[Chem. Pharm. Bull. 25(3) 413—419 (1977)]

UDC 615.451.3.076.9 : 541.18.08

Regional Capacities of Gastrointestinal Absorption and Lymphatic Transport for Lipid-Soluble Dyes in Rats[1)]

Tetsuo Noguchi, Yuji Tokunaga, Hideyuki Ichikawa, Shozo Muranishi, and Hitoshi Sezaki

Faculty of Pharmaceutical Sciences, Kyoto University[2)]

(Received May 17, 1976)

The regional differences in the capacities of absorption and lymphatic transport for lipid-soluble dyes, Sudan Blue and Oil Red XO, from oil-in-water emulsions were investigated in rat's whole gastrointestinal tract. The absorptive capacity was the highest in duodenum, followed by jejunum, ileum, large intestine, and the lowest in stomach. This was well compatible with the regional absorption of vehicle oil, triolein. Lymphatic transport of Sudan Blue was recognized only in duodenal and jejunal regions. This fact was supported by the observation of lymphatic staining, which also suggested the defect of usual lymphatic experiments dealed only the drained lymph fluids.

It is also documented in this paper that lipid-soluble dyes are taken up into the intestinal epithelial cells from emulsions by an energy-independent process, since little or no inhibition was observed in various pretreated duodenum except the decrease of lymphatic transport in acetone washed intestine.

However, severe damage in the villi was observed light microscopically when pretreated with high concentration (80%) of acetone, the absorption of Sudan Blue was significantly decreased in the case. These results suggest that the normality of macromorphological structure of mucosal surface is important for the absorption of lipid-soluble drugs from oil-in-water emulsions.

Keywords——rat; lipid-soluble compounds; oil-in-water emulsion; regional capacity; lymphatic transport; energy-independent process; surface structure; gastrointestinal absorption

The intestinal absorption of dietary fat has been studied extensively, and it is now well established that following the intraluminal lipolysis by pancreatic lipase, the lipolytic products are dispersed in bile salt mixed micelles, then taken up by the intestinal epithelial cells, most of them are reesterified into complex lipids within the endoplasmic reticulum and thereafter secreted into intestinal lymph in the form of triglyceride-rich lipoproteins (chylomicrons). Mishkin, *et al.*,[3)] using everted hamster intestinal sacs, suggested that the reversible binding of fatty acid by the intestinal mucosa might be due to a property of its superficial components and that it might be independent of the esterifying capacity of the tissue. They also showed that fatty acid esterification was faster in proximal than in distal segment.[4)] These are well compatible with the report of Ockner and Manning[5)] that supernatant fatty acid-binding protein (FABP) concentration in mucosa from proximal and middle thirds of jejuno-ileum significantly exceeds that from distal third. However, relatively few works related to regional absorption of the intestine have been done concerning lipid-soluble drugs. Only the lipid-soluble vitamins such as vitamin E and vitamin K were reported to be absorbed by the passive diffusion process

1) *a*) This paper constitutes the 8th report in a series of "Mechanism of the Intestinal Absorption of Drugs from Oil-in-Water Emulsions"; *b*) Preceding paper, Part VII: T. Noguchi, Y. Jinguji, T. Kimura, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 782 (1975).
2) Location: *Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto.*
3) S. Mishkin, M. Yalovsky, and J.I. Kessler, *J. Lipid. Res.*, **13**, 155 (1972).
4) S. Mishkin, R. Wener, M. Yalovsky, and J.I. Kessler, *Am. J. Physiol.*, **227**, 771 (1972).
5) R.K. Ockner and J.A. Manning, *J. Clin. Invest.*, **54**, 326 (1974).

most rapidly in the medial and in the distal portion of the small intestine, respectively.[6]

In this paper, the regional capacities of gastrointestinal absorption and lymphatic transport from oil-in-water emulsions over the whole gastrointestinal tract were estimated using two lipid-soluble dyes, Oil Red XO and Sudan Blue, as model compounds. Also an attempt was made to gain further insight into uptake mechanism of lipid-soluble compounds from the emulsion system.

