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Mechanism of the Intestinal Absorption of Drugs from Oil-in-Water Emulsions. VII.[1] Role of Bile in the Lymphatic Transport of Lipid-Soluble Compounds from Triolein Emulsions

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Lymphatic transport of lipid-soluble dye, Sudan Blue, and vitamin A acetate from oil-in-water emulsions was investigated using *in situ* loop method by the rat small intestine. A natural oil, triolein, which was known to be transported mainly through lymphatic pathway, was chosen as an oil phase of the emulsion.

Lymphatic transport of Sudan Blue and vitamin A acetate was very small in the absence of bile. When sodium taurocholate and egg phosphatidylcholine were co-administered with emulsions, lymphatic transport of lipid-soluble compounds were recovered even in bile fistula rats. Addition of one of them could not recover completely the lymphatic transport of lipid-soluble compounds.

It was concluded that both bile salts and phosphatidylcholine were necessary for the lymphatic transport of the fat and lipid-soluble compound which interact with oil administered intraluminally. However, overall contribution of lymphatic pathway to their disappearance from the small intestinal lumen is very small, and the main route of their absorption is thought to be the portal pathway.

It is well known that orally administered drugs are transported *via* portal and lymphatic pathway following the absorption from the intestine. Many studies have been done about the portal transport of drugs, however, relatively little work has been reported concerning lymphatic ones.[3,4]

In a previous report from this laboratory,[1] the correlation between the absorption of oil and that of lipid-soluble dyes were examined. However these dyes were not transported *via* lymph, while natural oils were known to be transported mainly through lymphatic pathway. There is now considerable evidence that vitamin A is transported *via* lymph and the portal vein.[5] Also Sudan Blue, a lipid-soluble dye, has been reported to be transported *via* lymph.[6] In this study, these compounds were chosen as model drugs and their intestinal absorption from oil-in-water emulsions was investigated with special reference to the obligatory role of bile to gain further insight into the basic mechanism of lymphatic transport of drugs.

Experimental

Materials——Sudan Blue was obtained from Tokyo Kasei Co., Ltd. and vitamin A acetate from Sigma Chemical Co., Ltd. Egg phosphatidylcholine was purchased from Merck and Co., Ltd. Triolein-^{131}I and sodium taurocholate were the same as those described in the previous paper.[1] Other chemicals used were of reagent grade quality.

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Animal Experiments——Male Wistar rats weighing 180—240 g were used in all the experiments. The absorption experiments using small intestinal loop were carried out similarly as described in the previous paper.[1] Major intestinal lymphatic was cannulated by the haparin-filled polyethylene cannulae (i.d. 0.50 mm, o.d. 0.80 mm, Dural Plastics and Eng. Pty. Ltd., Australia). A drop of tissue cement, Aron Alpha A® (Sankyo Co., Ltd., Japan), was applied to the hole in the lymphatic to seal it and to fix the cannulae in place.[7] The accessory lymphatic is intentionally disrupted by forceps and occluded with the cement to increase the return through the cannulated main lymphatic. To secure the cannulation 10 mm^2 of abdominal muscle was attached on the cement. The lymph was collected every hour in the heparinized tube. In some cases common bile duct was cannulated in the usual way.

Analytical Methods——Sudan Blue remained in the collected luminal fluid or in the intestinal tissue homogenate was extracted with benzene. Benzene extract was determined spectrophotometrically at 646 mμ. Vitamin A acetate remained in the collected luminal fluid, in the intestinal tissue, or transported to lymph was extracted with cyclohexane and determined fluorometrically, excited at 340 mμ and recorded at 485 mμ.[8] The distribution of vitamin A acetate and Sudan Blue was investigated by Sephadex G-200 column chromatography (column length 40 cm, diameter 1.8 cm). One milliliter of lymph collected from the first to the fourth hour was eluted with saline after preliminary centrifugation at 3000 rpm for 5 min. The turbidity of the eluted fraction was measured at 560 mμ. This turbidity of lymph was regarded to be equal to the concentration of chylomicron, for, the radioactivity of triolein-^{131}I administered in the small intestinal loop was parallel to the turbidity.

