Modification of Intestinal Absorption of Drugs by Lipoidal Adjuvants

Shozo Muranishi[1]

Abstract: The use of various lipids for the modification of intestinal absorption of lipophilic, hydrophilic, or macromolecular drugs is reviewed. The influence of lipids on drug absorption varies with the structure and physical state of incorporated lipids. The mechanisms of drug absorption across the intestine, that involve lipids in the state of emulsions, liposomes, and micelles, are discussed. The use of fusogenic lipids in the micellar state can be most effective in enabling the absorption of poorly absorbed drugs, such as antibiotics and macromolecules. Moreover, within the gastrointestinal tract their promoting ability is greatest in the colorectal region. Fusogenic lipids are also useful for selective lymphatic delivery of drugs with a macromolecular carrier.

The enteral route is a common method of drug administration because it is the safest and most convenient. Oral administration is the most useful general route of ingestion and the most economical, but it incurs the following deficiencies: Drugs in the gastrointestinal tract may be degraded by the highly acidic gastric environment, by the enzymes of the mucosa or by the liver before they enter the systemic circulation. Many highly polar drugs and macromolecular drugs may not be absorbed because of insufficient lipophilicity and too large molecular size. Rectal administration is also frequently useful, because approximately half of the absorbed drug does not pass through the liver before entry into the systemic circulation. The disadvantage of rectal administration also includes the inability of many highly polar drugs and macromolecular drugs to be absorbed across the colorectal barrier membrane.

Although the absorption mechanism of most drugs from the colorectum is similar to that in the upper part of the gastrointestine (1, 2), some types of drugs, like antibiotics and antitumor agents, seem to have a somewhat different mechanism according to the absorption site within the entire gastrointestine. Some authors reported that tetracycline, an orally absorbable antibiotic, cannot be absorbed through the rectal membrane (3); furthermore, the bioavailability from a rectal dose of bacampicillin, a produrg of ampicillin, is much lower than that from the oral dose (4). 5-Fluorouracil is not actively absorbed from the colorectal area, as opposed to the small intestine (5, 6).

In the process of designing pharmaceutical formulations for enteral application, the factors summarized in Table I are generally required to optimize their biopharmaceutical properties. To attain therapeutic activity of the drug in the biophase, novel pharmaceutical modifications may be considered.

Absorption and transport processes of drugs administered via the gastrointestinal tract are schematically depicted in Fig. 1. They can be classified into intraluminal phenomena during the exposure phase within the lumen (Fig. 1a) and mucosal uptake phenomena (Fig. 1b). The pharmaceutical modification in the former case implies controlled release, pH dependent-dissolution, timed disintegration, etc.; modification in the latter case implies improvement of absorption, selective direction into the lymphatic system occurring in the intestinal tissues.

Table I. Factors Required for Optimizing the Biopharmaceutical Properties of Drugs.

1. Compounds that enhance the physical properties of the desired dosage form to improve solubility or crystallinity.
2. Chemical stability-enhancing compounds to improve stability in the gastrointestinal tract.
3. Enzyme resistant or inhibiting compounds to reduce drug metabolism in the gastro-intestinal lumen and by the mucous membrane.
4. Transport facilitating compounds to improve absorption into the blood circulation.
5. Targeting-enhancing compounds to allow direct entry into the lymphatic system.
6. Bypassing the liver to avoid 'first-pass' elimination of high clearance drugs.
7. Controlled release of drugs to attain a prolonged drug action.

The use of lipids in biopharmaceutical modification has been studied by many authors, including Carrigan (7), Ogata (8–11), Noguchi (12–16), Yamahira (17, 18), and Nakamoto (19); Davis has reviewed the therapeutic uses of emulsion systems (20). Liposomes, smaller lipid particles, have also been considered as an oral dosage system that can entrap lipid-soluble and water-soluble drugs (21, 22). Lipids are a heterogenous group of organic compounds that have large aliphatic or aromatic hydrocarbon components, or both, and that may or may not have water-soluble groups attached. Typical lipids are represented in Fig. 2, classified on the basis of their chemical formulas.

In this review, biopharmaceutical modifications, with the use of lipids to enhance drug absorption, and mucosal uptake phenomena (as in Fig. 1b) are discussed.

Dispersed Systems and Drug Absorption

Emulsions have been used for many years for oral and topical administration of lipid-soluble substances. Indoxol showed enhanced bioavailability when administered as an O/W emulsion with the drug dissolved in the oil phase (23). Surprisingly, water-soluble macromolecules, such as insulin and heparin, were also shown to be absorbed to some extent when administered per os in emulsion form (24–26). These findings stimulated interest in the potential of emulsions, not only to act as vehicles for drugs, but also as drug delivery systems that modify absorption. Lipids and related dispersed systems include emulsions, liposomes, ufasomes, and micelles. Their size orders are as follows:

emulsions	liposomes (or ufasomes)	micelles
200~10 000 nm	25~1000 nm	3~100 nm

[1] Kyoto Pharmaceutical University, Department of Biopharmaceutics, Misasagi, Yamashina, Kyoto 607, Japan

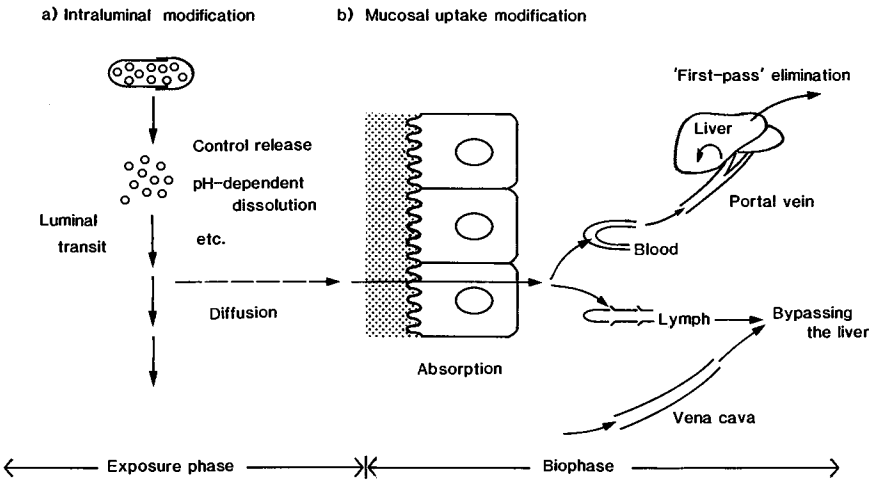

Fig. 1 Sites of adjuvant modification of drug transport via the intestine.

