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INTESTINAL LIPID ABSORPTION AND TRANSPORT

Cam T. Phan and Patrick Tso

Department of Pathology, University of Cincinnati Medical Center, 231 Albert Sabin Way, Cincinnati, OH 45267

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1. ABSTRACT

The purpose of this review is to update the reader on our current knowledge of the digestion, uptake, and transport of dietary lipid. In particular, it discusses how intestinal lipid transporters may play a role in the uptake of lipids by the enterocytes, and how chylomicrons are formed in the enterocytes and packaged for export into the lymphatic system through exocytosis. The classification and properties of lipids is first described followed by a discussion of structured lipids and their role in human nutrition. Digestion of triacylglycerols takes place in the stomach aided by the enzyme gastric lipase. The origin and properties of lingual and gastric lipase are reviewed. Most digestion of triacylglycerols by pancreatic lipase occurs in the intestinal lumen. Similarly, digestion of cholesteryl ester and phospholipids also takes place in the intestinal lumen. This review describes in considerable detail the uptake of lipid digestion products by the enterocytes, particularly the role of recently identified lipid transporters. The intracellular trafficking and the resynthesis of complex lipids from the lipid digestion products are talked about, particularly within the context of the recently generated knockout mouse that lacks the key lipid reesterification enzymes. Finally, the mechanisms of the formation and secretion of chylomicrons is described and clinical disorders discussed.

2. INTRODUCTION

Many past reviews have been written on the digestion and absorption of lipid and the formation of chylomicrons by the small intestine (1, 2, 3, 4). This review will update our current knowledge on the digestion, uptake, and transport of dietary lipid. In particular, it will discuss how intestinal lipid transporters may play a role in the uptake of lipids by the enterocytes and how chylomicrons are formed in the enterocytes and packaged for export into the lymphatic system through exocytosis.

3. DIETARY LIPIDS

In simple empirical terms, dietary fat is that part of the diet that can be extracted by organic solvents (5). Consequently, dietary fat is comprised of an array of

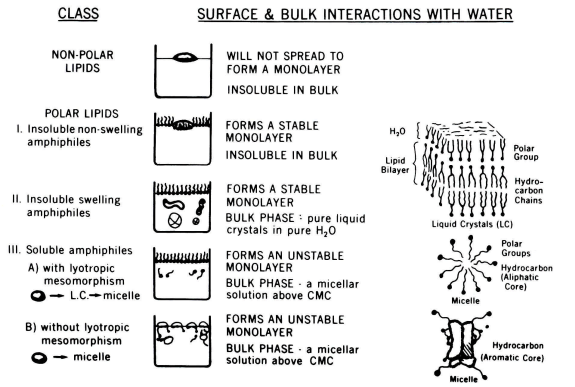

Figure 1. Classification of lipids based on their ability to interact with water. Non-polar Lipids: octadecane, carotene, squalene, cholesteryl oleate, cholesteryl linoleate, and paraffin oil. Polar Lipids: I. - triacylglycerols, diacylglycerols, long-chain protonated fatty acids, and fat-soluble vitamins. II. - phospholipids, monoacylglycerols, monoethers, and alpha-hydroxy fatty acids. IIIA. - sodium salts of long-chain fatty acids, many anionic, cationic and nonionic detergents, and lysophosphatidylcholine. IIIB. - bile salts, sulfated bile alcohols, and saponins (6).

compounds, from the highly non-polar hydrocarbons to the highly polar phospholipids (PL) and glycolipids. The classification of these various lipids and their behavior in an aqueous system has been superbly reviewed by Carey and Small (6). Whether a lipid is classified as a polar or non-polar lipid depends on its interaction with water. Non-polar lipids are insoluble in the bulk water phase and therefore will not interact with water (figure 1). Examples of non-polar lipids are cholesteryl ester (CE), hydrocarbons, and carotene. The polar lipids are made up of three classes: I) insoluble non-swelling amphiphiles; II) insoluble swelling amphiphiles; and III) soluble amphiphiles. The insoluble non-swelling amphiphiles include triacylglycerol (TG), diacylglycerol (DG), non-ionized long-chain fatty acids (FA), cholesterol, and fat-soluble vitamins. When these water insoluble amphiphiles are added to water, a thin film of lipid molecules, called a monolayer, is formed. Non-swelling amphiphiles are so named because they interact little with water in the bulk phase. A second group of lipids is the insoluble swelling amphiphiles and includes monoacylglycerols (MG), ionized FAs, and PLs. In addition to forming a monolayer on the surface of water, this group of lipids has the unique ability to interact with water to form a laminated lipid-water structure referred to as liquid crystal. In the liquid crystal state, non-polar groups of lipid molecules face one another sandwiching water between the polar groups. This unique lipid property is referred to as "swelling," thus the name swelling amphiphiles. The swelling amphiphiles possess strong polar groups, rendering the molecules soluble in water at low concentrations. This group of lipids can be divided further into soluble amphiphiles with lyotropic mesomorphism and those without. Sodium salts of long-chain FAs are an example of the soluble amphiphiles with lyotropic mesomorphism. When the concentration of these lipids reaches the critical micellar concentration, the monomers aggregate to form micelles. In the micelle, the polar group of the monomers faces outside, and the non-polar group faces inside. However, before sodium oleate molecules form micelles, they form an intermediate liquid crystal phase, and this phenomenon is referred to as lyotropic mesomorphism. In contrast, soluble amphiphiles without lyotropic mesomorphism do not undergo the intermediate liquid crystal phase before micelle form. An example of soluble amphiphiles without mesomorphism is bile salt secreted by the liver.

This review will mostly focus on the digestion, absorption, and transport of TG, the predominant dietary lipid. Readers interested in cholesterol absorption are referred to Dawson and Rudel (7).

Dietary fat constitutes a significant source of calories in the Western diet—as much as 30 % of total caloric intake, or 90 - 100 grams per day. Dietary intake of fat has received considerable attention in the last few decades since diets high in fat have been linked to high blood lipids, especially cholesterol and increased risk of coronary heart disease (8, 9, 10). It has generally been accepted that diets rich in saturated FAs are more cholesterolemic (raises blood cholesterol) than diets rich in polyunsaturated FAs (11). Trans-fatty acids have double bonds; yet they behave more like saturated FAs and considered just as atherogenic as saturated fat (12, 13). The Surgeon General currently recommends that overall fat consumption be reduced to an average of 30 percent or less of total caloric intake, with saturated fat consisting of 10 percent or less. This standard has also been adopted by the American Heart Association.

