MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY
Vol. 28, No. 9, pp. 1047–1058, 2002

REVIEW

The Role of Lymphatic Transport in Enhancing Oral Protein and Peptide Drug Delivery

Kishor M. Wasan

Division of Pharmaceutics and Biopharmaceutics, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3
Fax: (604) 822-3035; E-mail: kwasan@interchange.ubc.ca

ABSTRACT

The gastrointestinal lymphatic system is a specific transport pathway through which dietary lipids, fat-soluble vitamins, and water-insoluble peptide-type molecules (e.g., cyclosporine A) can gain access to the systemic circulation. Drugs transported by way of the gastrointestinal lymphatic system bypass the liver and avoid potential hepatic first-pass metabolism. Lymphatic delivery of immunomodulatory and low therapeutic index protein and peptide drugs used in the treatment of cancer cell metastases and HIV presents an opportunity to maximize therapeutic benefit while minimizing general systemic drug exposure. Furthermore, lymphatic drug transport may promote drug incorporation into the body's lipid-handling system, thus offering the potential to manipulate drug distribution and residence time within the body. This review article will discuss the potential utilization of lymphatic transport in enhancing the oral absorption of protein- and peptide-like drugs.

Key Words: *Lymphatic drug transport; Protein-like drugs; Peptide-like drugs; Lipid absorption*

INTRODUCTION

Over 65% of commercially available drugs are formulated for oral administration.[1] However, one of the major factors limiting the effectiveness of orally administered drugs is poor absorption from the gastrointestinal tract or extensive pre-systemic clearance through hepatic first-pass metabolism.[2] For poorly

DOI: 10.1081/DDC-120014573
0363-9045 (Print); 1520-5762 (Online)

www.dekker.com

water-soluble drugs, the slow dissolution rate in the primarily aqueous contents of the gastrointestinal tract presents a significant barrier to absorption.[3,4] One strategy for improving the absorption of these drugs involves administration in a lipid-based delivery system, which presents the drug to the gastrointestinal tract in a solubilized form, thus eliminating poor aqueous solubility, and slows dissolution rate as barriers to absorption.

The gastrointestinal lymphatic system is a specific transport pathway through which dietary lipids,[5–7] fat-soluble vitamins, and water-insoluble peptide-type molecules [e.g., cyclosporine A (CSA)][8,9] can gain access to the systemic circulation.[10] Drugs transported by way of the gastrointestinal lymphatic system bypass the liver and avoid potential hepatic first-pass metabolism. Lymphatic delivery of immunomodulatory agents and low therapeutic index drugs used in the treatment of cancer cell metastases and HIV presents an opportunity to maximize therapeutic benefit while minimizing general systemic drug exposure.[11,12] Furthermore, lymphatic drug transport may promote drug incorporation into the body's lipid-handling system, thus offering the potential to manipulate drug distribution and residence time within the body.

LIPID ABSORPTION FROM THE SMALL INTESTINE AND THE LYMPHATIC SYSTEM

Lipid Absorption from the Small Intestine

Most of the lipids are absorbed from the jejunum region of the small intestine, with the exception of bile salts, which remain in the small intestine lumen in order to facilitate further digestion (Fig. 1).[13–15] The bile salts are finally absorbed in the distal ileum,[14] and are transported back to the liver by the portal blood in a cycle that constitutes the enterohepatic circulation.[15–17]

Lipid absorption occurs when the micellar solution of lipids comes into contact with the microvillus membrane of the enterocytes.[18] The lipids are transported across the enterocyte membrane by primarily an energy-independent process, which relies on the maintenance of an inward diffusion gradient. This gradient can partly be achieved by the attachment of the fatty acids to specific intracellular binding proteins. However, the ultimate driving force for absorption probably comes from the rapid re-esterification of the lipids, which is an ATP-dependent process depending upon activation of fatty acids to acyl-CoA esters.[18]

The major digestive products of triglycerides (TG) are monoglyceride and fatty acid, while the major digestive product of biliary and dietary phosphatidylcholine is lysophosphatidylcholine. These digestive products are absorbed primarily by the enterocytes through simple diffusion. However, the absorption of cholesterol by the enterocytes is specific, since β-sitosterol (a plant sterol), a molecule that bears considerable resemblance to cholesterol, is poorly absorbed. This specificity requires energy, as the deprivation of blood supply results in free permeability of different sterols.[18] Recent studies by Repa et al.[19] have suggested that oxysterol receptors and the bile acid receptor are retinoid X receptor heterodimeric partners that mediate cholesterol uptake by regulating expression of the reverse cholesterol transporter, ABC1, and the rate-limiting enzyme of bile acid synthesis, CYP7A1, respectively. These heterodimers serve as key regulators of cholesterol homeostasis by governing reverse cholesterol transport from peripheral tissues, bile acid synthesis in liver, and cholesterol absorption within the intestine.[19]

After entry into the enterocytes, monoglycerides, fatty acids, and cholesterol are transported within the cell to the endoplasmic reticulum by fatty acid-binding protein and sterol carrier protein, respectively. Through the monoglyceride pathway, the digestive byproducts of triglycerides, monoglycerides, and fatty acids are resynthesized to form triglyceride in the endoplasmic reticulum. This triglyceride is then transported to the Golgi apparatus, where it is packaged into chylomicrons and released into the lymphatics (Fig. 2).[16–18]

The transport and metabolism of the absorbed cholesterol is much lower than that of triacylglycerols. The estimated half-life for absorbed cholesterol in the enterocyte is about 12 hr. During absorption the cholesterol becomes incorporated into the membranes of the enterocytes and diluted with endogenous cholesterol. A large proportion of the cholesterol that is transported from the enterocyte is esterified, mainly with oleic acid. The rate of esterification of cholesterol may regulate the rate of lymphatic transport of cholesterol. Two enzymes have been proposed to be involved in the esterification, cholesterol esterase and acyl-CoA cholesterol acyltransferase (ACAT).

