

Mechanism of Intestinal Absorption of Drugs from Oil in Water Emulsions.I¹⁾KIICHIRO KAKEMI (the late), HITOSHI SEZAKI, SHOZO MURANISHI,
HIROYASU OGATA and SATOSHI ISEMURA*Faculty of Pharmaceutical Sciences, Kyoto University²⁾*

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Mechanism of the absorption of drugs from oil in water emulsions was studied in the rat large intestine. Synthesized esters of fatty acids and phthalic acid-0.1% Polysorbate 80 was chosen as the emulsion system, the oil/water volume ratio was varied from 0 to 1, and the absorption from emulsions of sulfapyridine, salicylamide, and of acetanilide was investigated using *in situ* recirculation and loop techniques.

In the absorption of drugs having partition coefficients of larger than one, amount of drugs in aqueous phase rather than their concentrations was a critical factor for the absorption from oil in water emulsions.

In the absorption of poorly oil-soluble drugs, drug absorption from emulsions was larger than the one from aqueous solutions when the overall volume of emulsions was kept constant, which suggested the importance of absolute volume of aqueous phase in the absorption of drugs from such systems.

Usually drugs dissolved in oil were absorbed mainly *via* aqueous phase.

Emulsions are one of the dosage forms which have been used since early days. During the past decade, several publications have shown that emulsions possess an advantage for drug bioavailability. Wagner *et al.*³⁾ studied the effect of dosage forms on the serum levels of indoxole, and showed that the drug was more absorbed from emulsions in comparison with other pharmaceutical dosage forms. Lewis, *et al.*⁴⁾ evaluated emulsions as one of the useful forms for vitamin A absorption. Furthermore, it has been reported recently that the intestinal absorption of otherwise unabsorbable macromolecules such as insulin⁵⁾ and heparin⁶⁾ was enhanced by incorporating them into the form of emulsions.

Due to the complexity of the system as well as the experimental difficulties, however, past work on the mechanism of absorption from this biopharmaceutically important system has been relatively sparse. The purpose of this paper was to make clear the mechanism and the factors affecting the intestinal absorption of drugs from oil in water emulsion system. In this study large intestine was chosen as absorptive lumen, since drug absorption from this part of the intestine was found to be generally less complex than the rest of the intestine⁷⁾ and should allow closer and more meaningful study of the factors influencing it.

Experimental

Materials—Polysorbate 80 (Tokyo Kasei Kogyo Co., Ltd.). Isopropyl palmitate, ethyl laurate, and diethyl phthalate were purified by redistillation under reduced pressure. All other chemicals used were reagent grade quality.

1) Presented in part to the 87th Annual Meeting of Pharmaceutical Society of Japan, Kyoto, April 1967.

2) Location: *Yoshida-shimoadachi-cho, Sakyo-ku, Kyoto.*

3) J.G. Wagner, E.S. Gerard and D.G. Kaiser, *Clin. Pharmacol. Therap.*, **7**, 610 (1966).

4) J.M. Lewis, S.Q. Cohan and A. Messina, *Pediatrics*, **5**, 425 (1950).

5) R.H. Engel, S.J. Riggi and M.J. Fahrenbach, *Nature*, **291**, 856 (1968).

6) R.H. Engel and M.J. Fahrenbach, *Proc. Soc. Exptl. Biol. Med.*, **129**, 772 (1968); R.H. Engel and S.J. Riggi, *J. Pharm. Sci.*, **58**, 1372 (1969).

7) a) K. Kakemi, T. Arita and S. Muranishi, *Chem. Pharm. Bull.* (Tokyo), **13**, 861, (1965); b) K. Kakemi, T. Arita, R. Hori, R. Konishi, K. Nishimura, H. Matsui and T. Nishimura, *ibid.*, **17**, 255 (1969).