4. STRUCTURED LIPIDS

In the past decade, there has been increased effort to modify either chemically or biologically the FA profile of naturally occurring TGs to obtain a particular physical property or physiological function. Structured triglycerides (STG) are currently being manufactured for their potential clinical benefits and for their lower fat-producing value. The clinical application of STGs has been ably reviewed by Heird *et al* (14) and more recently by Bell *et al* (15). Beneficial effects include improved absorption of linoleic acid in cystic fibrosis patients (16, 17) and a protein sparing effect in STG based safflower oil (18) and fish oil (19). An exciting new approach to optimize the metabolic benefits of specific lipid mixtures has been the development of STGs. STGs are produced by the chemical interesterification of both medium- and long-chain FAs incorporated on the same glycerol These TG molecules are chemically distinct and offer unique advantages from their constituent physical mixture of medium-chain (MCT) and long-chain (LCT) TGs. For example, STGs that contain medium-chain FAs may provide a useful vehicle for rapid hydrolysis and absorption due to the smaller molecular size and greater water solubility than LCTs. Although STGs retain some characteristics of MCTs and LCTs, they may provide an alternative lipid source that could overcome the gastrointestinal intolerance related to the sole use of MCT or LCT in patients with malabsorptive diseases. Preliminary data from our laboratory has demonstrated that STG promotes the lymphatic absorption of both vitamin A

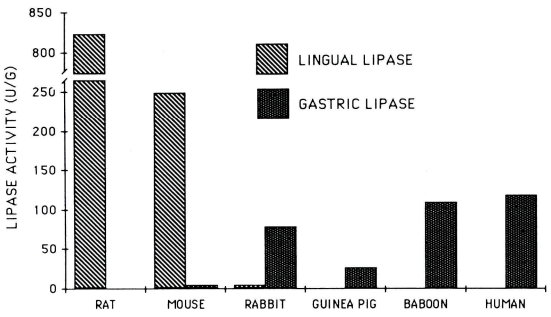

Figure 2. Lingual and gastric lipase activity levels in different species. Data are mean ± SEM of 2 to 3 animals for each species (276).

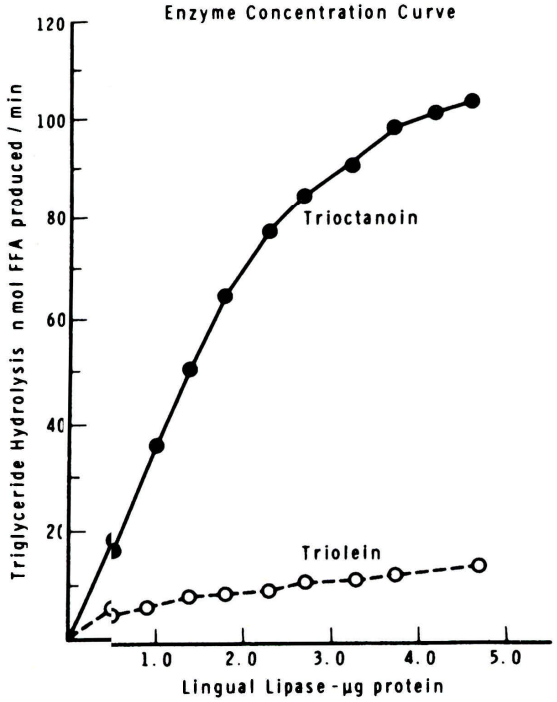

Figure 3. Hydrolysis of medium- (C 8:0) and long- (C 18:1) chain triglyceride by rat lingual lipase. The assay system contained 1 micromole of either glycerol tri(^{3}H)oleate or tri(^{14}C)octanoate; incubation was for 30 min. (29).

backbone by hydrolysis and random reesterification (20, 21). and vitamin E better than the constituent physical mixture with similar FA composition. It is tempting to speculate that STGs may also be used to enhance the lymphatic absorption of lipid soluble drugs. This very exciting aspect of STGs has yet to be explored.

A second reason for the recent upsurge in the chemical modification of TG's is that STGs produce fat that has lower caloric value. Examples of these STGs include Caprenin, produced by the Proctor and Gamble Company, and Salatrim, produced by the Nabisco Company. Caprenin is comprised of STGs containing C8:0, C10:0, and C22:0 (behanic acid). These medium-chain FAs provide fewer calories on a molar basis than long-chain FAs. Additionally, behanic acid is very poorly absorbed by the small intestine and forms calcium soap in the intestinal lumen. Caprenin was sold as a low calorie fat suitable for making chocolate and cookies. Salatrim is prepared by the interesterification of C2:0, C3:0, and C4:0 with hydrogenated canola, soybean, cottonseed, or sunflower oil. The predominant long-chain FA present in Salatrim is stearic acid. Short-chain FAs provide fewer calories per mole, and stearic acid is poorly absorbed by the small intestine (22). Salatrim is thus marketed as shortening with reduced calories to replace conventional oils.

5. DIGESTION OF DIETARY LIPIDS AND STRUCTURED LIPIDS

5.1. Gastric lumen

The digestion of dietary lipids and STGs begins in the stomach. The enzymes involved in the digestion process are lingual lipase, secreted by the salivary gland, and gastric lipase, secreted by the gastric mucosa. These enzymes have been called the acid lipases because their activities are highest in acidic medium. Controversy has existed for some time as to whether acid lipase is only secreted by the salivary gland or whether it is also secreted by the stomach. Interested readers are referred to the excellent review by Hamosh (23), which summarizes the historical development and series of experiments that have led to our current concept that these lipases are different enzymes. Interestingly, considerable difference exists among species in their relative abundance of lingual and gastric lipase. For example, rats possess predominantly lingual lipase, whereas humans possess predominantly gastric lipase (figure 2). Lingual lipase is secreted by the serous glands, also known as von Ebner's glands (24, 25, 26). Its major products of TG digestion are DGs and FAs (27, 28). Liao *et al* (29) determined that lingual lipase hydrolyzes TGs containing medium-chain FAs faster than those containing long-chain FAs (figure 3). Furthermore, Liao *et al* showed that TGs containing short-chain FAs are hydrolyzed even faster by lingual lipase than TG containing medium-chain FAs. Lingual lipase hydrolyzes neither phosphatidylcholine (PC) or cholesterol esterase (29, 30). The pH optimum of lingual lipase is probably around 4, but the enzyme is still quite active at pH 6 – 6.5 (28, 30). The enzyme therefore works well in the stomach, and probably still continues to digest TG in the upper duodenum where the pH is between 6 to 7. Lingual lipase preferentially hydrolyzes FA at the sn-3 position to produce DGs, regardless of the FA present (31, 32).

The distribution of gastric lipase in different parts of the human stomach is shown in figure 4 (33). The highest activity of gastric lipase can be detected in the fundus of the stomach. This has been confirmed in an independent study conducted during the post-mortem examination of two healthy human subjects (34) and in the stomachs of three different animal species. Human gastric lipase shares many characteristics of lingual lipase. For instance, human gastric lipase has a pH optimum ranging from 3 to 6.0 (33, 35), and it hydrolyzes medium-chain TGs better than long-chain TGs (29). Furthermore, the main hydrolytic products of gastric lipase are DGs and FAs (33, 35, 36). Gastric lipase does not hydrolyze PL and cholesterol esterase.

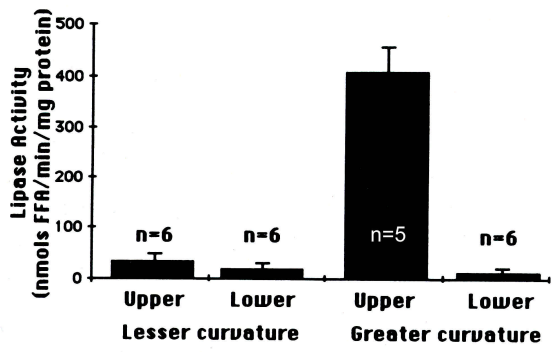

Figure 4. Localization of lipase in the human stomach—the level of lipolytic activity in the gastric mucosa of four sampling sites (33).