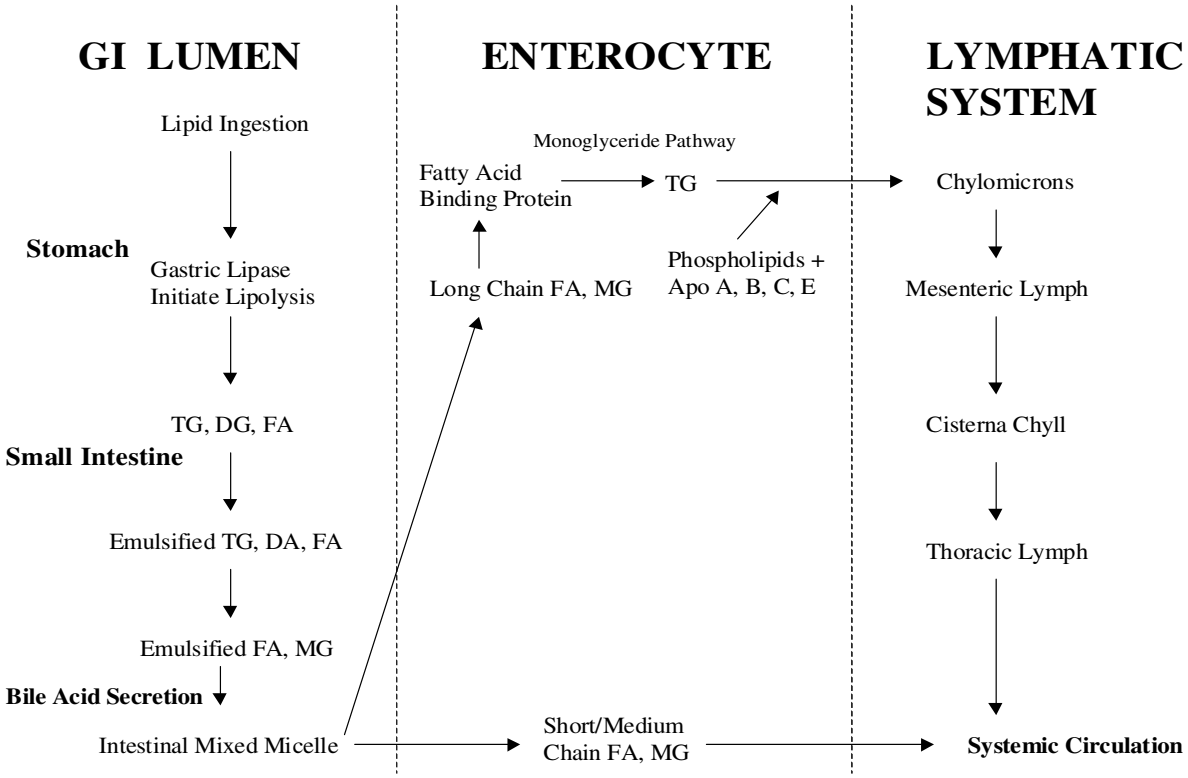

Figure 1. Triglyceride digestion and absorption in the small intestine. TG, triglyceride; MG, monoglyceride; DG, diglyceride; FA, fatty acids (From Ref. [2]).

In studies investigating intestinal absorption of cholesterol conducted in the early twentieth century, it was reported that adding cholesterol to the diet increased the concentration of cholesterol in intestinal lymph.[20] However, not until the 1950s was the quantitative significance of this lymphatic pathway known.[21] Biggs and colleagues demonstrated that following an intragastric dose of [^{3}H]cholesterol, very little isotropically labeled cholesterol appeared in the plasma of rats with thoracic lymph duct cannulas.[22] Chaikoff and coworkers recovered greater than 94% of absorbed labeled cholesterol in the thoracic lymph duct of rats.[23] Similar results have been reported in rabbits[24] and in a human subject with chyluria,[25] confirming that in mammals absorbed cholesterol is transported by the intestinal lymphatics and not by the portal system.

Intestinal Lymphatic System

The lymphatic system is an elaborate network of specialized vessels distributed throughout the vascular regions of the body. The primary and well-recognized function of the lymphatics is to drain the capillary beds and return extracellular fluid to the systemic circulation, thus maintaining the body's water balance. However, the structure and function of the lymphatics throughout the body are not uniform, and in specific areas the lymphatics perform a specialized role.[26–28] For example, the intestinal lymphatic system is responsible for the transport of dietary fat[29] and lipid-soluble vitamins to the systemic circulation.[26–28]

UTILIZATION OF LYMPHATIC SYSTEM IN THE DELIVERY OF PROTEIN- AND PEPTIDE-LIKE DRUGS

Deak and Csaky[30] originally designed a set of experiments to study the factors that regulate the absorption of nutrients and drugs into the intestinal lymphatic system in normal and cirrhotic rats. In these studies test compounds were administered by

