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Absorption of Lipids

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10.1 INTRODUCTION

Different lipid molecules vary in their interaction with water and with each other. The main purpose of this chapter is to consider how these differences influence successive events in absorption, from chemical digestion to transport away from the absorptive cell. Intraluminal events will be emphasised, including uptake from the lumen because these events are now better understood at a molecular physicochemical level both in health and disease. Other

sources¹⁻³ should be consulted for comprehensive reviews of absorption of particular lipid classes.

10.2 PHYSICOCHEMICAL BACKGROUND

A brief descriptive account follows. For more detailed information, the reader may consult a number of excellent reviews^{4-6,37}.

10.2.1 Water as a solvent

Water is a highly polar solvent in which electrons are distributed asymmetrically over individual molecules. The strong intermolecular attraction between the electron-sparse regions of the hydrogen atoms of one water molecule and the electron-dense vicinity of oxygen atoms in adjacent water molecules makes hydrogen bonding a major force in the interaction between water molecules. For a component to be water-soluble, it must be ionised or possess enough strongly-polar hydrophilic groups so that the over-all attraction of the molecule for water is not outweighed by the attraction of water molecules for each other. The latter interaction tends to 'squeeze' the component out of solution. The tendency may be augmented by attractive forces between the added component molecules or between their parts. A solute-solute interaction of this type plays a role in liquid crystal formation and in micelle formation (to be discussed later).

10.2.2 Lipid-water interaction

Various interactions between lipid and water molecules are categorised in Table 10.1, after Small's classification⁵.

Non-polar lipids and insoluble polar lipids are virtually insoluble in a bulk aqueous phase because their polar hydrophilic groups are too weak relative to the non-polar parts of the molecule. The water-water interaction predominates, extruding lipid from the watery environment to form a separate

Table 10.1 Classification of lipid interactions with water
(By courtesy of J. Amer. Oil Chemists Soc.)

<i>Class</i>	<i>Examples</i>
(A) Non-polar lipids	Long-chain and cyclic hydrocarbons
(B) Polar lipids	
(1) Insoluble non-swelling amphiphiles	Triglycerides, diglycerides, long-chain non-ionised fatty acids, cholesterol
(2) Insoluble swelling amphiphiles	Monoglycerides, 'acid soaps', lecithin
(3) Soluble amphiphiles	(a) Soaps of long-chain fatty acids, lysolecithin (b) Bile salts

phase. Short-range attractive forces between non-polar parts of adjacent molecules help maintain lipid aggregation.

Few lipids of importance in mammalian physiology are completely non-polar, i.e., have a symmetrical electron distribution over the molecule and no polar groups. Most have some polar features. Moreover, there is spatial separation of polar, hydrophilic and non-polar, hydrophobic or lipophilic parts of lipid molecules. Thus, lipids have a dual affinity—hence the term 'amphiphile'—and a tendency to orient in relation to water molecules. At an interface, amphiphiles form a film, with polar parts orientated toward the aqueous phase. In the bulk aqueous phase, the molecules aggregate so that non-polar groups are excluded as much as possible from interaction with water.

Insoluble non-swelling amphiphiles have a weak hydrophilic region, e.g. ester bonds in long-chain triglycerides. At body temperature, the mixture of fatty acids in the usual dietary lipids is above its melting point. The fatty acid chains are fluid either as non-ionised fatty acid or as esterified fats. In the bulk aqueous phase, dietary triglycerides aggregate as an oil with no fixed orientation of the molecules. Solid non-swelling amphiphiles, such as cholesterol, dissolve in the oil. Any excess lipid forms solid aggregates held together by short-range forces between non-polar parts of adjacent molecules.

While insoluble non-swelling amphiphiles may exist in a bulk aqueous phase as a liquid oil or as solid crystals, the characteristic state of swelling amphiphiles is that of liquid crystals. The basis of this liquid crystalline structure is a bilayer in which the polar hydrophilic heads are on the outside and the non-polar paraffin groups are arranged tail-to-tail inside the bilayer. Alternating lipid bilayers and layers of water form a lamellar structure.

Three factors promote formation of liquid crystals. The first is molecular configuration, namely, a strong polar, hydrophilic head relative to a non-polar tail. One can grasp the salient features of this molecular configuration by comparing a long-chain non-ionised fatty acid above its melting point (a non-swelling amphiphile with one CO_2H polar group) with its homologous monoglyceride. The monoglyceride is a swelling amphiphile, with two hydroxyl groups and one ester bond at the head end of the molecule and the same paraffin tail as the fatty acid. Secondly, lack of strong repulsion between head groups that would favour small spherical structures or dissolution rather than a bilayer. Thirdly, the paraffin tails in the bilayer are liquid. When water contacts a non-hydrated swelling amphiphile, it penetrates between the hydrophilic head groups and between the hydrophilic surfaces of adjacent lamellae, causing swelling and a sliding of lamellae over one another to generate the myelin figures seen under the microscope.

Swelling amphiphiles, such as phospholipids and long-chain monoglycerides, are not sufficiently polar to exist as free molecules in solution in more than trace concentrations. Lawrence⁷ pointed out, however, that water may be considered to dissolve in liquid crystalline aggregates. Swelling amphiphiles are too polar to dissolve readily in oil droplets composed of non-swelling amphiphiles but are orientated at the surface with their hydrophilic heads outwards. However, certain non-swelling amphiphiles are readily incorporated or dissolved in liquid crystalline lamellae of swelling amphiphiles. Important examples are cholesterol and non-ionised long-chain fatty acids;

their polarity and dimensions allow them to interdigitate with swelling amphiphiles, such as phospholipid and long-chain monoglyceride.

10.2.3 Bile acid–water interactions

In soluble amphiphiles (detergents), the interaction of the polar groups with water is strong enough relative to the hydrophobic portion to render the entire molecule soluble in water. For example, lecithin, with a strongly hydrophilic phosphorylcholine head but two hydrophobic paraffin chains, is a swelling amphiphile. Lysolecithin has one paraffin chain less and additional hydrophilic properties (hydroxyl group instead of one of the ester bonds) at the end. It is a soluble amphiphile.

Soluble amphiphiles exist in monomolecular solution in the bulk aqueous phase only up to a certain concentration, the critical micellar concentration. Above this, their amphiphilic nature asserts itself by the spontaneous formation of aggregates, micelles, the polar groups facing out toward the water, and the non-polar portion facing the interior and shielded from water. The aggregates are small enough not to scatter transmitted light, so that a micellar solution appears as clear as water. Such a micellar solution also contains monomers at the critical micellar concentration. Monomers in the surrounding water and aggregated molecules in the micelles are in thermodynamic equilibrium, exchanging rapidly with one another. Thus, a micelle should be looked upon as a 'flickering cluster'⁸ or cloud, whose size and structure remains constant although the component molecules are continuously entering and leaving.

Most of the common detergents are 'head-and-tail' linear types of amphiphiles, tending to aggregate in spherical micelles. Bile acids, the major intestinal detergent, are 'two-faced' planar amphiphiles with the fixed polar hydroxyl groups on one side of the sterol nucleus, the other side non-polar, and a short hydrophilic tail. In a simple bile acid micelle, the molecules appear to aggregate with the non-polar sides of the sterol nuclei back-to-back. This proposed configuration helped explain how bile salts interact with lipids in micellar solubilisation.

10.2.4 Bile acid–lipid–water interactions

Like other detergents, bile acids interact below the critical micellar concentration with insoluble oil, composed, for example, of non-swelling amphiphiles such as triglycerides. They coat the oil–water interface with a hydrophilic layer, thereby lowering the interfacial tension and permitting the formation of small droplets by shearing forces. The layer is also negatively charged due to the bile acid anions, creating repulsion between droplets and delaying coalescence into large globules. However, bile acids are inefficient emulsifiers in the absence of swelling amphiphiles⁹.

Above the critical micellar concentration, bile acids interact primarily with swelling amphiphiles, such as phospholipid and monoglyceride. These are solubilised in much higher concentrations in mixed bile acid micelles than in

the spherical micelles formed by 'head-and-tail' detergents, such as fatty acid anions or lysolecithin. This difference is probably due to the mode of molecular aggregation. Evidence suggests that a micelle composed of bile acid and swelling amphiphile is structured like a disc punched out of a lipid bilayer (analogous to a liquid crystal) and coated around its edge by bile acids, hydrophilic side outwards. The upper and lower surfaces of the disc (as in a liquid crystal) are also hydrophilic, due to the polar heads of the swelling amphiphiles. The whole aggregate is small enough to be in effect, water-soluble.

The mixed bile acid-swelling amphiphile micelle can further interact with certain non-swelling amphiphiles, notably cholesterol and non-ionised long-chain fatty acids. These are solubilised poorly in the simple bile acid micelle but can be solubilised to form a considerable proportion of the mixed micelle. Presumably this occurs (as in the liquid crystal) by interdigitating with swelling amphiphile molecules. Non-polar lipids or bulky, non-swelling amphiphiles, such as triglyceride, are solubilised in small amounts in mixed micelles. They are probably accommodated in the 'liquid' non-polar core formed by fluid paraffin chains of the swelling amphiphiles.