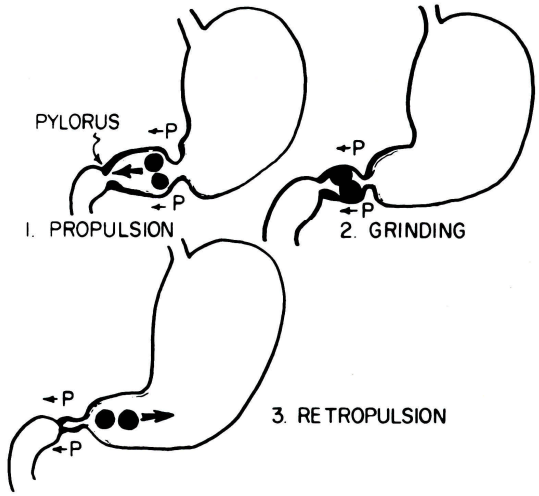

Figure 5. Diagrammatic representation of the consequences of antral peristalsis (277).

Rat lingual lipase was the first acidic lipase to be cloned (37). It is a glycoprotein with a molecular weight of 52,000 kDa. Rat lingual lipase has 377 amino acid residues and bears little homology to pancreatic lipase. Human gastric lipase was the second acid lipase to be cloned. It has 379 amino acid residues (38). There is considerable homology (78%) in the amino acid sequence of rat lingual lipase and human gastric lipase. Similar to rat lingual lipase, human gastric lipase shares little homology with pancreatic lipase.

The digestion of TG by both lingual and gastric lipase in the stomach plays an important role in lipid digestion, particularly in neonates. Milk fat, the primary source of nourishment for neonates, contains considerable medium-chain TG, and acid lipases work more efficiently with medium-chain TG than long-chain TG. Furthermore, the pancreatic lipase system is not fully developed in the neonate (39); thus, lingual and gastric lipases consequently play a crucial role in the normal digestion of milk fat.

Lingual and gastric lipases also play an important role in lipid digestion in adults. This is evidenced in cystic fibrosis patients, who maintain the ability to absorb dietary lipid although pancreatic lipase secretion is markedly or completely inhibited (40). Carriere *et al* (41) recently demonstrated under optimized assay conditions that human gastric lipase specific activity was much lower *in vivo* than *in vitro*. Although low, the specific activity observed *in vivo* was sufficient to digest a significant portion of the TG ingested.

The stomach is the major site for emulsification of dietary fat, an important prerequisite for efficient hydrolysis by pancreatic lipase. Gastric chyme is propelled forward through the antrum to the pylorus via the peristaltic waves of the corpus (figure 5). Liquid is squirted into the duodenum along with small solid particles in the chyme. The pylorus closes, and the antrum contracts forcefully grinding solid particles. As result of these contractions, the antral contents are retropelled from the terminal antrum back into the corpus. The squirting of antral contents into the duodenum, the grinding action of the antrum, and the retropulsion of antral contents back to the corpus provides most of the mechanical action involved in the initial emulsification of dietary TG. The DG and FA resulting from the action of acid lipases in the stomach and the PL that is normally present in the diet further aids the emulsification of dietary fat.

5.2. Intestinal lumen

The lipid emulsion enters the small intestine as fine lipid droplets that are less than 0.5 micromoles in diameter (42, 43). The combined action of bile and pancreatic juice brings about a marked change in the chemical and physical form of the ingested lipid emulsion. Most of the digestion of TG is brought about by pancreatic lipase in the upper part of the intestinal lumen. The pancreatic lipase works at the interface between oil and aqueous phases (44, 45). Desnuelle (46) expanded on these observations as well as those made by Schonheyder and Volqvartiz in 1946 (47). Monolayer technique, eloquently detailed by Verger (48), has greatly advanced our understanding of how the oil/water interface affects lipolysis. Spreading the lipid as a monomolecular film at the air/water interface has allowed the effect of physicochemical properties of the interface to be determined as well as the surface area on the rate of lipolysis. Only a small amount of lipid is required in the monolayer technique, an advantage when using rare synthetic lipids. A problem with the monolayer technique, however, is the denaturation of enzymes at the lipid/water interface (49, 50). The velocity of lipolysis is dependent on those factors that modify the physicochemical properties of the interface as well as the surface area (51, 52, 53).

Pancreatic lipase acts mainly on the sn-1 and sn-3 positions of the TG molecule to release 2-monoacylglycerol (2-MG) and free FAs (52, 54, 55, 56). Although 1-monoacylglycerol (1-MG) is formed from 2-MG through isomerization in an aqueous medium, 2-MG is probably the predominant form in which MG is absorbed by the small intestine since the formation of 1-MG occurs slower than the uptake of 2-MG by the small intestine (57). Further hydrolysis of the 1- or 2-MG by pancreatic lipase results in the formation of glycerol and FA (58). The enzyme

(pancreatic lipase) works more efficiently with 1-MG than with 2-MG. However, since the absorptive rate is very fast, most of the 2-MG is absorbed before degrading or isomerizing to form 1-MG.

Pancreatic lipase (EC 3.1.1.3) is abundant in pancreatic juice, 2 - 3 % of the total protein that is present (59). Its high concentration in pancreatic secretions and its high catalytic efficiency ensures the efficient digestion of dietary fat. Consequently, only very severe pancreatic deficiency results in malabsorption of fat. Pancreatic lipase has been purified from a number of species, including humans (60, 61, 62, 63). Porcine lipase has been sequenced, and it has 449 amino acid residues (64). Porcine pancreatic lipase is a glycoprotein with a carbohydrate chain of about 2000 attached to asparagine at position 166 of the protein (65, 66). For pancreatic lipase to work, it has to anchor itself at the interface. The serine residue at the 152 position of the peptide plays an important role in the binding site (67).

Pure pancreatic lipase works extremely inefficiently in a bile salt-lipid mixture, yet lipase present in pancreatic juice hydrolyzes TG extremely efficiently. This observation led to the discovery of the cofactor called colipase. In 1969, Morgan *et al* made the crucial observation that colipase is only necessary for lipase activity if bile salt is present (68). The laboratories of Dr. Borgstrom in Lund and Dr. Desnuelle at Marseille produced much of our understanding of the biochemistry of colipase and its interaction with pancreatic lipase. Colipase has been purified from a number of animal species (69, 70, 71, 72). The mechanism of how lipase works has been discussed in detail by Erlanson-Albertsson (73). TG lipid droplets covered with bile salts are not accessible to pancreatic lipase. However, the binding of the colipase to the TG/aqueous interface allows the lipase molecule to bind to the lipid/aqueous interface. Lipase binds with colipase with a 1:1 molar ratio (74).

Colipase is secreted by the pancreas as a procolipase (75). After entering the small intestinal lumen, the procolipase is activated by the cleavage of a pentapeptide from the N-terminus (73, 76, 77). Colipase from a number of species, including humans, has been cloned (75, 78, 79). The gene is located in chromosome 6 (80, 81). The amino acid sequence of some regions of the procolipase molecule is highly conserved, and these regions are the lipid-binding domain, the lipase-binding domain, and the activation peptide. The lipid-binding domain of procolipase consists of two regions, amino acids 6 - 9 (66, 82) and amino acids 53-59 (83, 84, 85). The lipase-binding domain of procolipase probably involves asparagine at position 72 and glutamine at 15 (73, 86).

Erlanson-Albertson and Larsson (82, 87) made the extremely interesting observation that the pentapeptide cleaved from the procolipase by trypsin, called enterostatin, seems to be a specific satiety signal for the ingestion of fat.

