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The Significance of Vehicle Oil Metabolism in the Absorption Process of Lipid-Soluble Compounds[1]

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The significance of vehicle oil metabolism in the absorption of lipid-soluble compounds from oil-in-water emulsions was investigated in rat small intestine using lipid-soluble dyes, Sudan Blue and Oil Red XO, and vitamin A acetate as model compounds. Although little difference was observed among their initial uptake rates, negligible amount of lipid-soluble compounds was absorbed from oleic acid emulsions during the first hour whereas an obvious amount of them was absorbed from triolein emulsions. From oleic acid-triolein mixed emulsions their absorption were decreased as oleic acid-triolein ratio was increased.

These results suggested that in the presence of oleic acid lipid-soluble compounds were pooled in some components of epithelial cells. This was well supported by the distribution experiments of Sudan Blue and Oil Red XO into the brush border fraction of the epithelial cells. In the case of oleic acid emulsions, about three to five times as much of dyes were bound to the brush border fraction as compared with triolein emulsions. This phenomenon was considered to be the reflection of the affinity of lipid-soluble compounds to oils, which was partly indicated by the solubilities of the former in the later.

It seems probable that the saturation of absorption of lipid-soluble dyes from triolein emulsions, described in our previous paper, is mainly due to the strong affinity of dyes to the membrane components in the presence of oleic acid which was produced from the hydrolysis of triolein.

Keywords——lipid-soluble compounds; oil-in-water emulsion; oleic acid; rat; initial uptake; strong affinity to the brush border; lipolysis

In previous papers from this laboratory,[1b,3] the mechanism of the intestinal absorption and the lymphatic transport of lipid-soluble compounds from oil-in-water emulsions and the factors affecting on them have been investigated in the rat. The absorption characteristics of lipid-soluble dyes used as model compounds was demonstrated as the reflection of that of oils. From emulsions using triolein as an oil phase, these dyes were absorbed faster in the early stage and slower in the later stage than from emulsions using tributyrin as an oil phase or from micellar solutions, from which they were absorbed monoexponentially.

The observed saturation phenomenon in the absorption rate in the case of triolein emulsions was attributed to the saturation of the adsorption on the mucosal surface, where the hydrolysis of the adsorbed triolein occurred slower than that of tributyrin.[3a] Dawson, *et al.*[4] have studied the absorption of oleic acid from micellar and nonmicellar taurocholate solutions using closed loops of rat jejunum *in vivo*, and reported that the absorption from the former was greater than that from the latter. Similar results were shown by Knobel[5] while, on the contrary other

1) *a*) This paper constitutes the 9th report in the series of "Mechanism of the Intestinal Absorption of Drugs from Oil-in-Water Emulsions"; *b*) Preceding paper, Part VIII: T. Noguchi, Y. Tokunaga, H. Ichikawa, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **25**, 413 (1976).
2) Location: *Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto, Japan.*
3) *a*) T. Noguchi, C. Takahashi, T. Kimura, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 775 (1975); *b*) T. Noguchi, Y. Jinguji, T. Kimura, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 782 (1975).
4) A.M. Dawson and J.P.W. Webb, *Proc. Soc. Exptl. Biol. Med.*, **142**, 906 (1973).
5) L.K. Knobel, *Am. J. Physiol.*, **223**, 255 (1972).

workers have reported different ones.[6] In those experiments, however, relatively high concentration of bile salts was used as compared with the concentration of lipids. Accordingly, much micelles existed beside oil droplets in the emulsions, and the physical properties as well as the mixture ratios of emulsions and micelles were considered to be different under various experimental conditions.

On the contrary we have used relatively low concentration of Tween-80 as an emulsifier to minimize micellar phase. In this paper, the significance of vehicle oil lipolysis was investigated from the standpoints of the uptake rate of vehicle oils containing their metabolites and of the distribution rate of lipid-soluble compounds from vehicle oils to the absorptive membrane and to the cytoplasmic proteins which participate in cellular transport of lipid-soluble compounds. The mechanism of the saturation in the absorption of lipid-soluble compounds from triolein emulsions were discussed from the viewpoint of vehicle oil lipolysis.