Experimental

Materials——Oil Red XO and Sudan Blue were obtained from Tokyo Kasei Co., Ltd., and triolein-^{131}I from Daiichi Radioisotope Labs., Ltd. Triolein and Sudan Black B were purchased from Sigma Chemical Co., Ltd. and Merck and Co., Ltd., respectively. Those were used as supplied. Other chamicals used were of reagent grade quality.

Preparation of Emulsions——Every oil-in-water emulsion contains 1 ml of triolein, 10 mg of a dye, 0.05 g of Tween-80 in 25 ml. Distilled water was used as the water layer. The mixture of those components was shaken vigorously and sonicated at 20 kHz, 100W for 5 min by the sonicator (No. 5202, Ohtake Seisakusho, Japan) under ice cooling.

Animal Experiments——(1) *In situ* loop method was used to estimate the regional capacities for absorption and lymphatic transport. Male Wistar rats weighing 190—220 g were fasted for overnight. Under sodium pentobarbital anesthesia the abdomen was exposed by a middle line incision. Cardiac and pyloric orifices were ligated, and the small intestine was cannulated at 1 cm from the end of pylorous. After washing out the intestinal contents with saline warmed at 37° the cannula was removed and the small intestine was divided into the following three loops by ligature; from pylorus to Treitz (duodenal loop), the following 10 cm length from Treitz (jejunal loop), and from ileocaecal valve (ileal loop). The common bile duct was ligated before washing in case of need. Also large intestine (colon and rectum) was used for loop in the same manner. Emulsions were instilled in these loops and incubated for two hours. The volume of emulsions were 2.0 (stomach), 0.3 (duodenum), 1.0 (jejunum), 1.0 (ileum), and 1.5 ml (large intestine) respectively. As soon as the incubation period was over, the loops were removed and dyes remained were determined. Rats were not fasted except the stomach loop experiments. Major intestinal lymphatic cannulation was carried out as described in the previous paper.[1b]

(2) *In vitro* everted sac method was used to study the effects of various pretreatments on the uptake of Sudan Blue. After *in situ* pretreatments with various reagents the duodenum from pylorus end to Treitz was everted in the usual manner, tied both ends, and incubated for 10 min at 37° in 5 ml of test emulsions. Sudan Blue taken up into duodenal tissue was then assayed after washing the tissue with water.

(3) Initial uptake of dyes with or without 2,4-dinitrophenol (DNP) pretreatment was investigated using the whole small intestinal loop. The whole small intestine was used for loop in the same manner described in the previous paper.[7] Five milliliters of emulsion were infused into the loop for one minute, and after infusion the infused emulsions were rapidly washed out with 5 ml of water 4 times. The combined washes were collected to estimate the remained dyes.

Analytical Methods——The dye remained in the collected luminal fluid or in the intestinal tissue homogenate was extracted with benzene or chloroform. The dye in the lymphatic fluid was also extracted with benzene. All extracts in the organic solvents were determined spectrophotometrically. Radioactivity of triolein-^{131}I was measured by the well-type γ-ray scintilation counter (Model ATS-521, Fujitsu Co., Ltd., Japan) after being solubilized by alkali.

Results

Regional Capacities of Gastrointestinal Absorption and Lymphatic Transport

Two lipid-soluble dyes were used to investigate the regional differences of absorptive capacities in bile duct ligated rats. Both are almost insoluble in water and soluble in oil, but their physiological characteristics are different. Sudan Blue is absorbed *via* both lyphatic and portal pathway whereas Oil Red XO is absorbed mainly by portal route.[1b,7] Fig. 1 shows the absorption of these dyes and their vehicle oil, triolein, from various regions examined in

6) D. Hollander, E. Rim, and K.S. Muralidhara, *Gastroent.*, **68**, 1492 (1975); D. Hollander and T.C. Truscott, *Am. J. Physiol.*, **226**, 1516 (1974); *etc.*

7) T. Noguchi, C. Takahashi, T. Kimura, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 775 (1975).