5.3. Digestion of phospholipid

The digestion of PL occurs in the small intestine. Both lingual and gastric lipases are incapable of digesting PL. In bile, PL (predominantly PC) is found in mixed micelles along with cholesterol and bile salts. Once in the intestinal lumen, the luminal PC will distribute between the mixed micelles and the TG droplets, but PC tends to favor the micellar phase over the oil phase (88). PC is then acted upon by pancreatic phospholipase A_2 (PLA_2) (EC 3.1.1.4) at the sn-2 position to yield a FA and lysophosphatidylcholine (LPC) (89, 90). Some phospholipase A_1 (PLA_1) activity may also be present in pancreatic juice. This is thought to be due to pancreatic lipase (91). The characteristics of PLA_2 have been studied thoroughly. It was first purified and characterized from porcine pancreas (92). It is secreted as an anionic zymogen, which is activated by tryptic cleavage (92, 93), has a molecular weight of approximately 14,000 (92), requires calcium for activation (94, 95), and its activity requires the presence of bile salts (96). It has multiple isoforms (97, 98) and apparently requires a 2:1 bile salt to PC molar ratio for optimal activity (99). It is heat stable, with a pH optimum of 8 - 9 (96).

Phospholipase from porcine, canine, and human species have been sequenced (100, 101, 102, 103). Recently, Richmond and Hui (104) reported the genomic organizing of the PLA_2 gene of the mouse and demonstrated that the mouse protein is highly homologous to the rat, dog, and human enzyme. Pancreatic PLA_2 is secreted as a proenzyme that is activated in the small intestine by the tryptic cleavage of an N-terminal heptapeptide (105). Although the bulk of intestinal PLA_2 activity is derived from pancreatic juice, there is probably some contribution from the intestinal mucosa (106), where the enzyme is concentrated in the brush border.

5.4. Digestion of cholesteryl ester

Most dietary cholesterol is present as the free sterol, with only 10 - 15 % as the sterol ester (107). Cholesteryl ester entering the small intestine must first be hydrolyzed before free cholesterol can be absorbed. The enzyme involved in the hydrolyzation process is cholesterol esterase (3.1.1.13). Cholesterol esterase is also called carboxylic ester hydrolase or sterol ester hydrolase. It has been purified from porcine (108, 109), rat (110), and human pancreas (111, 112). Human cholesterol esterase has a molecular weight of about 100 kDa (111), whereas rat cholesterol esterase has a molecular weight of 70 kDa (110). Rat, bovine, and human pancreatic cholesterol esterase has been cloned (113, 114, 115). It seems to be well conserved and shares 78% homology in both the rat and human. Using site-directed mutagenesis, DiPersio *et al* (116) demonstrated that substitution of the serine at position 194 with threonine or alanine abolished enzyme activity, thus showing that serine at 194 is in the catalytic site of pancreatic cholesterol esterase. Using a similar approach, DiPersio *et al* (117) further demonstrated that histidine in position 435 is probably a component of the catalytic triad of this enzyme as well. The third component is aspartic acid at position 320 (118). It is likely that human cholesterol esterase shares the same catalytic sites as that of the rat.

Human cholesterol esterase has a broad specificity and can hydrolyze TG, CEs, and phosphoglycerides (119), but has little activity against glycerides. Cholesterol esterase activity is greatly enhanced by the presence of bile salts, particularly trihydroxy bile salts such as sodium cholate. A unique property of cholesterol esterase is its self-association.

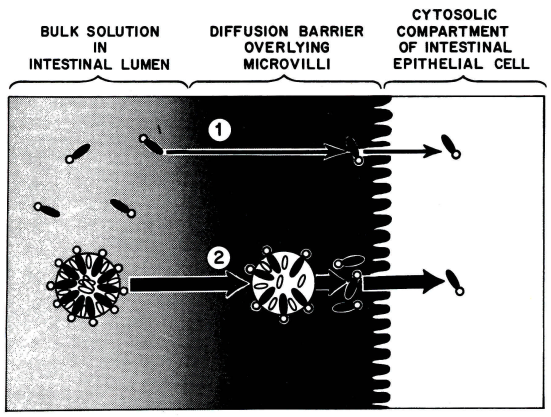

Figure 6. Diagrammatic representation of the effect of bile salt micelles (or vesicles) in overcoming the diffusion barrier resistance by the unstirred water layer. In the absence of bile acids, individual lipid molecules must diffuse across the barriers overlying the microvillus border of the intestinal epithelial cells (arrow 1). Hence, uptake of these molecules is largely diffusion limited. In the presence of bile acids (arrow 2), large amounts of these lipid molecules are delivered directly to the aqueous-membrane interface so that the rate of uptake is greatly enhanced (124).

For instance, the presence of trihydroxy bile salts promotes the self-aggregation of the enzyme into polymeric form (110). The self-association of cholesterol esterase protects the enzyme from proteolytic inactivation, thus retaining its activity. Cholesterol esterase isolated from the pancreas exists mainly as dimers and tetramers (112).

6. UPTAKE OF DIETARY LIPIDS BY ENTEROCYTES

Much of our current understanding of the micellar solubilization and uptake of dietary lipids comes from the work of Hofmann and Borgstrom, who discovered the uptake of lipid digestion products by enterocytes (120, 121). This concept was challenged by Carey and his associates, who discovered the co-existence of unilamellar liposomes with bile salt-lipid mixed micelles in the small intestine (43). Only recently has the uptake of lipid digestion products by enterocytes been accepted as a passive process. However, recent work by several investigators has raised the possibility that some lipids may be taken up by the enterocytes via carrier-mediated processes that are energy dependent. This theory is reviewed in section 6.3.

6.1. Importance of micellar solubilization

Understanding the importance of micellar solubilization of 2-MG and FA in their uptake by enterocytes requires discussion of the role of the unstirred water layer—a concept introduced by Dietschy and his colleagues (122, 123, 124). Shown in figure 6, the brush-border membrane of enterocytes is separated from the bulk fluid phase in the intestinal lumen by an unstirred water layer. This unstirred water layer mixes poorly with the bulk phase in the intestinal lumen. Consequently, solute molecules in the bulk phase gain access to the brush border membrane by diffusion across the unstirred water layer. For any molecule, the rate of uptake by the small intestine is dependent on the number of molecules that are in close proximity to the brush border membrane and therefore available for uptake. The solubility of FA and MG in an aqueous medium is extremely low; consequently, very few molecules gain access to the brush-border membrane (arrow 1). In contrast, the micellar solubilization of MG and FA greatly enhances the number of molecules available for uptake by the enterocytes (arrow 2). Micellar solubilization increases the aqueous concentration of FA and MG 100 to 1000 times. Despite the slower diffusive rate of the micelle relative to the monomolecular FA molecule (due to size), micellar solubilization still markedly enhances the diffusion of FA and MG molecules across the unstirred water layer.

Several investigators have demonstrated that trihydroxy bile acids are more effective in promoting cholesterol absorption than dihydroxy bile acids (125, 126, 127); however, the degree of solubilization was not measured in these experiments. Watt and Simmonds (127) showed a linear relationship between the amount of cholesterol taken up by the small intestine and the micellar cholesterol concentration, thus demonstrating the importance of micellar solubilization in the uptake of cholesterol. Using Pluronic F-68, a non-ionic surfactant in promoting the micellar solubilization of cholesterol but not its uptake by enterocytes, Watt and Simmonds demonstrated that the uptake of cholesterol by the enterocytes is dependent on the planar structure of the bile acid (127). Bile salt in intestinal cholesterol uptake thus extends beyond its role in solubilizing cholesterol. This is further illustrated by the fact that chenodeoxycholyl taurine (CDC-tau) is a better micellar solubilizer of cholesterol than cholyl taurine (C-tau), even though cholesterol uptake is significantly greater with C-tau than it is with CDC-tau (128, 129, 130).