Results

Intestinal Absorption and the Lymphatic Transport of Sudan Blue

In this paper the relation between the intestinal absorption and the lymphatic transport of lipid-soluble compounds was investigated. Sudan Blue was used mainly at the final concentration of 400 μg/ml (dose 2.0 mg). As shown in Fig. 1 there was a dose dependency in the absorption of Sudan Blue. However, this concentration was chosen due to sensitivity of the assay. There was no effect of the bile duct ligation. Lymphatic transport of Sudan Blue was studied in the various conditions (Fig. 2). If the bile duct was fistulized, the trans-

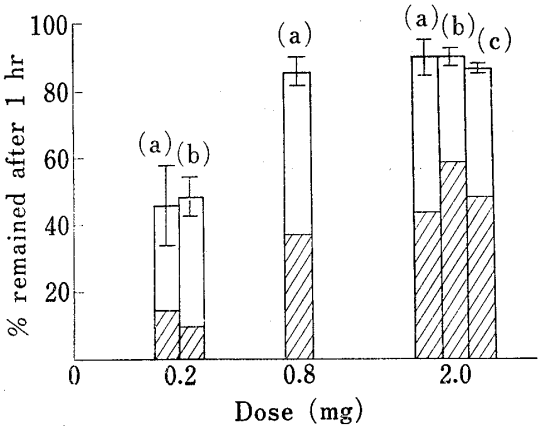

Fig. 1. Dose Dependency of the Absorption of Sudan Blue from Triolein Emulsions

(a) intact rats (b) bile duct ligated rats (c) bile duct ligated rats co-administered with 10 mM sodium taurocholate and 10 mg egg phosphatidylcholine.
Every emulsion contains 4 ml of triolein and 40 mg of Sudan Blue per 100 ml. Polysorbate-80 was adjusted to 0.2% (w/v). See Ref. 1 in detail.
□: Sudan Blue remained in intestinal tissue
▨: Sudan Blue remained in intestinal lumen
Results are expressed as the mean of at least 4 animals. The vertical bar indicates ±S.D. of total Sudan Blue remained.

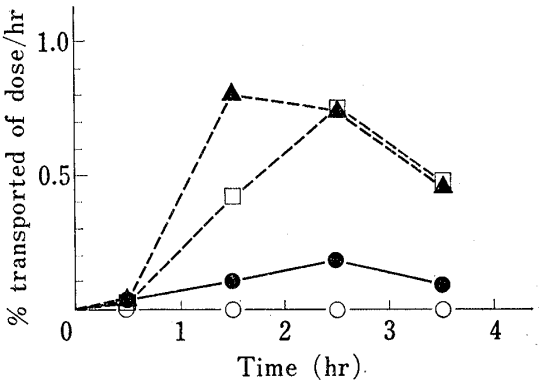

Fig. 2. Lymphatic Transport of Sudan Blue

Intact rats were administered two different doses, 0.8 mg (□) and 2.0 mg (▲). Bile fistula rats were administered same emulsions as Fig. 1 (○), with 20 mM sodium taurocholate (○), with 10 mg egg phosphatidylcholine (○), and with sodium taurocholate and egg phosphatiylcholine simultaneously (●). Results are expressed as the mean of at least 4 animals.

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port of Sudan Blue into lymph was almost completely blocked. This inhibition was not recovered by the addition of 20 mM of sodium taurocholate or 10 mg of egg phosphatidylcholine to the emulsion. However if these compounds were added simultaneously, the transport of Sudan Blue was somewhat recovered. When 0.8 mg of Sudan Blue was administered, the fraction of lymphatic transport was smaller than 2.0 mg. As is evident from Figs. 1 and 2, no good correlation has been noted between the absorption and the lymphatic transport of Sudan Blue. This may be attributed to the slow absorption of Sudan Blue. Therefore the intestinal absorption during three hours was investigated using the *in situ* small intestinal loop and the result was compared with the lymphatic transport for four hours. However, as is evident from Fig. 3, no significant difference was seen in the extent of intestinal absorption even during three hours.