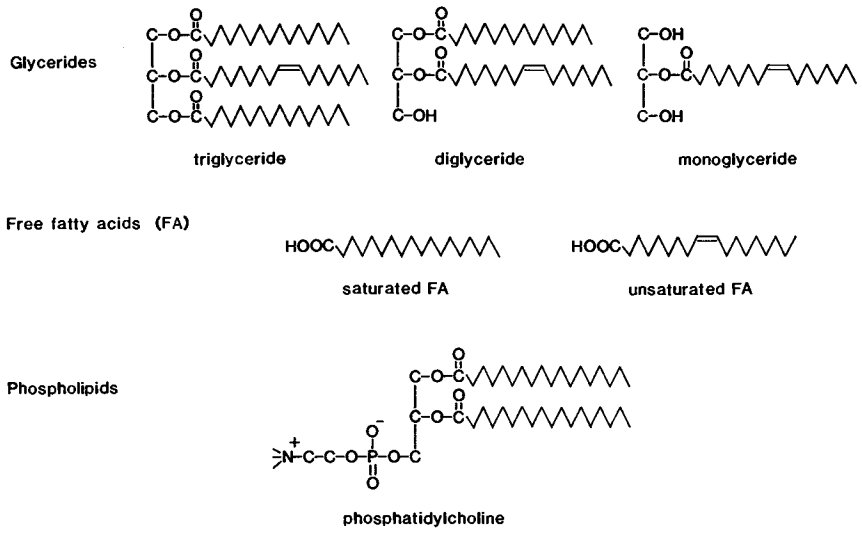

Fig. 2 Chemical classification of lipids.

Emulsions

The sizes of oil particles in O/W emulsions are generally larger by a factor of 10 than those of liposomes; it was therefore thought that an intact oil particle does not permeate the intestinal wall.

The mechanisms of intestinal absorption of drugs from O/W emulsion have been extensively studied *in vitro* and *in vivo*. In the first paper (8) of our series on the topic, the equation of Bean and Heman-Ackah (27) for a preservative-emulsion system was used as the starting point:

$$C_W = C_t \frac{\Phi + 1}{k\Phi + 1} \qquad (1)$$

This considers only the partition between the oil phase and external water phase, neglecting micellar interactions. Equation (*1*) can be written in terms of the amount of drug in the aqueous phase and in the emulsion:

$$A_W = A_t \frac{1}{P\Phi + 1} \qquad (2)$$

as $A_W = C_W \cdot V_W$ and $A_t = C_t (V_o + V_W)$ and $\Phi = V_o/V_W$.

The results of our initial study suggested that not only the concentration of drug, but also the absolute amount, has to be taken into account when considering the absorption process. Isopropyl palmitate and ethyl laurate, which represent unabsorbable oils, were used in absorption studies with the large intestine, and therefore, absorption of oil particles can be neglected.

On the other hand, the mechanism of the absorption process is expected to be more complicated in drug absorption from an oil-phase solution coexisting with a micelle phase, in which an excess of surface active agent is present. Ogata et al. (10) documented that two different oil-soluble drugs exert different effects on the absorption surface of the rat large intestine. In the one case (a) some of the drug is distributed into the aqueous phase, while in the other case (b) it is not. Typical examples of (a) and (b) are phenylbutazone and retinol acetate, respectively. Drugs of type (a) may be absorbed mainly via the aqueous phase without interaction with micelles or oil particles. On the other hand, for drugs of type (b), as micelles are competing for binding sites with oil particles on the mucosal layer, the drug absorption rate may depend not only on the amount of drug in the micellar phase, but also on the degree of adsorption of micelles onto the mucosal layer. Oil particles adsorbed on the mucosal layer interfere with the adsorption of micelles.

However, the oil-phase does not always work as an inert carrier or a simple reservoir. Recent studies have shown that the oil-phase of some natural oils exerts some biological effect of its own. Such examples include medium chain triglyceride

(17, 28) and oleic acid (29) which can be absorbed from the gastrointestine; their modification of drug absorption represents a separate mechanisms. Noguchi et al. (12) studied the absorption of the oil-soluble dye, Oil Red XO, from rat small intestine when administered as O/W emulsions of different oils. With tributyrin as an oil-phase, the dye was absorbed from the emulsion monoexponentially to the same extent as from a Polysorbate 80 micellar solution, despite the fact that the dye is very lipophilic and is not considered to be located in the aqueous phase. On the other hand, the dye was absorbed faster from the emulsion of triolein as an oil-phase in the early stage and slower in the later stage than from the tributyrin emulsion. These absorption characteristics of the dye were demonstrated to be related to the absorption of the oil-phase, which represents the third case (c) for the absorption of lipophilic drugs. Oil Red XO does not seem to move into systemic compartments together with oil, since it was not transported into the intestinal lymph even from triolein emulsion.

These three cases described above, (a), (b), and (c), are depicted in Fig. 3 which shows absorption of lipophilic drugs in oil with transportation through a micelle or aqueous phase.

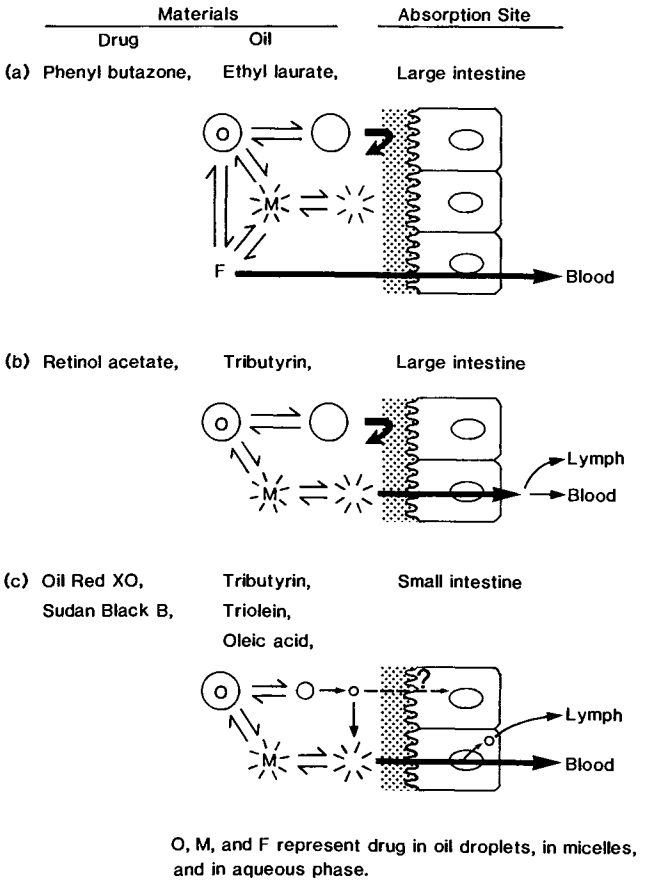

Fig. 3 Schematic representation of the absorption mechanism of oil-soluble drugs which are distributed in O/W emulsions.

