review

Intestinal absorption of fats

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SUMMARY Great progress in understanding the mechanisms of fat absorption by the mammalian intestine has been made in the past decade and a half. In contrast to the contentions of rival theorists of the century before this, it has been possible for many laboratories throughout the world to confirm observations made by use of the new tools available, and to come to agreement on current interpretations of the data. A brief survey of the historical background is presented to establish mainly a point of departure, after which some of the notable contributions of the postwar period and early Fifties are discussed in time sequence.

In developing the review of advances during the past decade, however, I have chosen to focus upon metabolic events occurring in the epithelial cells of the gut mucosa. For emphasis on these events a deviation has been made from the usual anatomic sequence of discussion in order to consider the glycerol moiety of the glycerides synthesized in the cells, the activation of fatty acids, and the pathway of direct esterification of monoglycerides. By this device I have attempted to underscore the importance of the components of the intraluminally formed micelles, and the intracellular partition based upon chain length and solubility. Also, it is hoped that the biochemical transformations may be borne in mind when considering the morphologic and fine structure changes associated with pinocytosis, possible micellar absorption of lipid, intracellular transport of the fat droplets, and the release of finished chylomicrons into the lacteal spaces.

Finally, some of the structural determinants of glyceride synthesis and the energetics of the over-all process of triglyceride absorption are mentioned before a statement of the present position is offered.

Compounds containing long hydrocarbon chains possess chemical and physical properties of great value to the mammalian organism, especially the two properties of yielding large amounts of energy upon oxidation and being insoluble in water. Fat, by virtue of its high energy yield per unit weight, provides a conveniently portable fuel. The low solubility of lipids makes them useful in membrane dividers separating cells and the myriad aqueous microcompartments essential to the existence of complex multicellular animals.

These valuable fatty substances are gathered from the environment, without altering or shortening the long chains of approximately 12 to 20 carbon atoms, by mechanisms which have evolved in the higher animals, including man, for efficiently absorbing sizable quantities of lipids. Most abundant in the natural plant and animal foodstuffs are the glycerides, or fats, although there are many other lipid substances of importance in mammalian diets, such as sterols, phospholipids, and the vitamins A, D, E, and K. It may seem astonishing that anything of significance should remain undiscovered in such an obviously important area as the digestion and absorption of fats, but many points do remain to be clarified. This review will be concerned primarily with intestinal triglyceride absorption, especially with the advances in our understanding of its mechanisms made in the years since World War II, and will refer only briefly to the literature of the past century.

During the past 16 years progress in this field has been impressive; the climate of controversy over great rival theories championed by outstanding individuals and manifested by strong opinions has changed into a generally cooperative advance. With the gradual unfolding of facts confirmed in several laboratories in Europe and the United States, all the older theories have become untenable in whole although verified in part. Critical to forward impetus in the field of fat absorption have been the development and exploitation of new tools, which have made possible the collection of previously unobtainable

data and the reevaluation of older work. The most powerful of these new tools have been (*a*) the electron microscope, which has revealed a whole new order of magnitude of cellular ultrastructure, (*b*) the general availability of radioactive isotopic tracer compounds, (*c*) the many chromatographic techniques for separation and identification of lipid compounds, (*d*) techniques of lymphatic cannulation and in vitro preparations of isolated intestinal tissue, (*e*) the preparative ultracentrifuge for separating cell parts and organelles, and finally (*f*) the techniques of intestinal mucosal biopsy, which have been revolutionary in their effect on the study of clinical disorders of fat absorption in humans.

Spearheading the effort to unravel the complexities of fat absorption for almost 15 years have been the laboratories in Sweden, directed by Bergström and by Borgström, whose incisive studies have provided consistently solid advances, and whose reviews (1–3) have brought much previous work into perspective. Other excellent reviews include the classic, encyclopedic work of Deuel (4) and the recent surveys of Wilson (5) and Johnston (6), in which these authors have incorporated their own important contributions into the most current concepts.

The over-all process of absorbing fats, beginning with the eating of a meal and culminating with the entrance of lymph chylomicrons into the venous blood, comprises the first of several major intercompartmental transfers of triglycerides in lipid metabolism. Fat is ingested into the lumen of the gastrointestinal tract mainly in the form of mixed triglycerides from meats, dairy products, or vegetable oils. In the vascular channels of lymph and blood, the lipid is found again as triglycerides in the form of chylomicrons suspended in the aqueous circulating fluids. It has taken over 100 years to resolve the conflict over whether dietary triglycerides are absorbed intact or whether they require hydrolysis to glycerol and fatty acids in the gut lumen for efficient absorption and resynthesis into triglycerides.