Finally, it must be emphasised that bile acids are detergents only in the anionic form. Non-ionised bile acids are insoluble non-swelling amphiphiles. The significance of the secretion of bile acids in bile as taurine or glycine conjugates is that the conjugates are virtually fully ionised at the slightly acid pH of the contents of the upper small intestinal. This is not true for unconjugated bile acids, which have a pK_a of *ca.* 6 compared with pK_a values of *ca.* 2 and 4 for taurine and glycine conjugates respectively.

10.3 DIGESTION

10.3.1 State of luminal lipids before digestion

The dietary lipids in man are primarily insoluble amphiphiles: triglycerides, phospholipid, cholesterol and plant sterols. The lipids are present in the stomach mainly as oil, because the proportion of unsaturated and other low-melting-point fatty acids in the usual dietary glycerides is sufficient to lower the melting point of the lipid mixture below body temperature.

No data are available on the degree of dispersion of oil as an emulsion by the time the lipids leave the stomach. Our food contains some constituents, such as soluble protein, which could assist emulsification, and some lipid is ingested with detergent additives. However, the efficiency of the gastric antrum as an emulsifying chamber, the effect of varying motility or biliary reflux and other factors have not been studied.

In the duodenum, the dietary oil mixes with a micellar solution of endogenous phospholipid and cholesterol secreted in the bile and with smaller amounts of other endogenous lipids. One should note that the micelles in bile are already almost saturated with phospholipid and cholesterol and that the major dietary lipid, triglyceride, is only slightly solubilised even in expanded bile salt-phospholipid-cholesterol micelles. In the absence of digestion, the micellar phase of intestinal contents would consist mainly of biliary lipid, with

the possible addition of small amounts of dietary phospholipids, sterol and fat-soluble vitamins.

10.3.2 Pancreatic lipolysis

Pancreatic enzymes are generally accepted to be the most important agents in lipid digestion. Pancreatic juice contains lipolytic activity against the major dietary esterified lipids—triglyceride, glycerophospholipids and cholesterol esters of long-chain fatty acids—as well as against water-soluble carboxylic esters of fat-soluble vitamins. The number of separate enzymes responsible for all these activities is unresolved. At least three have been isolated and purified with relative success: glycerol ester hydrolase or lipase, a carboxylic ester hydrolase and phospholipase

10.3.2.1 Lipase

Lipase, which normally accounts for virtually all digestion of dietary triglyceride, has two properties of outstanding physiological importance: (a) it acts only at an oil–water interface, and (b) the enzyme behaves towards its substrate, triglyceride, as a specific primary ester hydrolase, the products of digestion being free fatty acid (liberated from the 1- and 3-positions) and 2-monoglyceride. With artificial substrates *in vitro*, the specificity of lipase for glycerol esters or primary alcohol esters is not absolute¹⁰. It is appropriate here to consider the physicochemical and physiological implications of these two properties of lipase.

As an interfacial enzyme, lipase activity depends upon the area of the oil–water interface and, therefore, on the particle size distribution of the emulsion. More strictly, however, activity depends on the surface concentration of adsorbed enzyme and the surface concentration of sterically-accessible substrate (triglyceride). Thus, the initial velocity of lipolysis depends on factors modifying the physicochemical properties of the interface as well as its surface area¹¹.

Digestion by lipase converts an insoluble weakly-polar amphiphile, triglyceride, into a mixture of more polar products which behaves together as a swelling amphiphile. The mixture of 2-monoglycerides liberated from dietary fat has the properties already discussed for a swelling amphiphile. The behaviour of the mixed free fatty acids is more complex¹². For present purposes, the mixture of non-ionised and ionised long-chain fatty acids (acid soap) found at the slightly acid pH of upper small intestinal contents in the presence of bile acids may be included as a swelling amphiphile in addition to 2-monoglyceride.

In the absence of solubilisation, the digestion products would tend to slow hydrolysis. Being more polar, they would displace triglyceride from the interface, thereby reducing the availability of substrate, and they would possibly reduce lipase activity toward the remaining substrate by making the surface of the oil droplet more hydrophilic. These effects might be offset somewhat by

a co-operative action of swelling amphiphiles and bile acids, enhancing emulsification and increasing interfacial area. Nevertheless, a 'clean' hydrophobic interface would seem desirable for an optimal rate of lipolysis. The generation of swelling amphiphiles, which are readily solubilised by bile acid micelles, ensures rapid removal of digestion products from the interface.

A physicochemical basis for the interfacial activity of lipase and for its specificity as a primary ester hydrolase for triglycerides has been proposed by Brockerhoff¹³. Lipase behaves as a weak nucleophilic agent in attacking ester bonds. It is, therefore, efficient only in a non-polar environment shielded from water, i.e. at an oil-water interface. Reducing the hydrophobic properties of the interface would decrease activity. The presence of electron-donating groups adjacent to the site of hydrolysis (e.g., the hydroxyl groups on either side of the ester bond in 2-monoglyceride) would inhibit activity. Steric hindrance must be invoked, however, to explain why the 2-ester bond is not split first in triglyceride, i.e., while it has an electron-withdrawing ester group on either side.

10.3.2.2 *Interaction of lipase with bile acids: colipase*

Bile acids should promote triglyceride digestion by their emulsifying and solubilising action. Nevertheless, bile acids inhibit the activity of highly purified lipase above the critical micellar concentration. It is restored by a heat-stable low-molecular-weighted fraction first described by Baskys *et al.*¹⁴. This substance, colipase, has been purified to give an appreciation of its probable significance in intraluminal fat digestion¹⁵⁻¹⁷.

Colipase is an acid polypeptide, with a mol. wt. of *ca.* 10 000. It is heat-stable, due probably to its five disulphide bridges. In the presence of bile acids, it binds to lipase, maximally at pH 6 with a colipase:lipase molar ratio of 2:1. Parenthetically, colipase has a remarkably low concentration of aromatic residues. Thus, it is undetectable by absorption at 280 nm when one scans column effluent spectrophotometrically for peptides; this created some difficulties in isolating the active material.

The colipase-lipase complex has important physiological properties. Thus, lipase activity is no longer inhibited in the presence of bile acids above the critical micellar concentration, and the optimum pH for activity is shifted from pH 9 to 6. Lipase activity in duodenal contents resists inactivation by proteolytic enzymes much more than lipase activity in pancreatic juice¹⁸. This may also be due to the formation of the colipase-lipase complex in the presence of bile acids. Thus, colipase seems to confer properties on lipase which adapt it to function optimally under conditions encountered in the duodenum. Apparently, colipase itself does not enhance lipase activity. The maximum activity of a given amount of pure lipase is the same at pH 6 with colipase plus bile acids as at pH 9 with or without colipase in the absence of bile acids¹⁷.

10.3.2.3 *Esterase*

Activity of an esterase (mol. wt. 70 000) has been separated from lipase activity (mol. wt. 40 000) by Sephadex gel filtration of pancreatic juice¹⁹. It resembles

the esterase separated from lipase in pancreatic homogenates in that it lacks activity against insoluble esters. The enzyme in pancreatic juice, unlike intracellular esterase, requires bile acids for its activity²⁰. The pancreatic juice esterase is active, not only against soluble carboxylic esters, but also against insoluble esters when these are solubilised in bile acid micelles. Thus, it can split monoglyceride and even the minute quantities of triglyceride and cholesterol ester which are solubilised in mixed micelles¹⁹. The purified enzyme²¹ may be identical with cholesterol ester hydrolase previously isolated first as a dimer (mol. wt. 136 000)²² and then as a monomer²³, and considered to be a separate enzyme. A consensus must still be reached on a number of points, e.g. whether cholesterol ester hydrolase activity can be separated from soluble ester hydrolase activity and whether the sterol hydrolase activity has a more specific requirement for trihydroxy bile acids than the esterase²⁴. Bile acids appear to have a dual effect on both activities: protecting against proteolytic activity even below the critical micellar concentration and inducing reversible aggregation to higher-molecular-weight species.

Carboxylic ester hydrolase activity is usually measured against soluble esters that are absent from the diet. Its physiological function is obscure. As a cholesterol ester hydrolase, it would be active mainly against the small fraction of cholesterol ester which is solubilised. It may hydrolyse solubilised esters of fat soluble vitamins. Apparently, it plays no important part in triglyceride digestion. The proportions of 2-monoglyceride and free fatty acid in the intestinal contents and data on the mixture entering the absorptive cell are consistent with the predominance of lipase activity.

10.3.2.4 Phospholipase

The phospholipase of pancreatic juice is secreted as an inactive precursor. In the duodenum, the zymogen is activated by trypsin and protected by bile acid from further proteolysis. The pancreatic enzyme is phospholipase A², hydrolysing specifically at the 2 (β)-ester position²⁵ to 1(α)-acyl lysolecithin and one molecule of fatty acid. Studies in humans by Borgström and his colleagues²⁶ indicated that this pancreatic enzyme is the main agent of phospholipid digestion. Little or no hydrolysis of 1 (α)-ester occurs either in lecithin or 1-lysolecithin. Phospholipase A² activity varies in parallel with the other pancreatic enzymes. 1 (α)-Acyl lysolecithin constitutes up to 50–60% of the total choline glycerophosphatides in intestinal contents after a test meal.