Experimental

Materials——Oil Red XO and Sudan Blue were obtained from Tokyo Kasei Co., Ltd., and vitamin A acetate from Sigma Chemical Co., Ltd. Triolein (Sigma Chemical Co., Ltd.), monolein (Tokyo Kasei Co., Ltd.), oleic acid (Nakarai Chemicals, Ltd.), and egg lecithin (Merck and Co., Ltd.) were used as supplied. MCT-8 (trioctanoin) was supplied by Nisshin Seiyu Co., Ltd. Sodium taurocholate was synthesized by the method of Norman [7] with slight modification. Other chemicals were of reagent grade.

Preparation of Emulsions——Unless otherwise specified, emulsions containing 4% v/v of triolein, 400 μg/ml of drugs, and 0.2% w/v of Tween-80 in distilled water were prepared. Others containing 80 mM of oleic acid and 40 mM of monolein (instead of triolein) and 0.4 mg/ml of egg lecithin and 20 mM of sodium taurocholate (instead of Tween-80) were used. The mixture of these components was shaken vigorously and sonicated at 20 kHz, 100 W for 5 min by a sonicator (No. 5202, Ohtake Seisakush Japan) under ice cooling.

Animal Experiments——Male Wistar rats weighing 190—220 g were used in all experiments. Under sodium pentobarbital anesthesia, the whole small intestine was made into loop and served for absorption experiments in the same manner described in a previous paper.[3a] Cannulation of major intestinal lymphatic was also carried out similarly as described in a previous paper.[3b]

Distribution of Lipid-Soluble Dyes in the Epithelial Brush Border——The change of the distribution pattern of Sudan Blue and Oil Red XO was examined in the brush border fraction of the epithelial cells. After 30 min of incubation in the intestinal loop *in situ*, emulsions remaining in the lumen were washed out with cold water. Immediately, the small intestine was removed, everted, and the mucosa was scraped with a glass slide. Then, the mucosa was placed in 75 volumes of 5 mM EDTA buffer (pH 7.4) and homogenized in a glass homogenizer for 2 min. All experiments were performed at 4°. The brush border fraction was obtained by the method of Forstner, *et al.*,[8] and the dye distributed in it was determined spectrophotometrically. A portion of the total homogenates was used to determine the control value (100% of dose remained in the whole mucosal tissue).

Solubility of Dyes——The dyes were agitated in various solvents at 25° for two days, after that period of time no further changes in the concentration of dyes were observed. Samples were centrifuged then, the supernatant layers were extracted with organic solvents and the concentration of dyes was determined spectrophotometrically.

Analytical Methods——The dye and vitamin A acetate remained in the collected luminal fluid, in the intestinal tissue homogenate, or in the lymphatic fluid were determined spectrophotometrically or spectrofluorometrically after extraction with organic solvents. The procedures in details were described in our previous papers.[3] Luminal triolein and MCT-8 were determined by the modified method of Van Handel,[9] and oleic acid was estimated titrimetrically using phenolphthalein as an indicator.

Results

Absorption and Lymphatic Transport of Lipid-Soluble Compounds from Emulsions Containing either Triolein or Its Metabolites

It is well known that triolein is hydrolyzed intraluminally or at the surface of the epithelial cells, and the lipolytic products, monolein and oleic acid, are dispersed in bile salt mixed

6) J.D. Hamilton, *Biochim. Biophys. Acta*, **239**, 1 (1971); W.J. Simmonds, T.G. Redgrave, and R.L.S. Willix, *J. Clin. Invest.*, **47**, 1015 (1968).
7) A. Norman, *Arkiv. Kemi.*, **8**, 331 (1955).
8) G.G. Forstner, S.M. Sabesin, and K.J. Isselbacher, *Biochem. J.*, **106**, 381 (1968).
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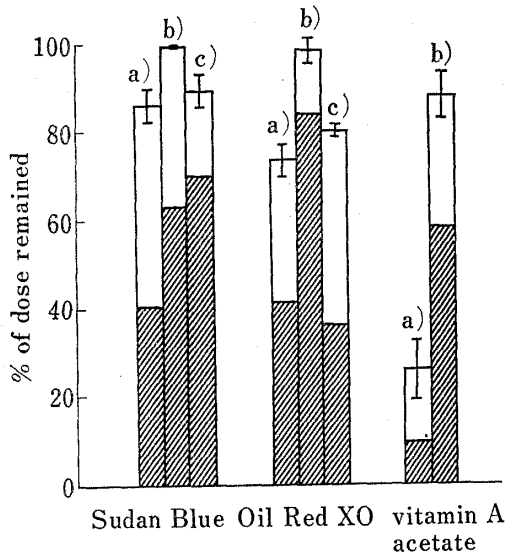