Background

Olof Rudbeck recorded in 1653 (7) his observations on the milky appearance of the thoracic duct after a fat meal. This was two centuries before Claude Bernard in 1855 lectured on his observations of the white opacification of mesenteric lacteals of dogs and rabbits during absorption of a fatty meal, and commented on the role of pancreatic juice in digesting neutral fats (8). These findings constituted a most important contribution indeed, and laid the foundation for progress in the latter nineteenth century. It had been noted that finely emulsified fat droplets could be seen in gut epithelial cells (9) and postulated that they simply filtered between tiny rod-like structures or through pores (10, 11) in the intestinal cell striated borders, as described by the microscopists of the midnineteenth century. This rather crude Particulate Theory developed and changed to the point that it was thought by Hoppe-Seyler (12) that a part of the fat was split by pancreatic lipase to glycerol and fatty acids, which formed soaps; these combined with bile which stabilized the emulsion passing through the tiny channels in the striated borders more or less directly into the lymph. In 1880, Munk (13) demonstrated that dogs could maintain weight quite well when fed free fatty acids instead of equivalent amounts of fats, and that free fatty acids were converted in the mucosa to neutral fats which appeared in the lymph. At the turn of the century Pflüger (14) vigorously championed the Lipolytic Theory, which held that fats were totally hydrolyzed to glycerol and fatty acids, which were absorbed as soaps or dissolved in bile. The site of resynthesis was thought by Moore (15) to be the intestinal mucosal cells, using energy from glucose oxidation to drive the endothermic synthetic reaction. The Lipolytic Theory held sway for about 40 more years, and was well set forth by Verzár and McDougall (16), and by Bloor (17).

Serious question concerning the extent of triglyceride hydrolysis was raised by Frazer (18) in 1938, based on his own chylomicron counts of portal and systemic blood during olive oil or oleic acid absorption. The earlier findings of Mellanby (19) of rapid lacteal opacification after giving fat emulsified with bile, supported by his own chylomicron data, led Frazer to propose his Partition Theory in an attempt to explain some of the apparently contradictory evidence. His experiments on the composition of chylomicrons (20), intestinal emulsification of fats and paraffins (21), differences between fat and fatty acid absorption (22), formation of fine stable emulsions composed of triglycerides, bile salts, and monoglycerides (23), and the formation of partial glycerides during fat digestion (24) led to strengthening of his arguments against the Lipolytic Theory. Major defects in the latter were the ideas that fat was completely hydrolyzed in the intestinal lumen (14), that paraffins were not absorbed (25), that the free luminal surface of the intestinal epithelial cells was a solid membrane (26), and that no significant amount of fatty material passed up the portal vein during absorption (27). Frazer also disagreed with the idea that adrenal hormones controlled phosphorylation of fat in the gut mucosal cells (28). His Partition Theory, advanced as an alternative working hypothesis and more fully elaborated in his 1946 review (29), was a more sophisticated version of the old Particulate Theory of the previous century. This concept prevailed generally until the work of the past 10 or 15 years showed it in turn to be inadequate in many respects, although it had great heuristic value. Use of the potent new methods mentioned above has led to no new sweeping theories or

major controversies, but to a broad reassessment of all the older ideas and much reliable new information which has been verified in many independent laboratories.

As a point of departure in our consideration of the succession of these illuminating findings, a summary of the key points in the Partition Theory may provide a basis for understanding the significance of the discoveries and work related to intestinal absorption of fat during the past 16 years. As expressed in Frazer's 1946 review (29), the Partition Theory proposed that:

> Intraluminal hydrolysis of triglycerides was partial, not complete (30, 24), and yielded fatty acids, diglycerides, monoglycerides, and later, some free glycerol, plus some residual triglycerides. Pancreatic lipase might be displaced from the oil–water interface by free fatty acids split off from glycerides, and thus would not produce complete hydrolysis under physiologic conditions.
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> The extent of hydrolysis depended on the type of fat eaten, the particular conditions in the intestine, normal functioning of the epithelial cells, and other metabolic activities.
>
> The luminal emulsion was most effectively dispersed by the *ternary* complex of fatty acids, bile salts, and monoglycerides, not by soaps, bile salt–fatty acid complexes, phospholipids, nor by any binary combination of any of these.
>
> The emulsion droplets of diameter less than 0.5 μ passed through the spindle-shaped "pores" or "canals" in the intestinal brush border described by Baker (31) and into the cells. Charged particle absorption was thought to be a process regulated by electrolytes in the membrane but not directly by adrenal hormones. Even paraffins could be absorbed well if emulsified to droplets of diameter less than 0.5 μ.
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> Once in the cells the droplets perhaps acquired a coating or admixture of newly synthesized phospholipids (32, 33), entered the lymph as chylomicrons, then were carried to the adipose tissue depots.
>
> Intracellular synthesis was not essential to absorption. Phosphorylation as a necessary stage in resynthesis (34), or related to adrenal hormones (28), was denied, although Frazer granted that phospholipids might be important to interfacial changes.
>
> Fatty acids liberated by hydrolysis, and separated from the unsplit fat emulsion droplets, entered the cells by an unknown mechanism along with other water-soluble compounds. Inside the intestinal mucosal cells, the fatty acids did not move with the fat droplets to the lymphatics, but were carried by the portal vein to the liver. This constituted an alternative pathway of fat absorption.