10.3.3 Gastric and pregastric lipolysis

Unesterified long-chain fatty acids are present in gastric contents in man after a test meal containing triglyceride²⁷. Lipolytic activity has been demonstrated in gastric contents in man when reflux of duodenal contents was shown to be negligible²⁸ and in rats after pancreatic juice and bile were diverted for several days²⁹. Activity is slight against long-chain triglycerides and moderate against medium-chain triglycerides. Activity against milk triglycerides in the stomach is said to be superior to that of pancreatic lipase. Although eluted from Sephadex gel in a mol. wt. fraction of *ca.* 40 000 (similar to pancreatic lipase),

the gastric activity is stable to low pH and is highly susceptible to tryptic inactivation, even in the presence of bile acids. Histochemistry in rats³⁰ and the reduction of activity with achlorhydria in man²⁸ are compatible with a gastric origin of the lipase. However, the contribution of a lingual lipase³¹ to the activity found in gastric contents has not yet been evaluated fully.

Whatever the source, it seems that the lipolytic activity in gastric juice contributes little to normal triglyceride digestion. A role has been suggested in the suckling animal and in pancreatic insufficiency.

10.3.4 Factors regulating digestion

The previous section dealt with the enzymology of the lipid hydrolases, particularly with the interactions of substrate, enzyme, bile acid and water. Enzymological studies are particularly concerned with initial reaction rates in a closed system, *in vitro*. Lipid digestion *in vivo* in the intestinal contents is an open system. To use Hofmann's simile³², the upper small intestine may be considered as a reaction vessel to which substrate, enzymes, fluid and detergent are continuously added, in which enzymes catalyse reactions at oil-water interfaces as well as in the bulk aqueous medium, and from which reaction products are continuously removed. The steps are interdependent physicochemically and physiologically. Thus, it is difficult to determine whether a given factor is affecting the rate of digestion directly and when the rate of digestion is a major determinant in the overall process.

10.3.4.1 Addition of substrate: gastric emptying

The regulation of gastric emptying is discussed in the chapter on motility. Under suitable conditions, a fairly precise adjustment of gastric emptying to triglyceride load can be demonstrated, e.g. in rats given a test meal of finely emulsified triglyceride in a fixed volume of saline³³. Presumably, this is mediated by solubilised fatty acid in the duodenal contents³⁴, reducing antral motility by neural and hormonal mechanisms. If these observations are generally applicable, they suggest that stomach emptying is regulated to give a fairly steady supply of lipid substrate to the duodenal reaction vessel. In a mixed meal, however, the lipid content is only one of several factors potentially influencing gastric emptying. Moreover, when the lipid is not pre-emulsified, it leaves the stomach after the main fluid bulk of the meal has been emptied. Suitable markers for the oil phase of gastric contents are now available³⁵, but whether the rate at which lipid leaves the stomach is specifically regulated, independent of the other constituents of the meal, has not been established.

10.3.4.2 Addition of enzyme: pancreatic secretion

Regulatory mechanisms are discussed in the chapters on pancreatic exocrine secretion and gastrointestinal hormones. Although these mechanisms could conceivably adjust secretion of lipolytic enzymes to the composition of the

duodenal contents continuously, it is uncertain that secretory control works in this way. The problem is analogous to that for gastric emptying. Thus, enzyme output in man is stimulated by essential amino acids or fatty acids when these materials are perfused steadily through the duodenum³⁶, but no evidence exists that the output of lipolytic enzymes is regulated independently. Moreover, in patients on a regimen of three formula meals per day, pancreatic secretion is turned on by the first meal and persists throughout the day at a steady near-maximal rate; it decreases to a minimum during the night (Go, Brunner, Hofmann and Summerskill, personal communication). This suggests that secretory control is geared to provide a continuous surplus of enzymes for duodenal digestion rather than to continuously adjust the enzyme supply to the state of digestion.

10.3.4.3 Addition of detergent: bile acid secretion

In man, on a regime of three homogenised formula meals per day, bile acid secretion varies more than enzyme output. Nevertheless, after the initial surge due to gall-bladder emptying, when the first meal of the day is taken, secretion into the duodenum becomes fairly steady, until digestive-absorptive activity ceases overnight³⁸.

10.3.4.4 Stabilisation of pH

The prevention of excessive duodenal acidity is important for regulation of lipid digestion. The mechanisms are discussed in the chapters on gastrointestinal hormones and gastric and pancreatic secretion. Continuous measurement of pH in man and animals³⁹⁻⁴⁰ indicates that digestion takes place mainly in a slightly acidic medium, with occasional swings to more-acid values early in the digestive period. The adverse effects of frequent short periods of high intraluminal acidity have been nicely documented in patients with gross hypersecretion of gastric acid due to a gastrin-producing tumour⁴¹. Lipase is irreversibly inactivated and solubilisation is impaired.

10.3.4.5 Rate-limiting factors

Little is known about the steps limiting the overall processes of digestion, solubilisation and uptake of lipids from the lumen—thereby determining the point at which a steady state is reached. Evidence has been mentioned which indicates that digestion *in vivo* proceeds with an excess of enzyme, in contrast to the excess of substrate usually used for enzymological studies. Borgström has suggested that the rates of hydrolysis and regeneration of ester bonds at the oil–water interface are rapid relative to the removal of triglyceride digestion products by absorption⁴². Thus lipolytic products rapidly accumulate until a balance is reached at which no net lipolysis would occur *in vitro*, while, *in vivo*, further generation of lipolytic products is determined by their absorption. However, when intestinal contents are incubated *in vitro*, net liberation of fatty acids continues, e.g. the concentration may rise by

30–60%⁴³. These findings suggest that absorption maintains the concentration of lipolytic products *in vivo* below the true equilibrium value for a closed system *in vitro*.

In man, assemblies of fine plastic tubes passed through the nose allow perfusion and concurrent sampling at a number of sites in the lumen of the small intestine. The composition and physical state of the contents can be measured serially for long periods. Unabsorbable markers and absorbable radioactive tracers, added to the meal or by perfusion into an intestinal segment, allow measurement of rates, e.g. enzyme output into duodenum or generation of lipolytic products. Such techniques may help to identify rate-limiting steps. A steady state can be set up by perfusion and then the system can be perturbed, e.g. by adding further enzyme to see whether enzyme supply is limiting the lipolytic rate⁴⁴.

10.3.4.6 Anatomical reserve

Normally, digestion and absorption of lipid are almost completed in the upper half of the small intestine. During overload, the digestive-absorptive process extends into the distal half of the small intestine. This reserve capacity must be taken into account when one compares the results of *in vitro* and *in vivo* studies.

10.4 SOLUBILISATION

10.4.1 Redistribution of lipids during digestion

The concentration of solubilised lipid is an important determinant of uptake and absorption rates. Lipid in the intestinal contents is distributed between an oil phase and an aqueous phase consisting of micelles and a monomolecular solution. Before digestion starts, nearly all the dietary lipid is in the oil phase, and the bile acid micelles in the aqueous phase contain mainly biliary phospholipid and cholesterol. Digestion initiates a complex redistribution of lipid between oil and aqueous phases. Lipase generates micellar solutes, i.e. monoglyceride and fatty acid, while phospholipase reduces the load of micellar solutes by converting lecithin, a swelling amphiphile, into lysolecithin. The latter is a soluble amphiphile, some of which leaves the micelles to enter a monomolecular solution. Absorption also continuously removes lipid solute from the micelles while the micellar solvents, conjugated bile acids, are absorbed very little in the upper small intestine. The distribution of lipid between oil phase and micelles during digestion is thus determined by rates of generation and final removal of micellar solute, as well as by factors which would determine the distribution at equilibrium in a closed system.

10.4.2 Equilibrium distribution between micelles and oil

At equilibrium in a closed system, the chemical potential of a non-ionised lipid would be the same in oil, monomolecular aqueous solution and micelles. Consider first pure non-ionised long-chain fatty acid dispersed as oil droplets

in water, in the absence of micelles. In this case, the tendency for fatty acid molecules to escape from oil to water would be very small, but maximal, since fatty acid is the only constituent of the oil (mole fraction = 1). An exceedingly low concentration of long-chain fatty acid molecules in water would offset the tendency to escape from oil to water. Nevertheless, the monomolecular aqueous concentration (10^{-5} mol l⁻¹ of water, 10^{-5} mol/55 mol water, mole fraction 5×10^{-6}) is a maximal value. The monomolecular solution would be fully saturated. If a second component, say triglyceride, were present in the oil, the mole fraction of fatty acid would be less than one, its chemical potential would usually be lowered, and the monomolecular concentration of fatty acid in water would decrease correspondingly below the 100% saturation value.

