestine and suggested that the absorption rate of the micelle of the surfactant is negligible in comparison with that of the single molecule forming no micelle.

The present paper describes the relationship between the concentration of the surfactant added to the drug solution and the rate of absorption of the drug from the small intestine. Salicylamide was used as a drug and polysorbate-80 as a surfactant.

It has been shown that the drug absorption from the intestine may be treated as a first order process.[5,6] When the absorption of the surfactant is negligible, (in this case the absorption oft he micelle is, of course, negligible,) the rate of absorption of the drug in the micellar solution depends on only the concentration of the free drug, as equation (1) shows.

$$\frac{dD}{dt} = -k_{Df} C_{Df} \tag{1}$$

where D is the amount of the total drug in the intestinal lumen, k_{Df} is the absorption rate constant of the free drug and C_{Df} is the concentration of the free drug in the intestinal lumen.

According to Shinoda, *et al.*,[7] the micellar aqueous solution can be treated by means of the pseudophase separation model, in which two phases are considered—— the dispersed micellar phase and the continuous aqueous phase surrounding the micelles. Nakagawa[8] obtained the partition constant of a dye between these two phases.

The partition constant, K, for the drug in the micellar solution is defined as follows[8] :

$$K = \frac{\frac{D_m}{S_m}}{\frac{D_f}{V_w}} = \frac{D_m \cdot f_w}{D_f \cdot C_{sm}}; \quad f_w = \frac{V_w}{V}, \quad C_{sm} = \frac{S_m}{V} \tag{2}$$

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1) K. Kakemi, T. Arita, S. Muranishi : The 84th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April, 1964 (Abstract p. 93).
2) *Idem* : The 25th Kinki Branch Annual Meeting of the Pharmaceutical Society of Japan, Kyoto, November, 1964 (Abstract p. 57).
3) G. Levy, R. H. Reuning : J. Pharm. Sci., **53**, 1471 (1964).
4) H. Nogami, J. Hasegawa, T. Fuwa : Personal communication.
5) H. Nogami, M. Hanano, H. Yamada : This Bulletin, **11**, 395 (1963).
6) T. Koizumi, T. Arita, K. Kakemi : *Ibid.*, **12**, 421 (1964).
7) K. Shinoda, E. Hutchinson : J. Phys. Chem., **66**, 577 (1962).
8) T. Nakagawa : Ann. Repts. Shionogi Research Lab., 8, 31 (1958).

where D_m is the amount of the drug in the micellar phase, D_f is the amount of the free drug, S_m is the amount of the surfactant forming the micelle, V_w is the volume of the continuous aqueous phase surrounding the micelles, V is the total volume of the micellar solution of the drug. f_w is the volume fraction of the aqueous phase to the total solution and C_{sm} is the concentration of the surfactant forming the micelle. From equation (2), the total amount of drug, D, is expressed in equation (3).

$$D=D_m+D_f=\left(\frac{C_{sm}}{f_w}K+1\right)D_f \tag{3}$$

When V is constant, equation (1) is,

$$\frac{dC_D}{dt}=-\frac{k_{Df}}{V}C_{Df} \tag{4}$$

where C_D is the total drug concentration in the intestine. From equation (3),

$$C_D=\frac{D}{V}=\left(\frac{C_{sm}}{f_w}K+1\right)C_{Df} \tag{5}$$

Inserting equation (5) into (4), we get equation (6).

$$\frac{dC_D}{dt}=-\frac{k_{Df}}{V}\cdot\frac{f_w}{C_{sm}K+f_w}\cdot C_D \tag{6}$$

When the total volume of the drug solution is constant and absorption of the surfactant is negligible, both f_w and C_{sm} are constant.