A particularly important point of Watt and Simmond's study (127) was that the uptake of cholesterol by a hydrophobic acceptor (silicone tubing) was the same for Pluronic F-68 and bile acids. This suggests that intestinal cholesterol absorption is an active process possibly mediated by a transporter. This supports an earlier finding by Sylven (131), who found that both cholesterol and beta-sitosterol (plant sterol) are present in the human diet, but cholesterol, unlike beta-sitosterol, is well absorbed by the small intestine. Sylven found that the ability of the small intestine to discriminate between cholesterol and beta-sitosterol absorption is energy-dependent and compromised due to deprivation of blood supply (131). The possibility that transporters are present at the brush border membrane is witnessed in patients with beta-sitosterolemia, a condition where the intestine fails to discriminate between cholesterol and beta-sitosterol (132, 133, 134). Although the mechanism of beta-sitosterolemia is not fully understood, Patel *et al* (135) reported that the genetic defect of beta-sitosterolemia is linked to chromosome 2p21, between microsatellite markers D2S1788 and D2S1352. Berge *et al* (136) recently reported that seven different mutations occurred in two adjacent genes responsible for encoding new members of the ABC transporter (ABCG5 and ABCG8) in sitosterolemia patients. Feeding cholesterol to mice upregulated these genes, thus suggesting that ABCG5

and ABCG8 work together to limit intestinal cholesterol absorption by cholesterol efflux by the small intestinal epithelial cells.

6.2. Importance of unilamellar vesicles

When the jejunal contents of humans digesting a lipid meal were ultracentrifuged, there was a solid particulate layer on the bottom of the tube followed by a clear micellar layer and an oily phase on the top (121). The oily phase mostly consisted of TG, partial glycerides, and FAs. The clear micellar phase consisted of bile salts, FAs, and MGs. However, when Porter and Saunders (137) carefully analyzed the aqueous phase after ultracentrifugation and after the intestinal contents had passed through a series of filters with progressively smaller pores (the smallest being 100 nm in diameter), they found that neither sample displayed a clear micellar phase. Instead, a slightly turbid phase was observed. Furthermore, a concentration gradient of lipids in the micellar phase was found. The importance of this observation was not realized until later when Patton and Carey observed the digestion of fat *in vitro* using light microscopy (138). Minimally, three phases were present: the oil phase (mainly TG, partial glycerides and FA), the calcium soap phase (Ca^{++} ions and protonated long-chain FA), and the viscous isotropic phase (MG and FA). They concluded that it is probably an oversimplification to divide the intestinal contents into an oil and a micellar phase.

Carey *et al* (43) proposed that when the bile salt concentration in the lumen exceeds the critical micellar concentration, the lipid in the intestinal lumen is incorporated into mixed micelles, and that these are probably in the form of large mixed disc-like micelles more or less saturated with lipids with a hydrodynamic radius of about 200 Å. When the amount of lipid in the aqueous phase increases further, the formation of liquid crystalline vesicles (liposomes) with a hydrodynamic radii of 400 - 600 Å eventually results (43, 139). This finding may have important pathophysiological implications. Patients with low intraluminal bile salt concentration (140), or those with bile fistulae (141), seem to have reasonably good fat absorption. Thus, Carey *et al* proposed that the liquid crystalline vesicles possibly play an important role in the uptake of FA and MG by enterocytes (43) in these diseased states. However, the relative role of the micelle and the liquid crystalline vesicle in the uptake of FA and MG by enterocytes is yet unresolved since these two different types of particles co-exist in the small intestinal lumen and constantly exchange MG, FA, and bile salt molecules. Nevertheless, the micellar hypothesis of fat absorption as first proposed by Hofmann and Borgstrom (121) still holds true with some modifications.

The aqueous phase may contain liquid crystalline vesicles in addition to mixed disc-shaped micelles (142). Both cholesterol and LPC are presumably incorporated into both the disc-shaped micelles and the liquid crystalline vesicles before their uptake by enterocytes. Shoemaker and Nichols (143) made an interesting observation that bile salts and lysophospholipids form submicellar aggregates. These submicellar aggregates co-exist with artificial PL vesicles in an aqueous medium. Recently, Shoemaker and Nichols provided convincing evidence that these submicellar aggregates serve as an efficient shuttle mechanism for the transfer of lysophospholipids between membranes (144). The existence of these submicellar aggregates in the intestinal lumen, and their role in delivering lysophospholipids to enterocytes for absorption, remain to be demonstrated.

6.3. Uptake of fat digestion products by enterocytes

Until very recently, it was generally believed that FA and MG are absorbed by the enterocytes through simple diffusion (1). Strauss demonstrated that the uptake of FA is passive and not temperature dependent (145). The MG and FA enter the enterocytes as monomers (1). Studies by Stremmel have indicated the existence of a FA binding protein associated with the brush border membrane, and that this protein plays a role in the uptake of FA by enterocytes (146). That FA may be taken up by enterocytes via a carrier-mediated process has been implied in an earlier finding by Chow and Hollander (147). They demonstrated that linoleate uptake by the small intestine indicates a concentration-dependent dual mechanism of transport. At low linoleate concentration, FA is taken up via a carrier dependent process, whereas at a higher lineolate concentration, FA is taken up predominantly by passive diffusion. Work by Stremmel has raised the possibility that some lipids, especially FA, may be taken up by enterocytes via carrier-mediated processes (146, 148). Although Stremmel mainly focused on FA uptake, he found that this FA binding protein was capable of transporting cholesterol, but not cholesterol esterase. This finding came after the discovery that a jejunal loop treated with the antibody against the FA binding protein significantly reduced cholesterol uptake by the loop. Stremmel *et al* (148) demonstrated that this protein is mainly present in the apical and lateral of the villus (in the region of the tight junction) and in the crypt. Consequently, the FA binding protein appears to be a plausible candidate transporter for a whole array of lipid molecules, including cholesterol. This conclusion, however, is of concern for two reasons. First, how similar is this transporter to the mitochondrial glutamic oxaloacetic transaminase, which is not involved in lipid absorption (149)? Second, why do the crypt cells express this protein when they are not involved in fat absorption?

Thurnhofer *et al* first described the presence of a binding protein in the small intestinal brush border membrane that facilitates the uptake of cholesterol by the small intestine (150, 151, 152, 153). The protein has an apparent molecular weight of slightly less than 14,000 (determined by SDS polyacrylamide gel electrophoresis) and a value of 11,500 (determined by gel filtration on Sephadex G-75SF). Using brush border membrane, Thurnhofer *et al* (152) demonstrated that cholesterol uptake is most effective from cholate mixed micelles and is characterized by half-times on the order of seconds. Proteolytic treatment of the brush border membrane vesicles significantly reduced the capacity of these vesicles to take up cholesterol. When polyclonal antibodies raised against the 14K protein were present in the incubation medium, it significantly reduced cholesterol uptake by these vesicles. This 14K protein was later identified as SCP-2, which is an intracellular protein (154). Thus, the identity of the plasma membrane-bound cholesterol transport protein, if it exists, remains obscure.