Work of the Past Sixteen Years

Confirmation of one point was made by Zilversmit, Chaikoff, and Entenman (35), who showed that phospholipid turnover was insufficient to account for all neutral fat absorption by means of synthesis via a phosphorylated intermediate. However, it was only a few years before the first wedge was driven into the framework of ideas associated with the Partition Theory. In 1949 the electron microscope studies of Granger and R. F. Baker (36) revealed that the striations in the epithelial cell borders were due not to canals as proposed by J. R. Baker (31), but to thousands of rod-like processes, as had been stated in 1857 (37) but not accepted. These structures, averaging less than a micron in length and about a tenth as much in width, covered the free surface of the intestinal mucosal epithelial cells, more than 1000 per cell, and increased the surface area of the cells by a factor of 10 to 30. A further flaw in the Partition Theory quickly appeared when Bloom, Chaikoff, Reinhardt, Entenman, and Dauben (38) reported that 83–93% of free palmitic acid labeled with C^{14} at the carboxyl position was absorbed, of which 70–92% was recovered from the thoracic duct lymph of rats, while only 0.2–0.4% reached the liver in the rats whose lymph was drained off by thoracic duct cannulae. These results depended upon the use of the increasingly available radioactive isotope C^{14} and the technique of cannulation of thoracic and intestinal lymph ducts developed by Bollman, Cain, and Grindlay (39), which made it possible to study absorption in the unanesthetized animal. Using this technique, Bloom, Chaikoff, Reinhardt, and Dauben showed that long-chain fatty acids were absorbed almost completely via the lymph, where they were incorporated into the phospholipids (40) as well as triglycerides, and that the partition of fatty acids between lymph and portal blood depended on chain length (41). They found that only 5–19% of the 10-carbon acid absorbed appeared in the lymph, compared to 84–95% of the 18-carbon acid. By studying the absorption of intermediate even-numbered fatty acids and pentadecanoic acid (42), and the concentration of isotope in the portal blood (43), it became clear that major proportions of fatty acids shorter than 12-carbon atoms were transported directly to the liver via the portal vein after absorption rather than being incorporated into lymph glycerides. It is of interest to note that this finding had been anticipated in 1913 by Raper (44), who showed the lesser tendency of shorter fatty acids to be absorbed as glycerides in the lymph, using iodine numbers and mean fatty acid molecular weights. He had proposed that there should be a gradual transition from one mode of absorption to the other, depending on chain length.

Another milestone of great importance was the demonstration by Mattson, Benedict, Martin, and Beck in 1952

(45) that pancreatic lipase in its hydrolytic attack on triglycerides appeared to be specific for the ester bonds at the outer two positions, so that the predominant cleavage products of fat were the 1,2-diglycerides and 2-monoglycerides, as well as free fatty acids. This was shown also for other triglycerides such as glyceryl tripropionate by Schønheyder and Volqvartz (46) in the same year, the result being confirmed and extended by Borgström (47, 48), Mattson and Beck (49), and Savary and Desnuelle (50). Formation of partial glycerides had been observed during the hydrolytic attack of pancreatic lipase on neutral fats by Artom and Reale in 1935 (30), and the special emulsifying properties of monoglycerides and bile salts on triglycerides had been demonstrated by Frazer, Schulman, and Stewart (23). Production of impressive quantities of monoglycerides was shown by Frazer and Sammons (24) during hydrolysis in vitro of olive oil suspensions by pancreatic lipase, with a rather small amount of complete cleavage of the triglycerides to free glycerol. The relative resistance of monoglycerides to attack by pancreatic lipase was further shown by Desnuelle, Naudet, and Rouzier (51, 52). Reversibility in vivo of triglyceride hydrolysis to lower glycerides and fatty acids was shown, although reesterification of glycerol with free fatty acids by pancreatic lipase in the intestine was not demonstrable (51, 53).

The Glyceryl Backbone of Glycerides

It was thought that glycerol once liberated by complete hydrolysis of a glyceride was irretrievably lost and could not be reincorporated into lymph fats. This was "proved" by several groups (54–57) who after feeding various forms of labeled glycerol along with fatty acids or triglycerides found negligible labeling of lymph glycerides, and the idea was supported by failure to find a glycerokinase in the intestinal mucosa (35, 38). Working on the assumption of no reincorporation of glycerol, many investigators carried out a great variety of experiments designed to determine the extent of hydrolysis of triglycerides prior to absorption. This knotty problem had been argued for over a century and continued to defy efforts to design a completely convincing experiment. It did emerge, however, that large amounts of fatty acids were liberated during digestion, on the order of 75% of those originally esterified to triglycerides (55, 59), but that probably less than half of the glycerides were split completely to free glycerol. Thus, it appeared that the major form in which fat was absorbed was as fatty acids, in terms of the relative number of lipid molecules entering the gut mucosa.