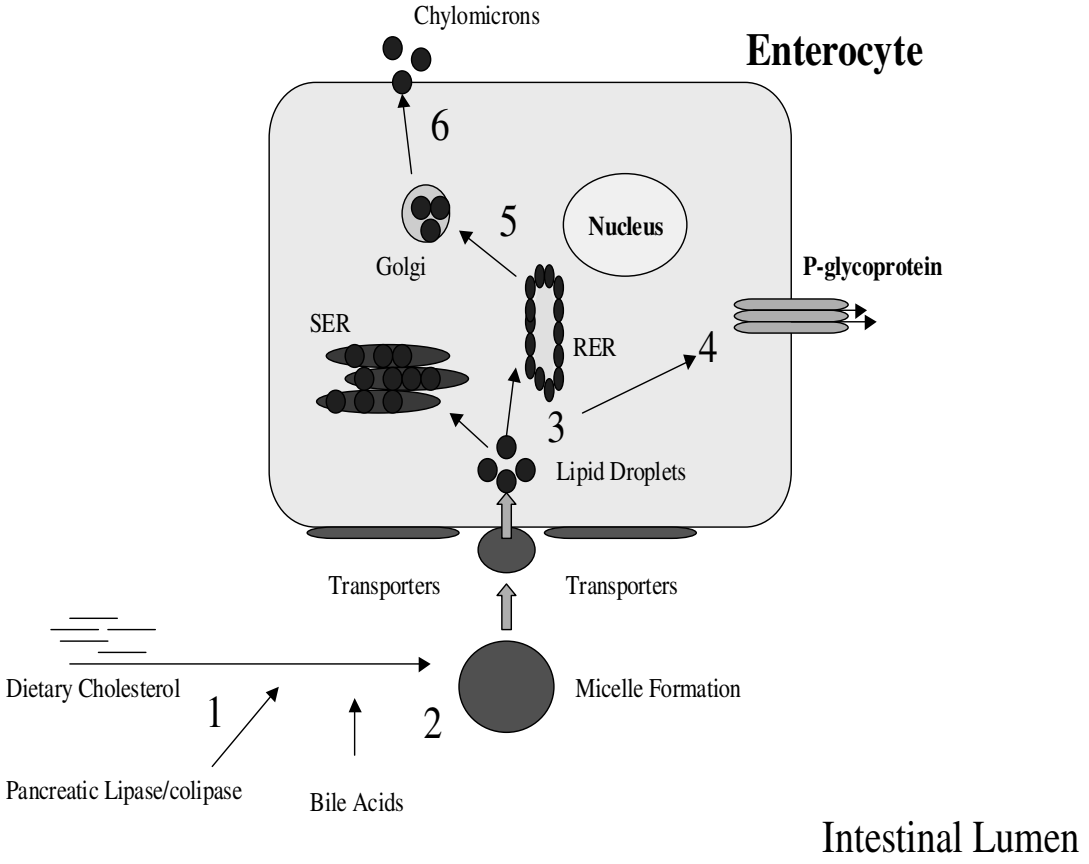

Figure 2. Transport of lipids through the enterocytes and the formation of chylomicrons. 1, Lipolysis with pancreatic lipase; 2, addition of bile salts for micelle formation; 3, lipid uptake from brush border membrane; 4, removal of lipids via *p*-glycoprotein; 5, transfer of lipid droplets to Golgi apparatus by fatty acid-binding protein and sterol carrier protein; 6, resynthesis of triglycerides and addition of phospholipids and apolipoproteins to form chylomicrons.

in situ jejunal luminal perfusion or systemic intravenous infusion, and concentrations within intestinal lymph, venous plasma, and intestinal perfusate were determined. They found that the absorption of compounds from the interstitial space into the intestinal lymphatic system is determined by the compound permeability through the capillary of the portal circulation and by their lipid solubility. Compounds too large to be absorbed into the portal circulation are primarily absorbed into the intestinal lymphatics.

The study of protein and peptide absorption via the lymphatics goes back nearly 30 years to the early work of Gallo-Torres[31] and recent work of Ikeda et al.[32] In these early studies the investigators found that up to 30% of the original dose of d-1,3,4-$^{3}H_{2}$-α-tocopheryl nicotinate,[31] alpha-, gamma-, and delta-tocotrienols, and alpha-tocopherol[32] is absorbed from the gastrointestinal tract through the lymphatic system. In recent years, a number of protein- and peptide-like compounds have been developed for oral administration. However, oral administration of these compounds is often limited by their instability in the gastrointestinal environment and poor absorption from the gut.[33]

To promote the absorption of these protein/peptide-like drugs, a number of groups have reported the use of unsaturated fatty acids with absorption-enhancing activities and less harmful properties to the gastrointestinal membranes in hydrolysates of natural oil.[33] These unsaturated fatty acids affect the permeability of these compounds and are associated with disorder in the membrane's interior due

to the interaction of these fatty acids with the polar head group of phospholipids.[33]

A second approach is the development of a drug delivery system that specifically targets the intestinal lymphatics.[33] One such delivery system was developed using the anticancer agent, Bleomycin. Bleomycin, by the combined effects of an ion-pair complex with dextran sulfate and an absorption enhancer (e.g., unsaturated fatty acids), was found in high concentrations within the lymph following administration. These high concentrations of drug may be due to a molecular sieving effect in the blood–lymph barrier in the intestinal tissue.[33]

A third approach in improving the intestinal absorption of protein/peptide-like compounds is by synthesizing novel lipophilic derivatives of peptides by a chemical modification with fatty acids, while maintaining their pharmacological activity.[33] This has been attempted with peptides such as thyrotropin-releasing hormone, tetragastrin, enkephalin, calcitonin, and insulin. In each of these cases the stability and permeability of these peptides were improved by acylation with some fatty acids having appropriate carbon numbers. This resulted in greater absorption of these compounds from the gastrointestinal tract via the lymphatic system.

Mimicking the absorption process of dietary fats, lipid conjugates and lipid microspheres have also been used to target the lymphatic route. Yanagawa et al.[34] demonstrated that when the polypeptide, CSA (an immunosuppresive agent widely used in organ transplantation) was incorporated into lipid microspheres consisting of olive and soybean oil and egg lecithin and administered orally to rats, CSA concentrations were 46 times greater in the lymph ducts 2 hr post-administration compared to conventional CSA therapy. Sato and colleagues further reported that emulsifying a CSA derivative, dihydrocyclosporine D, with a lipophilic emulsifier, milk fat globule membrane, significantly enhanced the blood and lymphatic fluid concentrations of the CSA derivative after intraduodenal dosing in rats.[35]

Taken together these studies report a number of strategies for improving the delivery and gastrointestinal absorption of protein- and peptide-like drugs via the intestinal lymphatic system. With further investigation and research the potential exists to use these approaches to help improve the therapeutic effectiveness of these types of drugs.

BIOLOGICAL AND PHARMACEUTICAL FACTORS AFFECTING LYMPHATIC DRUG DELIVERY

Contribution of Lymphatic Transport to the Increased Absorption of Water-Insoluble Drugs into the Systemic Circulation

The majority of orally administered drugs gain access to the systemic circulation by direct absorption into the portal blood (Fig. 3).[1] However, for some water-insoluble compounds, transport by way of the intestinal lymphatic system may provide an additional route of access to the systemic circulation.[5] Exogenous compounds absorbed through the intestinal lymph appear to be generally transported in association with the lipid core of intestinal lipoproteins (predominantly triglyceride-rich chylomicrons), thereby requiring co-administered lipid to stimulate lipoprotein formation. Delivery into the bloodstream by way of the intestinal lymphatics has been suspected to contribute to the overall absorption of a number of highly lipophilic compounds,[31,36–41] including CSA,[8,9] naftifine,[42] probucol,[43] mepitiostane,[44–48] halofantrine,[49–51] testosterone undecanoate,[36] and polychlorinated biphenyls.[52]

Lymph from the intestinal lymphatic system (as well as hepatic and lumbar lymph) drains through the thoracic lymph duct into the left internal jugular vein and then to the systemic circulation.[17,26,27] Thus, the transport of drug by way of the intestinal lymphatic system may increase the percentage of drug that can gain access to the systemic circulation. In addition, the process of intestinal lymphatic drug transport often continues over time periods longer than typically observed for drug absorption through the portal vein. Consequently, drug transport through the lymph may be utilized to prolong the time course of drug delivery to the systemic circulation. Preliminary findings published by Hauss et al.[53] suggest that the incorporation of a water-insoluble agent, ontazolast (a potent inhibitor of leukotriene B_4) into lipid-based formulations composed of a mixture of mono-, di-, and triglycerides increased the amount of drug reaching the systemic circulation (Table 1) and transported through the lymph (Table 2). Charman and colleagues have done similar work with another hydrophobic compound, halofantrine.[49,54]