A number of other proteins have also been demonstrated to bind lipids. These include GP330 (also called megalin), CD36, and caveolin. GP330, a member of the low density lipoprotein receptor gene family, is an endocytic receptor expressed in many absorptive epithelia, including the kidney proximal tubules, type II pneumocytes, mammary epithelium, and thyroid follicular cells (155). It has been demonstrated that GP330 is involved in the renal uptake of polybasic drugs (155, 156, 157) vitamin B_{12} (157), cholesterol carrying lipoproteins (158), albumin (159), and proteases (156). The fact that the forebrain of GP330 knockout mice failed to develop has been attributed to the deficient supply of cholesterol to the brain of the fetus through the maternal-fetal lipoprotein transport system (160). GP330 expression in the intestine is still uncertain (155). Moreover, whether GP330 participates in cholesterol uptake by the small intestine is unknown. It is unclear if GP330, or a related protein, is expressed in the small intestine (155). Recently, Hauser *et al* (161) provided evidence that the scavenger receptor BI is involved in intestinal uptake of cholesterol. In their most recent paper, Schulthess *et al* (162) demonstrated that the uptake of cholesterol mediated by scavenger receptors is inhibited by an amphipathic alpha-helix of 18 amino acids, lending further support to this receptor's involvement in intestinal cholesterol absorption.

A membrane transporter for FA has been identified in adipocytes. This FA transporter is inhibited by sulfo-N-succinimidyl derivatives of long-chain FAs (163, 164, 165, 166, 167) and by 4,4'-diisothiocyanostilbene-2-2'-sulfonate (DIDS). This protein has an apparent molecular weight of 88 kD, an isolectric point of 6.9, and has a strong sequence homology to CD36 and PAS IV. PAS IV is a protein enriched in the apical membranes of lipid secreting mammary cells during lactation. Cloning of this binding protein revealed that it bears 85% homology to CD36 present in human platelets and lactating mammary epithelia (164). Northern blot analysis revealed that the message is abundant in the heart, intestine, fat, muscle, and testis (165, 166, 167). Fatty-acid transporters have been localized to the brush border membrane, and its expression is highest in the jejunum, lower in the duodenum, and lowest in the ileum (167). These same investigators reported that a high-fat diet rich in long-chain FAs, but not medium chain FAs, resulted in increased fatty-acid transporter expression. Interested readers are referred to the review by Abumrad *et al* (168).

Other potentially binding proteins have been identified. One for long-chain FA has been cloned in adipocytes by Schaffer and Lodish (169); but whether this protein is expressed in the small intestine is unknown. Caveolin is another protein that binds cholesterol (170). Caveolin was the first reported protein associated with caveolae. Caveolae are non-clathrin-coated invaginations present on the surface to bind glycolipids (171), cholesterol (172), glycosyl-phosphatidylinositol (GPI)-linked proteins (173), and other proteins for potocytosis (174). It is currently unknown if caveolin plays a role in the intestinal absorption of cholesterol and FAs.

7. RESYNTHESIS OF TRIGLYCERIDES AND FORMATION OF CHYLOMICRONS

To date, it is not known how the various absorbed lipids migrate from the site of absorption to the endoplasmic reticulum, where biosynthesis of complex lipids takes place. A FA binding protein (FABP) present in the small intestine has been isolated and characterized by Ockner and Manning (175). It has been suggested that this FABP play an important role in the intracellular transport of absorbed FA. This is partly supported by the finding that FABP concentration is greater in villi than in crypts, the jejunum than the ileum, and the intestinal mucosa of animals fed a high-fat diet than those fed a low-fat diet (175, 176). We know there are at least two FABPs in enterocytes—the I-FABP and L-FABP. The letter preceding the FABP indicates the organ from which it was first isolated (I = intestine and L = liver). These two FABPs differ in their binding specificity. I-FABP binds strongly with FA, and L-FABP binds with long-chain FAs and with LPC, retinoids, bilirubin, carcinogens, and even selenium (177, 178, 179). Based on NMR binding studies, Cistola *et al* (180) speculated that the binding of I-FABP is involved in the intracellular transport of FA, while the L-FABP is involved in the intracellular transport of MG and LPC. However, none of these functions of FABP has been tested rigorously in *in vivo* animals. Binding proteins of FAs have been ably reviewed by Storch and Thumser (181).

Demonstrating *in vivo* functions of the FABPs has been very difficult. One strategy is to knockout the gene of a particular FABP. This, however, has not yielded definitive results since other FABPs often take over that function which the knocked out FABP once assumed. For example, when Shaughnessy *et al* (182) used the adipocyte FABP knockout mouse to study its function, the keratinocyte FABP functionally compensated for the absence of the adipocyte FABP. Using the I-FABP knockout mouse model, investigators found that males have higher plasma TG and greater body weight than wild types (183), thus implying that fat absorption is probably normal in these genetically modified animals. The female knockouts weighed more than wild types, but less than male knockouts. Determining whether lymph chylomicron (CM) composition is altered in the I-FABP knockout mouse would be interesting to study.

Two sterol carrier proteins, SCP-1 (47,000 mol wt) and SCP-2 (13,500 mol wt), have been isolated and characterized (184, 185). SCP-1 is important in the microsomal conversion of squalene to lanosterol (186), while SCP-2 (also known as the non-specific lipid transfer protein) participates in the microsomal conversion of lanosterol to cholesterol (187). Additionally, SCP-2 is involved in the intracellular transport of cholesterol from cytoplasmic lipid droplets to mitochondria and the translocation of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane (188). It also appears to be involved in the intracellular trafficking of PC (189, 190) and FAs (191), and SCP-2 has been proposed to play a role in peroxisomal FA-CoA binding (192).

7.1. Monoglycerides and fatty acid

2-MG and FA are reconstituted to form TG, mainly

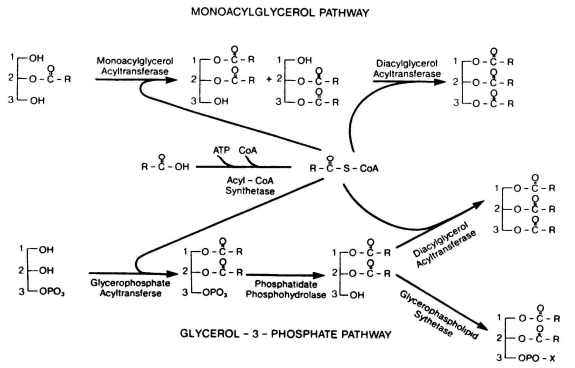

Figure 7. Pathways of triacylglycerol biosynthesis in the intestinal mucosa.

via the MG pathway. As shown in figure 7, 2-MG is reacylated into TG by the consecutive action of MG acyl transferase (MGAT) and DG acyltransferase (DGAT) (193, 194). The enzymes involved in this MG pathway are present in a complex called "triglyceride synthetase" (193, 195), and this complex has been purified by Lehner and Kuksis (196). It is thought that the synthesis of TG from DG is catalyzed by the enzyme acyl CoA:DGAT. The gene for this enzyme has been isolated, and a knockout mouse has been generated. Interestingly, this mouse can synthesize TG in the intestinal mucosa (197), thus raising the question whether another enzyme(s) is involved in the formation of TG from DG. This notion is supported by data from Oelkers *et al* (198) showing that a lecithin cholesterol acyltransferase-like gene produces an enzyme that mediates DG esterification in yeast. It is likely that a related protein is also present in the intestinal epithelial cells.