Fig. 1. Absorption of Lipid-Soluble Compounds from Triolein and Oleic Acid Emulsions

a) triolein emulsion, 1 hr incubation
b) oleic acid emulsion, 1 hr incubation
c) oleic acid emulsion, 3 hr incubation
□: remained in intestinal tissue
▨: remained in intestinal lumen
Each emulsion contains 4% v/v of oils, 0.2% w/v of Tween-80, and 400 μg/ml of drugs.
Results are expressed as the mean of at least 4 animals. The vertical bars indicate±S.D. of total compounds remained.

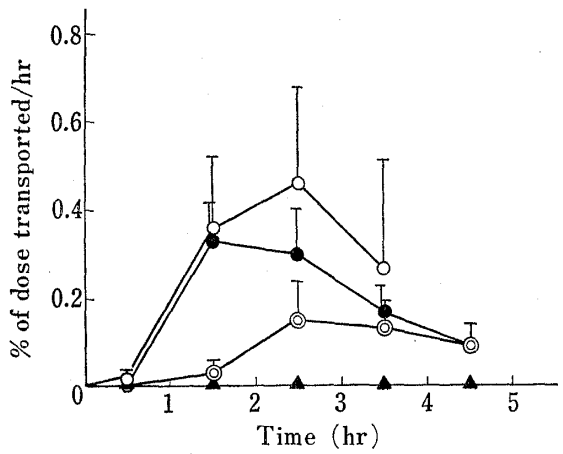

Fig. 2. Lymphatic Transport of Sudan Blue and Oil Red XO from Various Emulsions

○: Sudan Blue–triolein emulsion; ●: Sudan Blue–oleic acid emulsion; ◎: Sudan Blue–oleic acid, monolein emulsion containing Na-taurocholate and egg lecithin; ▲: Oil Red XO–oleic acid emulsion (2 animals)
Each emulsion contains 4% v/v of oils. 0.2% w/v of Tween-80, and 400 μg/ml of dyes except oleic acid–monolein mixed emulsion, which contains 80 mM of oleic acid, 40 mM of monolein, 0.4 mg/ml of egg lecithin, 20 mM of sodium taurocholate, and 400 μg/ml of dyes.
Results are expressed as the mean of at least 4 animals exclusive of Oil Red XO. The vertical bars indicate+S.D.

micelles, then taken up by the epithelial cells. It was, therefore, considered that the absorption of lipid-soluble compounds was faster from emulsions of triolein metabolites than from that of triolein. From this point of view, using lipid-soluble dyes, Sudan Blue and Oil Red XO, and vitamin A acetate as model compounds, the absorption from oleic acid emulsions was compared with that from triolein emulsions. As shown in Fig. 1, their absorption from oleic acid emulsions were surprisingly low (Fig. 1-(b)), and the absorption of both dyes in the first hour was negligible. Same result was observed about the absorption of Sudan Blue from oleic acid–monolein mixed emulsions containing egg lecithin and sodium taurocholate (where 99.0±0.6% of the administered dose was remained after one hour). However, a significant amount of these dyes was absorbed during 3 hr (Fig. 1-(c)). This slow absorption from oleic acid emulsion was also observed when vitamin A acetate was used. In the case of dyes, however, it was not reflected well on the lymphatic transport (Fig. 2). Only in the case of oleic acid–monolein emulsion, the lymphatic transport of Sudan Blue was significantly lower than the other two. Lymphatic transport of Oil Red XO was not observed when oleic acid as well as triolein emulsions[3a] were used.

Effect of Coexistence of Oleic Acid with Triolein

The following two possibilities could be considered from the above data: (1) The absorption of oleic acid from emulsions may be slower than that of triolein. In this case, the absorption of Sudan Blue from triolein emulsion should be inhibited by pretreatment with oleic acid due to the adsorption of its droplets to the epithelial cells. (2) Oleic acid may be bound strongly to the brush border with lipid-soluble compounds. In this case, pretreatments with triolein emulsion could not facilitate the absorption of Sudan Blue from oleic acid emulsion.