Thus,

$$\log\left(\frac{C_D}{C_D^0}\right)=\log\left(\frac{D}{D^0}\right)$$
$$=-\frac{0.434\times k_{Df}}{V}\cdot\frac{f_w}{C_{sm}K+f_w}\cdot t=-\frac{0.434}{V}kt \tag{7}$$

where C_D^0 and D^0 are the initial concentration and the initial amount of the total drug, respectively, and

$$k=\frac{f_w}{C_{sm}K+f_w}\cdot k_{Df} \tag{8}$$

When the concentration of the total surfactant, C_s, is much larger than that of the surfactant in the form of individual molecules, not forming the micelle, C_{sm} becomes nearly equal to C_s. Then, equation (9) is obtained,

$$k=\frac{f_w k_{Df}}{C_sK+f_w} \tag{9}$$

Equation (9) shows the relationship between the absorption rate constant of the drug in the micellar solution, k, and the total concentration of the surfactant, C_s.

Experimental

Determination of the Volume Fraction of the Aqueous Phase to the Total Solution——The volume fraction of the dispersed micellar phase to the total solution, ϕ, can be calculated using the following equation,[9]

9) E. Guth, R. Simha: Kolloid-Z., **74**, 147 (1936). Quated by A.E. Alexander, P. Johnson: "Colloid Science," Vol. I, 360 (1949), Oxford.

$$\eta_r = 1 + 2.5\phi + 14.1\phi^2 \quad (10)$$

where η_r is the relative viscosity of the micellar solution to the solvent. η_r was obtained from the measurements of density and kinematic viscosity for the solution containing 0.2 millimole of salicylamide, 0.9 g. of NaCl and 0, 2, 5 or 10 g., respectively, of polysorbate-80 per 100 ml., adjusted to pH 6.5 with 0.1*N* NaOH. The measurement of kinematic viscosity was carried out with a Ubbelohde viscometer at 37°. The mean value of the effective micellar volume, $(\phi/C_s)_{mean}$, was obtained. The volume fraction of the aqueous phase surrounding the micelles to the total solution, f_w, can be calculated using $f_w = 1-(\phi/C_s)_{mean} \times C_s$.

Determination of the Partition Constant of Salicylamide between the Micellar Phase and the Aqueous Phase——The amount of the drug in the micellar phase, D_m, is

$$D_m = D - D_f = D - C_{Dw} \cdot V_w \quad (11)$$

where C_{Dw} is the drug concentration in the aqueous phase surrounding the micelles, $C_{Dw} = D_f/V_w$. From the equations (2), (5) and (11), the partition constant, K, is

$$K = \left(\frac{C_D}{C_{Dw}} - f_w\right)\frac{1}{C_{sm}} \quad (12)$$

When $C_{sm} \fallingdotseq C_s$,

$$K = \left(\frac{C_D}{C_{Dw}} - f_w\right) \cdot \frac{1}{C_s} \quad (13)$$

Since C_s and C_D in the equation (13) can be given as the experimental condition, the partition constant is obtained when C_{Dw} is experimentally measured. In order to determine C_{Dw}, equilibrium dialysis experiments were carried out with Visking dializing tube (pore size 24 Å) for two systems a) and b).

a) Fifty ml. of the inner solution of the dializing tube contained 0.45 g. of NaCl and 2, 5 or 10 g. of polysorbate-80. The outer solution (50 ml.) contained 0.1 or 0.2 millimole of salicylamide and 0.45 g. of NaCl.

b) Fifty ml. of the inner solution contained 0.1 or 0.2 millimole of salicylamide, 0.45 g. of NaCl and 2, 5 or 10 g. of polysorbate-80. The outer solution (50 ml.) contained 0.45 g. of NaCl only.

Each solution was adjusted to pH 6.5 with 0.1*N* NaOH.

After standing at 37° for 44 hr., the concentration of the outer solution of each system was measured. The outer solution was taken out, 0.7% $FeCl_3$ (in 0.07*N* HNO_3) was added and it was diluted with distilled water. The absorbance of this solution was read on a spectrophotometer (Hitachi Co., Ltd. model EPU-2A) at 530 mμ. No effect of presence of polysorbate-80 on the result by this method was checked.