Preparation of Emulsions—Synthesized esters of fatty acids and phthalic acid—0.1% Polysorbate 80 was used as the emulsion system. Less than 50% (w/v) of isopropyl palmitate (IP), ethyl laurate (EL), and diethyl phthalate (DP) was added to water as dispersed phase. Overall concentration of Polysorbate 80 (PS-80) was kept constant at 0.1%, a minimum concentration to maintain emulsions stable during absorption experiments. Since the addition of any electrolyte to emulsions containing relatively small amount of the emulsifier was observed to accelerate the aggregation tendency of particles,⁹ distilled water was used as continuous phase. The mixture of oil, water, drug, and emulsifier was shaken vigorously and placed overnight at 37°. Emulsification of 100 ml of the mixture was followed by recirculation through homogenizer (Type HO/UA, Erweka-Apparatebau GmbH.) for ten minutes. The emulsions were placed at 37° for 30 minutes before absorption experiments.

Particle Size Distribution of Emulsions—Analysis of particle size distribution was carried out before and after the absorption experiments. Emulsions were diluted to obtain 2% (v/v) oil phase, and photomicrographs were taken immediately. Particle size distribution and average particle size were counted by Size Frequency Analyzer (Type SF-10 Shimadzu Seisakusho Ltd.).

Drug Assay Method in Emulsion System—The procedure was followed by two steps, the first step was demulsification which was carried out by adding electrolytes and heating. The second was extraction of drugs to aqueous phase after cooling and adjusting the pH of the mixture. Except acetanilide, emulsions were diluted to 5 times by volume with distilled water. One-half gram of sodium carbonate was added to 8 ml of the diluted emulsion. The diluted emulsion was heated in a water-bath for 5–10 minutes to dissolve sodium carbonate and cooled. The cooled mixture was shaken mechanically for 30 minutes, centrifuged, and the concentration of drug in aqueous phase was determined.

In the case of acetanilide, 2 g of sodium citrate was added instead of sodium carbonate. Before extraction, pH of the diluted emulsion was lowered less than 1.0 by adding 2 ml of conc. HCl.

Analytical Methods—Sulfonamides^{7(a)} and salicylamide⁹ were analyzed by the same method as described in the previous report from this laboratory. Acetanilide was determined as follows. Four ml of 5N HCl was added to 1 ml of test solution. The solution was boiled for 1 hr, cooled, and added 6 ml of 5N NaOH. After 5 ml of ethylene dichloride was added to 5 ml of the cooled solution, the mixture was shaken mechanically for 20 min. The optical density of the separated organic phase was determined at 290 m μ .

Apparent Partition Coefficient—Partition coefficient of a drug in emulsion system was determined as follows. Emulsions were put into Visking cellulose tubings, 24/32 inches in diameter, and ultracentrifuged at 3000 rpm for 1–2 hr. Drug concentration in clear filtrate (C_w) was determined. Overall drug concentration (C) was obtained by the procedure described elsewhere in this paper.

Absorption Experiment—Male Wistar rats weighing 170–200 g were used in all the experiments. The procedure of absorption experiments has been described in the previous paper except for the following minor modifications. The large intestine was exposed by a middle line abdominal incision, and cannulated at the end of the caecum with polyvinyl tubing. Glass cannula was then inserted into the anus, and was secured by a ligature at the other end. Twenty ml of sample solution was recirculated at a rate of 5 ml/min for 1 hr at 37°. The recirculated fluid was collected completely by washing out with fresh saline, and the amount of the drug in collected fluid was determined. When the rate of absorption of drugs was investigated as a function of aqueous phase volume of emulsions, the latter was kept constant (18 ml) and the speed of recirculation of emulsions was so adjusted that the aqueous phase of emulsions could maintain an equal frequency of contact to the absorptive membrane surface regardless of the overall volume of emulsions. In other words, when oil/water volume ratios were 1/3, 2/3, and 1, overall volume of emulsions and the speeds of recirculation were 24–7 ml/min, 30–8 ml/min, and 36–10 ml/min, respectively.

Result and Discussion

Particle Size Distribution of Emulsions

Particle size distribution of emulsions were determined before and after absorption experiments. Photomicrograph and particle size distribution curve of a typical system are shown in Fig. 1 and Fig. 2, respectively.

Numbers of droplets measured were 150–250 in each determination. The length of 20 μ was divided into 11 elements and the number of oil droplets (n), having diameter of X_n — X_{n+1} , were plotted at $(X_n + X_{n+1})/2$, where X_n is the length of n elements. Average diameter of emulsion droplets was obtained by calculating the area under the particle size distribution

8) A.P. Lemberger and N. Mourad, *J. Pharm. Sci.*, **54**, 229 (1965).