The addition of bile acid micelles to the system containing only fatty acid would not materially alter the concentration (percentage saturation) of the monomolecular solution of fatty acid, as long as an oil phase remains. In equilibrium with an oil composed of pure fatty acid, both micelles and monomolecular solution would be saturated with fatty acid. Saturation of the micelles is defined by the mole fraction of non-ionised fatty acid in the micelles, which is much higher than the mole fraction in monomolecular solution. It must be emphasised that only bile acid exceeding the critical micellar concentration acts as micellar solvent.

The concentration of micellar lipid per unit volume of water must be distinguished from the concentration (mole fraction) of lipid in the micelles. The former increases with increasing bile acid concentration, as long as undissolved fatty acid remains. The latter remains unchanged as long as the concentration (percentage saturation) of the monomolecular lipid solution in water remains unchanged. In our theoretical situation, when all the fatty acid has been solubilised, a further addition of bile acids would reduce the solute: solvent ratio in micelles below saturation value. A new equilibrium would be established with the monomolecular concentration reduced below saturation to an extent determined by the micellar solute: solvent ratio. In this way, addition of micellar solvent (detergent) may reduce the efficiency of a process in which single molecules of lipid participate, but not micellar aggregates. This can be demonstrated *in vitro* for diffusion through porous membranes impermeable to micelles⁴⁵. The concept may also be relevant to lipid uptake by absorptive cell membranes (to be discussed later).

When the system contains several micellar solutes, provided they are present only in micelles, in monomolecular solution and in the oil mixture, the same argument can be applied. But there are complications. The different lipids cannot usually be treated as if they were independently solubilised in micelles. First, there is a limit to the mole fraction of total swelling amphiphiles which can be solubilised; the presence of one variety limits the solubilising capacity for another. Second, the presence of swelling amphiphiles greatly enhances the solubilising capacity for certain non-swelling amphiphiles, e.g. cholesterol, as already discussed.

Fairly accurate measurements of concentrations in monomolecular solution are needed to substantiate some of the foregoing arguments. For most lipids, such concentrations are very low and data are not yet available for the complex types of system encountered in the intestinal contents.

10.4.3 Analysis of micellar lipid

The lipid concentration in the aqueous phase depends primarily on micellar solubilisation. Analysis of lipid in the aqueous phase, 'micellar lipid', is complicated by several possible artefacts. The aqueous phase is usually isolated by high-speed centrifugation (10^7 g min), to float the emulsified oil droplets to the surface, and by sampling the clear infranatant layer. If the duration of the centrifuged force is too low, finely-emulsified particles may remain in a seemingly clear infranatant⁴⁶. With more intense centrifugation, the infranatant may show a concentration gradient due to partial sedimentation of micelles⁴³⁻⁴⁶. With prolonged contact, micellar lipid is taken up by the walls of the cellulose nitrate tubes commonly used⁴⁶. Another complication is continued lipolytic activity during and after collection of intestinal contents. A considerable amount of fatty acid may be liberated by lipase, even during heating to inactive the enzyme. Phospholipase is resistant to heat. Moreover, freezing, thawing and heating of the sample before ultracentrifugation may alter the distribution of the lipid. Passage of intestinal contents through a series of filters of decreasing porosity has been shown to effect a quick and fairly clean separation of oil and aqueous phases and to minimise artefacts due to continued lipolysis⁴³. Clearly, measurements of the concentration and composition of micellar lipid in the intestinal contents must still be interpreted cautiously.

10.4.4 Steady-state distribution

In a previous part of this section, the distribution of lipid between oil and micelles was treated as an equilibrium in a closed system. In the intestinal contents *in situ*, a continuous net transfer of lipid occurs from oil to micelles and monomolecular solution and out of the aqueous phase into the absorbing cells. The driving force for this net flux of absorbable lipid is a difference in chemical potential so that, by definition, the oil and aqueous phase cannot be in equilibrium. While proof is lacking, it seems highly likely that lipid in the two aqueous states (monomolecular solution and micelles) is virtually in equilibrium. It is uncertain whether transfer of lipid between oil particles and aqueous phase involves no rate-limiting step; this step would lead to considerable divergence between lipid distribution predicted for a closed system and the distribution found for the same total concentration of lipid, detergent, etc., during absorption *in situ*. Higuchi and his colleagues have studied the role of an interfacial resistance in the transfer of lipid from emulsion particles to mixed micellar solutions⁴⁷⁻⁴⁹. They have observed differences, e.g. between cholesterol and betasitosterol which may be relevant for absorption *in vivo*.

10.5 UPTAKE FROM THE LUMEN

10.5.1 General features

Over-all, the intraluminal phase of digestion and absorption is an open system in which digestion products are continuously generated and removed, and the

lipid in oil and micelles is constantly redistributed. The aim of this discussion is to consider uptake as a passive process driven by the difference in chemical potential between absorbable lipid in the aqueous phase of intestinal contents and in the aqueous phase of the cytoplasm immediately inside the brush border of the absorptive cell. There are at least two major resistances to flux of lipid down this potential gradient—a diffusion barrier due to an unstirred aqueous layer on the luminal side of the brush border and a more complex resistance encountered in translocation of lipid through the cell membrane itself.

10.5.2 The unstirred layer

However vigorously the bulk of a liquid may be stirred, a relatively undisturbed layer remains adjacent to any surface in contact with the liquid. In this layer, mixing with the bulk of the fluid or transport of solute or solvent molecules can occur only by diffusion. The boundary between the unstirred layer and the well-mixed bulk of the liquid is indistinct. In the intermediate zone, the efficiency of mixing increases with increasing distance from the surface. The thickness of the combined unstirred and poorly-stirred layers is defined operationally as the equivalent thickness of a single layer in which transport occurs only by diffusion. This equivalent thickness can be measured by suddenly altering the composition of the bulk fluid and measuring the rate of change of a property limited by diffusion, such as a diffusion potential, when electrolyte concentration is changed, or a streaming potential for a non-penetrating solute such as sucrose^{50,51}. The estimated thickness of the unstirred layer varies with experimental conditions and has no fixed anatomical dimension.

10.5.3 The cell membrane

Transport of lipid through the cell membrane involves at least two steps, both of which might contribute a resistance to absorptive flux.

10.5.3.1 Partition

The first step is passage of lipid molecules from the aqueous environment on the luminal side of the cell membrane to the lipid environment just inside the luminal boundary of the cell membrane. A lipid molecule has less free energy in a lipid environment than in an aqueous medium. Roughly, the tendency to escape from lipid to water is less than from water to lipid. In a closed system, with no absorptive flux, an equilibrium would be established with the concentration of the lipid solute molecules (mole fraction) in the membrane much higher than the concentration (mole fraction) in the aqueous medium. By definition, the chemical potential of the solute would be the same in aqueous medium and in a cell membrane; the lower free energy per solute molecule in the membrane is balanced by the higher concentration. One can regard this as a partition between cell membrane lipids and the aqueous medium just exterior to the membrane. Partition of a lipid solute into the

membrane lipid ('solvent') would be decreased by hydrophilic attraction, mainly hydrogen bonding, between lipid solute and water. Attractive forces between lipid solute and cell membrane constituents would favour partition into the membrane.

During absorption, the system is open, with continuous flux of lipid from the aqueous medium into and through the cell membrane. The concentration of lipid solute within the cell membrane must therefore be lower than one would predict from partition in a closed system. If the resistance at the interface between luminal water and cell membrane were low, however, the discrepancy would be small. Whether this is the case cannot yet be determined.

10.5.3.2 Translocation

The second step is penetration of the membrane, that is, translocation through the membrane lipid. This step depends on prior partition, since the higher the concentration of lipid solute in the membrane on its luminal side, the greater the flux to the inner side of the membrane tends to be. For a given concentration gradient within the membrane, the flux is determined by resistances dependent on the size of the moving molecule and its interaction with the membrane 'solvent' lipid and other materials.

Passage of lipids into and through a cell membrane differs in important respects from uptake and diffusion through a bulk oil phase *in vitro*. For example, the membrane lipids are mainly amphiphiles and are part of an organised structure including protein. Also, they form a bilayer in which the distance for solute movement is only about twice the longest dimension of the absorbed dietary lipids. Nevertheless, as Diamond and Wright showed, a model based on partition and diffusion in non-aqueous solvents agrees well, quantitatively, with observed membrane selectivity for the different non-electrolytes. Their excellent review⁵² should be consulted for detail and for a more rigorous analysis of membrane permeability than that attempted here.

10.5.4 Resistance to diffusion and membrane penetration

The driving force transferring a given lipid between intraluminal fluid and intracellular fluid is the difference in chemical potential of the lipid in these two aqueous media or, as an approximation, the difference in aqueous concentration $C_1 - C_3$, where C_1 = concentration in the bulk aqueous phase and C_3 = concentration in intracellular fluid. The unstirred layer creates a resistance which depends on two factors. The first is the specific resistance met by the lipid in aqueous diffusion, that is $1/D$, where D = aqueous diffusion coefficient in the layer. This is usually assumed to equal the free diffusion coefficient, but may not. The second is the thickness of the unstirred layer, d . The resistance to diffusion through the unstirred layer is thus d/D .