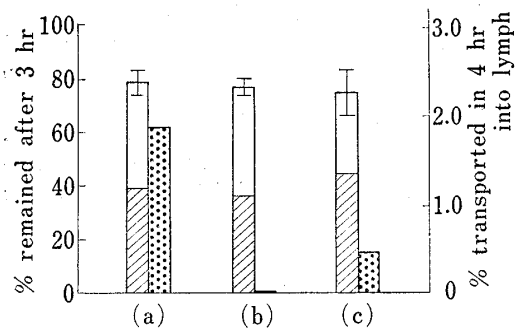

Fig. 3. Absorption and Lymphatic Transport of Sudan Blue

a) intact rats (b) bile fistula rats (c) bile fistula rats administered same emulsions as Fig. 1 with 20 mM sodium taurocholate and 10 mg egg phosphatidylcholine.
□: Sudan Blue remained in intestinal tissue
▨: Sudan Blue reamined in intestinal lumen
▩: Sudan Blue transported into intestinal lymph
Results are expressed as the mean of at least 4 animals. The vertical bar indicates ±S.D. of total Sudan Blue remained.

Intestinal Absorption and the Lymphatic Transport of Vitamin A Acetate

In the case of vitamin A acetate, 2.0 mg was administered in all the experiments. The time course of absorption of vitamin A acetate in the intact rat is shown in Fig. 4. During the first one hour, 34% of vitamin A acetate was absorbed, but reduction in absorption was observed for the following two hours. Tissue accumulation of vitamin A acetate was also studied at various conditions. It is well-documented that vitamin A acetate is metabolized in the intestinal lumen and in the epithelial cell and appears in the lymph as palmitate. Since vitamin A palmitate had the same fluolescence intensity as vitamin A acetate, the lymphatic transport of vitamin A acetate is expressed as the percentage of dose. Fig. 5 shows that the effect of bile duct fistulation is similar to the case of Sudan Blue. When 20 mM of sodium taurocholate and 10 mg of egg phosphatidylcholine were added to the emulsion simultaneously,

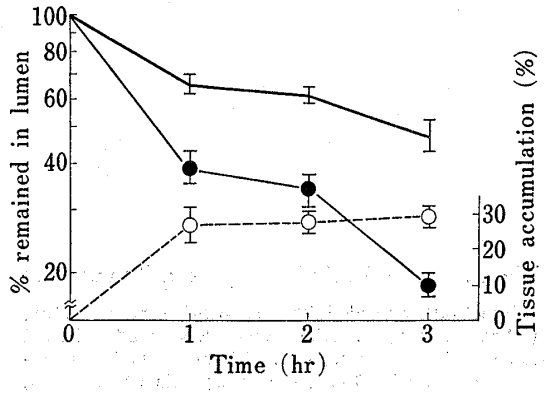

Fig. 4. Absorption of Vitamin A Acetate from Triolein Emulsions

Every emulsion contains 4 ml of triolein and 40 mg of vitamin A acetate per 100 ml. Polysorbate-80 was adjusted to 0.2% (w/v).
———: Overall disappearance curve of vitamin A acetate
—●—: vitamin A acetate remained in inteatinal lumen
---○---: vitamin A acetate accumulated in intestinal tissue
Results are expressed as the mean ±S.D. of at least 4 animals.

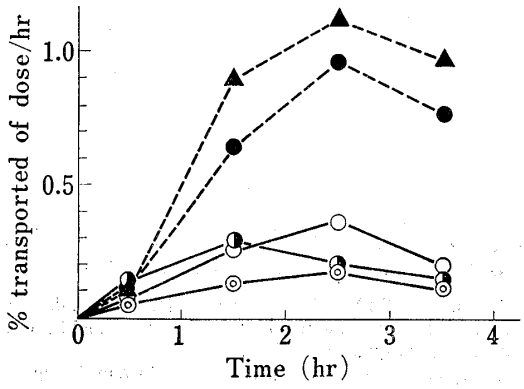

Fig. 5. Lymphatic Transport of Vitamin A Acetate

Same emulsions as Fig. 4 were administered to intact rats (▲), bile fistula rats (○), bile fistula rats with 20 mM sodium taurocholate and 10 mg egg phosphatidylcholine (●), bile fistula rats with 20 mM Polysorbate-80 and 10 mg egg phosphatidylcholine (◑), and bile fistula rats with 20 mM sodium taurocholate (◎).
Results are expressed as the mean of at least 4 animals

the lymphatic transport was almost completely recovered. However when 20 mM of Polysorbate-80 was used instead of sodium taurocholate, the lymphatic transport of vitamin A acetate was negligible and remained all the same as the rat with bile fistula. In Fig. 6, intestinal absorption during three hours is compared to the lymphatic transport during four hours. There seems to be no relationship between the two in all the cases. To clarify whether vitamin A is transported incorporated within the oil droplet, the lymphatic transport of vitamin A acetate from Polysorbate-80 solution was also investigated. As shown in Fig. 7, equal amount of vitamin A acetate was transported into lymph from the Polysorbate-80 micellar solution. It was also recognized from Fig. 7 that the peak of the lymphatic transport of vitamin A acetate was attained faster from the micellar solution than from the emulsion.