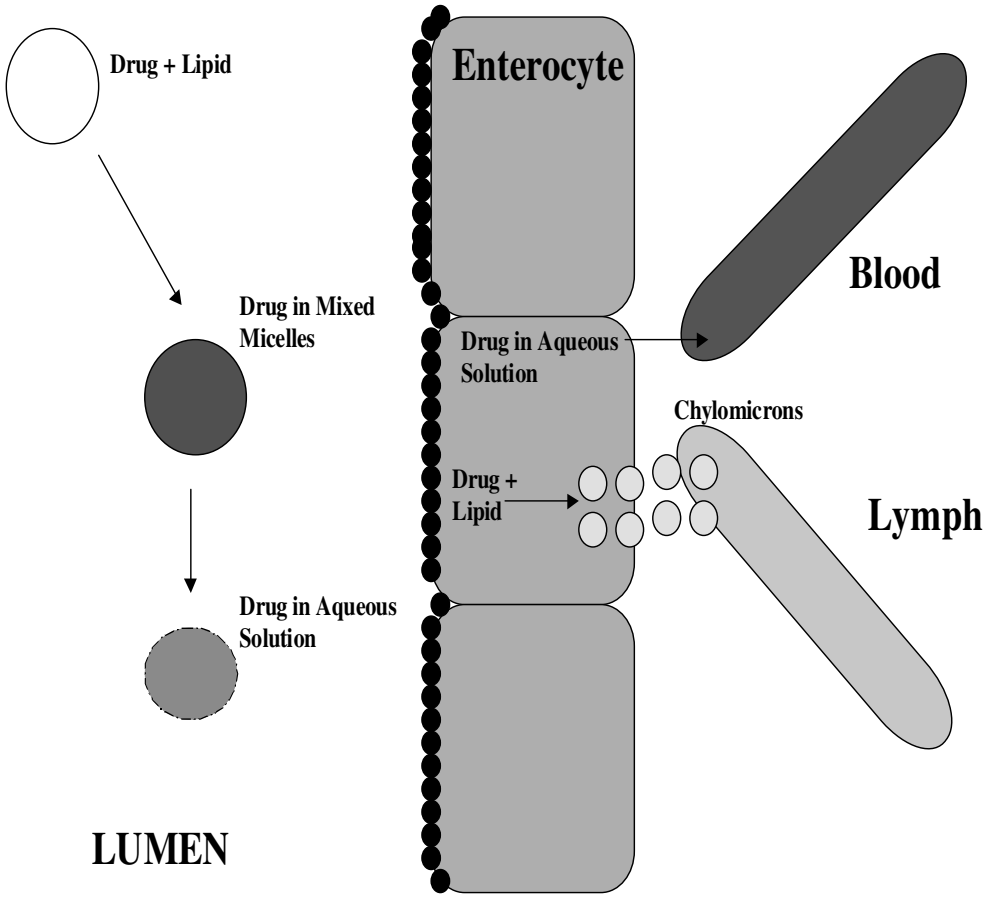

Figure 3. Transport of drug in lipid from the human into the lymph via the enterocyte.

Evaluation and Assessment of Intestinal Lymphatic Transport

A number of animal models have been described for the assessment of intestinal lymphatic drug transport.[1,2,11,53,55,56] Lymphatic transport studies are commonly first conducted in the laboratory rat, with larger more complicated models (e.g., dog or pig) subsequently investigated.[1,2,11,56] However, the utility of lymph fistulation in large animals is limited by considerable logistical and economic constraints. Ideally, sampling strategies for lymphatic transport studies should provide the capacity to estimate both the extent of lymphatic transport and the extent of portal blood absorption in order to estimate the overall bioavailability of the drug/formulation. This strategy enables the unambiguous determination of the extent of lymphatic transport, relative to absorption via the portal blood, and the total bioavailability of the drug/formulation. As lymphatic transport can be affected by experimental factors such as the site of lymphatic cannulation and the period of fasting prior to dosing,[1,2,5,10,11,56–59] it is important to standardize procedures when comparing studies.

The triple-cannulated anesthetized rat model (where the mesenteric lymph duct, jugular vein, and duodenum are accessed) has been used for the assessment of lymphatic transport.[53,55] General anesthesia precludes oral dosing in the anesthetized model, and consequently drug and lipid formulations are administered intraduodenally.[1,2,11,56] This limitation thus circumvents the inherent emulsifying action of the stomach and the potential effects of lipids on gastric emptying. Thus, the conscious rat model best represents the in vivo situation in terms of both lack

Table 1

Selected Plasma Non-compartmental Pharmacokinetics Parameters After Oral Gavage Administration of Ontazolast (100 mg/kg) to Rats in Various Formulations[a]

Formulation into Blood	$AUC_{(0-8\,hr)}$ (ng hr/mL)	T_{max} (hr)	C_{max} (ng/mL)	Absorption (% of original dose)
Suspension ($n=3$)	65±15	3.5±1.3	16±2.3	0.5
Peceol ($n=6$)	528±68*	4.6±0.6	137±34*	5.3
SEDDS 50/50 ($n=6$)	752±236	2.0±0.24	164±35*	7.0
SEDDS 20/80 ($n=6$)	877±104*	1.8±0.5	345±83*	7.8

[a]$AUC_{(0-8hr)}$ for 2 mg/kg intravenous dose is 240±40 ng hr/mL. Peceol is lymphotropic solubilizing agent comprised of a mixture of mono- and diglycerides of oleic acid, which closely resembles the end-products of intestinal lipid digestion.[4] SEDDS is a self-emulsifying drug delivery system, 50/50 represents equal proportions of Peceol and Gelucire 44/14 and 20/80 represents two parts of Peceol for every eight parts of Gelucire 44/14. Gelucire is a liquid excipient, which contains 19% mono-, di-, and triglycerides and 81% PEG 1500 mono- and diesters and free PEG 1500.[3] Data reported as mean ± standard error of the mean.
*$p < 0.05$ vs. suspension formulation using normal scores ranks.
Source: Ref. [53].