The second resistance is that encountered on passage into and through the cell membrane. More than one process is involved, but the overall resistance may be expressed as $1/P$, where P = permeability coefficient of the membrane

to the molecule concerned. This may be treated as a resistance in series with that of the unstirred layer. The total resistance to uptake into the cell is therefore $(d/D + 1/P)$, and the amount transported (flux per unit area) depends on:

$$\frac{C_1 - C_3}{(d/D + 1/P)}$$

The relative importance of the two resistance terms varies with the type of molecule and the experimental conditions. The resistance of the unstirred layer predominates if d/D is large relative to $1/P$, that is, with a thick unstirred layer (d large) and a slowly diffusing molecule or aggregate (D small) or with a high permeability of lipid through the cell membrane (P large). Under such conditions, most of the fall in chemical potential (concentration), $C_1 - C_3$, occurs across the unstirred layer ($C_1 - C_2$) and only a small proportion across the cell membrane ($C_2 - C_3$). The converse is true when the membrane resistance predominates (Figure 10.1).

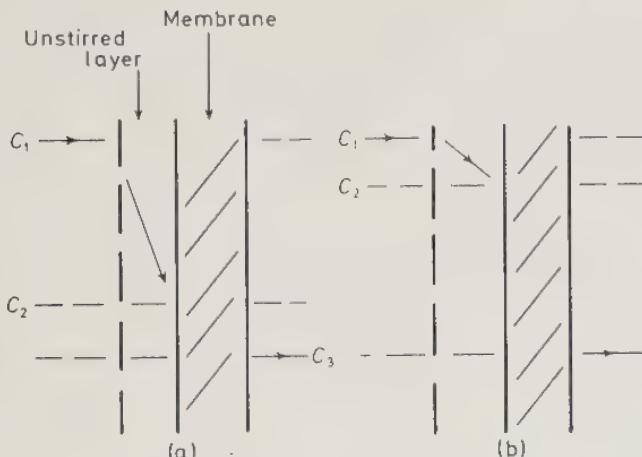


Figure 10.1 Aqueous concentration gradient (A) when diffusion through unstirred layer is the rate-limiting step and (B) when membrane permeability is the rate-limiting step. C_1 = concentration in bulk aqueous phase (well stirred); C_2 = concentration immediately external to cell membrane; and C_3 = concentration in aqueous cytosol. It is assumed that intracellular concentration, C_3 , is maintained at a constant low value.

This section has dealt with the restricted case in which diffusion across the unstirred layer and penetration of the cell membrane is monomolecular. When micelles are also present, the aqueous concentration gradient and the resistances to diffusion and membrane penetration must be treated separately for each physical species. This is done in the next section, which also considers the complication introduced when both micelles and monomers diffuse freely across the unstirred layer but only monomers penetrate the cell membrane.

10.6 SOLUBILISATION AND UPTAKE

10.6.1 *In vitro*

Micellar solubilisation unquestionably promotes lipid absorption. The uptake of long-chain fatty acids by everted sacs or rings of small intestine has repeatedly been shown to proceed more rapidly from micellar bile acid solution than from emulsions without bile acids^{53,54}. When everted sacs were incubated in media containing a fixed total amount of fatty acid with varying amounts of bile acid, uptake increased *pari passu* with the curve for solubilisation⁵⁵. Uptake varied linearly with the concentration of solubilised fatty acid when this was altered by three methods: (a) by increasing bile acid with a fixed total amount of fatty acid, (b) by varying fatty acid in the presence of a fixed excess of bile acid, so that all the fatty acid was solubilised; and (c) by adding a non-absorbable oil, so that less fatty acid was micellar and more was in the oil with constant amounts of bile acid and total fatty acid. The slope of the line relating uptake to solubilised concentration was much the same, regardless of how solubilisation was altered⁵⁶⁻⁵⁸.

These observations indicate that micellar solubilisation accelerates uptake by increasing the lipid concentration in aqueous dispersion which provides the driving force for uptake. The amount of insoluble lipid, even in finely dispersed oil droplets, contributes little.

10.6.1.1 *Micellar transport across the unstirred layer*

Although solubilisation increases the effective concentration gradient for uptake, the resistance to aqueous diffusion of solubilised molecules is higher, i.e. the diffusion coefficient is lower. This is due to the greater size of micellar aggregates compared with single molecules in solution. The diffusion coefficient for micellar lipid is lower despite the rapid exchange of individual molecules between micelles and monomolecular solution. Since the ratio of the number of solubilised molecules to the number in monomolecular solution is high at any instant, any given molecule must reside in micelles most of the time, subject to the constraints on movement of the micellar aggregate.

When lipid in micellar solution diffuses across an unstirred layer, the total flux (J_{total}) equals $J_{\text{mic}} + J_{\text{mono}}$, where J_{mic} and J_{mono} are the fluxes of lipid in micelles and in monomolecular solution, respectively.

$$\text{Flux} = \frac{\text{Driving force}}{\text{Resistance}} - \frac{(C_1 - C_2)}{(d/D)} = (C_1 - C_2) \times D/d$$

where $C_1 - C_2$ = difference in concentration across the unstirred layer, d = thickness of the layer and D = aqueous diffusion coefficient. Thus,

$$J_{\text{total}} = (C_1 - C_2)_{\text{mic}} \times \frac{D_{\text{mic}}}{d} + (C_1 - C_2)_{\text{mono}} \times \frac{D_{\text{mono}}}{d}$$

Micellar solubilisation greatly increases the aqueous concentration of certain lipids. For example, in a 10 mM micellar solution of long-chain fatty acids, the ratio $(C_1)_{\text{mic}}/(C_1)_{\text{mono}} = \frac{10^{-2}M}{10^{-5}M} = 1000:1$. The resistance to

diffusion ($1/D$), on the other hand, is $(\text{mol. wt.})^{\frac{1}{2}}$ for molecules of small-to-moderate size and $(\text{mol. wt.})^{\frac{3}{2}}$ for the large micellar aggregate. No reliable measurements of micellar weight are available for mixed micelles in the intestinal contents⁶. Assuming a fairly large value (125 000) the ratio of resistance to diffusion of an 18-carbon fatty acid (micelles: monomolecular solution) = $(125\ 000)^{\frac{1}{2}} - (280)^{\frac{1}{2}} = 3:1$. Thus, micellar solubilisation in this example increases the driving force for diffusion *ca.* 1000-fold, while the resistance increases only *ca.* threefold.

No great importance should be attached to the number in the example given. It serves merely to emphasise that, for molecules such as long-chain fatty acids which have a low concentration in monomolecular solution but are readily solubilised, diffusion flux is greatly increased by solubilisation and most of the lipid is transported in micelles.

(a) *Rate-limiting effect*—As noted previously, the total resistance to uptake of a lipid may be considered the sum of the resistance to diffusion across the unstirred layer and a resistance term attributable to the cell membrane. That is, total resistance equals $(d/D + 1/P)$, where d = thickness of the unstirred layer and D and P = aqueous diffusion coefficient and membrane permeability coefficient, respectively.

If uptake is unaffected by altering d/D , clearly the resistance to diffusion across the unstirred layer is not a substantial fraction of the total resistance. Wilson, Sallee and Dietschy⁵⁹ showed that this was true for a monomolecular solution of short- and medium-chain (6- and 8-carbon) fatty acids. Uptake was unaffected by vigorous stirring, which would reduce the effective thickness of the unstirred layer to a minimum.

Resistance to micellar diffusion of long-chain fatty acid contributed substantially to the resistance to uptake from micellar solution. This was shown by the lower uptake flux per unit concentration in unstirred micellar solution compared with flux from a monomolecular solution of short- and medium-chain fatty acids. It was also demonstrated by a considerable increase in uptake when the micellar medium was stirred. Strictly speaking, these observations do not prove that d/D constitutes a major part of the resistance to uptake from well-stirred micellar solutions (d at its minimum value). However, the conclusions of Wilson *et al.*⁵⁹ are consistent with the data of others⁵⁸, who showed that differences in uptake from two types of well-stirred micellar solutions could be correlated with differences in the diffusion coefficients.

Apparently the efficiency of uptake, that is uptake flux per unit concentration in the aqueous phase, is less from micellar solution due to the diffusional resistance of the unstirred layer. The effective thickness of the layer and the size of the micelles, therefore, modifies uptake from micellar solution. However, most lipid digestion products have a very low concentration in a saturated monomolecular solution. This limitation is removed by solubilisation, which greatly increases total uptake, despite the unstirred layer.

10.6.1.2 *Micelles and membrane penetration*

Enhanced diffusion might not be the only way in which uptake is promoted by solubilisation. Other possibilities are (i) increased membrane permeability; (ii) penetration of the cell membrane by intact micellar aggregates, and (iii) a metabolic effect of bile acids within the absorptive cells. The first two possibilities will be considered here and the third in a later section.

(a) *Permeability*—Evidence exists that bile acids, particularly in high concentration, increase the permeability of the brush border to small water-soluble molecules⁶⁰. No evidence definitely excludes a similar effect on permeability to lipid. Nevertheless, several findings argue against an important permeability effect of bile acids in lipid uptake: (i) solubilised lipid, such as monoglyceride, inhibits the effect of bile acid on permeability to small water-soluble molecules⁶⁰; (ii) the relationship between uptake and solubilised fatty acid concentration is unaffected by bile acid concentration⁵⁸; (iii) damage to cells by unconjugated dihydroxy bile acids is associated with a decrease, not an increase, in fatty acid uptake⁵³; (iv) when fatty acid is completely solubilised, a further increase in conjugated bile acids may result in a decreased uptake⁶¹ (see later).