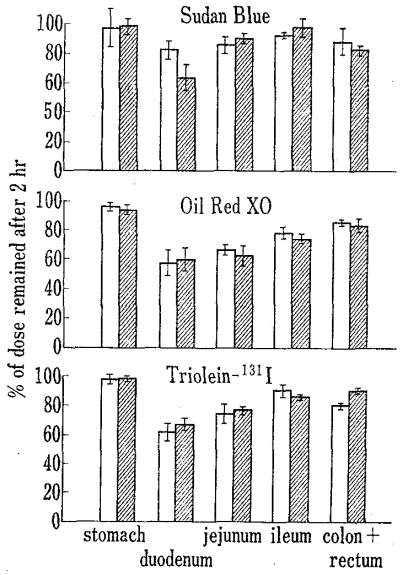

Fig. 1. Percentage of Dose Remaining of SudanBlue, Oil Red XO, and Triolein-^{131}I Two Hours After Administration into Various Regions of Bile Duct Ligated Rats

□: without bile; ▨: with 20% of rat's bile
An ordinate represents the overall remaining (remained in the lumen and in the tissue) of the materials indicated in the upper side.
Results are expressed as the mean±S.D. of at least 5 animals.

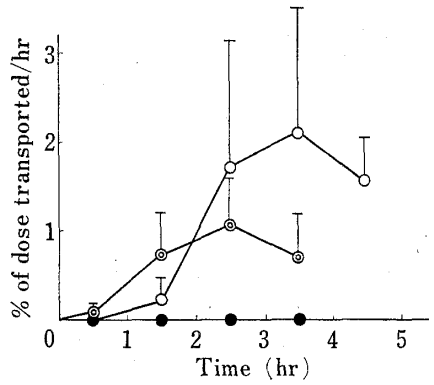

Fig. 2. Lymphatic Transport of Sudan Blue from Various Regions of Small Intestine

⊙: duodenum; ○: duodenum+10 cm jejunum; ●: 13 cm ileum
The dose of emulsion was 0.3 ml for duodenum and 1.3 ml for others. Same result was obtained when bile was introduced to ileum with by-passing.
Results are expressed as the mean of at least 3 animals. The vertical bar indicates+S.D. Statistically significant differences are not observed between the lymphatic transport in duodenum and that in duodenum+10 cm jejunum during 2—3 hr after administration.

bile duct ligated rats. Moreover, 20% of rat's bile collected from another rat was mixed in emulsion for the purpose of studying the effect of bile components under the same condition. No absorption of the dyes and triolein was observed from the stomach loop. The absorption was largest in the duodenal region, and decreased in order in jejunum, ileum, and large intestine. No significant difference was seen between the experiments without and with rat's bile, except in the absorption of Sudan Blue from duodenum ($p<0.01$) and that of thiolein-^{131}I from large intestine ($p<0.01$). The absorption of Sudan Blue in the duodenal region was decreased without bile. This fact suggested that the upper small intestine, especially duodenum, has higher capacity of lymphatic transport for Sudan Blue. It is recognizable from the previous reports of this laboratory[1b] and of others[8] that the absence of bile components resulted in significant impairment of lymphatic transport of lipid-soluble compounds from small intestine.

The lymphatic transport of Sudan Blue was investigated following the infusion of emulsion into loops made from various regions. As shown in Fig. 2, the capacity of lymphatic transport for Sudan Blue in duodenum+upper jejunum is 1.4 times of that in duodenum. On the other hand, no lymphatic transport was observed in the case of lower ileum regardless of the bile introduction. This is agreement with the recent report of Sabesin, *et al.*[9] that distal intestine was intrinsically defective of chylomicron secretion. These results were discussed later with regard to the observation of lymphatic staining by Sudan Black B.

8) *a*) N. Gallagher, J. Webb, and A.M. Dawson, *Clin. Sci.*, **29**, 73 (1965); *b*) P.J.A. O'Doherty, G. Kakis, and A. Kuksis, *Lipids*, **8**, 249 (1973); *c*) R. Nishigaki, S. Awazu, M. Hanano, and T. Fuwa, The 96th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, April, 1976.
9) S.M. Sabesin, P.R. Holt, and S.B. Clark, *Lipids*, **10**, 840 (1975).