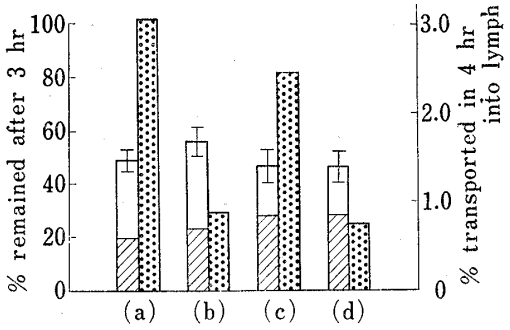

Fig. 6. Absorption and Lymphatic Transport of Vitamin A Acetate

(a) intact rats (b) bile fistula rats (c) bile fistula rats administered emulsions with 20 mM sodium taurocholate and 10 mg egg phosphatidylcholine (d) Bile fistula rats administered emulsions with 20 mM Polysorbate-80 and 10 mg egg phosphatidylcholine. Emulsions were the same as Fig. 4.
□: vitamin A acetate remained in intestinal tissue
▨: vitamin A acetate remained in intestinal lumen
⁞⁞: vitamin A acetate transported into intestinal lymph
Results are expressed as the mean of at least 4 animals. The vertical bar indicates ±S.D. of total vitamin A acetate remained.

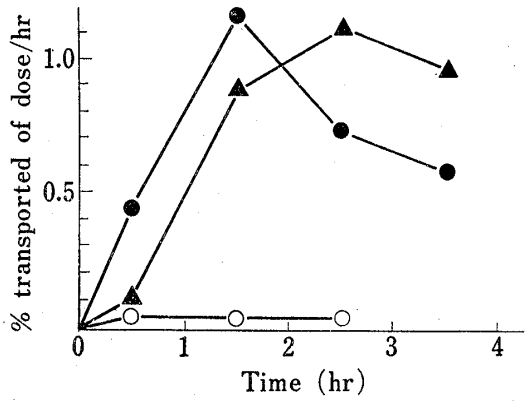

Fig. 7. Lymphatic Transport of Vitamin A Acetate from Emulsions and Micellar Solutions

Intact rats administered same triolein emulsions as Fig. 4 (▲), 4% Polysorbate-80 solutions (●), and bile fistula rats administered 4% polysorbate-80 solutions (○).
Results are expressed as the mean of at least 3 animals.

The Distribution of Vitamin A in the Lymph

It may well be considered that triolein is transported into the lymph being enveloped in the chylomicron. To clarify the interaction of triolein and lipid-soluble compounds during

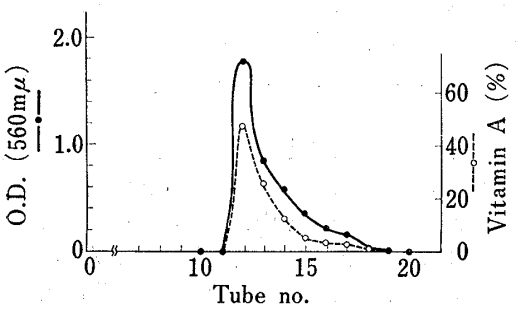

Fig. 8. Distribution of Vitamin A in the Lymph After Administration of Vitamin A Acetate as Triolein Emulsion

Emulsion was the same as Fig. 4. Vitamin A was presented as the percentage of whole elution fluid by Sephadex G-200 column chromatography. Whole vitamin A in the lymph was recovered from the elution fluid.