(b) *Micellar or monomolecular penetration*—Presumably, uptake would be promoted if micelles were taken up and penetrated the cell membrane as intact aggregates. Direct evidence from electron microscopy is contradictory⁶²⁻⁶⁴. It seems unlikely that micellar aggregates passing through the membrane at the time of fixation could survive processing of tissue for electron microscopy. Nor does it seem likely that the labile hydrophilic micellar aggregate would remain intact *in vivo* during passage from an aqueous medium into the lipid environment of the cell membrane.

If the components of micelles were absorbed in the proportions in which they were present in the micelles, this would be strong indirect evidence for micellar penetration. Some results are consistent with this view⁵⁹, but evidence favouring independent uptake of micellar components is accumulating. The ratio for uptake of two lipid solutes does not agree consistently with the ratio in the micellar medium⁶⁵. The relation of uptake to concentration solubilised has also been shown to be linear but different for two lipids (fatty acid and glyceryl monoether) when each was the only micellar solute. Furthermore, the proportion in which they were taken up from various micellar mixtures could be predicted from the data for independent uptake⁵⁷.

Evidence for independent uptake of different micellar solutes does not, of course, exclude interaction of micelle with membrane before the lipid solutes pass through. If this occurred, the type of micellar solvent might be important for uptake. Cholesterol absorption has been shown to have a specific requirement for bile acid³ and cholesterol is taken up poorly or not at all when solubilised in other detergents⁵⁸. Apparently, this is not true for fatty acids. Even for cholesterol, the effect of temperature on uptake is consistent with a process of low activation energy, such as diffusion⁶⁶, rather than with the higher activation energies of specific types of absorption interaction at surfaces.

(c) *Protein binding*—Most evidence favours uptake of lipid as single molecules. This conclusion is consistent with a process involving partition

between water and lipid followed by diffusion in lipid, as in Diamond and Wright's model⁵². In other models, lipid interaction with a mixture of binding sites of low affinity and high affinity has been postulated. The evidence for such models rests rather heavily on data for efflux of labelled lipid after an uptake period⁶⁷. Several difficulties attend the interpretation of such evidence. For example, washout from the unstirred layer could contribute to a 'rapid efflux' component. Also the specific activity of the pools contributing to efflux is unknown. This contrasts with influx from a large 'pool' of incubation medium in which the specific activity changes very little. In addition, it would be difficult to distinguish between the effect of higher affinity sites on efflux curves and the effect of longer mean free path for diffusion. The latter situation might occur if the solution between the lower halves of the villi were less well stirred than that at the tips. In both cases, efflux would be accelerated by increasing the affinity (decreasing the chemical potential) of the medium for lipid, as might occur when albumin was added to bind fatty acid or detergent concentration was raised above the critical micellar concentration.

The concept that protein on or in the cell membrane may influence lipid uptake should not be discarded merely because the evidence is inconclusive. The cell membrane is a highly-organised lipoprotein structure, not simply a lipid film. The high degree of specificity in absorption of steroids, at least *in vivo*, has been ascribed to lipid-binding proteins⁶⁸. Whether these are intracellular or reside in the cell membrane remains undetermined.

One must also remember, however, that apparently small differences in molecular structure may have large effects on lipid:water partition⁵² or on penetration of a lipid interface. For example, the plant sterol, β -sitosterol, is transferred much more slowly than cholesterol from oil droplets to water⁴⁹, although the plant sterol differs from cholesterol only in addition of a methyl group at C-24 in the side chain. The two sterols have a similar solubility in mixed bile acid-swelling amphiphile micelles. Whether a specificity of uptake or penetration analogous to that for the cell membrane can be obtained with more organised lipid structures, such as liquid crystals or artificial bilayers, needs further study. Detailed information and references on the specificity for absorption of sterols and fat-soluble vitamins may be found in a fine review by Borgström¹.

(d) *Micellar transport and monomolecular gradient*—If micelles do not penetrate the cell membrane and if the resistance to diffusion across the unstirred layer does not limit the uptake from monomolecular solution, it is difficult to explain why uptake is much greater from micellar solution than from saturated monomolecular solution in the absences of micelles. The ultimate driving force for uptake must be the concentration of the penetrating species, i.e. monomers, not micelles. Micellar lipid could, therefore, promote absorption by maintaining a higher monomolecular concentration at the luminal interface of the brush border, after diffusion across the unstirred layer. Since the maximum monomer concentration is that of a saturated monomolecular solution, no enhancement by solubilisation could occur if the unstirred layer contributed no significant resistance to monomer diffusion, relative to the cell membrane resistance. Some mechanism involving lipid loading of the membrane directly from micelles would have to be invoked.

On the other hand, monomolecular penetration would be consistent with

decreased uptake found when an excess of micellar solvent is present, e.g. when more bile acid is added to a micellar solution of lipid⁶¹. In such circumstances, the concentration in monomolecular solution, which is in equilibrium with the micelles, must fall as excess of micellar solvent renders the micelles unsaturated with lipid. The driving force for uptake is thus lower, although the total aqueous concentration of lipid is unchanged.

10.6.2 *In vivo*

In vitro preparations have both limitations and advantages in elucidating mechanisms of lipid absorption⁵⁸. *In vitro* preparations are incomplete models of the *in vivo* process, since they lack the capacity to extrude absorbed lipid from cells as chylomicrons and cannot sustain a steady uptake for long periods. Function starts to deteriorate when the segment is removed, losing its lymph and blood drainage and supply of blood-borne nutrients. Structural disorganisation may be apparent in less than 1 h. Rate-limiting processes may differ from those in the living animal and metabolic handling of absorbed products may be distorted. Selectivity of absorption, e.g. for sterols, may be impaired¹. The advantage of *in vitro* preparations, particularly in studying transfer from lumen to cell membrane, are that the absorptive area is standardised and the physical state of the absorbate can be closely controlled.

In vivo, quantitative studies comparable to those *in vitro* are difficult to perform. Separation of oil and aqueous phases of human intestinal contents gave the first clear evidence of micellar solubilisation as a key mechanism in lipid absorption⁶⁹. Since then, bile acid deficiency, defective solubilisation and lipid malabsorption have been correlated in a variety of pathological conditions⁷⁰⁻⁷². However, a continuous gradation of absorption rates with solubilised concentration, e.g. of long-chain fatty acids, has not been demonstrated in man or animals. Indeed, steady-state lymphatic absorption of long-chain fatty acid in rats with bile fistula was as efficient with duodenal infusions containing a trace of micellar lipid as when all the lipid was solubilised. Absorption was moderately depressed when no micellar lipid was present⁷³.

Discrepancies between *in vivo* and *in vitro* results may be explained at least partly by differences in the absorptive situation. Normally, lipid is almost completely absorbed before the distal half of the small intestine is reached²⁷. Thus, the distal absorptive area remains in reserve. Measures of overall absorption, such as lymphatic output in animals or balance studies in man reveal no absorptive deficit until the reserve area is utilised fully⁷⁴. Similarly, if bile acids are deficient but not absent from the contents of the upper small intestine, progressive absorption of fluid may raise the concentration enough to permit solubilisation before the bile acids are actively absorbed in the distal ileum. Such adaptations have been documented by Knoebel⁷⁵.

We have little information on the kinetics of lipid uptake *in vivo* comparable to that obtained *in vitro*. In man, preliminary reports claim that long-chain fatty acid uptake from a perfused intestinal segment is slower in the absence of micelles⁷⁶ and that uptake in different patients correlates with the concentration solubilised⁷⁷. Under steady-state conditions, the components of mixed micelles are taken up at different rates by perfused jejunal segments.

Monoglyceride and fatty acid is absorbed faster than cholesterol, and taurine-conjugated bile acids are not absorbed, a finding consistent with uptake of single molecule but not of micellar aggregates⁷⁸.

Many animal studies have been reported on absorption from closed segments or continuously perfused segments, *in situ* but usually under anaesthesia. Data suitable for kinetic analysis, however, have come mainly from pharmacological studies of lipid-soluble drugs of low molecular weight⁷⁹. Preliminary reports indicate that uptake of long-chain fatty acid from a closed segment varies with solubilised concentration and that different lipids are taken up at independent rates from the same mixed micelles⁵⁸.

Although the evidence is incomplete, apparently when the absorptive area and physicochemical state of the absorbate can be standardised, *in vivo* results are similar to *in vitro* data. They are consistent with the concepts that solubilisation enhances the total delivery of lipid by diffusion to the absorptive surface but that uptake and penetration of the cell membrane occurs ultimately from a monomolecular solution. Nevertheless, the mechanisms of uptake by isolated gut *in vitro* and intact gut *in situ* may not be identical. In particular, an apparently obligatory role of bile acids in cholesterol absorption and a high degree of selectivity for different sterols are more clearly defined *in vivo*. The *in vitro* intracellular events, briefly discussed in the next section, may differ in some respects from those *in vivo*.