To examine these possibilities intestinal loops were pretreated with either oleic acid or triolein before the absorption experiments. As shown in Table I, though the pretreated emul-

Table I. Effect of Pretreatment with Oils on the Absorption of Sudan Blue from Triolein and Oleic Acid Emulsions

Oil component of test emulsion	Pretreatment	% remained after 1 hour		
		In lumen	In tissue	Overall
Triolein	none	64.0±6.0	17.9±2.9	81.8±4.0
	oleic acid emulsion	69.2±2.2	13.9±3.6	83.1±1.4
Oleic acid	none	66.9±3.3	35.2±3.4	102.2±0.8
	triolein emulsion	82.3±2.5	18.7±1.1	101.0±1.8

Each emulsion contains 4% v/v of oils, 0.4 mg/ml of egg lecithin, and 20 mM of sodium taurocholate. Only test emulsions contain 400 μg/ml of Sudan Blue. Five milliliters of emulsions were administered for pretreatment in the loop and was incubated for 30 min. As soon as the incubation period was over, the emulsion was pushed out by air to maintain adsorbed emulsions intact, then 5 ml of test emulsions were admistered and was incubated for one hour.

Results are expressed as the mean±S.D. of at least 4 animals.

sions were pushed out by air, overall absorption was not significantly different from control experiments. Next, the absorption of Sudan Blue and Oil Red XO was investigated from various ratios of oleic acid–triolein mixed emulsions. Results are shown in Fig. 3. Although the pattern is different between two dyes, the absorption and the disappearance of both dyes from the intestinal lumen were decreased as the ratio of oleic acid to triolein was increased. These results are in agreement with our previous observation[3a] as the disappearance of Oil Red XO from the intestinal lumen was saturated after 30 min of incubation, when half of triolein was considered to be hydrolyzed.

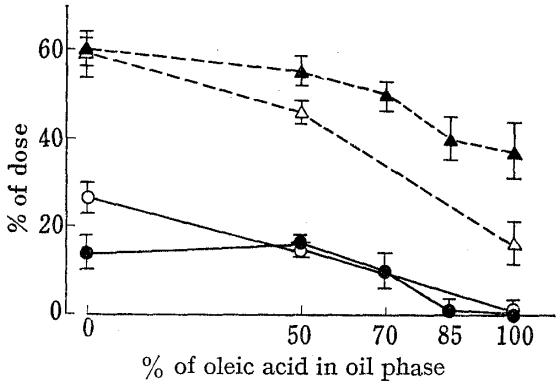

Fig. 3. Effect of Concentration of Oleic Acid on the Absorption of Sudan Blue and Oil Red XO from Oleic Acid-Triolein Mixed Emulsion

●: Sudan Blue absorbed; ○: Oil Red XO absorbed; ▲: Sudan Blue disappeared from intestinal lumen; △: Oil Red XO disappeared from intestinal lumen

Five milliliters of emulsions, which contain 4% v/v of oils, 0.2% w/v of Tween-80, and 400 μg/ml of dyes, were administered in the intestinal loop and was incubated for one hour.

Results are expressed as the mean±S.D. of at least 4 animals.

Table II. Initial Uptake of Lipid-Soluble Compounds and Their Vehicle Oils from Various Emulsions *in Situ*

Emulsion system		Uptake/min (% of dose)	Absorption/hr[a] (% of dose)
Compound	Oil		
Sudan Blue	triolein	11.3±5.4	14.1±3.8
Oil Red XO	triolein	16.3±3.8	26.3±3.5
—	triolein	6.4±1.5	—
Sudan Blue	oleic acid	12.0±2.7	0.5±0.2
Oil Red XO	oleic acid	9.2±2.5	1.4±2.1
—	oleic acid	13.9±3.4	—
Sudan Blue	MCT-8	2.4±1.0	2.3±0.7
Oil Red XO	MCT-8	10.7±0.4	6.8±2.1
—	MCT-8	3.6±2.5	—

a) Absorption of dyes for one hour was calculated from the same data as Fig. 1.

Each emulsion contains 4% v/v of oils, 0.2% w/v of Tween-80, and 400 μg/ml of dyes. Emulsions were instilled into the small intestinal loop for one minute at the rate of 5 ml/min. At the end of the infusion period the luminal emulsions were rapidly washed out with 5 ml of water 4 times.