Table 2

Effect of Formulation on the Lymphatic Transport of Ontazolast and Triglyceride in Rats Following a 100 mg/kg Oral Gavage Dose of Ontazolast[a]

	Amount (μg) of Ontazolast Transported Lymphatically by Time Interval (mL)			Total Lymph Output (mL)	
Formulation	0–5 hr	5–8 hr	8–24 hr	0–8 hr	8–24 hr
Suspension ($n=3$)	4.8±2	1.1±0.5	1.7±0.1	24.5±5.6	67.1±27.8
Peceol ($n=6$)	217±45*	45±4*	12±4*	34.3±7.2	75.6±15.2
SEDDS 50/50 ($n=6$)	192±8*	16±8*	6±4	23.9±4.0	52.5±8.7
SEDDS 20/80 ($n=6$)	157±29*	13±4*	11±4*	17.3±4.1	65.7±15.2

[a]Peceol and SEDDS are described in Table 1. Data reported as mean±standard error of the mean.
*$p < 0.05$ vs. suspension formulation using normal scores ranks.
Source: Ref. [53].

of anesthetic effects and the ability to orally administer drug formulations. Previously reported methods for collecting lymph from the rat required total restraint of the animal and fluid replacement, by intravenous or intraduodenal infusion, to maintain lymph output.[13,60,61] Hauss and colleagues have developed a rat model to allow collection of mesenteric lymph for five days from conscious, minimally restrained animals with a fully patent cannulae and no signs of physical distress.[53,55] This model obviates the need for total restraint or general anesthesia, both of which are known to influence intestinal lymphatic transport of test compounds in unpredictable ways.[55] Animals are provided free access to an electrolyte solution, which they consume in sufficient quantity to maintain adequate lymph output without the need for the previously required infusions for fluid replacement. The rat is the appropriate experimental animal to investigate oral absorption and lymphatic transport because intestinal characteristics (e.g., anatomical, metabolic, and biochemical characteristics) of these animals are similar to those found in humans.[57,58,62,63] Specifically, the intestinal processing and absorption of dietary lipids are similar in rats and humans.[64]

Proposed Mechanisms That Govern the Lymphatic Transport of Water-Insoluble Drugs

Although the mechanisms by which drugs gain access into the intestinal lymphatic system through the enterocyte are not fully elucidated, there is growing evidence that supports our hypothesis that the majority of drugs transported by the lymphatics are associated with the triglyceride core of chylomicrons.[18,59,65–68] In addition, Charman and Stella[69] proposed two important factors that appear to be prerequisites for the lymphatic transport of water-insoluble drugs, the drug's diffusion/partition behavior and lipid solubility.

Diffusion and Partition Behaviour of Water-Insoluble Drugs

The extent of a drug's partitioning between the portal blood and intestinal lymph may be estimated from a comparison of the relative rates of drug mass transfer by each route. In this regard, the rate of fluid flow in the intestinal lymphatic system is approximately 500-fold less than that in the portal blood,[27,70,71] and during peak lipid transport, the lipid content of the lymph is only of the order of 1–2% (w/v). Thus, the effective mass ratio between lymph lipid and the portal blood is of the order of 1:50,000. Consequently, the selective lymphatic transport of small molecular weight, water-soluble drugs is unlikely if the route of absorption (portal blood vs. lymph) is governed by the relative rates of fluid flow. However, this ratio suggests that for similar extents of absorption and transport by the portal blood and intestinal lymph (not taking into account metabolic conversion, chemical stability, and/or bioavailability considerations), a candidate molecule should have a log octanol/water partition coefficient ($\log P$) in the region of 5 (highly water-insoluble). Hauss et al.[53] reported that when ontazolast, which has an octanol/water $\log P = 4.0$ was incorporated into lipid-based formulations composed of a mixture of mono-, di-, and triglycerides, a significantly greater amount of drug was transported by the lymph than suspension control. Caliph et al.[54] studied the effects of short-, medium-, and long-chain fatty acid-based vehicles on the absolute oral bioavailability and intestinal lymphatic transport of halofantrine. They reported that increases in lymphatic drug transport appeared to correlate with increases in lymphatic lipid transport.[54]

These initial studies provide evidence that lymphatic transport contributes to the overall oral absorption of water-insoluble compounds incorporated into lipid-based formulations. However, a more comprehensive investigation of these initial findings needs to be done.

Lipid Solubility of Water-Insoluble Drugs

In addition to a high partition coefficient being a prerequisite for lymphatic transport, lipid solubility is a further important parameter to consider. Charman and Stella[69] reported the relationship between lipid solubility and lymphatic transport of two highly water-insoluble compounds (DDT and hexachlorobenzene, HCB) which have similar octanol/water partition coefficients yet different solubilities. Both compounds would be regarded as highly lipophilic, as evidenced by their high octanol/water partition coefficients (DDT, 6.2 vs. HCB, 6.5), however, the 13-fold higher triglyceride solubility of DDT compared with HCB (DDT, 9.75 ± 0.15 vs. HCB, 0.75 ± 0.05 solubility) in peanut oil (g solute/100 mL) is reflected in the 14.6-fold increase in the extent of intestinal lymph transport reported in an anesthetized rat model.[69,72] Hauss et al.[53] had previously observed that when ontazolast concentration in the lymph was correlated to chylomicron triglyceride in the lymph, self-emulsifying drug delivery system formulations (consisting mainly of mixed triglycerides), which promote more rapid absorption of ontazolast, also favored lymphatic drug transport (Table 3). These findings suggest that solubility in chylomicron triglycerides may be a determining factor for promoting lymphatic transport.

Taken together, these studies provide *preliminary* evidence that triglyceride solubility may play a major role in promoting the lymphatic transport and increased oral absorption of lipophilic compounds.

CLINICAL SIGNIFICANCE

Understanding the potential mechanisms responsible for the increased gastrointestinal absorption of water-insoluble compounds when incorporated into lipid-based delivery vesicles[60,61] helps to improve the effectiveness of these compounds. Furthermore, improving the effectiveness of these compounds by increasing their ability to be absorbed from the

Table 3

Effect of Formulation on the Lymphatic Transport of Triglyceride (TG) and Ontazolast in Rats Following a 100 mg/kg Oral Gavage Dose of Ontazolast[a]

Formulation	Cumulative Amount of TG (mg) Transported in Mesenteric Lymph (Exogenous and Endogenous Lipid)		Total TG Transported from Exogenous Lipid (% of dose, 0–8 hr)	Total (μg, 0–8 hr)
	0–5 hr	5–8 hr		
Suspension ($n=4$)	21.9±4.9	52.8±17.9	ND	6.0±2.0
Peceol ($n=6$)	128.9±26.9*	210.7±39.9*	51.8±10.8	260±40*
SEDDS 50/50 ($n=6$)	101.0±6.7*	158.1±10.8*	38.3±1.4	220±10*
SEDDS 20/80 ($n=6$)	67.2±7.0*	131.0±11.3*	31.1±2.5	170±30*

[a]Peceol and SEDDS are described in Table 1. ND, non-detectable. Data reported as mean ± standard error of the mean.
*$p < 0.05$ vs. suspension formulation using normal scores ranks.
Source: Ref. [53].

intestine could decrease the number of times patients are required to take these drugs, and thus improve their compliance. In the larger perspective, these studies will have broad implications for the way we formulate and evaluate the bioavailability of lipid-based drug entities. In contrast to dissolution rate, the effects on the gastrointestinal membrane permeability, transit time, or the gut wall metabolism of the drug compound, the lymphatic transport of drugs has been overlooked and is not well understood. Hence the role of lymphatic transport as a possible means of increased systemic blood drug levels needs to be further addressed.