Liposomes

Liposomes are smectic mesophases of phospholipids organized into bilayers that are generally more micronized than oil emulsions. In the recent past, there has been an interest in liposomes for oral use as an *in vivo* drug carrier system. Several reports documented increased blood levels of substances, such as insulin, entrapped in liposomes; in comparison to free drugs, augmented physiological effects have been observed (30–33). However, liposomes are easily degraded by the bile salts in the intestine (34). Moreover, evidence is lacking that liposomes can be transported intact through the gut wall or that complexes resulting from the interaction of liposomes with the intestinal mucosa can penetrate the intestine.

In our recent study (35), the intestinal absorption of carboxyfluorescein (CF), a poorly absorbable and water-soluble marker entrapped in liposomes, was investigated *in vivo* using bile fistula rats. The CF disappearance from the gut and the resulting plasma concentrations showed no difference or even slightly lower absorption with liposomal administration compared to free CF administration. Since phospholipids, phosphatidylcholine and phosphatidylethanolamine, are poorly absorbed, the probability of absorption of a drug entrapped in liposomes may be rather slight. However, these results did not rule out the possibility that a drug entrapped in liposomes may be transported through the intestinal wall if the liposome fuses with the intestinal mucosa or after the drug is released from the liposome at the absorption site.

In contrast to the results with liposomes, CF entrapped in ufasomes, which are a constituent of oleic acid, was shown to be absorbed to some extent (36).

Micelles

The size of small micelles that are formed from amphiphiles is generally smaller than that of liposomes. However, it is probably incorrect to regard micelles as rigid structures with a precise shape because they represent dynamic structures with a liquid core. The intestinal absorption of lipophilic drugs entrapped in micelles is discussed below.

Less harmful surfactants such as Polysorbate 80 have been used for micelle formation in animal experiments (37–39). Since monomers or some congeners of such surfactants are poorly absorbable (38), intact micelles are thought to not permeate the gut easily, although this remains in question. Retinol acetate and Oil Red XO entrapped in micelles of Polysorbate 80 were shown to be continuously absorbed from the intestine (12, 38). Even though the diffusion coefficient of micelles in the aqueous boundary layer next to the mucosa may be smaller than that of a free drug, micelles could penetrate the boundary layer and enable delivery of an incorporated drug to the epithelial cells (micelle-mediated mechanism).

Furthermore, that the initial absorption of retinol acetate in micelles is initiated by the adsorption maximum onto the surface of the mucosa has become clear from the fact that the

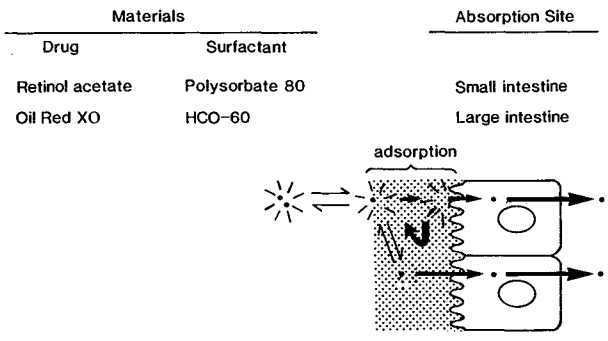

Fig. 4 Proposed mechanism of intestinal absorption of oil-soluble drugs from micellar solution.

absorption maximum of retinol acetate was coincident with the adsorption maximum of Polysorbate 80 (38). The processes involved in the intestinal absorption of lipophilic drugs in micellar solution is depicted in Fig. 4. It is therefore expected that micellar solubilization of an insoluble drug would serve to enhance the absorption rate over a micronized suspension formula.

Enhanced Absorption of Water-Soluble, Poorly Absorbed Drugs by Lipid Surfactant Mixed Micelles

Among therapeutic drugs, there are many water-soluble, poorly absorbed drugs with low or high molecular weights. A sophisticated drug delivery system via the enteral route is desirable for such drugs. Several studies demonstrated the enhancement of oral absorption by the addition of surfactant (40) and EDTA (41, 42), use of O/W emulsion (24), and entrapment in liposome (30, 32). These are indicated by increased blood levels of macromolecular drugs, notably insulin or heparin.

Macromolecular Drugs

It is generally accepted that the gut is impermeable to a large molecule such as heparin, a mucopolysaccharide with a molecular weight above 6,000. However, we previously found that monoolein-bile salt mixed micelles (MM) enhanced intestinal absorption of heparin (43–45); this was more predominant than the effect of an oil emulsion reported by Engel et al. (24). Windsor et al. (41) and Engel et al. (17, 40) used EDTA and surfactant to promote the intestinal absorption of heparin in their earlier studies; however, EDTA and sodium lauryl sulfate are known to be rather harmful to the mucosa. In our first study, the biological activity of heparin in plasma was measured after coadministration of adjuvants unharmful to the mucosa, tauro- or glycocholate and monoolein, resulting in a marked increase in heparin plasma concentrations (43).

Table II shows the increased absorption of heparin from a rat small intestinal and large intestinal (colorectal) loop by the addition of monoolein-taurocholate MM. The increase in large intestinal absorption was found to be much greater than that in the small intestine. The relation between the initial concentration of MM and the biological activity of heparin is shown in Fig. 5. The effective concentration of monoolein incorporated in MM to potentiate the absorption of the macromolecule from the large intestine was in the range of 10 mM, while a 4-fold higher concentration was needed in the small intestine. Peak plasma concentrations were reached in a shorter time after the administration of ^{35}S-heparin to the large intestine than to the small intestine, indicating that the enhancing effect of MM occurs earlier in the large intestine. The higher sensitivity of colorectal mucous membrane to MM is a noteworthy property when considering rectal administration.

Table II. Plasma Clearing Factor Activity* after Administration of Heparin to the Small Intestine and the Large Intestine (44, 45).