Determination of the Rate of Absorption from the Rat Small Intestine——The test solution (100 ml.) contained 0.9 g. of NaCl, 0.2 millimole of salicylamide and 0, 2, 5 or 10 g., respectively, of polysorbate-80, adjusted to pH 6.5 with 0.1*N* NaOH. The drug-free test solution (100 ml.) contained 0.9 g. of NaCl and 10 g. of polysorbate-80, adjusted to pH 6.5 with 0.1*N* NaOH.

According to the recirculating perfusion method of Schanker, *et al.*,[10] the decrease of amount of the drug remaining in the perfusion solution was regarded as the amount absorbed. Wistar strain male rats (270±20 g.) were fasted for about 24 hr. prior to the experiments but were allowed free access to water. The animals were anesthetized by the intraperitoneal injection of 1.25% pentobarbital sodium parenteral solution (0.5 ml./100 g. body wt.). The small intestine was exposed by a midline abdominal incision and cannulated at the duodenal and ileal ends with polyethylene cannulae having inside diameter of 2.5 mm. and outside diameter of 3.5 mm. The intestine was replaced in the abdomen, the incision was closed and these cannulae were joined to a perfusion pump. The small intestine was first cleared of particulate matter by perfusion with 100 ml. of 0.9% NaCl solution maintained at 37°. Then, 100 ml. of the test solution was recirculatingly perfused from duodenum to ileum at a rate of 10 ml. per min.

After perfusion for the time previously designed, the test solution was transferred into a measuring cylinder and the volume of the solution was read. Then the intestinal lumen was washed by perfusion with 100 ml. of 0.9% NaCl. The washing solution was added to the recovered test solution and the volume of the solution was made up to 500 ml. with distilled water. After filtration, 10 ml. of this solution was pipetted into a 20 ml. measuring flask and 1 ml. of 0.7% $FeCl_3$ (in 0.07*N* HNO_3) was added. The volume was made up to 20 ml. with distilled water. The absorbance of the solution was read on a Hitachi EPU-2A spectrophotometer at 530 mμ.

10) L. S. Schanker, *et al.* : J. Pharmacol. Exptl. Therap., **125**, 275 (1958).

The amount of polysorbate-80 remaining in the drug-free solution was determined by UV absorption at 232 mμ. The blank test was carried out by perfusion of 0.9% NaCl solution under the same condition. UV absorption of the blank was negligible.

The experiments for each solution were repeated three times or more.

Results and Discussion

The results of measurement for the viscosity are shown in Table I.

Table I. Volume Fraction of Aqueous Phase to Total Solution

C_s [a]	η_r	ϕ	ϕ/C_s [b]	f_w [c]
0.020	1.124	0.0397	1.99	0.96
0.050	1.379	0.0975	1.95	0.90
0.100	2.253	0.2223	2.22	0.80

a) g./ml.
b) The mean value of ϕ/C_s is 2.0 (ml./g.).
c) f_w is calculated from $f_w=1-2.0\,C_s$.

The values of ϕ/C_s indicate that the effective micellar volume of the surfactant is independent of the concentration within the range of these experimental condition, (average : 2.0 ml./g.). Thus the volume fraction of the aqueous phase to the total solution is calculated from $f_w=1-2.0\ C_s$.

The results of equilibrium dialysis are shown in Table II.

Table II. Partition Constant of Salicylamide between Micellar Phase and Aqueous Phase

C_s [a]	C_D [b]	C_{Dw} [b]	$C_{Dw(mean)}$ [b]	K [c]
0.020	1.00	(a) 0.60 (b) 0.60	0.60	35
0.050	1.00	(a) 0.43 (b) 0.42	0.43	29
0.050	2.00	(a) 0.82 (b) 0.82	0.82	31
0.100	1.00	(a) 0.25 (b) 0.25	0.25	32

a) g./ml. *b*) micromole/ml. *c*) The mean value of K is 32 (ml./g.).