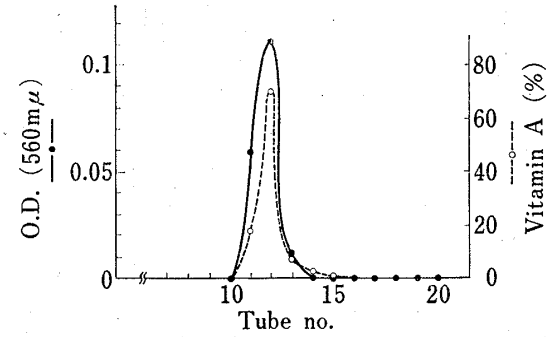

Fig. 9. Distribution of Vitamin A in the Lymph After Administration of Vitamin A Acetate as Polysorbate-80 Micellar Solution

The same dose of vitamin A acetate as Fig. 8 was administered as 4% Polysorbate-80 solution. Vitamin A was presented as Fig. 8.

the lymphatic transport, the distribution of lipid-soluble compounds was investigated using Sephadex G-200 column chromatography. The results are shown in Figs. 8 and 9. Triolein-^{131}I was detected at the same peak of OD (560 mμ), the turbidity, which thought to be proportional to the fraction of the chylomicron. Sudan Blue had the same pattern with triolein-^{131}I. When vitamin A acetate was administered as micellar solution, the lymph was almost clear, pale white. However the elution pattern of this lymph indicated that even from micellar solution vitamin A acetate was transported in the fraction of chylomicron (Fig. 9).

Discussion

Present findings confirm that absence of bile components results in significant impairment of lymphatic transport of Sudan Blue and vitamin A acetate from triolein emulsion. Recently, O'Doherty, *et al.*[9] reported that fat release from intestinal mucosa was effected by the feeding of phosphatidylcholine or choline, which play an important role in triglyceride transport by providing surfactant phosphatidylcholine for the chylomicron envelope and by supporting mucosal protein biosynthesis. In their studies, however, the micellar solution containing 800 mg of monoolein and free fatty acids and 1% of taurocholic acid was administered by stomach tube for every preparation, even for control sham operated rats.

In this paper, phosphatidylcholine did not affect the lymphatic transport of Sudan Blue and vitamin A acetate at all in the absence of sodium taurocholate (Figs. 2 and 5). The converse applies for sodium taurocholate which did not affect the lymphatic transport of them in the absence of phosphatidylcholine. It was also reported by O'Brien, *et al.*[10] that the activities of microsomal acyl-CoA synthetase were restored to normal levels in bile fistula rats by the infusion of sodium taurocholate and phosphatidylcholine and that infusion of sodium taurocholate alone resulted in some increase in specific enzyme activities toward the normal range. It was then concluded that both bile salts and phosphatidylcholine were necessary for the lymphatic transport of the fat administered intraluminally.

In the previous paper,[1] it was demonstrated that Oil Red XO did interact with triolein at the stage of uptake into the mucosal cell but not at the lymphatic transport from the mucosal cell. It has been made clear from Sephadex G-200 column chromatography that Sudan Blue and vitamin A interact with oils even at the stage of lymphatic transport, for, they were enveloped in the chylomicron. This result is compatible with the report of Inoue, *et al.*[6] that Sudan Blue was chiefly distributed in the chylomicron in the lymph, which was demonstrated by the ultracentrifugation method. The fact that the lymphatic transport of Sudan Blue and vitamin A acetate were affected by the bile components strongly supports the view that these lipid-soluble compounds interact with oil even in the epithelial cells and are transported *via* lymph enveloped in the chylomicron. Even from micellar solution, vitamin A acetate was transported *via* the intestinal lymph to the same extent as from triolein emulsions (Fig. 7). From Fig. 9 it is conceivable that vitamin A is dissolved in the endogeneous triglycerides and is enveloped in the chylomicron.

Though highly lipid-soluble compounds such as Sudan Blue and vitamin A acetate have been proved to be transported *via* lymph interacting with oils, its overall contribution to their disappearance from the small intestine is very small (Fig. 3 and 6). These observations suggest that even highly lipid-soluble compounds like Sudan Blue and vitamin A acetate, the main route of absorption is thought to be the portal pathway. However it is still unknown why dyes used in the previous experiments such as Oil Red XO and Sudan Black B having high lipid solubility were not transported *via* lymph at all.

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10) J.B. Rodgers, R. Tandon, and R.J. O'Brien, *Biochim. Biophys. Acta*, **326**, 345 (1973).