ACKNOWLEDGMENTS

I would like to acknowledge Dr. David Hauss for the many discussions we had pertaining to the use of lymphatic transport for the delivery of water-insoluble compounds. Support from the Canadian Institutes of Health Research is acknowledged.

REFERENCES

1. Pouton, C.W.; Charman, W.N. The Potential of Oily Formulations for Drug Delivery to the Gastrointestinal Tract. Adv. Drug Del. Rev. **1997**, *25*, 1–2.
2. Porter, C.J.H.; Charman, W.N. Uptake of Drugs into the Intestinal Lymphatics After Oral Administration. Adv. Drug Del. Rev. **1997**, *25*, 71–89.
3. Serajuddin, A.T.M.; Sheen, P.C.; Mufson, D.; Berstein, D.F.; Augustine, M.A. Effect of Vehicle Amphiphilicity on the Dissolution and Bioavailability of a Poorly Water-Soluble Drug from Solid Dispersions. J. Pharm. Sci. **1988**, *77*, 414–417.
4. Sheen, P.C.; Kim, E.I.; Petillo, J.J.; Serajuddin, A.T.M. Bioavailability of a Poorly Water-Soluble Drug from Tablet and Solid Dispersion in Humans. J. Pharm. Sci. **1991**, *80*, 712–714.
5. Hofmann, A.F.; Borgstrom, B. Physico-chemical State of Lipids in Intestinal Content During Their Digestion and Absorption. Feb. Proc. **1986**, 21, 43–50.
6. Shiau, Y.-F. Mechanism of Intestinal Fat Absorption. Am. J. Physiol. **1981**, *240*, G1–G9.
7. Borgstrom, B.; Dahlqvist, A.; Lundh, G.; Sjovall, J. Studies of Intestinal Digestion and Absorption in the Human. J. Clin. Invest. **1957**, *36*, 1121–1136.
8. Ueda, C.T.; Lemaire, M.; Gsell, G.; Nussbaumer, K. Intestinal Lymphatic Absorption of Cyclosporin A Following Oral Administration in an Olive Oil Solution to Rats. Biopharm. Drug Dispos. **1983**, *4*, 113–124.
9. Reymond, J.P.; Sucker, H.; Vonderscher, J. In Vivo Model for Ciclosporin Intestinal Absorption in Lipid Vehicles. Pharm. Res. **1988**, *5*, 677–679.
10. Thomson, A.B.R.; Keelan, M.; Garg, M.L.; Clandinin, M.T. Intestinal Aspects of Lipid Absorption: In Review. Can. J. Physiol. Pharmacol. **1989**, *67*, 179–191.
11. Charman, W.N.; Porter, C.J.H. Lipophilic Prodrugs Designed for Intestinal Lymphatic Transport. Adv. Drug Del. Rev. **1996**, *19*, 149–169.
12. Tucker, G. Drug Delivery: Lymphing Along? Lancet **1993**, *341*, 1314–1315.
13. Carey, M.C.; Small, D.M.; Bliss, C.M. Lipid Digestion and Absorption. Ann. Rev. Physiol. **1983**, *45*, 651–677.
14. Thomson, A.B.; Schoeller, C.; Keelan, M.; Smith, L.; Clandinin, M.T. Lipid Absorption: Passing Through the Unstirred Layers, Brush Border Membrane, and Beyond. Can. J. Physiol. Pharmacol. **1993**, *71*, 531–555.