9) K. Käkemi, T. Arita and H. Yamashina, *Yakuzaigaku*, **21**, 97 (1961).

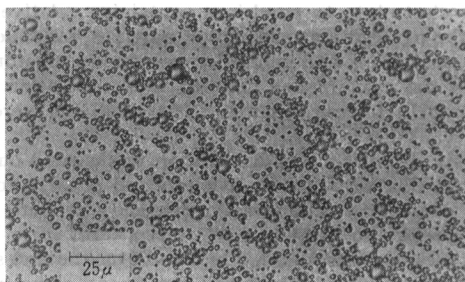


Fig. 1. Photomicrograph of Typical Emulsion before Absorption Experiment

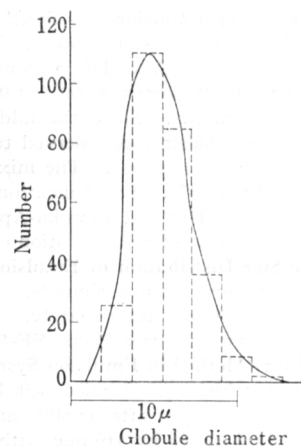


Fig. 2. Typical Particle Size Distribution Curve of Emulsion before Absorption Experiment

curve. These analysis and calculation were attained by operating the Analyzer connecting analog computer.

Table I shows average diameter of various emulsions before and after absorption experiments. Average diameter of 4–6 μ seems a little larger than those of ordinary emulsions. This is presumably because a low concentration of the emulsifier was used in this study to minimize the effect of micellar phase on drug absorption. As shown in the table average diameters of oil droplets scarcely altered during absorption experiment. However, particle size did increase to some extent in emulsions having larger ϕ values. Such differences were, however, not very large and it seems reasonable to assumed that particle size of emulsions used was almost identical under the experiential conditions.

TABLE I. Average Diameters of Emulsions of Three Kinds of Oils at Initial and Final Time of Absorption Experiments

Oil: Water ratio	Average diameter (μ)					
	IPP		EL		DP	
	I ^{a)}	F ^{b)}	I	F	I	F
1/9	4.0	4.5	4.2	4.4	3.8	3.8
1/3	5.5	4.9	5.0	5.0	4.8	5.4
2/3	5.5	5.8	5.8	5.6	5.2	5.4
1/1	5.8	6.2	6.0	6.0	6.0	6.2

a) average diameter at initial time of absorption experiment

b) average diameter at final time of absorption experiment

Partition Coefficient in Emulsion Systems

Bean and Heman-Ackah reported¹⁰⁾ that, in an emulsion system, drug concentration in aqueous phase was related to the overall concentration of the drug by the expression;

$$C_w = C \left(\frac{\phi + 1}{K\phi + 1} \right) \quad \text{Eq. (1)}$$

10) H.S. Bean and S.M. Heman-Ackah, *J. Pharm. Pharmacol.*, **16**, Suppl. 58T (1964).

where C_w , C , K , and ϕ represent concentration of drug in aqueous phase, overall concentration of drug, oil/water partition coefficient, and oil/water volume ratio respectively.

TABLE II. Distribution of Salicylamide to Oil and Water at 37°

Oil		C_w/C	
		Absence of emulsifier	Presence of ^{a)} emulsifier
Isopropyl palmitate	1/3	0.745	0.728
	1/1	0.593	0.555
Ethyl laurate	1/3	0.467	0.456
	1/1	0.305	0.277

a) 0.1% polysorbate 80

As is seen from Table II, however, the ratios of C_w to C of salicylamide in the presence of emulsifier are a little smaller than those without the emulsifier. This seems to be due to the fact that above expression can generally applicable to an emulsion without emulsifier and that in the present ultracentrifugation method, drugs interacted with emulsifier micelles were found in oily phase but not in aqueous phase. As the differences of partition coefficients of these cases were small, the partition coefficients in the absence of the emulsifier shown in Table III, was substituted for the ones in emulsion system in this paper.