	Small intestine	Large intestine
Control	0.020	0.009
10 mM Na taurocholate	**	0.017
10 mM monoolein-Na taurocholate	0.035	0.214
40 mM Na glycocholate	0.049	–
40 mM monoolein-Na glycocholate	0.231	–
40 mM monoolein-Na taurocholate	0.229	0.349
Trioctanoin emulsion	0.068	0.011

*plasma sample at 30 min after administration
**not determined

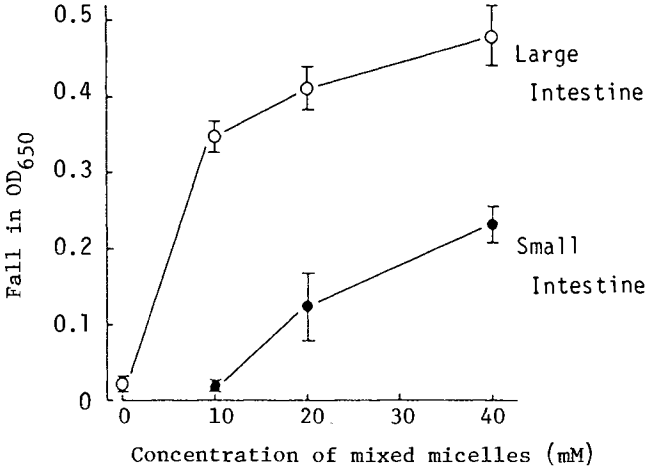

Fig. 5 Effect of the concentration of monoolein-Na taurocholate mixed micelles on plasma-clearing factor activity after the administration of heparin (45).

Although a 10 mM taurocholate micellar solution alone did not cause a marked increase, the addition of more than 2.5 mM monoolein enhanced absorption. Therefore, the important component in enhancing absorption is not the bile salt, but monoolein. Oleic acid, instead of monoolein, also caused a marked effect regardless of the surfactant used. However, triglycerides, such as trioctanoin and triolein, were not effective in improving heparin absorption. Such polar lipids can be absorbed in the presence of surfactant from the large intestine, penetrating the epithelial cells. Monoolein or oleic acid disappeared from the large intestinal lumen within the initial 15 min (Fig. 6). This disappearance appears to be correlated with the absorption rate of heparin.

Enhanced absorption via the rectal route by coadministration of polar lipid-surfactant MM has been observed with other macromolecular compounds, insulin (46), interferon (47), dextrans (48) and dextran sulfate (49).

Low Molecular Weight Drugs

Aminoglycosides, such as streptomycin and gentamycin, and β-lactam antibiotics, like cefazolin, are typical drugs that are poorly absorbed in the gastrointestinal tract, and therefore, have been limited to parenteral use. We have studied the enhancement of absorption of these drugs by polar lipid-surfactant MM (50). The influence of MM on the absorption of antibiotics was similar to the results obtained with heparin, namely, the effect was much stronger and faster in the loop of large intestine than in the small intestine. The effects of MM on the absorption of 5-fluorouracil were also examined in the stomach. However, even at a higher MM concentration (40 mM) absorption was increased only two fold (6).

In summary, the mucosal sensitivity to polar lipid-surfactant MM along the entire gastrointestine has the following rank order: large intestine > small intestine > stomach. The fact

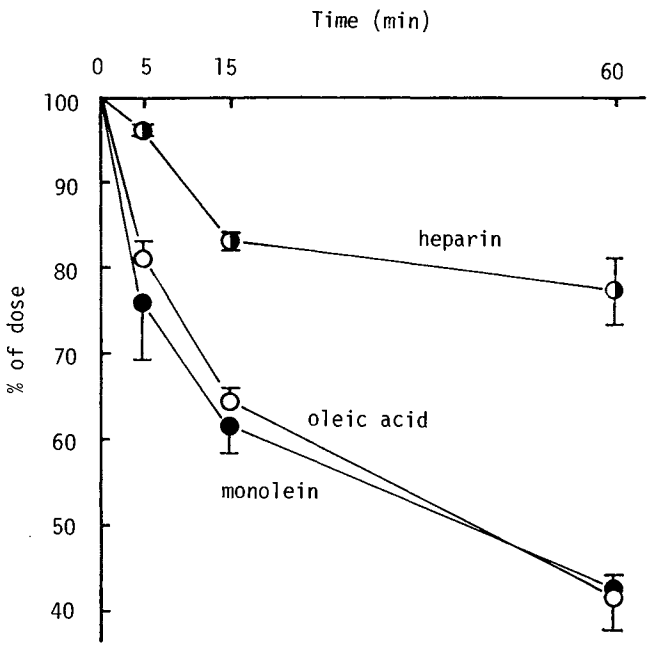

Fig. 6 Disappearance of ^{35}S-heparin and lipid (monoolein or oleic acid) from the lumen after the administration of taurocholate mixed micellar solutions into the large intestine (45).

that the colorectal area possesses the highest sensitivity is very attractive for designing formulae for poorly absorbed drugs.

Various factors would increase the absorption of poorly absorbed drugs as depicted in Fig. 7 when administered via oral and rectal routes. Oral administration of drugs generally involves transit processes, e. g. gastric transit with dissolution in gastric juice, followed by fast intestinal transit. MM would be spread out and diluted during transit in the intestinal lumen; therefore, the enhancement effect would decrease because the effectiveness of MM is concentration dependent. With rectal drug administration, transit time is slower since movement in the lower part of the intestine is slower. Moreover, much less fluid is secreted in the colorectum, and MM, therefore, should be diluted less in the lumen. Hence, rectal drug administration is more amenable to enhancing the absorption of normally poorly absorbed drugs with lipid surfactant MM.

Significance of Fusogenic Lipids

The enhancement effects by innocuous surfactants alone, e. g. tauro-, glycocholate, and Polysorbate 80, are not remarkable and rather slow in the intestinal absorption of drugs (51–53). However, polar lipid-bile salt MM enhance the absorption of streptomycin much more than bile salt alone. Table III shows the plasma concentration of streptomycin shortly after large intestinal administration of taurocholate mixed-micellar solutions containing various lipids. The plasma concentrations reached a peak in 15 min, which is very early after administration (54).

Table III. Plasma Concentration of Streptomycin at 15 min after Administration of 10 mM Taurocholate Mixed-Micellar Solution to the Large Intestine (54).

