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Mechanism of the Intestinal Absorption of Drugs from Oil-in-Water Emulsions. VI.[1] Absorption of Lipid-Soluble Dyes from Tributyrin and Triolein Emulsions in Rat Small Intestine

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Absorption characteristics of highly lipid-soluble dyes from oil-in-water emulsions was studied in the rat small intestine. Short-chain triglyceride or long-chain triglyceride was chosen as an oil phase and absorption of oils as well as dyes (Oil Red XO and Sudan Black B) was investigated using *in situ* loop method.

From emulsions using tributyrin as an oil phase, Oil Red XO was absorbed monoexponentially to the same extent as from Polysorbate-80 micellar solution despite the fact that the dye is very lipophilic and is not considerable to be localized in the aqueous phase. On the other hand, from emulsions using triolein as an oil phase, Oil Red XO was absorbed faster in the early stage and slower in the later stage than from tributyrin emulsions. These absorption characteristics of Oil Red XO was demonstrated as the reflection of that of oils.

Oil Red XO does not seem to move into inner compartments with oil, for it was not transported into intestinal lymph even from triolein emulsions.

In the previous papers from this laboratory,[3] the mechanism and the factors affecting the intestinal absorption of drugs from oil-in-water emulsion system have been investigated using the rat large intestine. Synthetic oils employed in those experiments could be considered practically nonabsorbable from the large intestine. On the other hand, it is well established that natural vegetable oils are absorbed from the small intestine after hydrolysis and solubilization.

Hamilton[4] suggested that oleic acid could be absorbed directly from emulsion droplets without passing through an intermediary micellar phase in the rat jejunum. Carrigan[5] studied the gastrointestinal absorption of micronized griseofulvin from an oil-in-water emulsion dosage form and reported that bioavailability of micronized griseofulvin could be increased as compared to an aqueous suspension. Still the mechanisms by which the presence of triglyceride in the emulsion markedly enhances the absorption of griseofulvin have not been fully understood.

It is of considerable interest from the standpoint of drug delivery to use oil-in-water emulsions for improving the bioavailability of highly lipid-soluble drugs which are poorly absorbed owing to their low solubilities in water. In this paper, lipid-soluble azo-dyes and

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4) J.D. Hamilton, *Biochim. Biophys. Acta*, **239**, 1 (1971).
5) P.J. Carrigan and T.R. Bates, *J. Pharm. Sci.*, **62**, 1477 (1973).

natural oils were used as model drugs and oil phase respectively and the relationship between the absorption of oils and that of dyes was studied in the rat small intestine.

Experimental

Materials——Oil Red XO was obtained from Tokyo Kasei Co., Ltd. and Sudan Black B from Merck and Co., Ltd. Triolein-^{131}I was purchased from Daiichi Radioisotope Labs., Ltd. Sodium taurocholate was synthesized by the method of Norman.[6] Other chemicals used were of reagent grade quality.

Preparation of Emulsions——Overall concentration of Polysorbate-80 was kept constant at 0.2% (w/v) to maintain micellar phase as little as possible. The mixture of oil, water, drug, and the emulsifier was shaken vigorously and 25 ml of mixture was sonicated at 20 kHz, 100 W for 10 min by the sonicator (No. 5202, Ohtake Seisakusho) under ice cooling. The oil droplet of emulsions thus made had the uniform particle size (about 3 μ).

Animal Experiments——Male Wistar rats weighing 160—220 g were used in all the experiments. Under sodium pentobarbital anesthesia the small intestine was exposed by a middle line abdominal incision, and cannulated at the end of pylorus with a glass cannulae. Another cannulae was inserted into the ending of ileum. After washing out the intestinal contents with saline warmed at 37°, the loop was made by ligating the intestine at the very ends of cannulaes. Five milliliters of sample solution were injected into the loop, and were incubated at 37°. As soon as the incubation time was over, the luminal fluid was collected completely by washing out with distilled water and the amount of the dye in the collected fluid was determined. At the end of the washing, the loop was removed, and the dye remained in the intestinal tissue was determined. To investigate the metabolism of Oil Red XO during absorption experiment, *in vitro* everted sac method was used. The small intestine was everted in the usual manner and three equal loops were made, then incubated two hours at 37° in 10 ml of emulsion with or without bubbling O_2/CO_2 gas (95: 5). Metabolism of Oil Red XO was also examined by paper chromatography by the following solvent systems; butanol: glacial acetic acid: water (40: 12: 34), pyridine: isopentyl alcohol: water (7: 7: 6), propyl alcohol: isopentyl alcohol: glacial acetic acid: water (4: 1: 1: 3).