Meanwhile, an important reaction had been demonstrated by Kornberg and Pricer, who showed that liver microsomal particles catalyzed the esterification of glycerophosphate to phosphatidic acid (60) as well as the activation of free fatty acids to fatty acyl coenzyme A thiolesters (61). The importance of glycerophosphate as an intermediate in the incorporation of inorganic phosphate into phospholipids in liver mitochondria was demonstrated also by Kennedy (62) at about the same time. Dihydroxyacetone derived from glucose had been suggested by Reiser and Williams (63) as a possible substrate for esterification in the gut mucosa, and they were able to show that doubly labeled palmitoxyhydroxyacetone was converted in part into lymph glycerides. Later (64), Buell and Reiser showed that dihydroxyacetone phosphate and glycerophosphate, but not free glycerol, acted as precursors for glyceride glycerol synthesized in the mucosa. The free glycerol liberated from triglycerides in the intestinal lumen appeared to be extensively oxidized, as shown by Gidez and Karnovsky (65). The supposition that glucose provided the glycerophosphate for glyceride synthesis was convincingly substantiated by Dawson and Isselbacher (66), who demonstrated labeling of glyceride glycerol but not of fatty acids when slices of rat jejunum were incubated with glucose-C^{14}. They showed further the marked stimulation of incorporation of glucose carbon into glyceride glycerol upon addition of fatty acids or especially upon adding conjugated bile salts. Buchs and Favarger (67) had shown also that label from glucose-U-C^{14} administered intravenously to rats fed oleic acid appeared 12 minutes later in the glycerol portion of intestinal and liver lipids but almost none appeared in the fatty acid moiety. Further, they provided evidence which shook the idea that free glycerol was not utilized for intestinal fat synthesis by showing high specific activities of lipid glycerol after intravenous injection of glycerol-1-C^{14} during oleic acid ingestion. Comparable quantitative incorporation and even higher specific activities were found in the intestinal mucosa compared to those of the liver, and glycerol was better incorporated than glucose under these conditions. They showed again the poor incorporation of free glycerol fed along with fatty acids, which they thought to be due to more rapid absorption of the water-soluble glycerol. By administering glycerol-C^{14} mixed with oleic acid and taurocholate directly into duodenal segments of rats, however, Saunders and Dawson (68) were able to recover 12–31% of the glycerol radioactivity in lymph lipids. The importance of this observation was to emphasize the need to allow the glycerol and fatty acids to enter the intestinal cell simultaneously in order to observe maximal incorporation of the more soluble glycerol.

Although some phosphorylated compounds containing C^{14} were noted during these experiments, they were not identified. It was shown by Haessler and Isselbacher (69) and by Clark and Hübscher (70) that the intestinal mucosal cells do contain a glycerokinase, despite earlier reports to the contrary. This enzyme produces L-glycerol-3-phosphate from glycerol using energy from the phos-

phorylytic cleavage of ATP and is now more properly designated (71) as ATP:glycerol phosphotransferase [2.7.1.30]. It appears to be localized in the cytoplasm of small intestinal epithelial cells (72); the specific activity of the glycerokinase of the intestinal cell sap appears greater in the rat than in the hamster, somewhat less than the liver cytoplasmic enzyme in both species, and greater in the lower than in the upper small bowel. Free glycerol can also be incorporated into lipids by intestinal homogenates, and this process may be stimulated by fatty acids (68) or by conjugated bile salts (73). Holt has shown incorporation of labeled glycerol into lymph lipids of the human in a case of chyluria (74). A possible explanation for earlier failures to demonstrate an intestinal glycerokinase may be provided by the finding of a very active glycerophosphatase in the same tissue (70, 72), which under certain conditions might mask the glycerokinase activity.

The implication of these recent observations is that the old assumption of no reutilization of glycerol after total hydrolysis of glycerides is invalid, and that calculations concerning the extent of digestive hydrolysis of fats will have to be made in consideration of this point.

The Activation of Fatty Acids

Fatty acids are made up of a long, rather inert hydrocarbon "tail" and a terminal carboxyl group which represents the only reactive group or chemical "handle" present. In all the known metabolic reactions of fatty acids, such as chain lengthening, oxidation, or esterification, the fatty acids have been shown to be first "activated" to coenzyme A (CoA or CoASH) derivatives. Kornberg and Pricer had found (75) using guinea pig liver that the enzymatic esterification of glycerophosphate by fatty acids to produce phosphatidic acids required participation of adenosine triphosphate (ATP) and CoA. They reasoned that these requirements pointed to the first step in the esterification process being an activation of the fatty acid to an acyl CoA, analogous to the acetate activation reported earlier (76), and found an enzyme which catalyzed the conversion of long-chain saturated and unsaturated monocarboxylic acids to their CoA thiolesters. The latter derivatives were isolated from the reaction mixture along with inorganic pyrophosphate (PPi) split off from ATP as a result of the action of the enzyme present in the guinea pig liver microsomal fraction. This enzyme did not activate the short-chain fatty acids; it was later termed a fatty acid thiokinase, or fatty acyl CoA synthetase, and more recently (71) fatty acid: CoA ligase (AMP) [6.2.1.3]. The reaction (Fig. 1) is reversible, requires magnesium ion to bind phosphate groups, and in the analogous case of acetate, synthetic acetyl adenylate can replace ATP and acetate, as shown by Berg (77). However, during the reaction no free acetyl adenylate could be demonstrated to accumulate in the absence of CoA, so that Ingraham and Green (78) postulated an intermediate enzyme complex with the reactants. These points have been summarized and discussed by Cornforth (79) and are thought to apply equally well to long-chain fatty acids. It should be pointed out that this transformation utilizes part of the energy released by the pyrophosphate cleavage of ATP to create the reactive thiolester compound with CoASH, and that the resulting fatty acyl SCoA derivative not only is much more chemically reactive than the fatty acid but has also become water-soluble at neutral pH by virtue of many polar groups on the large CoA moiety (Fig. 2).