Composition	μg/ml	Composition	$C_{m:n}$	μg/ml
None	<1.5	Caprylic acid	$C_{8:0}$	<1.5
Na taurocholate	<1.5	Lauric acid	$C_{12:0}$	5.0
Monoolein	14.2	Myristic acid	$C_{14:0}$	3.4
Diolein	<1.5	Palmitic acid	$C_{16:0}$	<1.5
Triolein	<1.5	Palmitoleic acid	$C_{16:1}$	7.7
Oleyl alcohol	<1.5	Oleic acid	$C_{18:1}$	14.9
Methyl oleate	<1.5	Linoleic acid	$C_{18:2}$	14.4
		Linolenic acid	$C_{18:3}$	13.2

Administered dose of streptomycin was 4 mg/200 g rat

Among the lipids used in MM, unsaturated fatty acids and their monoglycerides enhanced the intestinal absorption of streptomycin more than saturated fatty acids. The lower the melting point of the fatty acid, the more the drug absorption was increased. Methyl oleate, oleyl alcohol, diolein and triolein did not increase absorption. Other reports confirm that the

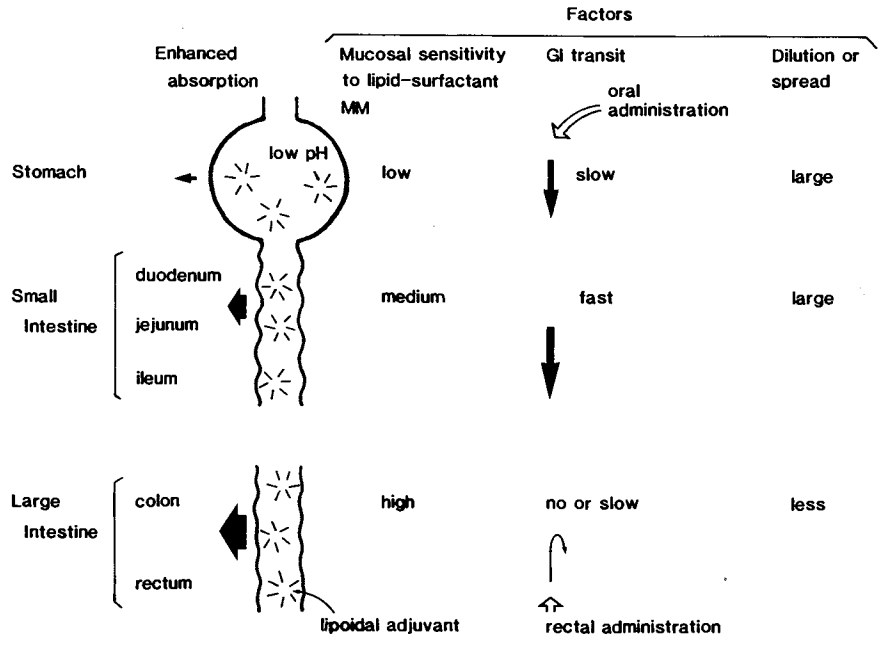

Fig. 7 Schematic representation of the factors that cause enhanced absorption in gastrointestinal by coadministration of lipoidal adjuvants.

colonic absorption of oxalate, an unabsorbable dietary substance, is not enhanced by taurocholate or octanoate, but it is enhanced by unsaturated fatty acids, oleate and recinoleate (55, 56).

Consequently, lipids that have a polar head and a low melting carbon chain are possibly capable of enhancing the intestinal absorption of poorly absorbed drugs. Interestingly, those lipids that enhanced the intestinal absorption of drug are identical to fusogenic lipids in a report on erythrocytes fusion (57). Maggio and Lucy (58) suggested that the presence of a fusogenic lipid with low melting point might produce an increase in the permeability of the lipid bilayer. Although we have found an enhancement of absorption by fusogenic lipids in the micellar state of an innocuous surfactant, it is likely that a lipid is essential for absorption, while a surfactant mainly contributes to solubilizing the lipids. The micellar state may facilitate the incorporation of the polar lipid component of MM into the mucosal membrane.

It is interesting to note that mixed micellar solution of egg lecithin and glycocholate did not cause any enhancement of small intestinal absorption of streptomycin (50). Egg lecithin is a major component of liposomes that has been investigated as a carrier of orally given drugs. It may seem improbable that promotion of intestinal drug absorption can be expected from liposomes. Our recent study (35) showed that within the first 60 min after administering liposome-entrapped carboxyfluorescein to the small intestine, its plasma concentration was lower than after administration of the free compound (Fig. 8). In contrast to liposomes, the enhancement effect of oleic acid-taurocholate MM on the absorption of carboxyfluorescein was striking as shown in the same figure.

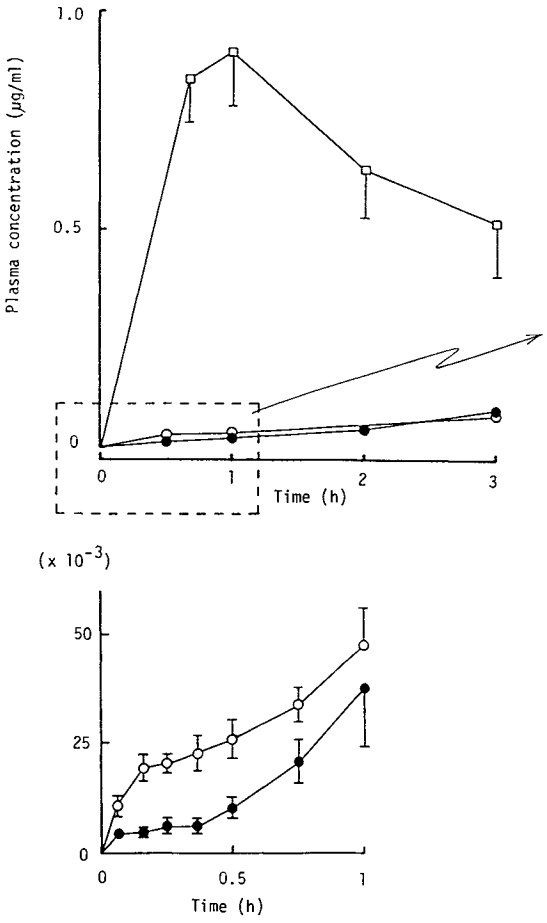

Fig. 8 Comparison of plasma concentrations of carboxyfluorescein (CF) following administration of free compound and CF-entrapped in liposomes and with mixed micelles to the small intestine (35).
□: CF/oleic acid (40 mM) + Na taurocholate (40 mM)
●: CF/egg phosphatidylcholine-liposomes
○: Free CF