Effect of Various Pretreatments

The data presented above indicate that absorption and lymphatic transport of Sudan Blue were the most predominent in the duodenal region. As noted, other investigators[3,10] have shown that the initial uptake of fatty acids from micellr solution is not energy dependent, since it occurs in the presence of metabolic inhibitors. It is probable, however, that the affiinity of oil droplets to the membrane components should play an important role in the uptake of lipid-soluble compounds from emulsions. Also it has been reported that the thickness of the unstirred water layer adjacent to the mucosal surface has a considerable effect on the rate of lipid uptake by the intestine.[11] To examine these facts in the emulsion systems, the effect of various pretreatments on the uptake of Sudan Blue in rat's everted duodenal sac was studied. The results are summarized in Table I. The pretreatment of 50% or 80% acetone caused marked decrease in the uptake of Sudan Blue, suggesting that the lipoidal surface components may play an important role in the uptake of lipid-soluble compounds from emulsion systems. Boiling perfectly inhibited the uptake of the dye. This result is fairly different from the fact that the uptake of fatty acids from micellar solution occurs even with boiled or dead tissue.[10]

On the other hand, as shown in Table I, the pretreatment with 1 mM DNP caused no significant inhibition. Thus, the uptake of lipid-soluble compounds from emulsions as well as from

Table I. Effect of Pretreatment on the Uptake of Sudan Blue in Rat's Evarted Duodenal Sac

Pretreatment	Uptake/10 min (μg)	
	Pretreated	Control
50% acetone[a]	21.2±9.4	52.4± 7.1
80% acetone[a]	15.4±6.0	64.3±14.6
100°, 7 min[b]	0	46.3± 6.6
1 mg/ml papain[c]	43.4±3.1	52.6±12.8
1% pronase[c]	35.9±8.1	38.2± 3.5
1 mM DNP[d]	44.1±7.2	30.0± 5.7

a) Duodenal loop was incubated with acetone-water for 10 min *in situ* before uptake experiment.
b) Everted duodenal sac was heated in 100° water for 7 min before uptake experiment.
c) Duodenal loop was incubated with enzyme solutions for 30 min *in situ* before uptake experiment.
d) Duodenal loop was incubated with 1 mM DNP solution for 30 min *in situ*, and uptake experiment was carried out under the same concentration of DNP.
Results are expressed as the mean±S.D. of at least 4 animals.

Table II. Effect of Pretreatment on the Absorption of Sudan Blue from Rat's Duodenal Loop

Pretreatment	% remained after 2 hr
50% acetone	81.6± 6.7
80% acetone	86.7± 7.4
Control	72.8±10.4
1 mg/ml Papain	72.0± 3.1
Control	67.0± 3.3

The condition of the pretreatment was the same as Table I. Sudan Blue remained in the lumen and in the tissue after incubation for two hours was represented as % of administred dose.
Results are expressed as the mean±S.D. of at least 3 animals.

10) J.M. Johnston and B. Borgström, *Biochim. Biophys. Acta*, **84**, 412 (1964).
11) B.E. Lukie, H. Westergaard, and J.M. Dietschy, *Gastroent.*, **67**, 652 (1974).

micellar solutions is considered to be an energy-independent process. The structure of surface protein seems to play a minor role in the uptake of Sudan Blue, since neither 1 mg/ml papain nor 1% pronase could cause the significant inhibition of uptake. It may be concluded, therefore, that the normality of macromorphological surface structure is important for the absorption of Sudan Blue from emulsion systems. These results were confirmed by *in situ* experiments (Tables II and III). As shown in Table II, however, the extent of the inhibition of the absorption by acetone pretreatment is relatively small as compared with the result of *in vitro* experiments. In the case of 50% acetone pretreatment no significant reduction from the control level was observed. To examine the effect of DNP pretreatment *in situ*, a short time infusion method was applied, since the animals are hard to be survived for a longer period owing to the high toxicity of DNP (Table III). This method is useful to estimate the initial uptake rate of dyes, since it may well be considered that the normality of epithelial cells in the everted sac is suspicious and that portal as well as lymphatic transport of dyes does not arise in such a short time. No significant reduction of uptake was also observed in the case of Oil Red XO emulsion.