Analytical Methods——(1) Oil Red XO and Sudan Black B: The dye remained in the collected luminal fluid was extracted with chloroform. The dye remained in the intestinal tissue was extracted with chloroform after being homogenized by a glass homogenizer. Chloroform extract was determined spectrophotometrically at 484 mμ for Oil Red XO. In the case of Sudan Black B, 2 ml of chloroform extract was diluted with 3 ml of absolute ethanol so that the absorbance peak would shift from 1000 to 600 mμ.

(2) Triglycerides: Triglyceride was determined by the modified method of Van Handel.[7] Chloroform extract containing triglyceride was evaporated at 85° and chloroform was removed perfectly under vacuum condition for 30 min. The residual triglyceride was hydrolyzed by potassium hydroxide and the product glycerol was oxidized by sodium periodate to formaldehyde. The latter was colored with chromatropic acid, and determined spectrophotometrically at 570 mμ after adding thiourea to lower the blank.

(3) Butyric Acid: Butyric acid was measured by gas-liquid chromatography (Model GC-5A, Shimadzu Seisakusho, Japan) utilizing direct aqueous injection.[8] The collected luminal fluid or the tissue homogenate was preparatory delipidated with the equal volume of chloroform and centrifuged. Five milliliters of the supernatant were deproteinized with 1 ml of 25% metaphosphoric acid. After centrifugation at 3000 rpm for 30 minutes, 3 ml of supernatant was pipetted and 1 ml of 20 mM caproic acid was added to it as the internal standard. Two microliters of this mixture were injected. The column substrate was FFAP on nonacid washed 60/80 Chromosorb W. The oven temperature was maintained at 145° and the injection port at 240°. Hydrogen flow to the flame head was maintained at 40 ml/min. Nitrogen carrier gas was set at approximately 40 ml/min. Sensitivity was set at range 10^2 and attenuation 8.

(4) Triolein-^{131}I: Radioactivity of triolein-^{131}I remained in the collected luminal fluid was measured by the well-type γ-ray scintilation counter (Model ATS-621, Fujitsu Co., Ltd., Japan) after being extracted with benzene. Whole small intestine was solubilized by alkali treatment and an aliquot was used for measuring the radioactivity remained in the intestinal tissue. Biodegradation of triolein-^{131}I was checked by Silica Gel thin-layer chromatography in petroleum ether: ether: glacial acetic acid (80: 30: 1) and subsequent radioactive scanning.

Results

Preliminary *in vitro* experiment using everted rat intestine revealed that no biotransformation of Oil Red XO takes place during the intestinal absorption study (Table I). To

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7) C. Naito, M. Usui, K. Kohayakawa, H. Okaniwa, and T. Ichida, *Igaku no Ayumi*, **57**, 551 (1966).
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simplify the experimental condition, the common bile duct has been ligated frequently. The effect of the bile duct ligation on the absorption of Oil Red XO from water-in-oil emulsions was investigated using three oils. As shown in Table II, the absorption of Oil Red XO from emulsions in bile duct ligated rats was no less than in intact rats. Therefore, bile duct ligation was adopted for all the *in situ* absorption experiments in this paper.

TABLE I. Metabolism of Oil Red XO in the Rat Small Intestine *in Vitro*

Condition	% remained after 2 hr incubation		
	in lumen	in tissue	total
with O_2/CO_2 (95:5)	74.1±6.8	30.6±7.4	104.7±1.6
without O_2/CO_2	77.2±2.8	26.6±3.2	103.8±1.5

Results are expressed as the mean ± S.D. of at least 4 animals. Every emulsion contains 4 ml of tributyrin and 4 mg of Oil Red XO per 100 ml. See text in detail.