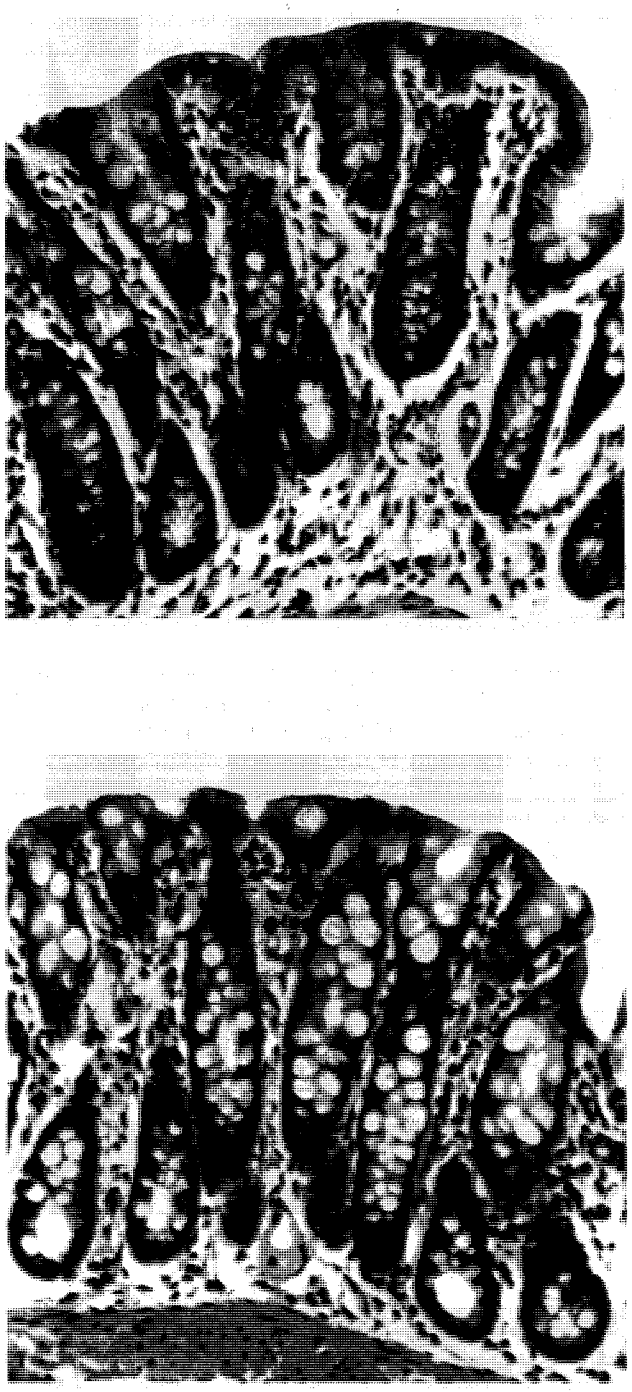

Fig. 9 Photomicrographs (H-E stain, x 200 in original photograph) of the rat colon.

The effect of fusogenic lipids on the intestinal mucosa is not only a result of influx, but also of outflux of drug permeation. An exorption (blood to lumen) study of sulfanilic acid revealed that oleic acid, linoleic acid, and their monoglycerides in the lumen were able to permeate the gut, while lauric and palmitic acids were less effective. These results suggest that fusogenic lipids influence both fluxes of drug permeation. Moreover, the most important finding is that the alteration of the mucosal barrier permeability caused by the lipids is reversible and transient. Microscopic observations showed no severe damage, such as disruption and loss of surface mucous cells, on exposure to the MM (Fig. 9).

Fusogenic lipids, therefore, may promise better intestinal absorption of poorly absorbed drugs. Development of new dosage forms, such as a rectal suppository, is now being carried out.

Mechanism of Enhanced Permeability by Fusogenic Lipid

The above experimental results of the absorption enhancement by fusogenic lipid MM suggest that enhancement is mostly due to alterations of the mucosal membrane permeability caused by the incorporation of the lipid component of MM. Fatty acids and monoglycerides are a minor component of biological membranes; however, they have been considered to play an important role in regulating many physiological functions. The mechanism of the action of lipids on the mucosal membrane is discussed below.

Effect on the Permeability of Brush Border Membrane and Artificial Liposomal Membrane

For drug permeability to occur in the intestinal mucosa, there may be a critical barrier of brush border membrane of epithelial cells. Brush border membranes, isolated according to the method of Kessler et al. (59), were used for studying the effect of lipid-bile salt MM on the permeability of biomembranes (60). Although brush border membrane vesicles are somewhat leaky, the permeability of sulfanilic acid was increased by the addition of MM (Table IV), indicating that uptake of fusogenic lipid in the epithelial membrane caused an enhancement of membrane permeability.

Table IV. Effect of the Treatment with Lipid Micellar Solution on the Permeability of Small Intestinal Brush Border Membranes of Rat.

Treatment solution	K of sulfanilic acid (10^{-2} min^{-1})*
None	2.13 ± 0.08
10 mM Na taurocholate	4.38 ± 0.46
10 mM monoolein – Na taurocholate	6.69 ± 1.17
10 mM oleic acid – Na taurocholate	8.57 ± 1.02

*at 25°C

Liposomes have been extensively investigated as biological membrane models. Membrane permeability properties, the alteration of membrane permeability, and fragilitiy of membranes have been studied with this model membrane (34, 61, 62). In the study on the permeability of liposomal membrane, phenol red, bromphenol blue, sulfanilic acid, cephazolin and procainamide ethobromide (PAEB) were used as impermeable compounds, as well as aminoglycosides (63). The incorporation of monoolein into liposomes reconstituted from egg phosphatidylcholine increased the release rates of these compounds through the liposomal membrane. As shown in Table V, unsaturated fatty acids such as oleic acid and linoleic acid markedly enhanced the release rate of PAEB, while saturated fatty acid caused less increase in the release rate. Diolein, triolein, oleyl alcohol and methyl oleate had no effect on the release rate of phenol red. Thus, a close correlation has been found between the enhancement of the intestinal absorption of drugs induced by MM and the alteration of the permeability of liposomal membrane by incorporation of a lipid component.

Table V. Effect of the Incorporation of Various Lipids on the Release Rate of Procainamide Ethobromide (PAEB) Through Liposomal Membranes (63).

Lipid	m.p. (° C)	K ($10^{-3}$$min^{-1}$)*
None		0.00
Monoolein	35	13.1
Lauric acid	43–45	7.83
Myristic acid	53–55	5.38
Palmitic acid	60–68	3.78
Stearic acid	64–70	3.66
Palmitoleic acid	−1 – +1	9.19
Oleic acid	8–10	11.7
Linoleic acid	−5	13.0
Linolenic acid	−11	15.5

*37° C

However, our liposome work failed to elucidate the alteration of the mucosal membrane permeability to anionic compounds induced by unsaturated fatty acid MM. Monoolein enhanced the release rate of both anionic and cationic drugs. One may have anticipated that the release of these anions from fatty acid-incorporated liposomes is inhibited by the repulsion between the anion and anionic layers of fatty acid on the membrane surface. However, the charge of a fatty acid may be eliminated in the mucosal membrane by the membrane acidification or a binding to the membrane protein. If this is correct the alteration in the mucosal membrane permeability to these anions may indeed be induced by unsaturated fatty acid MM.