Several studies have demonstrated that the enzymes involved in the MG pathway are located on the cytoplasmic surface of the endoplasmic reticulum (199). This finding has important bearing on our understanding of the intracellular packaging of lipoproteins. The data seem to indicate that TG is formed at the cytoplasmic surface of the endoplasmic reticulum, and that TG enters the cisternae of the endoplasmic reticulum. Because TG has a low solubility in PL bilayers (~ 3 mol %), Small (200) postulated that TG molecules saturate the membrane rapidly. Once the solubility of TG is exceeded, the TG splits the bilayer and forms a small lens. As the lens grows, it bulges into the cytoplasmic side or the cisternal side of the endoplasmic reticulum. Finally, this protrusion pinches off the membrane and forms lipid droplets in the cytoplasm or precursors of lipoproteins in the cisternae of the endoplasmic reticulum. This is an interesting hypothesis that warrants further investigation. Using ultrastructural studies, Wetterau and Zilversmit (201, 202, 203) demonstrated that a protein in the liver, small intestine, and several other organs promotes the transfer of TG and cholesterol esterase between membranes. The small intestine and liver have the highest TG transfer activity, and both are active in packaging TG-rich lipoproteins. Therefore, Wetterau and Zilversmit proposed this transfer activity to play a role in the intracellular packaging of lipoproteins.

A second pathway in intestinal mucosa that forms TG is the alpha-glycerophosphate pathway (193, 194). This pathway involves the stepwise acylation of glycerol-3-phosphate to form phosphatidic acid (figure **7**). In the presence of phosphatidate phosphohydrolase, phosphatidic acid is hydrolyzed to form DG, which is then converted to TG. The relative importance of the MG-pathway and the alpha-glycerophosphate pathway depends on the supply of 2-MG and FA. During normal lipid absorption, when 2MG is sufficiently present, the 2-MG pathway facilitates the conversion of 2-MG and FA to form TG and aids in inhibiting the alpha-glycerophosphate pathway (193, 194, 204). Conversely, when the supply of 2-MG is lacking or insufficient, the alpha-glycerophosphate pathway becomes the major pathway for the formation of TG.

7.2. Phospholipids

Some absorbed LPC is reacylated to form PC (205, 206, 207, 208). The remaining absorbed LPC is hydrolyzed to form glycero-3-phosphorylcholine (209). The liberated FA is used for TG synthesis, while the glycero-3-phosphorylcholine is readily transported via the portal blood for use in the liver (210). Another reaction occurring in the intestinal mucosa is the combining of two molecules of LPC to yield one molecule of PC and one molecule of glycero-3-phosphorylcholine (106, 211).

7.3. Cholesterol

Exogenous cholesterol absorbed by enterocytes enters a free cholesterol pool within the enterocytes that also contains cholesterol from endogenous sources. Endogenous cholesterol is derived from three sources: 1) non-dietary cholesterol absorbed from the lumen (biliary cholesterol and cholesterol from cells shed from the intestinal mucosa), 2) cholesterol derived from plasma lipoproteins (212), and 3) cholesterol synthesized *de novo* (213, 214, 215). The enterocytes treat each of these sources of cholesterol differently. For instance, cholesterol derived from the intestinal lumen does not mix evenly with the free cholesterol pool and is preferentially esterified in the enterocytes for export into lymph as TG-rich lipoproteins (216, 217, 218). Stange and Dietschy (217) found that very little newly synthesized cholesterol is transported into lymph during fasting. However, during active lipid absorption, significantly more newly synthesized cholesterol is incorporated into the TG-rich lipoproteins that are transported in lymph. Readers interested in cholesterol synthesis and the uptake of lipoproteins by the small intestine are referred to the articles and reviews of Spady *et al* (212), Dietschy *et al* (219), Stange and Dietschy (217), and Spady *et al* (220).

Cholesterol is transported mainly as esterified cholesterol and almost exclusively by the lymphatic system. The rate of esterification of cholesterol regulates its lymphatic transport (221). Two proposed enzymes involved in cholesterol esterification are cholesterol esterase (222, 223) and acyl-CoA cholesterol acyltransferase (ACAT) (222, 224, 225). The distribution and regulation of ACAT in the small intestinal epithelium has been studied in considerable detail. The rat jejunum and ileum have high specific activities of ACAT, with the jejunum displaying slightly higher activity than the ileum.

Conversely, the specific activity of ACAT in the guinea pig, rabbit, and human is significantly higher in the jejunum than in the ileum (226, 227, 228). This enzyme is stimulated by the feeding of a high cholesterol diet (226, 227). However, it is not clear whether this increase in ACAT activity is caused by the high cholesterol diet or by the increase in microsomal cholesterol that is isolated from these animals for the purpose of conducting the ACAT bioassay.

Using immunocytochemistry, Gallo *et al* (229) demonstrated that intracellular cholesterol esterase is derived from pancreatic cholesterol esterase. A later study by Field (230) confirmed the presence of a cholesterol esterase in the enterocyte. It is still unclear how pancreatic cholesterol esterase is taken up by the enterocyte. Most investigators believe that ACAT plays a more important role in mucosal cholesterol esterification than cholesterol esterase. This is supported by several substantiated observations. First, ACAT displays the highest activity in the segment of the small intestine that is most actively involved in cholesterol absorption (226, 227, 228). Second, the activity that is present in the intestinal epithelium adequately accounts for all of the cholesterol esterase transported by the small intestine (231). Third, Watt and Simmonds (232) failed to demonstrate an effect of the diversion of pancreatic juice (the origin of mucosal cholesterol esterase) on the absorption and esterification of luminal dietary cholesterol in lymph fistula rats. Finally, employing a number of specific ACAT inhibitors, several studies have shown a reduction in lymphatic cholesterol output (222, 233, 234, 235). ACAT inhibitors continue to be synthesized, and interested readers are referred to Lee *et al* (236) for additional information.

Chang and associates have played a pivotal role in the purification and identification of ACAT proteins (237, 238, 239). Two have been identified—ACAT-1 and ACAT-2. Using immunohistochemistry, Chang *et al* (238) reported that ACAT-1 is present in both fetal and adult hepatocytes, and ACAT-2 is present only in fetal hepatocytes. Further, Chang *et al* (238) found that the ACAT activity of the small intestine can be immunodepleted by antibodies raised against ACAT-2, and that most of ACAT-2 is located at the apices of the villi. The role of ACAT-2 in intestinal cholesterol absorption is supported by the findings of Buhman *et al* (240), who reported that ACAT-2 knockout mice are resistant to diet-induced hypercholesterolemia due to defective cholesterol esterification and absorption by the small intestinal cells.