From the agreement of C_{Dw}-values obtained for both systems (a) and (b) in each condition, it is clear that the systems have been in equilibrium. The critical micelle concentration (c.m.c.) of polysorbate-80 in 0.9% NaCl solution (pH 6.5) at 37° which was estimated by the method of solubilization of sudan III, was 0.035 to 0.065% (w/v). The partition constant can be calculated using equation (13), since in these experiments the total concentration of the surfactant is much larger than the c.m.c., which is usually considered to be nearly equal to the concentration of the single molecule of the surfactant. The partition constant of salicylamide between the micellar phase and the aqueous phase is almost independent of concentration of the drug and/or the surfactant in these experiments, (average : 32 ml./g.).

In the absorption experiments, the volume of perfusion solution was approximately constant. When the logarithm of residual ratio of salicylamide in the surfactant-free solution was plotted against time, a straight line was obtained, as shown in Fig. 1.

The absorption rate constant of the free drug, k_{Df}, was calculated from equation (14) by the method of least squares.

$$\log\left(\frac{D_f}{D_f^0}\right) = -0.434\frac{k_{Df}}{V}t \qquad (14)$$

where V was the volume of the perfusion solution and D_f^0 was the initial amount of the free drug in it. k_{Df} was 1.92.

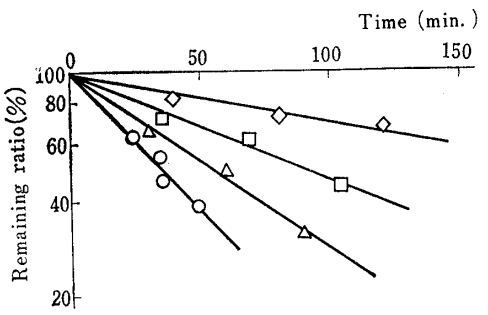

Fig. 1. Logarithmic Plot of Salicylamide Remaining in Perfusion Solution Containing Polysorbate-80 in Various Concentration

Initial salicylamide concentration is 2 mM.
—○— Surfactant-free solution
—△— 2% Polysorbate-80 solution
—□— 5% Polysorbate-80 solution
—◇— 10% Polysorbate-80 solution

The pKa of salicylamide which was obtained in 0.9% NaCl solution at 37° with a potentiograph E-336 (Metrohm A.G.) was 8.2 and the final pH of the perfusion solution was 6.5 to 6.6. Thus, in these experiments the ionized form of salicylamide was negligible.

When the initial concentration of the drug in the perfusion solution was 1 mM or 4 mM (*i.e.* half or twice the concentration in the above case), k_{Df} was 1.75 or 1.80, respectively. Also from the fact that the approximately equal values for k_{Df} were obtained, it might be reasonable to regard the intestinal absorption in these experiments as a first order process.

The consistency of the positions of peaks on the ultraviolet absorption curve of the perfusion solution after the experiment and those of the initial solution was confirmed. Therefore, it was impossible that salicylamide had been decomposed in the intestinal lumen.

The residual ratio of polysorbate-80 in the drug-free solution after perfusion for 90 minutes was 95%. From the results, the absorption of polysorbate-80 might be negligible under the experimental condition. Thus, the concentration of surfactant, C_s, and the volume fraction of the aqueous phase, f_w, are constant during perfusion.

It might be possible that polysorbate-80 caused damage to the small intestine in the experiment. In order to know whether the damage, if it occurred, modified the rate of absorption of salicylamide from the intestine or not, the absorption rate of the drug was measured by perfusion of the surfactant-free solution for 35 minutes after perfusion of 10% polysorbate-80 solution for 90 minutes. The remaining ratio of salicylamide from the intestine pretreated by polysorbate-80 was 48.6% (average) and that from the intestine not pretreated was 50.4% (average). There is no significant difference between them. Therefore, it seems that polysorbate-80 has not caused the irreversible damage having effects on the rate of absorption of salicylamide.