Results are expressed as the mean±S.D. of at least 4 animals.

Initial Uptake of Lipid-Soluble Dyes and Their Vehicle Oils

To obtain further insight about the difference between oleic acid and triolein emulsions, the uptake of Sudan Blue from these emulsions were studied using *in vitro* everted duodenal sacs. During 10 min 46.0±10.6 μg and 46.3±6.6 μg of Sudan Blue were taken up from oleic acid and triolein emulsions, respectively. No significant difference was noted between the two. Therefore, using the whole small intestinal loop *in situ*, initial uptake of dyes and oils

was measured. As shown in Table II, in the cases of triolein and MCT-8 emulsions, the absorption of dyes during one hour was well reflected by their initial uptake within one minute. On the contrary, there was no relationship in the case of oleic acid emulsion. Although the absorption of dyes from oleic acid emulsions were much smaller than that from triolein emulsions, their uptake was about the same between triolein and oleic acid emulsions.

It is also noticeable that in some cases the uptake of dyes was greater than that of their vehicle oils. This is demonstrated by the uptake of Sudan Blue from triolein emulsion and that of Oil Red XO from triolein and MCT-8 emulsions. These results, suggest the existence of a special mechanism in the absorption process of dyes from oleic acid emulsions, which causes the accumulation of lipid-soluble dyes in the mucosal tissue.

Differences in Distribution of Dyes to the Brush Border Fraction

As the idea that the saturation of the mucosal surface evokes the suppression of the absorption of lipid-soluble compounds from oleic acid emulsions was denied from Tables I and II, it can be considered that the compound could move into inner compartments of the epithelial cells less easily owing to its binding to the brush border in the presence of oleic acid. To elucidate this concept, the small intestine was removed 30 min after instillation of emulsions and the brush border was fractionated. As shown in Table III, the percentage of dyes distributed in the brush border fraction was different between triolein and oleic acid emulsions. In the case of oleic acid emulsions about three times of Sudan Blue and five times of Oil Red XO were bound to the brush border as compared with that of triolein emulsions.

Table III. Distribution of Sudan Blue and Oil Red XO in the Brush Border Fraction

Emulsion system		Dye in the brush border fraction[a]
Compound	Oil	
Sudan Blue	triolein	2.0±0.8
	oleic acid	6.6±2.5
Oil Red XO	triolein	2.3±0.7
	oleic acid	10.4±2.5

a) expressed as the percentage of accumulated dyes in the mucosal tissue±S.D.

Five milliliters of emulsions, which contain 4% v/v of oils, 0.2% w/v of Tween-80, and 400 μg/ml of dyes, were instilled into the intestinal loop and were incubated for 30 min. As soon as the incubation period was over, administered emulsions were washed out. Then the intestine was everted, and after being scraped the mucosa the brush border was fractionated in the usual way. See detail in the text.

Discussion

In the case of a drug possessing an oil/water partition coefficient of less than one, absorption from oil-in-water emusions through the large intestinal membrane usually took place after being released from the oil into the luminal fluid.[10] However, in the case of lipidsoluble but water-insoluble drugs, the absorption process is considered to be consisted of the following steps: (1) adsorption of vehicle oils to the absorptive membrane, (2) partition of drugs into membrane lipids with simultaneous partition into hydrolyzed oils, (3) release from membrane lipids or from hydrolyzed oils and movement into inner compartments, such as microsomes, by binding to cytoplasmic proteins, (4) transport into portal or lymphatic system according to the property of the drugs.

The first step is thought to be controlled only by the property of the oil, including its metabolism. As described in our previous paper,[3a] the amount absorbed of Oil Red XO from tributyrin emulsion during one hour was greater than that from triolein emulsion due to the rapid metabolism of tributyrin. The absorption of Sudan Blue and Oil Red XO from

MCT-8 emulsion was slower than that from triolein emulsion probably due to the slower uptake rate of MCT-8 (Table II).

The second step is considered to be drug dependent process and seems to be very complicated, for, both vehicle oils and their metabolites are absorbable. The drug-dependency of this step has been elucidated by many facts. Concentration dependency has been observed in the absorption of Sudan Blue contrary to Oil Red XO.[3a] Significant amount of Oil Red XO was absorbed from squalane which was reported to be unabsorbable from rat's intestine.[11] The uptake of Oil Red XO from MCT-8 emulsion was faster than Sudan Blue and MCT-8 itself (Table II).