8. ASSEMBLY OF INTESTINAL LIPOPROTEINS

Despite the wealth of information gathered over the last thirty years about intestinal lipid absorption, the mechanism of the intracellular assembly, modification, and secretion of lipoproteins from the small intestinal epithelial cells is not fully understood. This is partly due to the complexity of the processes involved and lack of good experimental models to study the various steps. Intestinal cell culture systems (e.g. CaCo-2 cells) have been used extensively to study the formation and secretion of lipoproteins (3, 241, 242, 243, 244) and the genetic expression and post-translational modification of apolipoproteins (245). During fasting, very low density lipoproteins (VLDL) are the only lipoproteins produced by the small intestine (246, 247, 248). After a meal, the small intestine produces predominantly CMs (248, 249). Currently, the separation of intestinal CMs and VLDLs is based primarily on operational criteria. Lipoproteins that have a Svedberg flotation (S_f) rate exceeding 400 are classified as CMs; those with a S_f rate of 20-400 are defined as VLDLs (250). Numerous studies support the idea that intestinal CMs and VLDLs represent lipoproteins produced by two separate pathways. Ockner *et al* (246) demonstrated that the intraduodenal infusion of palmitate causes a marked increase in VLDL transport, but that VLDL output remains unchanged when oleate and linoleate are infused. In contrast, CM output is markedly increased when oleate and linoleate are infused. The FA composition of the VLDL TG is different from the CM-TG, suggesting the presence of a different pathway for VLDL and CM assembly. An ultrastructural and biochemical study conducted by Mahley *et al* (251) showed that intestinal Golgi vesicles contain either CMs or VLDL particles, and that little mixing of particle size occurs, thus suggesting separate biosynthetic pathways for these lipoproteins. Further evidence is provided by Vahouny *et al* (252), who demonstrated that puromycin had no significant effect on the incorporation of radioactive leucine into VLDL peptides in male rats. In contrast, however, the incorporation of radioactive leucine into CM peptides was markedly inhibited.

Studies conducted by Tso *et al* (253, 254) have shown that rats fed as little as 0.5 mg/hour of Pluronic L-81 (L-81, a hydrophobic surfactant) have markedly impaired lymphatic transport of TG and cholesterol. They further demonstrated that L-81 inhibited lipid transport by inhibiting the formation of intestinal CMs, but not the formation of VLDL particles. Using this unique tool, Tso *et al* further showed that intraduodenal infusion of egg PC in rats resulted mostly in the lymphatic transport of VLDLs, and that this transport was not affected by the administration of L-81 (255). However, when triolein was infused, the lymphatic transport of lipid was inhibited because the feeding of triolein resulted primarily in the formation of CMs (253, 254). These results led Tso *et al* to propose two pathways for the formation of intestinal lipoproteins—one that forms predominantly CMs, and one that forms predominantly VLDLs (figure 8) (255). Moreover, Tso *et al* proposed that the pathway forming predominantly CMs is inhibited by L-81, but the pathway forming predominately VLDLs is not, and that pre-CM and pre-VLDL particles are packaged in the Golgi apparatus into either pre-CM and pre-VLDL-containing vesicles. The Golgi-derived vesicles containing either pre-CMs or pre-VLDLs probably correspond to those vesicles observed by Mahley *et al* (251). This hypothesis is supported by the study conducted by Nutting *et al* (256) where appearance time for CMs in control rats and VLDLs in L-81 treated rats was measured. The appearance time, defined as the time between placement of radioactive FA into the intestinal lumen and the appearance of radioactive lipid in lymph, was 10.8 min in control rats—significantly shorter than the 16.2 min in L-81-treated rats. This difference in appearance time further supports the hypothesis that CMs

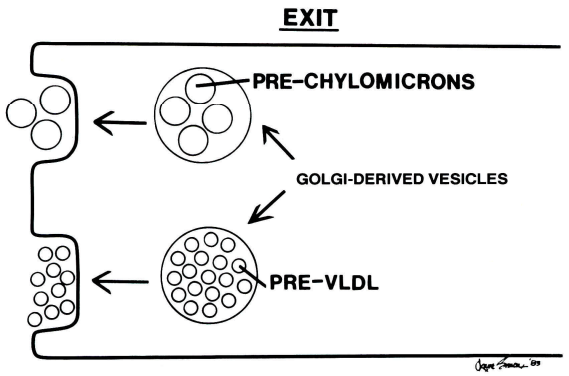

Figure 8. Packaging and secretion of intestinal chylomicrons (CM) and very low-density lipoproteins (VLDL). The diagram depicts Golgi derived vesicles containing either pre-CM or pre-VLDL particles. Little mixing of pre-CM and pre-VLDL particles in these vesicles occurs.

and VLDLs are packaged separately in enterocytes. Finally, using Caco-2 cells, Luchoomun and Hussain (257) also demonstrated that L-81 inhibits CM but not VLDL formation by these cells.

Based on their Caco-2 studies, Hussain and his colleagues have proposed a sequential assembly model to study VLDL and CM formation (258). This model will provide information on the assembly of primordial lipoprotein particles, the synthesis of TG-rich lipid droplets, and the core expansion involving the fusion of primordial lipoproteins with lipid droplets. Readers interested in the details of this model are referred to the review by Hussain (3).

9. CLINICAL DISORDERS OF INTESTINAL LIPID TRANSPORT

In humans, the liver secretes only apolipoprotein B-100 (apo B-100), and the small intestine secretes only apolipoprotein B-48 (apo B-48) (259, 260, 261). Both apo B-100 and apo B-48 are encoded by the same gene (262, 263). The biogenesis of apo B-48 involves a unique mechanism by which the CAA codon encoding Gln at 2153 of the apo B-100 mRNA is changed to UAA (stop codon) and thus forms apo B-48. Rat apo B-48 has a molecular weight of about 240,000 kDa (264) and is an extremely hydrophobic protein (265). Each CM or VLDL particle has one apo B-48 (266). We know that apo B is required for the formation of CM, which has been demonstrated by apo B knockout animals. We do not know, however, whether the production of apo B is physiologically rate-limiting in forming CM by the enterocytes. Data from several laboratories indicate that the supply of apo B is probably not the rate-limiting step for forming CM. Hayashi *et al* (267) demonstrated that apo B output in lymph, an indication of the number of CM produced by the small intestine, does not change after intraduodenal infusion of lipid, despite the fact that lymphatic TG output increases seven- to eightfold. These data support the finding by Davidson *et al* (270) that no increase in apo B synthesis results from lipid absorption.

Abetalipoproteinemia is a rare genetic disorder involving the complete failure of the liver and gut to make TG-rich lipoproteins (271). It has previously been thought that abetalipoproteinemic patients have a problem synthesizing apo B. Glickman *et al* (261) reported that apo B synthesis, as determined by ^{3}H leucine incorporation, decreased in abetalipoproteinemic patients but was not abolished, thus suggesting that failure to synthesize apo B by the gut and liver may not be the reason abetalipoproteinemic patients do not produce CM and VLDL. This was later confirmed by the finding that abetalipoproteinemia results from the mutation of the microsomal TG transfer protein gene (272, 273).

Anderson's disease, named after the investigator who first reported it, is another disorder in the formation of CMs by the small intestine (274, 275, 276). Anderson's disease is also known as chylomicron retention disorder because although observable CM particles are in the enterocytes, there are none in the intercellular space. This suggests that the secretion of CMs is defective among patients who have this disease. In a recent study, Dannoura *et al* carefully demonstrated that patients with Anderson's disease do not have a defect in those genes that carry known apoproteins or microsomal TG transfer protein, thus suggesting that this diseased condition is caused by a an unknown factor crucial for the secretion of chylomicrons (277). Currently, no animal model is available to study this perplexing disorder.

10. ACKNOWLEDGEMENTS

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Send correspondence to: Patrick Tso, Ph.D., Department of Pathology, University of Cincinnati Medical Center, 231 Albert Sabin Way (ML 0529), Cincinnati, OH 45267, Tel: 513- 558-2151, Fax: 513-558-2141, E-mail: tsopp@email.uc.edu