TABLE II. Effect of Bile Duct Ligation on the Absorption of Oil Red XO from Various Emulsions

Oil phase	% absorbed in 2 hr	
	intact	bile duct ligated
Tributyrin	55.8±1.6	91.0± 1.6
Olive oil	49.8±6.4	53.4± 8.9
Isopropylpalmitate	40.6±5.4	39.9±15.7

Every emulsion contains 4 ml of oil and 4 mg of Oil Red XO per 100 ml. Polysorbate-80 was adjusted to 0.2% (w/v).
Results are expressed as the mean ± S.D. of at least 4 animals.

Tributyrin Emulsion System

Comparison was made between the absorption of two lipid-soluble azo-dyes, Oil Red XO and Sudan Black B. The absorption characteristics of these dyes from the same oil-in-water emulsion system seems different (Fig. 1). Though Oil Red XO as well as Sudan Black B was absorbed from the small intestinal loop according to the first-order process, the absorption rate differed very much. Since the former was absorbed faster and can be determined easier than the latter, Oil Red XO was chosen as the model drug in the following experiments.

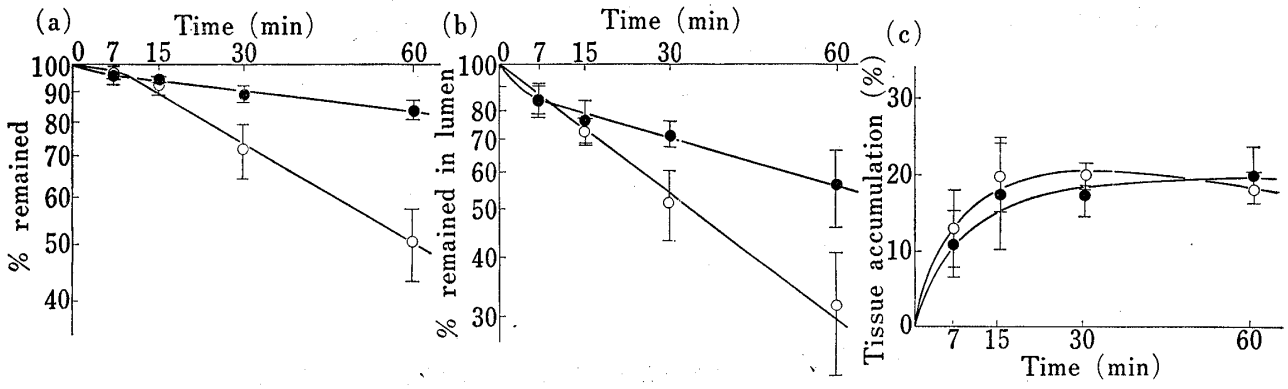

Fig. 1. Absorption of Oil Red XO and Sudan Black B from Tributyrin Emulsions

(a) overall disappearance of Oil Red XO and Sudan Black B from intestinal lumen and tissue
(b) disappearance of Oil Red XO and Sudan Black B from intestinal lumen
(c) accumulation of Oil Red XO and Sudan Black B in intestinal tissue
Every emulsion contains 4 ml of tributyrin and 4 mg of Oil Red XO (○) or Sudan Black B (●) per 100 ml
Results are expressed as the mean±S.D. of at least 3 animals.

The absence of the dose dependency in the absorption of Oil Red XO is shown in Table III. At the higher doses, Oil Red XO seems to be well absorbed, but no statistically significant difference is seen among the concentrations examined. On the other hand, the concentration of tributyrin has some effects on the absorption of Oil Red XO as shown in Table IV. The higher the concentration of tributyrin is, the less the absorption of Oil Red XO becomes.