10.7 INTRACELLULAR EVENTS

10.7.1 Absorbed lipid mixture

Table 10.2 shows the components of the absorbed lipid mixture and the lipids from which they are derived in the lumen.

Table 10.2

<i>Original lipid</i>	<i>Absorbed components</i>
Long-chain triglyceride	2-Monoglyceride, free fatty acid
Medium-chain triglyceride	Some triglyceride ⁸⁰ , free fatty acid, glycerol
Cholesterol and cholesterol esters	Cholesterol, free fatty acid
Glycerophospholipid	Lysophospholipid, free fatty acid ^{26, 81}
Sphingomyelin, cerebroside	Undigested ¹
Fat-soluble vitamins:	Mainly unesterified ¹
Some as esters or provitamin (A)	

The mixture of absorbable components enters the absorptive cell (enterocyte) in a fine dispersion, probably monomolecular. The components leave the enterocyte mainly in particulate form, as chylomicrons and very-low-density lipoprotein, after complex metabolic changes involving re-esterification and incorporation of non-dietary components. This intracellular phase of

lipid absorption will be only outlined here. Johnston's fine review contains a detailed discussion².

10.7.2 Metabolism

10.7.2.1 Activation of fatty acid

An essential preliminary to incorporation of absorbed free fatty acids into glycerides is the formation of thio-esters with coenzyme A, under the influence of fatty acid thiokinase. The fatty acid-CoA complex is water-soluble and has a detergent structure, with a strongly hydrophilic head group attached to the paraffin chain of the fatty acid⁶². This suggests a role in fatty acid transport from the brush-border membrane to the intracellular membranes, where esterification occurs. However, the thiokinase appears to be located in the microsomal fraction (that is, on the endoplasmic reticulum), whereas for efficient transport one would have expected it to reside on the intracellular side of the brush-border membrane or in the cell sap. There is little activity against short- and medium-chain fatty acids, which pass rapidly, unesterified, through to portal blood (see later for a discussion).

10.7.2.2 Re-esterification of partial glycerides

Both triglycerides and the major phospholipid, lecithin, are incompletely hydrolysed in the lumen and absorbed as the partial glycerides, 2-monoglycerides, and 1-acyl lysolecithin, respectively, together with free fatty acids. In the lumen, incomplete hydrolysis is important for dispersion and uptake, as discussed previously. In the enterocyte, the absorbed partial glycerides provide a potential shortcut for the reconstitution of triglyceride and phospholipid. The demonstration that this biochemical bonus was utilised, by acylation of 2-monoglyceride to triglyceride, was a major advance in research². Evidence suggests that the monoglyceride pathway could account for most of the triglyceride resynthesised in the enterocyte during normal absorption. Less is known about the reconstitution of lecithin from absorbed 1-acyl lysolecithin. Experiments with selectively-labelled phospholipid and analysis of fatty acid distributed in 1- and 2-positions of chylomicron phospholipid in lymph suggest that direct acylation makes a significant contribution⁶¹.

10.7.2.3 Hydrolysis

Partial glycerides may also undergo hydrolysis in the enterocyte. A mono-glyceride hydrolase has been demonstrated, but acylation of long-chain monoglycerides normally predominates². Hydrolysis of ester bonds can be a significant factor in the isolated intestine, *in vitro*, and possibly in the normal enterocyte for any medium-chain triglyceride absorbed intact. Sphingomyelins are thought to be taken up intact and split by intracellular or brush-border enzymes¹.

10.7.2.4 Glyceride synthesis

In liver and adipose tissue, triglyceride is synthesised from L- α -glycerophosphate, which is acylated to phosphatidic acid, dephosphorylated to diglyceride and then acylated to triglyceride. The mucosa of the small intestine can also use this pathway. In the enterocyte, L- α -glycerophosphate is generated mainly by glycolysis although, contrary to earlier belief, there is some capacity of glycerokinase to phosphorylate glycerol. The α -glycerophosphate pathway also diverges at phosphatidic acid and diglyceride for phospholipid synthesis.

Thus the enterocyte possesses alternative pathways for the synthesis of both triglyceride and phospholipid. Factors determining the extent of utilisation of the alternative modes of synthesis are not yet understood. The composition of the absorbed lipid mixture may play a part. For example, if fat is absorbed mainly as free fatty acid in animals with bile fistula, as has been suggested¹, the α -glycerophosphate pathway must be used in such circumstances.

10.7.2.5 Cholesterol

Cholesterol esters of long-chain fatty acids are absorbed only after hydrolysis to free cholesterol. Nevertheless, much of the cholesterol recovered in lymph after absorption is esterified. Esterification is thought to occur relatively late in the assembly of the chylomicron, through a small pool of cholesterol ester with a rapid turnover.

10.7.2.6 Non-dietary (endogenous) components

Even at the peak of fat absorption, the absorbed lipid mixture transported in lymph contains a considerable proportion of non-dietary components. The sources of endogenous lipids and the extent to which they participate in lipid turnover by the enterocyte are discussed in detail elsewhere⁸³. Biliary cholesterol and phospholipid make a major contribution. Mucosal synthesis is also a significant source of endogenous cholesterol. Endogenous fatty acids are derived mainly from the lumen. In chylomicrons after a fatty meal the proportion of endogenous fatty acid is greater in phospholipid than in cholesterol esters and it is least in triglycerides.

10.7.2.7 Role of bile acids

Metabolism of absorbed lipid in the enterocytes and assembly of transport particles (chylomicrons, very-low-density lipoprotein) is coordinated with intraluminal events, since lymphatic output of absorbed lipid varies *pari*

passu with uptake from the lumen, after a time lag of *ca.* 30 min. To a large extent, the coordinating mechanisms could be non-specific, mediated by changing flux of lipid into the enterocyte and by the balance of the absorbed mixture.

A more specific role has been claimed for bile acids. Their presence in the lumen inhibits cholesterol synthesis *de novo* by ileal mucosa⁶⁴. In addition, evidence has been advanced for an intracellular effect of bile acids, specific to the trihydroxy acid conjugates, promoting esterification of absorbed cholesterol³.

A specific effect of conjugated bile acids in promoting triglyceride synthesis has been claimed⁵³, but the evidence is equivocal^{63, 65}. In most experiments, non-specific effects have not been excluded. Solubilisation itself could accelerate synthesis by enhancing uptake of fatty acid and ensuring uptake of monoglyceride⁵⁸. Decreased activity of mucosal enzymes, fatty acid thiokinase and monoglyceride acylase, has been demonstrated in animals with bile fistula; it could be partly reversed by returning bile to the lumen⁶⁶.

On the other hand, unconjugated dihydroxy bile acids, particularly deoxycholic acid, inhibit many intracellular processes *in vitro*⁶⁷, but have less effect *in vivo*⁶⁸. Bacterial deconjugation sufficient to produce an intracellular effect may occur normally in the ileum of some animals, such as rats⁶⁷, but not in man. Pathologically, fatty acid malabsorption is associated with bacterial overgrowth, e.g. in the 'blind loop' syndrome. However, the pathogenic mechanisms are complex, including defective micelle formation by unconjugated bile acids⁷⁰.

10.7.3 Assembly and extrusion of transport particles

A fairly detailed discussion covering this topic and the remainder of the chapter has recently appeared⁶³. Hence, only an outline will be given here, including some more recent findings.

10.7.3.1 Transfer to sites of synthesis

Electron microscopy suggests that lipid is transferred across the cytosol beneath the brush border in fine dispersion, presumably water-miscible^{63, 64}. As has already been mentioned, fatty acids could be transported as water-soluble coenzyme A complexes. Alternatively, binding to a soluble protein has been suggested. A protein fraction, with a mol. wt. of *ca.* 12 000, has recently been isolated by gel filtration from jejunal mucosa and other tissues. This fraction is present in the organelle-free supernatant of homogenates. It has a high affinity for unsaturated long-chain fatty acids and other properties similar to Z-protein isolated from liver cytosol⁶⁹. Proteins binding sterols⁶⁸ and carotenoids⁹⁰ have also been described. Much work remains to be done (e.g. competitive binding assays, tests of enzymatic activity) before an intracellular transport function can be ascribed to such protein factors.

10.7.3.2 Assembly of lipid

The metabolic pathways for the lipid components of the intracellular particles have been mentioned. Little is known about how or where in the cell these components are brought together. Most of the enzymes concerned in triglyceride synthesis are microsomal, i.e. in the endoplasmic reticulum. Labelled lipid droplets appear rapidly in the smooth endoplasmic reticulum vesicles and cisterns and labelled fatty acid is incorporated into triglyceride with comparable speed, after exposure of the mucosa to radioactive fatty acids⁹¹. This suggests assembly within the endoplasmic reticulum.