TABLE III. Apparent Partition Coefficients^{a)} of Drugs Investigated between Oils and water at 37°

Drugs	pK_a		Oils		
			IPP	EL	DP
Salicylamide	8.3 ^{b)}		2.37	5.56	32.2
Sulfapyridine	2.58	8.43 ^{c)}	0.089	0.25	9.97
Sulfanilamide	2.36	10.43 ^{c)}	0.034		
Acetanilide	0.3 ^{d)}		1.05		

a) at neutral pH

b) K. Kakemi, T. Arita and H. Yamashina, *Yakuzaigaku*, **21**, 97 (1961)

c) T. Koizumi, T. Arita and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 413 (1964)

d) P.A. Shore, B.B. Brodie and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **119**, 361 (1957)

Drug Absorption from Emulsion System

In an emulsion system, drugs partition between oil and aqueous phases and relation of drug concentration in aqueous phase and in overall emulsions in such a system has been given by Eq. (1). From Eq. (1), amount of drug in aqueous phase is written by Eq. (2), where M_w and M represent the amount of drug in aqueous phase and the total amount of drug in emulsions respectively.

$$M_w = M \left(\frac{1}{K\phi + 1} \right) \quad \text{Eq. (2)}$$

Fig. 3 illustrates the schematic profiles of concentrations and amount of drugs in aqueous phase versus their oil concentrations. Bean^{10,11)} and Heman-Ackah¹²⁾ reported that concentration of preservatives in aqueous phase parallel to their microbiological actions and

11) H.S. Bean, J.P. Richards and J. Thomas, *Boll. Chim. Farm.*, **101**, 339 (1962).

12) S.M. Heman-Ackah and G.H. Konning, *J. Pharm. Pharmacol.*, **19**, Suppl., 189S (1967).

pointed out that K and ϕ were main factors which govern the effectiveness of preservatives in emulsions. However, in the case of absorption from emulsions, drugs are transported through the absorptive membrane and not only concentration but their absolute amount have to be taken into considerations.

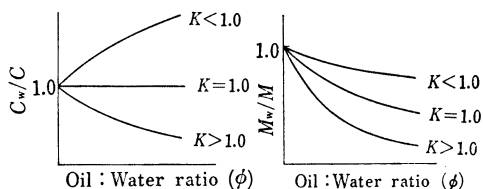


Fig. 3. Schematic Representation of Drug Distribution as a Function of Oil: Water Volume Ratio (ϕ)

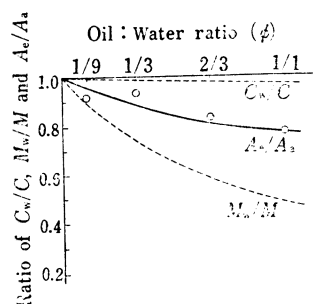


Fig. 4. Absorption of Acetanilide from Isopropyl palmitate Emulsion ($K=1.05$)

Fig. 4 shows the absorption of acetanilide from IPP emulsion, where A_e and A_a represent % absorbed from emulsion and % absorbed from aqueous solution, respectively. As is evident from the figure, absorption of acetanilide from emulsions decreased with the increase of ϕ . This phenomenon is quite different from the case of preservatives in emulsions reported by Bean *et al.* Since partition coefficient of acetanilide between IPP and water was 1.05, C_w/C was almost unity over the wide range of ϕ while M_w/M decreased with the increase of ϕ . It appeared that amount of drugs in aqueous phase rather than their concentration was a

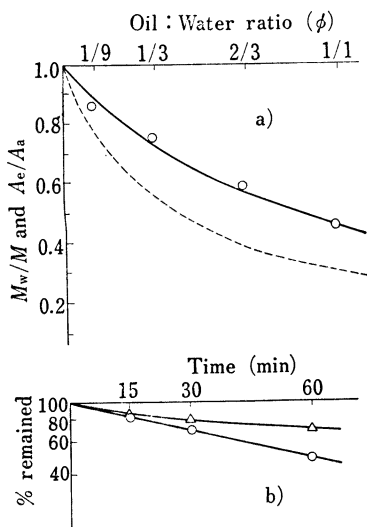


Fig. 5. Salicylamide Absorption from Emulsion System at More Than Unity Partition Coefficient

- a) M_w/M and A_e/A_a in IPP Emulsion ($K=2.37$) as a Function of ϕ , -----: M_w/M ; ———: A_e/A_a .
b) Semilogarithmic Plots of % remained against Time for IPP Emulsion, —○—: $\phi=1/3$; —△—: $\phi=1/1$.