Johnston, using everted sacs and segments of hamster small intestine, showed that fatty acids could be esterified in the intestinal mucosa (80), and that triglycerides were the major product (81) in the intestinal wall and serosal fluid. Jedeikin and Weinhouse had also showed that intestinal slices were active in esterifying fatty acids (82). Using homogenates of rat small intestinal mucosa, Dawson and Isselbacher (83) demonstrated that incorporation of C^{14}-labeled fatty acids into neutral fat was dependent upon CoA, ATP, and magnesium ions. They showed further (84) that short-chain fatty acids such as octanoate were not well incorporated, that suppression of lipase activity with fluoride and polyoxyethylene

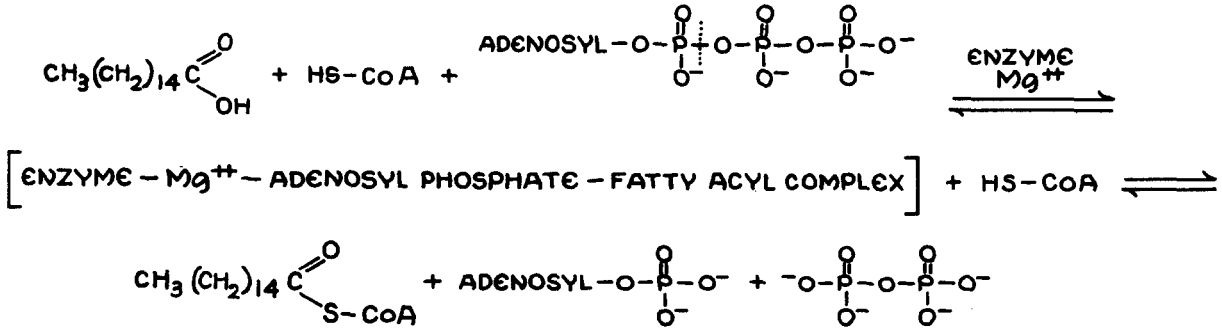

FIG. 1. The activation of fatty acids: proposed mechanism for the formation of fatty acyl thiolesters. Note that ATP is split into AMP plus pyrophosphate, and that the new bond formed is a carbonyl to sulfur link, or thiolester.

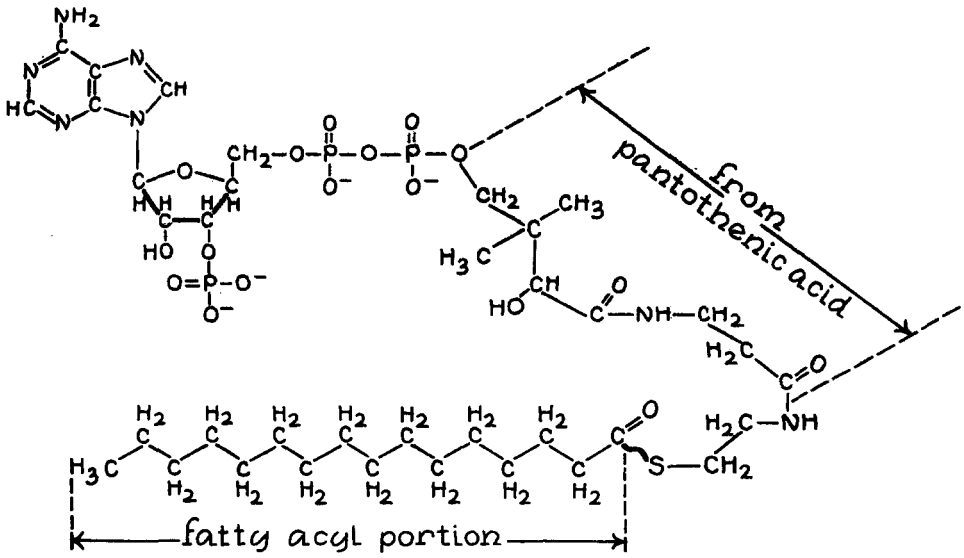

FIG. 2. An expanded structural diagram of palmitoyl-SCoA, to emphasize the relatively large bulk of the water-soluble, polar CoA moiety compared to the nonpolar, hydrocarbon "tail" of the fatty acyl portion.

sorbitan monooleate (Tween 80) enhanced incorporation, and that proximal small gut homogenates were several times as active as those from the ileum.

At almost the same time Clark and Hübscher (85) independently reported synthesis of tri- and diglycerides from labeled palmitate in a system containing rabbit small intestinal mitochondria. Their results were dependent on the presence in the system of CoA, ATP, and Mg^{++} ions. By adding glycerophosphate they were able to produce a marked stimulation of this glyceride synthesis, but added 1-monostearin or 1,2-dipalmitin produced lesser degrees of stimulation. The addition of glycerophosphate to similar reaction mixtures containing rat intestinal mitochondria did not produce much stimulation of palmitate incorporation into glycerides, although the rat intestinal alkaline phosphatase activity was 14 times as great as that of the rabbit. They also demonstrated the formation of fatty acyl CoA compounds in rabbit mitochondria, using a hydroxamate assay system (61). Study of the rat proximal jejunal epithelial cell fractions by Senior and Isselbacher (86) disclosed even higher specific activity of the fatty acid : CoA ligase in the microsomal fraction than in mitochondria, and very little in the cell sap. These findings were in contrast to those of Kornberg and Pricer (61), who found in guinea pigs considerable liver cell sap activity and no appreciable activity in any fraction in tissues other than liver. The fatty acid-activating enzyme of rat intestine was found to be thermolabile, more active in microsomes from duodenum and jejunum than in those from ileum, much less active toward fatty acids of less than 12 carbons, and dependent upon CoA, ATP, and Mg^{++}. This same substrate specificity and characteristics have been confirmed for hog intestinal mucosa by Ailhaud, Sarda, and Desnuelle (87) and the subcellular localization has been confirmed as in the microsomal fraction by workers in the same laboratory (88).