It is well known that the fatty acid binding protein (FABP) exists in the epithelial cellls of the small intestine, and that FABP binds to unsaturated fatty acids more specifically than to saturated fatty acids.[12] From this point of view, the absorption of oleic acid was compared with that of triolein, which had to be hydrolyzed before binding to FABP. Although there were many reports[13] that triolein was absorbed as much as oleic acid was, their absorption were compared in long time experiments. However, as shown in Fig. 1, in the first hour the absorption of lipid-soluble compounds from oleic acid emulsion was slower than that from triolein emulsion, though the initial uptake and the lymphatic transport of them were almost equal from both emulsions (Table II and Fig. 2).

These results suggest the existence of strong bindings which immobilize lipid-soluble compounds as well as oleic acid into inner compartments. The result of Table III strongly supports this idea. It can be well considered that when such a strong binding proceeds in the third step, the following fourth step must be also affected by it and resultant absorption will be reduced. The strength of this binding would be partly related to the solubility of drugs in vehicle oils. As shown in Table IV, the solubility of Sudan Blue in oleic acid is three times of that in triolein, while the solubility of Oil Red XO in oleic acid is less than twice of that in triolein. These properties also reflected well in the uptake pattern shown in Table II. The solubility of Oil Red XO in MCT-8 was almost equal to that in triolein, and the uptake of Oil Red XO from triolein and MCT-8 emulsions were both faster than vehicle oils. On the other hand, the uptake of Sudan Blue from MCT-8 emulsion was almost equal to that of MCT-8 probably due to the high solubility of Sudan Blue in MCT-8 compared with that in triolein. Moreover, it is interesting that the uptake of MCT-8 proceeded slower than triolein (Table II), though MCT-8 was reported to be absorbed without hydrolysis.[14]

Table IV. Solubilities of Sudan Blue and Oil Red XO to Various Solvents

Solvent	Solubility (mg/ml)	
	Sudan Blue	Oil Red XO
Water	0.0000	0.000
Triolein	10.1	22.0
Oleic acid	29.5	36.5
Oleic acid+monolein(2:1)	12.5	24.0
MCT-8	30.6	25.5

The samples were agitated at 25° for two days after that period of time no further changes in concentration of dyes were observed.

10) K. Kakemi, H. Sezaki, S. Muranishi, H. Ogata, and S. Isemura, *Chem. Pharm. Bull.* (Tokyo), **20**, 708 (1972); *etc.*
11) P.W. Albro and L. Fishbein, *Biochim. Biophys. Acta*, **219**, 437 (1970).
12) R.K. Ockner, J.P. Pittman, and J.L. Yager, *Gastroent.*, **62**, 981 (1972).
13) N. Gallagher, J. Webb, and A.M. Dawson, *Clin. Sci.*, **29**, 73 (1965).
14) K.J. Isselbacher, *Gastroent.*, **50**, 78 (1966).

From the above discussion, it seems probable that the saturation of absorption of lipid-soluble dyes from triolein emulsions described in our previous paper[3a] is mainly due to the strong affinity of dyes to the brush border components in the presence of oleic acid hydrolyzed from triolein. However, in the case of oral administration, triolein and oleic acid are almost completely absorbed from the small intestine, although the absorption of oleic acid proceeds more slowly than that of triolein (Fig. 1). Therefore, from the pharmaceutical point of view, oleic acid can be considered as a useful vehicle to sustain the release of lipid-soluble drugs from the epithelial cells to the circulation, consequently prolong their effects. Moreover, the lymphatic transport of Sudan Blue was not significantly decreased in the case of oleic acid emulsion compared with triolein emulsion (Fig. 2), though its absorption was severely reduced (Fig. 1). This means that the portion of lymphatic route was increased in the absorption of Sudan Blue from oleic acid emulsion. Lipid-soluble dyes transported *via* portal route seems to be highly metabolized in the liver and excreted in bile.[3a] So, the increased portion of lymphatic transport observed in oleic acid emulsion can contribute to the higher bioavailability.

As to the fourth step, the mechanism which decides the transport route, the lymphatic or portal one, is still unclear and is now under examination.