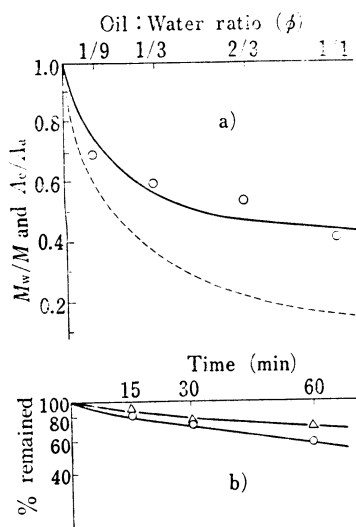


Fig. 6. Salicylamide Absorption from Emulsion System at More Than Unity Partition Coefficient

- a) M_w/M and A_e/A_a in EL Emulsion ($K=5.56$) as a Function of ϕ , -----: M_w/M ; ———: A_e/A_a .
b) Semilogarithmic Plots of % remained against Time for EL Emulsion, —○—: $\phi=1/3$; —△—: $\phi=1/1$.

dominant factor which governed the degree of absorption, and that drugs were absorbed mainly *via* aqueous phase, not directly from oily phase.

Absorption of a drug having partition coefficient of more than unity was studied with salicylamide and the absorption patterns from IPP, EL, and DP emulsions are shown in Fig. 5, 6, and 7. As is seen from the figures, the larger the partition coefficient and oil: water volume ratios were, the smaller was the amount of drugs in aqueous phase, and the less drug was absorbed. Since the loss of drug in aqueous phase is supplied from oil phase during absorption and the contribution of the direct route of absorption from oil phase is negligible, it seems natural that the absorption is generally much faster than the one estimated from the state of initial drug distribution. However, good correlation has been noted between the percentage of one-hour absorption and the amount of a drug in aqueous phase. This result supports the view that the amount of a drug in aqueous phase is a critical factor for the absorption from oil in water emulsions. Fig. 5, 6, and 7 also indicate the presence of two types of patterns in drug disappearance from emulsions, namely, the one which followed apparent first-order kinetics and the other did not. In the latter, initial ratios of M_w to M were 0.6, 0.4, and 0.2 and in the former, the ratios were close to 0 or to 1.0. On the basis of these observations, a mechanism of drug absorption from emulsions can be proposed. It seems that drugs dissolved in oil phase are absorbed mainly *via* aqueous phase and that the absorption proceeds with first-order kinetics when the initial amount of drug in aqueous phase is fairly large in comparison with the amount of total drugs in the system or when very little drug exists in aqueous phase

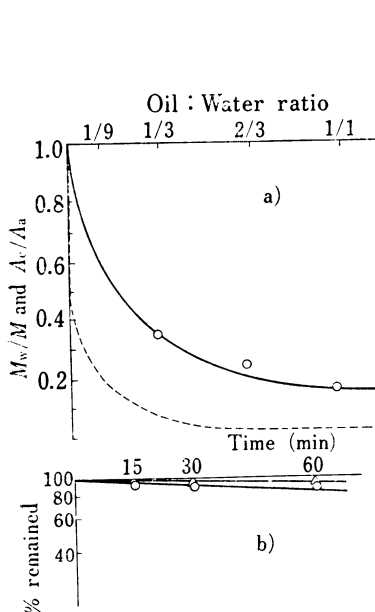


Fig. 7. Salicylamide Absorption from Emulsion System at More Than Unity Partition Coefficient

- a) M_w/M and A_e/A_a in DP Emulsion ($K=32.2$) as a Function of ϕ , —: M_w/M ; —: A_e/A_a .
b) Semilogarithmic Plots of % remained against Time for DP Emulsion, —○—: $\phi=1/3$; —△—: $\phi=1/1$.