Thus, the first step in glyceride synthesis from fatty acids in the intestine, as elsewhere, seems to be the activation to fatty acyl CoA, using energy from ATP. It is notable that the pathway of synthesis here is distinct from that of breakdown, and that the process is not simply a reversal of the effect of pancreatic lipase, consistent with the finding that other synthetic pathways are different from those of breakdown in the case of glycogen, fatty acids, etc. in several tissues. It seems reasonable to postulate that the partition of fatty acids between the esterified form in lymph and the free (probably albumin-bound) form in portal blood may be a function of whether or not they are activated to the CoA derivatives, although this is difficult to prove in vivo.

Monoglycerides: A Shunt Pathway

The demonstration by Kornberg and Pricer (60) and by Kennedy (62) that L-glycerol-3-phosphate was esterified in liver by fatty acyl thiolesters to form phosphatidic acid led to the supposition that the same pathway to glyceride synthesis was probably active in the intestinal mucosa. Indeed, Johnston and Bearden showed incorporation of P^{32} into phosphatidic acid when everted hamster gut sacs were incubated with palmitate and $NaH_2P^{32}O_4$ (89). Conversion of phosphatidic acid to 1,2-diglycerides by the enzyme phosphatidate phosphatase, or L-phosphatidate phosphohydrolase [3.1.3.4] was shown to occur in

the gut mucosa by the same authors (90) and by Coleman and Hübscher (91) at about the same time. The same sort of reaction had been shown in liver tissue in the earlier studies of Smith, Weiss, and Kennedy (92) and by Stein and Shapiro (93). However, Clark and Hübscher (85) had noticed that 1-monostearin and 1,2-dipalmitin stimulated incorporation of C^{14}-labeled palmitate into di- and triglycerides when rabbit small gut mucosal mitochondria were incubated with these substrates in the presence of cofactors ATP, CoA, and Mg^{++}. This stimulation was almost as marked as that observed when glycerol-3-phosphate was added, and suggested an additional pathway of direct monoglyceride esterification to diglyceride. In a more complete description of their work (94), they reported net synthesis of diglycerides and some triglycerides in rabbit gut mitochondria using DL-glycerol-3-phosphate as an acceptor for the labeled palmitate, which was activated to the thiolester in the presence of the cofactors. Addition of unlabeled phosphatidic acid reduced incorporation, while inhibition of phosphatidate phosphatase by polyoxyethylene sorbitan monolaurate (Tween 20) reduced incorporation of palmitate into neutral glycerides by almost 90%. In contrast to this, Tween 20 did not at all inhibit the incorporation of palmitate-1-C^{14} into glycerides using monoolein as an acceptor, which was evidence for the direct acylation of monoglycerides to diglycerides without phosphatidic acid intermediates being necessary.

Substantial amounts of monoglycerides were known to be formed during digestion of fats (30, 24, 45, 55, 95). Reiser and Williams (63), as well as Skipski, Morehouse, and Deuel (96) had shown by labeling both the glycerol and fatty acid portions of monoglycerides that the monoglycerides were absorbed and incorporated into lymph triglycerides, at least in part. Since hydrolysis of absorbed monoglyceride would produce free glycerol, shown earlier to be poorly incorporated into lymph triglycerides, the direct esterification of monoglyceride to higher glycerides seemed reasonable (94).

Direct demonstration of the acylation of monoglycerides by synthetic palmitoyl CoA was reported very shortly afterward by Senior and Isselbacher (98, 99), using rat intestinal epithelial cell microsomes. In the rat intestinal mucosa, the esterification of 1-monoglycerides by palmitoyl-1-C^{14} CoA was catalyzed best by the microsomal fraction rather than the mitochondria, and the effect was not additive. Monoolein appeared to be esterified preferentially to diglycerides, but monopalmitin yielded mainly triglycerides. Since transesterification could have accounted for labeling of glycerides without necessarily producing net synthesis, experiments were carried out with unlabeled palmitoyl CoA and DL-glyceryl-1(3)-C^{14}-1-palmitate (monopalmitin-C^{14}). Both labeling of di- and triglycerides and net ester bond synthesis were shown to occur. No esterification of free glycerol-1-C^{14} could be demonstrated, so preliminary hydrolysis of the monoglyceride seemed excluded as a mechanism. Further, added ATP did not stimulate formation of higher glycerides, and no significant amounts of phospholipid intermediates were detected at any time during the synthesis of higher glycerides from monopalmitin-C^{14} and palmitoyl CoA. Clark and Hübscher (97) later found the monoglyceride-esterifying enzymes in the cell sap of rat intestinal cells, as well as associated with membrane structures.