Table III. Effect of DNP Pretreatment on the Initial Uptake of Sudan Blue and Oil Red XO from Triolein Emulsion *in Situ*

Compound	Uptake/min (% of dose)	
	Pretreated	Control
Sudan Blue	11.5±2.5	11.3±5.4
Oil Red XO	18.7±1.9	16.3±3.8

Pretreatment with DNP was carried out using 0.1 mM DNP-saline for washing instead of saline. Whole small intestinal loop was washed with 20 ml of DNP-saline twice. After 2 min emulsion was infused into the loop. See detail in the text.
Results are expressed as the mean±S.D. of at least 4 animals,

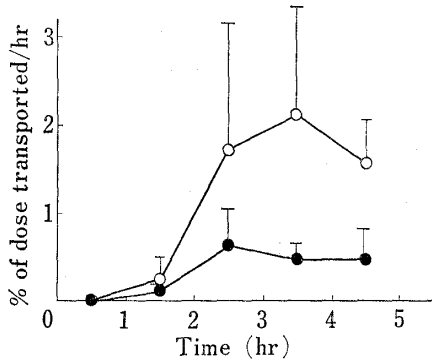

Fig. 3. Effect of Acetone Pretreatment on the Lymphatic Transport of Sudan Blue from Upper Small Intestine

○: control; ●: 50% acetone pretreated
1.3 milliliters of emulsion were administered into the intestinal loop, made up from duodenum and 10 cm of jejunum.
Results are expressed as the mean of at least 3 animals. The vertical bar indicates+S.D. Statistically significant differences are not observed between the lymphatic transport of control and that of 50% acetone pretreated rats during 0—2 hr after administration.

To investigate the effect of acetone pretreatment in detail, the lymphatic transport of Sudan Blue was examined using upper small intestine. As is evident from Fig. 3, the extent of lymphatic transport of Sudan Blue was decreased below one-thirds of the control with 50% acetone pretreatment although the disappearance from intestinal lumen and intestinal tissue was reduced only two-thirds of the control (Table II).

Discussion

Present experiments clarified the regional differences in the capacities of absorption and lymphatic transport for lipid-soluble dyes from oil-in-water emulsion systems. The absorptive capacity was the highest in duodenum, followed by jejunum, ileum, large intestine, and the lowest in stomach (Fig. 1). Ockner, *et al.*[5] reported that supernatant FABP concentration in mucosa from proximal and middle thirds of jejuno-ileum significantly exceeded that in duodenum and in distal thirds, expressed as micrograms per gram tissue. This result seems to be incompatible with ours in the point that absorption of dyes and triolein from duodenum was greater than that from jejunum. However, FABP was considered to work in the process

of lymphatic transport and there are some evidence that dyes used in this paper were absorbed mainly *via* portal route[1b] and that as much as 15% of absorbed oleic acid was transported *via* the portal system even in intact rat, 50% of which were in the form of free fatty acid.[8a]

Moreover, we have another evidence by macroscopic observation of lymphatic staining that the usual methods of lymphatic experiments, dealing only the drained lymph, could not offer the true aspect of lymphatic transport of lipid-soluble compounds. Owing to its high solubility in oils and its distinguishable color, Sudan Black B was infused as triolein emulsions into intestinal loop to estimate the movement of dyes transported into lymphatics. The group of lymph nodes into which lymph is drained from duodenum was stained with Sudan Black B within 30 min, sometimes within a few minutes. On the other hand, it takes about one hour to recognize the color in lymph nodes into which lymph is drained from jejunum and ileum, and after one hour the dye began to be recognizable in the cannulated polyethylene tube. These observation indicated that dyes entrapped in chylomicrons might be accumulated in the lymph nodes, suggesting that it takes much time to drain through the cannulated tube thereby causing underestimation of the rate of lymphatic transport when low dose of emulsion was administered. This concept can explain the result shown in Fig. 2. However, from the standpoint of overall absorption and lymphatic transport, jejunum may well be considered to be a major region since it has several times the length of duodenum.