15. Tso, P., Simmonds, W.J. The Absorption of Lipid and Lipoprotein Synthesis. Lab. Res. Meth. Biol. Med. **1984**, *10*, 191–216.
16. Tso, P.; Balint, J.A. Formation and Transport of Chylomicrons by Enterocytes to the Lymphatics. Am. J. Physiol. **1986**, *13*, G715–G726.
17. Tso, P. Intestinal Lipid Absorption. In *Physiology of the Gastrointestinal Tract*; Johnson, L.R., Ed.; Raven Press: New York, 1994; 1867–1908.
18. Davis, R.A.; Vance, J.E. Structure, Assembly and Secretion of Lipoproteins. In *Biochemistry of Lipids, Lipoproteins and Membranes*; Vance, D.E., Vance, J.E., Eds.; Elsevier: New York, 1996; 473–493.
19. Repa, J.J.; Turley, S.D.; Lobaccaro, J.M.A.; Medina, J.; Li, L.; Lustig, K.; Shan, B.; Heyman, R.A.; Dietschy, M.; Mangelsdorf, D.J. Regulation of Absorption and ABC1-Mediated Efflux of Cholesterol by RXR Heterodimers. Science **2000**, *289*, 1524–1529.
20. Mueller, J.H. The Mechanism of Cholesterol Absorption. J. Biol. Chem. **1916**, *27*, 463–480.
21. Wilson, M.D.; Rudel, L.L. Review of Cholesterol Absorption with Emphasis on Dietary and Biliary Cholesterol. J. Lipid Res. **1994**, *35*, 943–955.
22. Biggs, M.W.; Friedman, M.; Byers, S.O. Intestinal Lymphatic Transport of Absorbed Cholesterol. Proc. Soc. Exp. Biol. Med. **1951**, *78*, 641–643.
23. Chaikoff, I.L.; Bloom, B.; Siperstein, M.D.; Kiyasu, J.Y.; Reinhardt, W.O.; Dauben, W.G.; Eastham, J.F. C^{14}-Cholesterol. I. Lymphatic Transport of Absorbed Cholesterol-4-C^{14}. J. Biol. Chem. **1952**, *194*, 407–412.
24. Rudel, L.L.; Morris, M.D.; Felts, J.M. The Transport of Exogenous Cholesterol in the Rabbit. I. Role of Cholesterol Ester of Lymph Chylomicra and Lymph Very Low Density Lipoproteins in Absorption. J. Clin. Invest. **1972**, *51*, 2686–2692.
25. Hellman, L.; Frazell, E.L.; Rosenfeld, R.S. Direct Measurement of Cholesterol Absorption via the Thoracic Duct in Man. J. Clin. Invest. **1960**, *39*, 1288–1294.
26. Davidson, N.O. Cellular and Molecular Mechanisms of Small Intestinal Lipid Transport. In *Physiology of the Gastrointestinal Tract*; Johnson, L.R., Ed.; Raven Press: New York, 1994; 1909–1934.
27. Johnson, J.M. Triglyceride Biosynthesis in the Intestinal Mucosa. In *Lipid Absorption: Biochemical and Clinical Aspects*; Rommel, K., Goebell, H., Bohmer, R., Eds.; MTP Press: Lancaster, 1976; 85–94.
28. Zilversmit, D.B. The Composition and Structure of Lymph Chylomicrons in Dog, Rat and Man. J. Clin. Invest. **1965**, *44*, 1610–1622.
29. McDonald, G.B.; Weidman, M. Partitioning of Polar Fatty Acids into Lymph and Portal Vein After Intestinal Absorption in the Rat. Q. J. Exp. Physiol. **1987**, *72*, 153–159.
30. Deak, S.T.; Csaky, T.Z. Factors Regulating the Exchange of Nutrients and Drugs Between Lymph and Blood in the Small Intestine. Microcirc. Endothel. Lymph. **1984**, *1* (5), 569–588.
31. Gallo-Torres, H.E. Intestinal Absorption and Lymphatic Transport of d-1,3,4-$^{3}H_2$-α-Tocopheryl Nicotinate in the Rat. Int. J. Vitam. Nutr. Res. **1970**, *40*, 505–514.
32. Ikeda, I.; Imasato, Y.; Sasaki, E.; Sugano, M. Lymphatic Transport of Alpha-, Gamma- and Delta-Tocotrienols and Alpha-Tocopherol in Rats. Int. J. Vitam. Nutr. Res. **1996**, *66*, 217–221.
33. Muranishi, S. Delivery System Design for Improvement of Intestinal Absorption of Peptide Drugs. Yakugaku Zasshi **1997**, *117*, 394–414.
34. Yanagawa, A.; Iwayama, T.; Saotome, T.; Shoji, Y.; Takano, K.; Oka, H.; Nakagawa, T.; Mizushima, Y. Selective Transfer of Cyclosporin to Thoracic Lymphatic Systems by the Application of Lipid Microspheres. J. Microencaps. **1989**, *6*, 161–164.
35. Sato, H.; Liu, H.X.; Adachi, I.; Ueno, M.; Lemaire, M.; Horikoshi, I. Enhancement of the Intestinal Absorption of a Cyclosporine Derivative by Milk Fat Globule Membrane. Biol. Pharm. Bull. **1994**, *17*, 1526–1528.
36. Horst, H.J.; Holte, W.J.; Dennis, M.; Coert, A.; Geelen, J.; Voigt, K.D. Lymphatic Absorption and Metabolism of Orally Administered Testosterone Undecanoate in Man. Klin. Wschr. **1976**, *54*, 875–879.
37. Sugihara, J.; Furuuchi, S.; Ando, H.; Takashima, K.; Harigaya, S. Studies on the Intestinal Lymphatic Absorption of Drugs. II. Glyceride Prodrugs for Improving the Lymphatic Absorption of Naproxen and Nicotinic Acid. J. Pharmacobio-Dyn. **1988**, *11*, 555–562.
38. Sugihara, J.; Furuuchi, S. Lymphatic Absorption of Hypolipidemic Compound 1-*O*-[p-(Myristyloxy)-α-Methylcinnamoyl]Glycerol (LK-903). J. Pharmacobio-Dyn. **1988**, *11*, 121–130.
39. Adachi, I.; Liu, H.; Horkoshi, I.; Ueno, M. Possibility of Lymphatic Absorption of Epidermal Growth Factor from Intestine. Yakugaku Zasshi **1993**, *113*, 256–263.
40. Hauss, D.J.; Mehta, S.; Radebaugh, G.W. Targeted Lymphatic Transport and Modified Systemic Distribution of CI-976, a Lipophilic Lipid-Regulator Drug, via a Formulation Approach. Int. J. Pharm. **1994**, *108*, 85–93.
41. Barnwell, S.G.; Laudanski, T.; Story, M.J.; Mallinson, C.B.; Harris, R.J.; Cole, S.K.; Keating, M.; Attwood, D. Improved Oral Bioavailability of Propranolol in Healthy Human Volunteers Using a Liver Bypass Drug Delivery System Containing Oleic Acid. Int. J. Pharm. **1992**, *88*, 423–432.