Therefore, the pathway of direct acylation of mono- to diglycerides was clearly established. In the absence of palmitoyl CoA, free palmitate and CoA in equimolar amounts produced no higher glycerides, and none was formed when the microsomal fraction was inactivated by boiling beforehand. In fact, when the activated fatty acyl thiolesters were not present, the monopalmitin was split nearly completely to free glycerol and palmitate by the microsomes, indicating a competing glycerol monoester hydrolase to be active in the same subcellular fraction. Almost twice as much diglyceride was formed from 2-monopalmitin in 10 minutes as from DL-1-monopalmitin; no attempt was made to separate the 1,2- from the 1,3-diglycerides produced. Rabbit intestinal mucosal microsomes had the same capability and were more active than mitochondria in their ability to catalyze the transfer of fatty acyl groups from CoASH to monoglycerides. Attempts to purify or render soluble the monoglyceride acyl transferase from the microsomal fraction resulted only in loss of activity, as had occurred when this was tried (86) for the ATP:fatty acid ligase (AMP). Johnston and Brown (100) provided elegant additional evidence for the intact incorporation of monoglycerides into di- and triglycerides. Using palmitoyl CoA and DL-glyceryl-2-H^3 1-(palmitate-1′-C^{14}) with hamster intestinal mucosal homogenates, they obtained H^3 to C^{14} ratios of 1.90, 1.89, and 1.82 for the mono-, di-, and triglycerides, respectively.

Clark and Hübscher (94) had noted that monoolein stimulated palmitate incorporation into glycerides about three times as much as monopalmitin did, using rabbit gut mitochondria. This marked difference was not noted when the rat intestinal microsomal fraction (99) was used. In the latter studies, it was observed that addition of 2-monopalmitin increased incorporation of palmitoyl-1-C^{14} CoA into diglycerides almost twice as much as did addition of DL-1-monopalmitin. It remained for Johnston and Brown to show that the free primary hydroxyl groups of the monoglycerides were acylated before the free secondary hydroxyl groups (101), so that 1,3-diglycerides were the major products when 1-monoglycerides were esterified, and 1,2-diglycerides when 2-monoglycerides were used. No stereospecificity could be shown during

glyceride synthesis in the intestinal mucosa. The 2-monoglycerides were much more rapidly converted to diglycerides than were the 1-isomers. Curiously, they found that more triglycerides were synthesized from 1-monopalmitin than from 2-monopalmitin under the same conditions (102). Ailhaud, Samuel, and Desnuelle (103) confirmed the preferential esterification of the primary hydroxyl groups of the monoglyceride, but found opposite results with respect to triglyceride formation, that is, more from the 2- than the 1-monopalmitin, using rats.

When diglycerides are synthesized from L-glycerol-3-phosphate, however, definite stereoconfiguration is preserved about the 2-carbon of the glycerol. In studies using chicken liver, Weiss, Kennedy, and Kiyasu (104) showed that in the conversion of diglycerides to triglycerides this stereospecificity was important, and was even more so in phospholipid synthesis. This was confirmed by Goldman and Vagelos (105) using chicken adipose tissue. They found also that 1,2-diglycerides were better esterified to triglycerides than were 1,3-diglycerides. That these findings need not necessarily be true for intestinal triglyceride synthesis is obvious, and is emphasized by the fact that the monoglyceride acyl transferase system is very much less active in liver (99), adipose tissue (99), aorta (106), breast (107) and kidney (108) than it is in the intestinal mucosa. The direct pathway from monoglycerides to diglycerides appears to be a nonstereospecific shunt mechanism for producing large amounts of di- and triglycerides rapidly during absorption of a fatty meal. Thus it provides an alternative route of synthesis to that from glycerol phosphate, which is stereospecific and may be of relatively more importance in phospholipid synthesis. The two pathways are shown in Fig. 3, and the enzymes involved are designated or tentatively named.

Ether analogues of 2-monoglycerides, such as 2-glyceryl octadecyl-1-C^{14} ether, may also be esterified in rat intestine, as shown recently by Sherr, Swell, and Treadwell (109), and by Johnston and Borgström (110). Esterification of chimyl alcohol, 1-glyceryl cetyl ether, had previously been demonstrated in lymph lipids by Bergström and Blomstrand (249).

It had been noted, however, that if monoglycerides were not esterified to diglycerides by acyl CoA, they were split to free glycerol and fatty acids by enzymes also found in the microsomal fraction of rat intestinal mucosa (98). Even earlier, Tidwell and Johnston (111) had noted