On further reflection, the degree of PAEB permeability change in Table IV appears to be correlated with the melting point of incorporated lipids. Maggio and Lucy (58, 64) reported that the presence of a low melting fusogenic lipid in a mixed monolayer with phosphatidylcholine might facilitate molecular movement in the polar region of the phospholipid, and suggested that the presence of a low melting fusogenic lipid might produce an increase in the permeability of the lipid bilayer.

Physicochemical Aspects of the Interaction of Fusogenic Lipids with Biomembranes

To elucidate how the change in permeability was induced, the effect of fatty acids and monoglycerides on the physicochemical properties of the lecithin bilayer was investigated (65). The membrane fluidity was studied with the use of a spin label (5-nitroxide stearic acid), and the results are shown in Table VI. With respect to fatty acids, the degree of disorder of the

Table VI. Effect of Various Lipids on Motion Parameters of 5-nitroxide Stearic Acid Incorporated into Liposomal Membranes at 25° C (65).

Lipid*	2 *T* II (gauss)	Order parameter
None	49.6	0.57
Lauric acid	47.2	0.52
Myristic acid	48.0	0.54
Palmitic acid	48.2	0.54
Stearic acid	49.0	0.55
Palmitoleic acid	47.0	0.51
Oleic acid	47.4	0.52
Linoleic acid	46.8	0.50
Linolenic acid	46.7	0.50
Monoolein	49.7	0.58
Monopalmitin	51.0	0.60
Monostearin	50.4	0.60

*Equimolar mixture

membrane corresponds with the flexibility of the acyl chain of fatty acid which is represented by the melting or freezing point, i. e., the lower the melting point, the more the membrane is disordered. The *cis*-unsaturated and the shorter carbon chain fatty acids disorder the membrane's interior, but the longer saturated fatty acids do not. Karnovsky (66) has similarly shown with the fluorescence polarization technique, that the *cis*-unsaturated fatty acids disorder the lipid interior, while the *trans*-unsaturated and saturated fatty acids do not alter the bilayer interior.

Levine et al. (67) demonstrated that the packing of the lecithin bilayer appears to be determined by the close packing of the chains at the glycerol group, which is also likely to provide the main permeability barrier in the bilayer. According to Yeagle et al. (68) the surface of the lipid bilayer consists of an interlocking set of intermolecular electrostatic associations of the positively charged *N*-methyl groups with the negatively charged phosphate of neighboring lipids. The polar head group has also been suggested by these authors to play an important role in the maintenance of the bilayer configuration and the barrier properties.

The ESR spectrum of 3-doxyl-5α-cholestane incorporation into liposomal membranes is shown in Fig. 10 (65). The incorporation of fatty acids and monoglycerides broadened the outer hyperfine splitting of the probe, which indicated that the incorporated lipids order the polar region of the membranes.

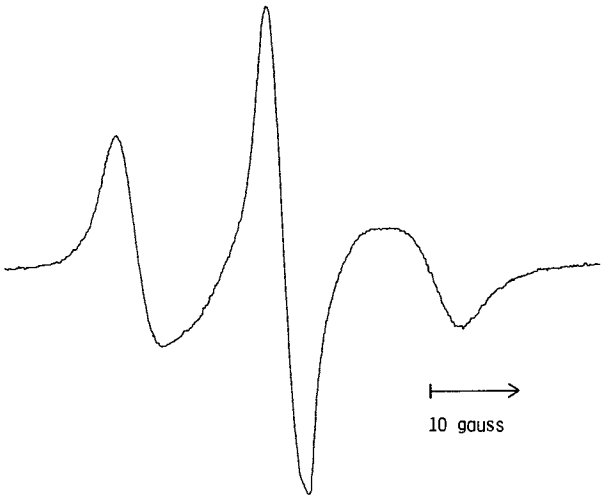

Fig. 10 Representative paramagnetic resonance spectrum for 3-doxyl-5α-cholestane in a dispersion of egg phosphatidylcholine (65).

Cullis et al. (69) found that the incorporation of oleic acid into erythrocyte ghost membranes broadens the characteristic ^{31}P-NMR spectrum of the bilayer structure in the absence of Ca^{2+}. The ^{1}H-NMR spectrum of egg phosphatidyl choline dispersed in pD 6.5 buffer solution (D_2O) is shown in Fig. 11. The incorporation of fatty acids and monoolein caused the peaks of the phosphate proton and of the olefine protons to disappear, and it broadened the choline methyl resonance. These investigations manifest the interaction of polar lipids with lecithin phosphate. Accordingly, the polar lipid head interacts with the lecithin phosphate; the interactive part may be the α-carbon hydrogen and the hydroxyl of glycerol for fatty acids and monoglycerides respectively. The flexible acyl chains

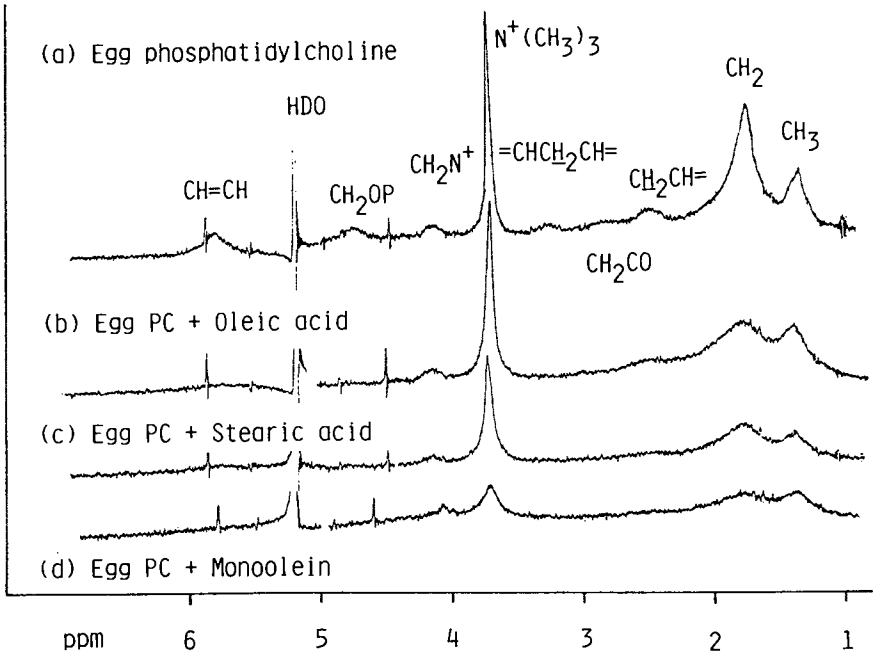

Fig. 11 100 MHz ^{1}H-NMR spectra of egg phosphatidylcholine-liposomes dispersed in pD 6.5 buffer solution (D_2O) (65).
(a) egg phosphatidylcholine; (b) 50 mol% oleic acid;
(c) 50 mol% stearic acid; (d) 50 mol% monoolein.

of the lipids and their interaction with the polar head of phosphatidyl choline may trigger a transient, 'corn'-shaped lipid complex in which the polar headgroup region is smaller than the one subtended towards the end of the acyl chain; the bilayer configuration destabilizes as a result.