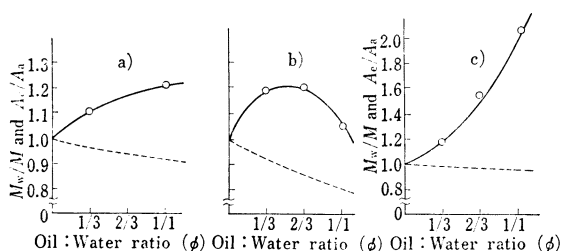


Fig. 8. Drug Absorptions from Emulsion Systems at Less Than Unity Partition Coefficient

- a) Sulfapyridine Absorption from IPP Emulsion ($K=0.089$)
b) Sulfapyridine Absorption from EL Emulsion ($K=0.25$)
c) Sulfanilamide Absorption from IPP Emulsion ($K=0.034$)
-----: M_w/M ; —: A_e/A_a

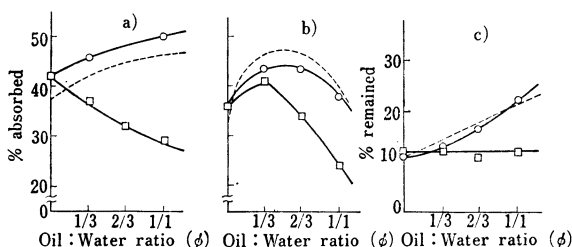


Fig. 9. Effect of Aqueous Phase Volume on Drug Absorption from Emulsion System at Less Than Unity Partition Coefficient

- a) Sulfapyridine Absorption from IPP Emulsion
b) Sulfapyridine Absorption from EL Emulsion
c) Sulfanilamide Absorption from IPP Emulsion
—□—: by constant aqueous phase volume procedure
—○—: by constant overall volume procedure
-----: by calculation with Eq. (3) using values of constant aqueous phase volume procedure.

initially. In these instances, absorption is more rate-limited by drug transfer from oil to water than the one from water to absorptive membrane. On the contrary, in an intermediate state between those cases, both the transfer of a drug from oil to water and that from water to membrane become rate limiting.

In the absorption of a drug having the partition coefficient of less than unity, an interesting phenomenon was noted as shown in Fig. 8. In every case, drug absorption from emulsions was larger than the one from aqueous solutions. This may not be due to an increase of aqueous phase drug concentration, because the absorption was likely enhanced when the initial drug concentration in aqueous phase was kept constant in the entire range of oil concentrations of emulsions investigated. Nogami, *et al.* reported¹³⁾ that the apparent absorption rate constant k' was represented by the following equation

$$k' = \frac{1}{2.303} \cdot \frac{k}{v} \quad \text{Eq. (3)}$$

where k is the intrinsic absorption rate constant independent of V , the volume of the perfusion solution. Perrin¹⁴⁾ and Martin¹⁵⁾ also pointed out that the volume of compartments had to be taken into account to analyze such drug transfer data. If total volume of emulsions is maintained constant at any oil concentrations, aqueous phase volume decreases with the increase of oil concentration. Accordingly, apparent absorption rate constant from aqueous phase will be increased as presumed from Eq. (3). To confirm such effect, aqueous phase volume of emulsions was maintained equal to that of the control experiment (absorption from aqueous solution) and the speed of recirculation through intestine was adjusted so as to make the frequency of contact with absorptive membrane equal to that in the control experiment. As shown in Fig. 9 (c), absorption of sulfanilamide from IPP emulsions, in which K value was 0.034, did not change significantly over wide range of oil concentrations. Under these conditions, it is evident, therefore, that, as presumed above, apparent absorption rate constant from aqueous phase is more clearly demonstrated in the system having partition coefficient of less than unity. Fig. 9 (a) and (b) show the similar patterns in sulfapyridine absorption from IPP and EL emulsions respectively. Based on the intrinsic absorption rate constants obtained from the constant aqueous phase volume technique, the values of % absorbed were calculated by Eq. (3) and are shown in Fig. 9 (a), (b), and (c). These values agreed well with the observed ones when the overall emulsion volume was kept constant. These results again support the view that the absolute volume of aqueous phase is one of the critical factors in the absorption of poorly oil-soluble drugs from oil in water emulsions.

13) H. Nogami, M. Hanano and H. Yamada, *Chem. Pharm. Bull.* (Tokyo), **16**, 389 (1968).

14) J. Perrin, *J. Pharm. Sci.*, **56**, 411 (1967); *idem*, *J. Pharm. Pharmacol.*, **19**, 25 (1967).

15) S.A. Khalli and A.N. Martin, *J. Pharm. Sci.*, **56**, 1225 (1967).