Table III. Dose Dependency of the Absorption of Oil Red XO from Tributyrin Emulsions

Dose (mcg/rat)	% remained after 2 hr			% absorbed in 2 hr
	in lumen	in tissue	total	
100	50.6±11.4	16.7±4.7	67.3± 7.1	32.7± 7.1
200	35.6± 5.7	18.2±2.2	53.8± 3.6	46.2± 3.6
400	29.7± 3.1	19.3±2.5	49.0± 2.8	51.0± 2.8
800	31.1±16.5	16.1±2.4	47.2±14.8	52.8±14.8

Concentration of tributyrin was kept constant at 4% (v/v).
Results are expressed as the mean±S.D. of at least 4 animals.

Table IV. Effect of Concentration of Tributyrin on the Absorption of Oil Red XO from Tributyrin Emulsions

Conc. of tributyrin % (v/v)	Oil Red XO remained after 2 hr (%)			Oil Red XO absorbed in 2 hr (%)
	in lumen	in tissue	total	
2	35.2±7.7	19.1±1.5	54.3±8.0	45.7±8.0
4	35.6±5.7	18.2±2.2	53.8±3.6	46.2±3.6
8	51.9±5.8	20.7±1.3	72.6±4.5	27.4±4.5
16	78.4±8.4	14.8±4.3	93.2±5.1	6.8±5.1

Concentration of Oil Red XO was kept constant at 4 mg per 100 ml of emulsions.
Results are expressed as the mean ± S.D. of at least 4 animals.

Triolein Emulsion System Compared with Tributyrin Emulsion System and Micellar Solution

When tributyrin, the short-chain triglyceride, was used as an oil phase, the absorption of a lipid-soluble dye proceeded by the first-order kinetics as mentioned elsewhere. The absorption of Oil Red XO from micellar solution was similar to that from tributyrin emulsions,

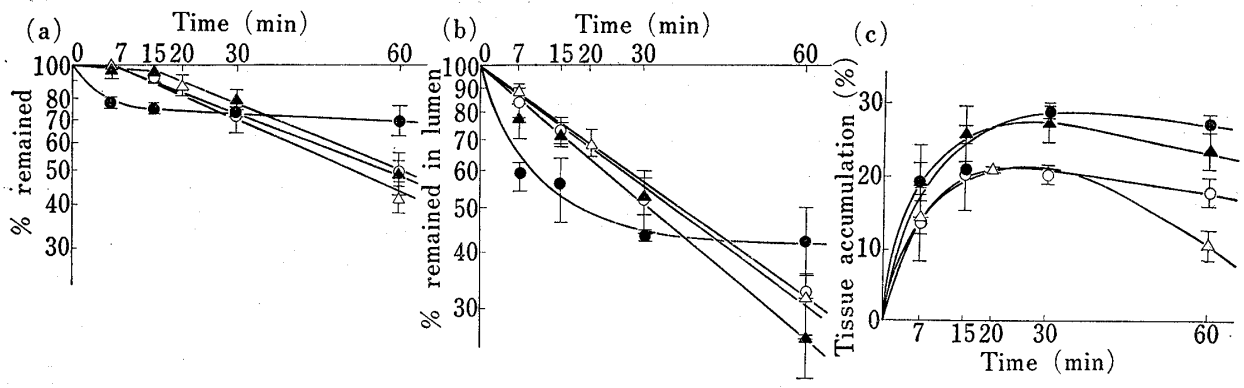

Fig. 2. Absorption of Oil Red XO from Emulsions and Micellar Solutions

(a) overall disappearance of Oil Red XO from intestinal tissue and lumen
(b) disappearance of Oil Red XO from intestinal lumen
(c) accumulation of Oil Red XO in intestinal tissue
Every emulsion contains 4 ml of oil and 4 mg of Oil Red XO per 100 ml. Results are expressed as the mean±S.D. of at least 3 animals.
○: Tributyrin emulsion; ●: Triolein emulsion; △: 4% Polysorbate-80 solution; ▲: Oleio acid-sodium taurocholate-monolein solution (10 mM: 20 mM: 5 mM).