Location of lipid-synthesising enzymes is not the sole factor determining appearance of lipid transport particles (very-low-density lipoprotein and chylomicrons) within the endoplasmic reticulum spaces. Such particles are scanty, absent, or varied in size when β -lipoprotein synthesis is defective, although some capacity to synthesise triglyceride is retained. Large lipid droplets, some without surrounding membranes, are seen in the cytoplasm in electron micrographs during fat absorption *in vitro*⁶³ and *in vivo*⁹². They are prominent in $\alpha\beta$ lipoproteinaemia in man⁹³ and in the upper ileum of rats during maximal fat absorption⁹⁴. The composition of these droplets is unknown. Speculatively, triglyceride may be synthesised on the cytoplasmic side of the endoplasmic reticulum but not translocated adequately into the cisterns unless enough apolipoprotein is available.

There is an increase in both the size and number of the chylomeric-sized particles from apical vesicles to lateral and basal exit sites. Whether composition also changes is uncertain, although an increase in the proportion of triglyceride⁹⁵ would be likely. The outlying cisterns and vacuoles of the Golgi apparatus contain transport particles during lipid absorption, whereas the medial cisterns and tubules seem to be more concerned with glycoprotein metabolism⁹⁶.

10.7.3.3 Protein components and extrusion

Good evidence exists that the protein as well as the lipid components of the transport particles in lymph (very-low-density lipoprotein, VLDL, and chylomicrons) originate in the intestinal epithelium^{83,97}, although both peptide and lipid components may exchange with soluble lipoproteins to some extent after extrusion from the cell. Lipid particles with the same size distribution as plasma VLDL are seen in the endoplasmic reticulum in fasting animals and man and, mixed with larger chylomeric size particles, after a fatty meal⁹⁸. Particles isolated from the mucosa during fasting have a size and composition like VLDL.

Whether protein is added to the polar lipid surface of chylomicrons inside the cell or after extrusion is more difficult to determine. Analysis of pelleted chylomeric 'membranes' supports an intracellular origin. So does the defect in chylomeric formation in $\alpha\beta$ lipoproteinaemia or with inhibitors of mucosal protein synthesis⁹⁷. In the former, a specific apolipoprotein deficiency has now been identified⁹⁹. This is the main apoprotein of β -lipoprotein (normal low-density lipoprotein) and a major constituent of VLDL.

Perhaps, as several workers have suggested, particulate lipid transport from the enterocyte depends primarily on the synthesis of VLDL. This suffices for particulate transport of endogenous lipid during fasting. After a fatty meal, as triglyceride synthesis increases relative to VLDL, chylomicrons form by VLDL aggregation. Similarly, the size of the chylomicrons increases with the triglyceride load or with relative deficiency of protein or strongly amphiphilic phospholipid to maintain a fine emulsion. An important technique which may help to test such a concept is the use of isolated enterocytes which retain the capacity to discharge lipid particles into the incubation medium¹⁰⁰.

Transport particles are generally believed to leave the cell by temporary fusion of endoplasmic reticulum vesicles or Golgi vacuoles with indentations (caveolae) in the lateral and basal cell membranes, from where they are discharged into tissue fluid. This has been affirmed by electron microscopic observations made by some investigators⁹⁸ but not by others⁶⁴. Experiments with an isolated enterocyte preparation, mentioned above, may offer some clues to the mechanism of extrusion and the factors affecting it.

10.8 LYMPHATIC AND VASCULAR TRANSPORT

After extrusion from enterocytes into tissue fluid, lipid is removed mainly by lymphatics, because most lipid leaves the cells as particles (VLDL and chylomicrons), to which lymphatics are freely permeable but not blood capillaries. The high permeability of lymphatic capillaries in the intestinal mucosa and throughout the body is usually attributed to lack of tight junctions between endothelial cells. A flap-valve action of overlapping cells has been suggested, so that tissue fluid and particles pass in unhindered but do not readily leak out again. This may be an oversimplification. The geometry of intercellular junctions is complex, often making it difficult to tell whether particles are passing between or through cells. Vesicle transport through cells may play a significant role^{98,105}.

The proportion of absorbed fatty acid and monoglyceride appearing in lymph depends on the extent to which it is incorporated into ester fat, mainly triglyceride. Hence, recovery in lymph is negligible for fatty acids with 8-carbon chains or smaller, low for 10-carbon chains, moderate (*ca.* 50%) for 12-carbon chains, and dominant (80% or more) for 14-carbon chains or longer⁸³. Absorbed cholesterol, whether esterified or not, is transported almost exclusively as particles in lymph. Apparently, the same is true for cholesterol synthesised in the intestinal mucosa. Some free cholesterol may be exchanged with soluble lipoproteins in tissue fluid and lymph¹⁰¹. Lymph probably transports most of the phospholipid synthesised or reassembled in the enterocytes. However, the complicated metabolism of glycerophosphoryl choline and fatty acid moieties makes it difficult to apportion nascent phospholipid between the transport pathways.

In recent years, advances in lipoprotein analyses have generated an interest in the respective roles of VLDL and chylomicrons as transport particles in lymph. Evidence exists that a large proportion of lipid is transported as VLDL during fasting¹⁰². After a fatty meal, much of the dietary lipid is transported

as chylomicrons. However, the relative importance of VLDL and chylomicron transport may vary with species, with type of lipid, and with composition and size of fatty meal. For example, VLDL transports nearly all absorbed cholesterol in the rabbit¹⁰³. In rats, a high proportion of saturated fatty acid (C 16:0) in the meal promotes triglyceride transport in VLDL¹⁰⁴. The size of chylomicron increases with increasing load of fat absorbed. The soluble lipoproteins (low-density lipoprotein and high-density lipoprotein) contribute little to lymphatic transport.

The subdivision of transport particles into VLDL and chylomicrons is an operational distinction, whereas the spectrum of particles may be continuous. Nevertheless, the distinction could be important. There seem to be differences in rate of clearance from the circulation and in metabolic fate of transported lipid between VLDL and chylomicrons¹⁰⁴ and between small and large chylomicrons. The rapidly growing body of knowledge concerning interchanges of lipid and apoprotein between particulate and soluble lipoproteins is outside the scope of this chapter.

Increased production of intestinal lymph is a well-known accompaniment of rapid fat absorption. The lymphagogue effect is potent. Evidence suggests that it may be mediated by a humoral agent acting on the microcirculation, but thus far none of the common vasoactive agents has been implicated⁸³.

Quantitative information is technically difficult to obtain on the proportion of absorbed lipid passing directly into the portal blood capillaries. An upper limit can be set by the discrepancy between amount absorbed and amount recovered in lymph. At one extreme lies cholesterol, which seems to be transported almost entirely in lymph. At the other extreme are short- and medium-chain fatty acids. These appear in lymph only in the small amounts to be expected from bulk drainage of tissue fluid into lymph and, in the case of medium-chain fatty acids, from a minor incorporation into mixed triglycerides. For long-chain fatty acids, as already noted, the discrepancy between absorption and lymphatic recovery is often small (less than 20%). Large differences should arouse suspicion that lymph is by-passing the cannulated duct, either in collateral channels or by lymphatic-venous anastomoses. Recovery of labelled long-chain fatty acid, mainly unesterified, in portal blood of rats with lymph fistula has provided direct evidence of vascular transport. Possibly, if re-esterification of long-chain fatty acids is slow or defective, a larger proportion may be transported in portal blood⁸³.

10.9 CONCLUSION

The absorption of lipids has been discussed as a physicochemical problem, and some current ideas have been summarised on the way in which interaction of lipid molecules with water and with other lipids influences their fate at each step in absorption.

In the lumen, triglyceride digestion and the entry of bile provide a supply of swelling amphiphiles and of detergents (bile acids), which are particularly well suited for micellar solubilisation of swelling amphiphiles. The latter, in turn, promote solubilisation of other lipid molecules. Thus, triglyceride digestion indirectly promotes absorption of other lipids.

Micellar solubilisation accelerates the delivery of lipid by diffusion across an unstirred layer. The large increase in aqueous lipid concentration with solubilisation outweighs the moderate decrease in diffusibility due to micellar aggregation.

Passage into and through the cell membrane is probably a monomolecular process. Small differences in hydrogen bonding or other hydrophilic properties of the molecule greatly modify the efficiency of transfer into membrane lipid. Small differences in molecular configuration could have a considerable effect on translocation of the molecule through the structured lipoprotein membrane.

The mode of transfer of molecules from lipid membrane to aqueous cytoplasm and the changes in physical state during metabolism are not understood. The binding of lipid by specific proteins may be significant. The end result is mainly a reaggregation of weakly polar lipid, stabilised as small particles (VLDL) or larger ones (chylomicrons) by a swelling amphiphile, phospholipid and a specific apoprotein. Synthesis of this apoprotein by the enterocyte is important for both assembly and extrusion of particles. After leaving the enterocyte, particulate lipid is removed by lymphatics, which are highly permeable. Some of the lipid, usually a small proportion, is removed in water-soluble form by the portal blood stream.

At present, any treatment of lipid absorption as a physicochemical problem will be speculative, incomplete, and—when attempted by a biologist—probably naive. Nevertheless, this account may serve to show how the molecular biophysics of lipids has already illuminated some aspects of absorption and has pointed out problems remaining to be solved.

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