Now, the absorption rate constant of salicylamide in the micellar solution, k, can be calculated for the various concentrations of polysorbate-80, C_s, from equation (9), by using the absorption rate constant of salicylamide in the surfactant-free solution, k_{Df}=1.92, the partition constant of salicylamide between the micellar phase and the

Table III. Absorption Rate Constant Calculated and Observed

C_s [a]	$k_{calc.}$ [b]	$k_{obs.}$ [b]	C_s [a]	$k_{calc.}$ [b]	$k_{obs.}$ [b]
0.00	—	1.92	0.05	0.69	0.79
0.02	1.15	1.26	0.10	0.39	0.33

a) g./ml.
b) ml./min.; for the intestinal perfusion, from duodenum to ileum, of the male rats weighing 250 to 290 g.

aqueous phase, K=32 and the volume fraction of the aqueous phase to the total solution, f_w=1-2.0 C_s. The calculated values of the absorption rate constants, $k_{calc.}$, are shown in Table Ⅲ.

The results of the perfusion experiments for the micellar solution of salicylamide are shown in Fig. 1. When the logarithm of residual ratio of salicylamide was plotted against time, a straight line was obtained. In each case, the slope was determined by the method of least squares and from the slope, the absorption rate constant, k, was obtained. These observed values, k_{obs}'s, are shown in Table Ⅲ. There is a quite agreement between calculated value and observed value of the absorption rate constant in Table Ⅲ.

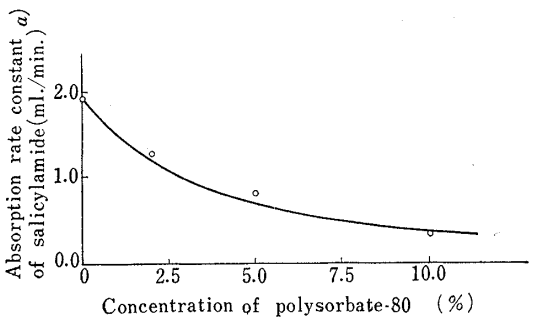

Fig. 2. Relationship between Concentration of Polysorbate-80 and Absorption Rate Constant of Salicylamide

——: Calculated curve ○: Observed value
a) For the intestinal perfusion, from duodenum to ileum, of the male rats weighing 250 to 290 g.

From these results, it appears that the relationship between the absorption rate constant of the drug and the concentration of the surfactant is expressed in equation (9). In Fig. 2, the absorption rate constant of salicylamide is plotted against the concentration of polysorbate-80.

The conclusions are as follows: In the model (1) that a micellar solution consists of two phases, one is a dispersed micellar phase and the other is a continuous aqueous phase surrounding the micelles, (2) that the partition ratio of the drug between the micellar phase and the aqueous phase is constant, and (3) that absorption of the drug incorporated in the micelle is negligible, the relationship between the absorption rate constant of a drug and the concentration of a surfactant is expressed in equation (8). When the total concentration of the surfactant is much larger than the concentration of monomolecular surfactant, the relationship is approximately expressed in equation (9). The model is shown in Fig. 3.

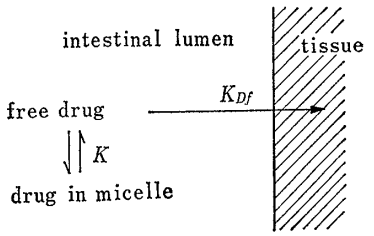

Fig. 3. Model of Absorption of Drug in Micellar Solution

This conclusion drawn from the experiments of salicylamide absorption from the small intestine supports the considerations by Kakemi, *et al.*,[1,2] and by Nogami, *et al.*[4]

Summary

1) The model of absorption of drug in micellar solution from the intestine is proposed. According to this model, the relationship between concentration of the surfactant and absorption rate constant of the drug was derived. The calculated values of the absorption rate constants from the equation were compared with the experimentally observed values.

2) The determination of absorption rate constant was carried out by the perfusion of salicylamide solutions containing polysorbate-80 in various concentrations through the rat small intestine *in situ*.

3) The experimental results indicate that there is a quite agreement between the observed value and the calculated value.

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