however, significant difference was seen in the case of triolein emulsion system as shown in Fig. 2. Faster uptake occurred in the first stage of absorption and after 15 minutes incubation, saturation was observed. Similar pattern was also noted when olive oil (J.P. VIII) was used as an oil phase. As oil phase dependent pattern of the absorption would be of interest, absorption of an oil itself was investigated and compared with that of Oil Red XO. The time course of the absorption of tributyrin shown in Fig. 3 demonstrates that tributyrin itself is absorbed according to the first-order process, whereas triolein does not disappear from the lumen monoexponentially. Its time course of absorption is very similar to the one of Oil Red XO from triolein emulsions. To investigate this difference in detail, metabolism of tributyrin and triolein during intestinal absorption was examined. Table V shows that tributyrin was metabolized to butyric acid at the luminal surface or inside of the epithelial cells and the hydrolysate was rapidly transported into portal blood. The metabolism of triolein was also examined using triolein-^{131}I. No significant change was observed by thinlayer chromatography of either of the intestinal fluid or of the intestinal tissue after 30 minutes incubation.

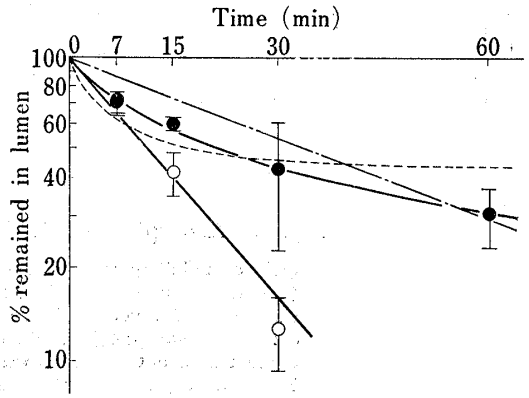

Fig. 3. Absorption of Triglycerides from Emulsions

Every emulsion contains 4 ml of oil and 4 mg of Oil Red XO per 100 ml. ○: tributyrin, ●: triolein, ——: Oil Red XO (tributyrin emulsion), ------: Oil Red XO (triolein emulsion).

Results are expressed as the mean±S.D. of at least 3 animals.

TABLE V. Metabolism of Tributyrin in the Small Intestine

	Initial amount of tributyrin (mmole)	Butyric acid remained after one hour (mmole)
In situ loop		
Collected luminal fluid	0	0
	682.1	159.2±16.1[a]
Tissue homogenate	0	0
	682.1	0
In vitro		
Collected luminal fluid[b]	1.71	56.7×10^{-3}
Tissue homogenate[c]	1.71	288.8×10^{-3}

a) expressed as the mean ± S.D. of 3 animals.
b) Five milliliters of saline were injected into the small intestinal loop and were incubated for one hour. After incubation luminal fluid was collected to 25 ml and 10 ml of the solution was reincubated with tributyrin for one hour at 37°.
c) Whole small intestine was homogenized and adjusted to 100 ml with water. Ten milliliters of the homogenates were reincubated with tributyrin for one hour at 37°. Tissue binding of produced butyric acid was negligible.

Effect of Concentration of Triolein on the Absorption of Oil Red XO and Triolein Itself

To observe further absorption behavior of Oil Red XO from triolein emulsions, intestinal absorption study was made at various concentrations of triolein. As shown in Fig. 4 and 5, less Oil Red XO and triolein were absorbed at higher concentrations of triolein. At a low concentration (1% triolein), however, the time course of Oil Red XO absorption became linear, while that of triolein remained nonlinear even at the concentration as low as 0.1%. Difference in the disappearance pattern of Oil Red XO and that of triolein is also reflected in the tissue accumulation. Percentage of tissue accumulation of triolein was almost indepen-