These findings suggest that the increase in membrane permeability caused by fusogenic lipids is associated with the disorder in the membrane's interior and the interaction of the incorporated lipid with the polar head group of phospholipid. However, the contribution of biological macromolecules in the mucous membrane, e.g. mucous glycoproteins and membrane-bound proteins, to the enhancement of intestinal permeability cannot be neglected and are now under study.

Lymphotropic Delivery of Drugs

After passage through the mucosal cells of the intestinal tract, there are both blood and lymph routes by which a compound may be transported before systemic distribution. The blood route is the primary one for most drugs (70); however, the lymph pathway is known to play an important role in the absorption of highly lipophilic structures, e.g. cholesterol (71), triglyceride (72), lipid soluble vitamins (73), DDT (74), Sudan Blue (13) and naftidine (75). Some efforts have been made to enable a lymphotropic delivery of hydrophilic compounds by designing a lipophilic prodrug (76), for the purpose of bypassing the liver or preventing lymph metastasis. However, long chain fatty acid 5-fluorouracil diglycerides designed in our laboratory did not show selective lymphotropic transport (77). Presently, it is difficult to design a lymphotropic compound for such a hydrophilic drug.

On the other hand macromolecules, such as proteins (78, 79), dextrans (48), dextran sulfate (80, 81) and enzymes (82), when absorbed intact, are transported exclusively by the lymphatic system. Lymphatic drug delivery was effected with a macromolecular carrier developed in our laboratory. There are, however, at least four requirements for directing a drug from the enteral route to the lymphatic system. First, drug carriers themselves should penetrate the intestinal barrier, such as epithelial cells. Second, the size and structure of lymphotropic carriers must be chosen according to the anatomical barrier of lymphatic and blood capillaries. Third, a highly specific and tight binding of drug to the carrier is required so as to achieve lymphatic delivery. Fourth, the binding complex should be dissociated to free drug in the lymphatic or circulatory system to allow drug action.

To begin, we considered the use of an ion-pair complex with dextran sulfate (DS) as the lymphotropic carrier, which is an anionic high-molecular weight compound. An anticancer agent, bleomycin, a cationic glycoprotein, was chosen for complexing with DS (Table VII), and a lipid-surfactant MM was used as an effective intestinal absorption-promoter for unabsorbable high molecular weight compounds (49, 80, 81, 83). When bleomycin was administered alone, monooleintaurocholate MM induced a marked absorption of bleomycin from the intestine; however, its concentrations in the blood and the lymph were alsmost identical. On the other hand, administering the bleomycin-DS complex together with the MM selectively produced a very high lymphatic concentration. This lymphotropic selectivity, observed by complexing bleomycin with DS, was more effective in the large intestine than in the small intestine; its mechanism may be due to a molecular sieving in the blood-lymph barrier in intestinal tissue (48).

Table VII. Design of a Complex for Lymphotropic Drug Delivery.

drug	lymphotropic carrier	type of complex
(a) bleomycin (BLM)	dextran sulfate (DS)	ion-pair
(b) 1-hexylcarbamoyl-5-fluorouracil	β-cyclodextrin polymer	hydrophobic inclusion

The bleomycin-DS complex remained stable in the lumen of the large intestine. Analysis of large intestine tissues, lymph, and plasma indicated that 55 %, 94 % and 95 %, respectively, of the complex was dissociated to free BLM. Therefore, the lymphotropic system (BLM – DS + MM) largely fulfills the previously mentioned four requirements. A schematics of the selective lymphotropic mechanism of this system, when administered to the large intestinal lumen, is shown in Fig. 12.

Another attempt with the same intent was made using 1-hexylcarbamoyl-5-fluorouracil (HCFU), which is a hydrophobic prodrug of 5-fluorouracil. We selected cyclodextrins which are already known to form non-covalent inclusion complexes and are expected to be applied in the field of pharmaceutical sciences as a molecular capsule. Since β-cyclodextrin (β-CD) is not large enough, β-cyclodextrin polymer (average molecular weight 10,000; poly β-CD) was chosen as a lymphotropic carrier in which the β-CD cavity can include

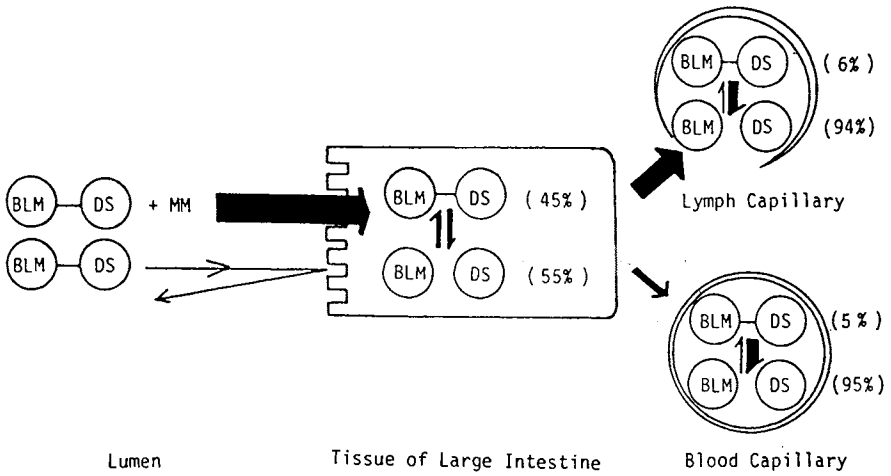

Fig. 12 Proposed mechanism for the selective lymphatic transfer of bleomycin from the large intestine by administration of a system containing bleomycin (BLM)-dextran sulfate (DS) + mixed micelles (MM) (49).

the lipophilic drug. The combined system (HCFU-poly β-CD + MM) increased the selective transfer of HCFU and 5-fluorouracil into the lymphatic system after large intestine administration (84).

This review points out that fusogenic lipids, such as unsaturated fatty acids and their monoglycerides, act on the biomembrane of the intestine to induce the absorption of poorly absorbed drugs. Such a system may be useful for polypeptides such as interferon (47) and insulin (46). Therapeutic applications of these delivery systems, including lymphotropicitiy, will continue to expand because numerous versatile macromolecular carriers, such as dextrane sulfate, β-cyclodextrin polymer and monoclonal antibodies, are available to enhance drug concentrations in the target system.

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