42. Grimus, R.C.; Schuster, I. The Role of the Lymphatic Transport in the Enteral Absorption of Naftifine by the Rat. Xenobiotica **1984**, *14*, 287–294.
43. Palin, K.J.; Wilson, C.J. The Effect of Different Oils on the Absorption of Probucol in the Rat. J. Pharm. Pharmacol. **1984**, *36*, 641–643.
44. Ichihashi, T.; Kinoshita, H.; Shimamura, K.; Yamada, H. Absorption and Disposition of Epithiosteroids in Rats (1): Route of Administration and Plasma Levels of Epitiostanol. Xenobiotica **1991**, *21*, 865–872.
45. Ichihashi, T.; Kinoshita, H.; Yamada, H. Absorption and Disposition of Epithiosteroids in Rats (2): Avoidance of First-Pass Metabolism of Mepitiostane by Lymphatic Absorption. Xenobiotica **1991**, *21*, 873–880.
46. Ichihashi, T.; Kinoshita, H.; Takagishi, Y.; Yamada, H. Effect of Oily Vehicles on Absorption of Mepitiostane by the Lymphatic System in Rats. J. Pharm. Pharmacol. **1992**, *44*, 560–564.
47. Ichihashi, T.; Kinoshita, H.; Takagishi, Y.; Yamada, H. Effect of Bile on Absorption of Mepitiostane by the Lymphatic System in Rats. J. Pharm. Pharmacol. **1992**, *44*, 566–569.
48. Ichihashi, T.; Kinoshita, H.; Takagishi, Y.; Yamada, H. Intrinsic Lymphatic Partition Rate of Mepitiostane, Epitiostanol and Oleic Acid Absorbed from Rat Intestine. Pharm. Res. **1991**, *8*, 1302–1306.
49. Porter, C.J.H.; Charman, S.A.; Charman, W.N. Lymphatic Transport of Halofantrine in the Triple-Cannulated Anaesthetized Rat Model: Effect of Lipid Vehicle Dispersion. J. Pharm. Sci. **1996**, *85*, 351–356.
50. Humberstone, A.J.; Porter, C.J.H.; Charman, W.N. A Physicochemical Basis for the Effect of Food on the Absolute Bioavailability of Halofantrine. J. Pharm. Sci. **1996**, *85*, 525–529.
51. Wasan, K.M.; Ramaswamy, M.; MacIntosh, M.; Porter, C.J.H.; Charman, W.N. Differences in the Lipoprotein Distribution of Halofantrine Are Regulated by Lipoprotein Apolar Lipid and Protein Concentration and Lipid Transfer Protein I Activity: In Vitro Studies in Normolipidemic and Dyslipidemic Human Plasmas. J. Pharm. Sci. **1999**, *88*, 185–190.
52. Busbee, D.L.; Yoo, J.-S.H.; Norman, J.O.; Joe, C.O. Polychlorinated Biphenyl Uptake and Transport by Lymph and Plasma Components. Proc. Soc. Exp. Biol. Med. **1985**, *179*, 116–122.
53. Hauss, D.J.; Fogal, S.E.; Ficorilli, J.V.; Price, C.A.; Roy, T.; Jayaraj, A.A.; Keirns, J.J. Lipid-Based Delivery Systems for Improving the Bioavailability and Lymphatic Transport of a Poorly Water-Soluble LTB_4 Inhibitor. J. Pharm. Sci. **1998**, *87*, 165–169.
54. Caliph, S.M.; Charman, W.N.; Porter, C.J. Effect of Short-, Medium-, and Long-Chain Fatty Acid-Based Vehicles on the Absolute Oral Bioavailability and Intestinal Lymphatic Transport of Halofantrine and Assessment of Mass Balance in Lymph-Cannulated and Non-Cannulated Rats. J. Pharm. Sci. **2000**, *89*, 1073–1084.
55. Hauss, D.J.; Fogal, S.E.; Ficorilli, J.V. Chronic Collection of Mesenteric Lymph from Conscious, Tethered Rats. Contemp. Top. Lab. Anim. Sci. **1998**, *37*, 56–58.
56. White, D.G.; Story, M.J.; Barnwell, S.G. An Experimental Animal Model for Studying the Effects of a Novel Lymphatic Drug Delivery System for Propranolol. Int. J. Pharm. **1991**, *69*, 169–174.
57. Kararli, T.T. Comparison of the Gastrointestinal Anatomy, Physiology, and Biochemistry of Humans and Commonly Used Laboratory Animals. Biopharm. Drug Dispos. **1995**, *16*, 351–380.
58. Levet-Trafit, B.; Gruyer, M.S.; Marjanovic, M.; Chou, R.C. Estimation of Oral Drug Absorption in Man Based on Intestine Permeability in Rats. Life Sci. **1996**, *58*, PL359–363.
59. Liu, H.-X.; Adachi, I.; Horkoshi, I.; Ueno, M. Mechanism of Promotion of Lymphatic Drug Absorption by Milk Fat Globule Membrane. Int. J. Pharm. **1995**, *118*, 55–64.
60. Chang, C.M.; Bodmeier, R. Binding of Drugs to Monoglyceride-Based Drug Delivery Systems. Int. J. Pharm. **1997**, *147*, 135–142.
61. Shah, N.H.; Carvajal, M.T.; Patel, C.I.; Infeld, M.H.; Malick, A.W. Self-emulsifying Drug Delivery Systems (SEDDS) with Polyglycolyzed Glycerides for Improving In Vitro Dissolution and Oral Absorption of Lipophilic Drugs. Int. J. Pharm. **1994**, *106*, 15–23.
62. Fagerholm, U.; Johansson, M.; Lennernas, H. Comparison Between Permeability Coefficients in Rat and Human Jejunum. Pharm. Res. **1996**, *13*, 1336–1342.
63. Soria, I.; Zimmerman, C.L. The Validation of the Intestinal Permeability Approach to Predict Oral Fraction of Dose Absorbed in Humans and Rats. Biopharm. Drug Dispos. **1996**, *17*, 817–818.
64. Pahl, M.V.; Oveisi, F.; Khamiseh, G.; Vaziri, N.D. Intestinal Absorption and Biliary Secretion of Cholesterol in Rats with Nephrotic Syndrome. Nephr., Dial., Transpl. **1998**, *13*, 1446–1451.
65. McDonald, G.B.; Saunders, D.R.; Weidman, M.; Fisher, L. Portal Venous Transport of Long-Chain Fatty Acids Absorbed from Rat Intestine. Am. J. Physiol. **1980**, *239*, G141–G150.
66. Bugaut, M.; Carlier, H. Role of Intestinal Hydrolases, Endogenous Substrates and Chyloportal Partition in Fat Absorption. In *Fat Absorption*; Kuksis, A., Ed.; CRC Press: Boca Raton, FL, 1987; Vol. 1, 197–232.
67. Simmonds, W.J. The Role of Micellar Solubilisation in Lipid Absorption. Aust. J. Exp. Biol. Med. Sci. **1972**, *50*, 403–421.

68. Muranishi, S. Drug Targeting Towards the Lymphatics. Adv. Drug Del. Res. **1991**, *21*, 1–38.
69. Charman, W.N.; Stella, V.J. Estimating the Maximal Potential for Intestinal Lymphatic Transport of Lipophilic Drug Molecules. Int. J. Pharm. **1986**, *34*, 175–178.
70. Bollman, J.L.; Cava, J.C.; Grindley, J.H. Techniques for the Collection of Lymph from the Liver, Small Intestine and Thoracic Duct of the Rat. J. Lab. Clin. Med. **1948**, *33*, 1349–1352.
71. Reininger, E.J.; Saperstein, L.A. Effect of Digestion on the Distribution of Blood Flow in the Rat. Science **1957**, *126*, 1176.
72. Sieber, S.M. The Lymphatic Absorption of p,p-DDT and Some Structurally-Related Compounds in the Rat. Pharmacology **1976**, *14*, 443–454.