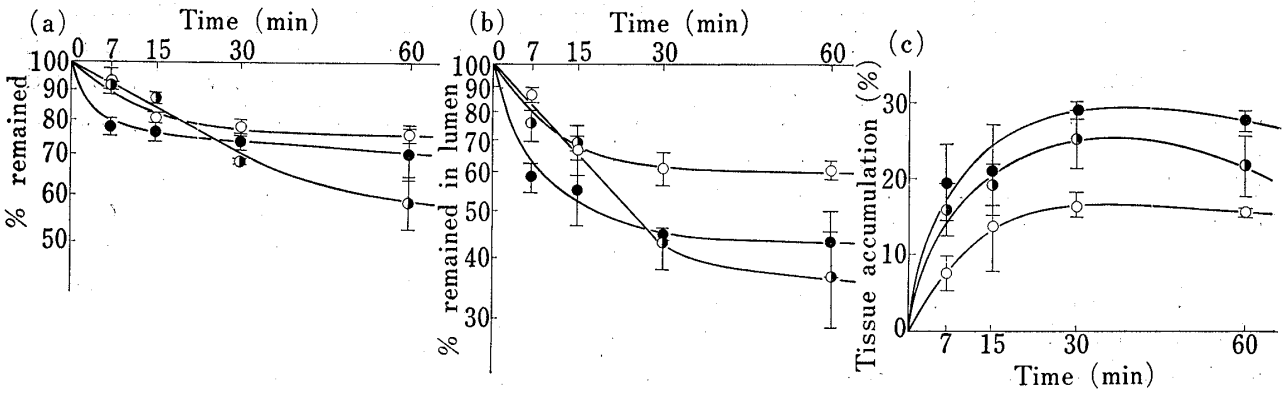

Fig. 4. Absorption of Oil Red XO at Various Concentration of Triolein

(a) overall disappearance of Oil Red XO from intestinal lumen and tissue
(b) disappearance of Oil Red XO from intestinal lumen
(c) accumulation of Oil Red XO in intestinal tissue
◑: 1% triolein; ●: 4% triolein; ○: 16% triolein
Concentration of Oil Red XO was kept constant at 4 mg per 100 ml of emulsions.
Results are expressed as the mean±S.D. of at least 3 animals.

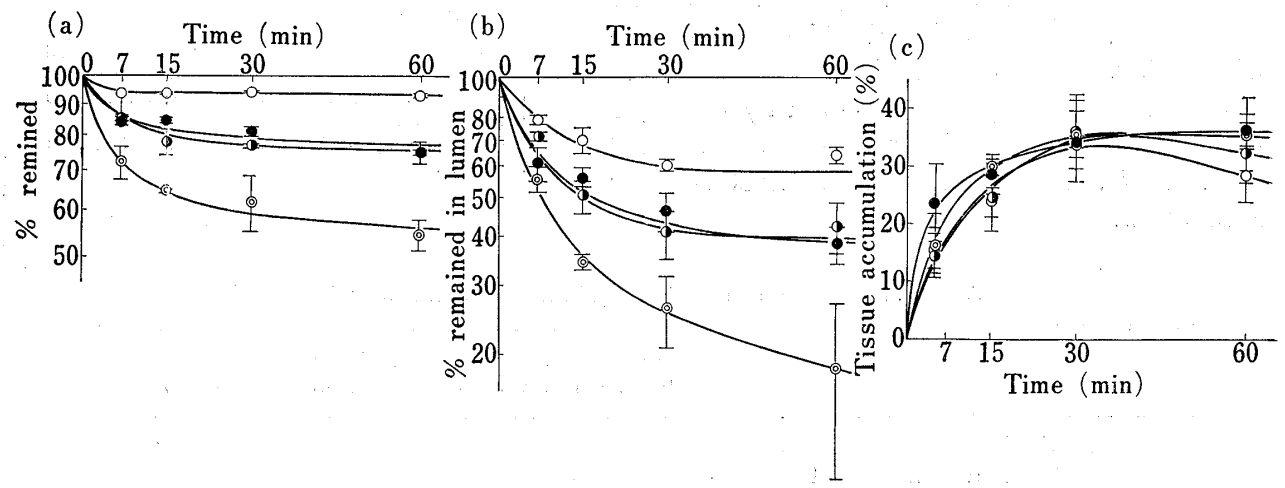

Fig. 5. Concentration Dependency of the Absorption of Triolein-^{131}I from Emulsions

(a) overall disappearance of triolein-^{131}I from intestinal lumen and tissue
(b) disappearance of triolein-^{131}I from intestinal lumen
(c) accumulation of triolein-^{131}I in intestinal tissue
◎: 0.1% triolein; ◑: 1% triolein; ●: 4% triolein; ○: 16% triolein
Concentration of Oil Red XO was kept constant at 4 mg per 100 ml of emulsions.
Results are expressed as the mean ± S.D. of at least 3 animals.

dent of the initial concentration of triolein (Fig. 5c), while that of Oil Red XO became lower with the increase of triolein concentration (Fig. 4c).