As to large intestine, dyes and triolein were absorbed from this region to the same extent as from ileum. As shown in Fig. 1, absorption of triolein-^{131}I was suppressed by the exsistence of rat's bile, and water absorption from administered emulsion was also observed to be inhibited at the same time (not shown in the figure). This fact agrees well with the recent report of Saunders, *et al.*,[12] stating that greater than 10 mM taurocholate caused severe alteration of colonic epithelium and inhibited water absorption.

The result of the absorption from stomach is consistent with previous reports by others[13] very well. Though the distal intestine was known to be adaptive to a high fat diet[14] or bile diversion,[15] the metabolism and transport of dietary fat from stomach probably proceed differently from that in the small intestine. Though the gastric mucosa is unable to produce chylomicra, it has another route of lipid clearance: the surface epithelial cells may be shed into the gastric cavity rapidly and then transported into the small intestine where final absorption of lipids occur.

Another purpose of this paper was to elucidate the uptake mechanism of lipid-soluble drugs from oil-in-water emulsions compared with that from micellar solutions It is now generally accepted that fatty acids are taken up into the intestinal epithelial cells from micellar solutions by an energy-independent process.[3,9,10] Redgrave[16] has described that the lymphatic recovery of lipids administered as emulsions were identical with that as micellar solutions. Our results indicated that also in the emulsion system the uptake of lipid occured by an energy-independent process (Tables I and II).

Rodgers and O'Connor[17] recently reported that intact molecule of phosphatidyl choline inhibits intestinal uptake of cholesterol and fatty acid from mixed micellar solutions under both *in vitro* and *in vivo* conditions. They suggested the possibility that the rate of penetration of micelles through the unstirred water layer adjacent to the mucosal surface might be expected to be decreased due to added phospholipid which expands the size of bile salt micelles.

12) D.R. Saunders, J.R. Hedges, J. Sillery, L. Esther, K. Matsumura, and C.E. Rubin, *Gastroent.*, **68**, 1236 (1975).
13) S.B. Clark, B. Brause, and P.R. Holt, *Gastroent.*, **56**, 214 (1969); H.F. Helander and T. Olivecrona, *Gastroent.*, **59**, 22 (1970); C.E. Stevens and C.P. Leblond, *Anat. Rec.*, **115**, 231 (1953).
14) A. Singh, J.A. Balint, R.H. Edmonds, and J.B. Rodgers, *Biochim. Biophys. Acta.*, **260**, 708 (1972).
15) S.B. Clark, B. Lawergren, and J.V. Martin, *Am. J. Physiol.*, **225**, 574 (1973).
16) T.G. Redgrave, *Quart. J. exp. Physiol.*, **52**, 130 (1967).
17) J.B. Rodgers and P.J. O'Connor, *Biochim. Biophys. Acta*, **409**, 192 (1975).

It was considered that without this unstirred water layer the uptake of lipid-soluble drugs from emulsions might be accelerated. Acetone-water and digestive enzymes were used to decrease the affinity of oil droplets to the absorptive membrane and to influence this layer simultaneously. However, no effect was observed *in vitro* and *in situ* experiments, except the case of 80% acetone–water pretreatment (Tables I and II).

The effect of these pretreatment was also investigated morphologically by light microscopy. Little or no damage was observed compared with control after pretreatments with 50% acetone–water, 1 mg/ml papain, and 1% pronase solutions. Still, there is a possibility that even 50% acetone pretreatment inhibits the uptake of bile components, since the lymphatic transport of Sudan Blue was decreased under such a condition (Fig. 3). Severe damage was noted in the villi when pretreated with 80% acetone–water. Villous tips were fully disrupted and many epithelial cells were detached everywhere. Common water–soluble drugs must be absorbed well from such a damaged intestine,[18] whereas absorption of a lipid-soluble dye from emulsions was reduced.

These result suggest that the normality of macro-morphological structure of mucosal surface is very important for the absorption of lipid-soluble drugs from oil-in–water emulsions, which occured by an energy independent process.

18) T. Nadai, R. Kondo, A. Tatematsu, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 1139 (1972).