Discussion

The present experiments have examined the mechanism of the absorption characteristics of lipid-soluble dyes from short-chain and long-chain triglyceride emulsions. The most striking finding is the marked difference in the time course of luminal disappearance of Oil Red XO from these two emulsions.

From the emulsion using tributyrin as an oil phase, Oil Red XO disappeared monoexponentially almost the same extent as from the Polysorbate-80 micellar solution (Fig. 2a). In this experiment bile duct was ligated and the luminal hydrolysis of tributyrin by pancreatic lipases can be neglected, although hydrolysis at the surface or inside of the epithelial cells may well be considered (Table V). After one hour incubation in the *in situ* small intestinal

loop, butyric acid appeared in the collected luminal fluid was 7.8% of the administered dose, however, no butyric acid remained in the tissue homogenate. In the *in vitro* incubation using tissue homogenate, 5.6% of the administered dose was metabolized to butyric acid. When the collected luminal fluid was used instead of the tissue homogenate, only a very small amount of butyric acid was found. These results suggest that tributyrin is metabolized at the luminal surface or inside of the epithelial cells and rapidly cleared *via* portal route. Since the disappearance of Oil Red XO from the intestinal lumen is monoexponential and seems to occur only from tributyrin oil droplets, metabolism of the latter, a short-chain triglyceride, would be very fast.

In the emulsion using triolein as an oil phase, the semilogarithmic plots of the percentage remained of Oil Red XO versus time were nonlinear. However, similarity of disappearance patterns of the vehicle oil and the dye was also observed as was the case of tributyrin. These results suggest that Oil Red XO is not absorbed *via* water phase but is absorbed directly from oil droplets following the adsorption of oil droplets to the epithelial cell surface. After the adsorption onto the membrane, however, Oil Red XO does not seem to move into inner compartments with the oil. This may be rationalized on the basis of the following reasons. The first reason is that Oil Red XO did not appear in the intestinal lymph although triolein is known to be transported into the intestinal lymph after it is enveloped in the chylomicrons. The second reason is that Oil Red XO was absorbed from 4%-squalane emulsion to a considerable extent (34.7 ± 5.4%/hr), despite the fact that squalane was not absorbed from the rat intestine.[9] The third reason is that tissue accumulation (%) of Oil Red XO was the lowest when the initial concentration of triolein was the highest (Fig. 4c), despite the fact that tissue accumulation (%) of triolein was almost independent on the initial concentration of triolein (Fig. 5c).

During the early stage of absorption, triolein is rapidly uptaken (Fig. 5b) and transported through the epithelial cells, as the overall disappearance (%) from the luminal fluid and from the tissue is rapid as shown in Fig. 5a. This rapid uptake of triolein during the early stage of absorption is compatible with the recent report of Clark, *et al.*[10] In the later stage of absorption, percentage absorption of triolein becomes smaller than that of tributyrin (Fig. 3). This is presumably because the rate of metabolism of triolein, its hydrolysis and subsequent chylomicron formation in the later stage of absorption becomes slower than in the earlier.

There is an evidence that once taken up into the epithelial cells, Oil Red XO is transported mainly *via* portal route and excreted into bile after being metabolized in the liver to a more water-soluble compound. It is of interest that such a highly lipophilic dye, Oil Red XO, is not transported into the intestinal lymph with the presence of triglyceride in the emulsion dosage form.

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10) S.B. Clark, B. Lawergren, and J.V. Martin, *Am. J. Physiol.*, **225**, 574 (1973).