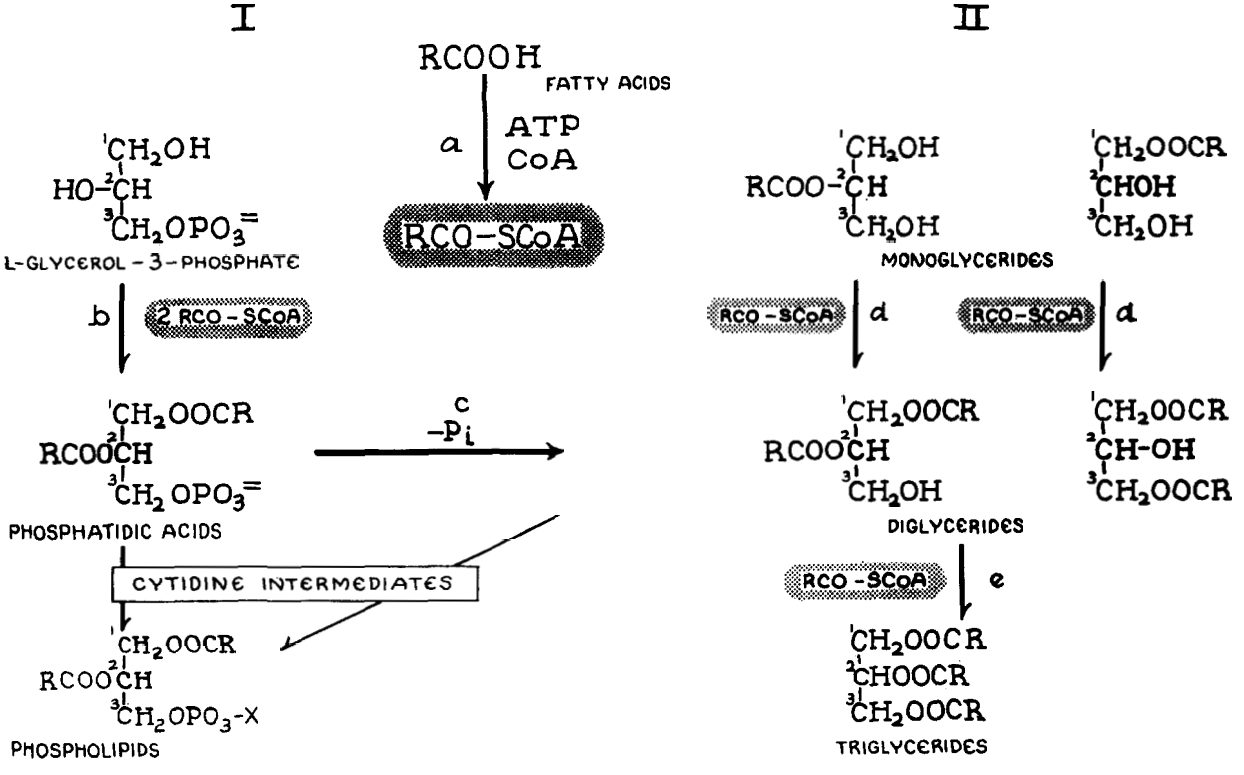

FIG. 3. The alternative pathways of diglyceride synthesis in the intestinal mucosa: I, The glycerol phosphate–phosphatidic acid pathway. II, The direct monoglyceride pathway. Enzymes involved are *a*, (fatty) acid:CoA ligase (AMP)[6.2.1.3], *b*, acyl CoA:L-glycerol-3-phosphate acyl transferase [2.3.1.15], *c*, L-α-phosphatidate phosphohydrolase [3.1.3.4], *d*, acylCoA:monoglyceride acyl transferase, *e*, acyl CoA:diglyceride acyl transferase. (Enzymes *d* and *e* have not been isolated and purified, nor officially named as yet.) The glyceride numbering system used here follows the well-established system in general use for the triose phosphates.[2]

splitting of monoglycerides incubated with everted sacs of hamster intestinal segments. DiNella, Meng, and Park (112) had reinvestigated the old idea of an intestinal mucosal lipase, and had described in hog intestinal homogenates a lipase which cleaved mono-, di-, and triolein at identical rates. This differed further from pancreatic lipase in that it was inhibited by serum albumin and was relatively insensitive to inhibition by dialysis, 0.01 M ethylenediaminetetraacetic acid, acetonitrile, and benzaldehyde. Further inquiry into the hydrolytic capacities of rat jejunal intestinal epithelial cells toward glycerides disclosed that the microsomal fraction showed a markedly greater ability to split monopalmitin than di- or tripalmitin, as reported by Senior and Isselbacher (113). Both 1- and 2-monoglycerides were hydrolyzed at roughly similar rates, depending on conditions in the suspending medium, and the enzymatic specific activity in the cell seemed greatest in the microsomal and mitochondrial fractions, with a little in the cell sap. Using 5 mM sodium taurocholate to suspend DL-1-monoglycerides, the microsomal fraction showed a hydrolytic activity which was maximal toward monoglycerides of medium chain length of around 10 carbons, and less for monobutyrin and monostearin. Because of these substrate specificities the enzyme was tentatively called monoglyceride lipase (113), although more correctly it should be designated as a glycerol monoester hydrolase.

McPherson, Askins, and Pope (114) had also found that homogenates of rat small gut hydrolyzed monoglycerides of long-chain saturated and unsaturated fatty acids much more than corresponding higher glycerides, and had no specificity for 1- or 2-monoglyceride isomers. Schmidt, Bessman, and Thannhauser (115) had commented upon intestinal mucosal hydrolysis of 1-monoglycerides, and the splitting of monoglycerides during triglyceride synthesis was noticed by Hübscher and Clark (116) as well. Tidwell and Johnston (117) had observed, however, that everted hamster gut sacs split monoacetin considerably more rapidly than monopalmitin, which would suggest that the enzyme which splits monoglycerides is more like the aliesterases reported in other tissues such as liver and kidney. Recently, Pope and Tidwell (118) have reported 200-fold purification from chicken intestinal mucosal microsomes of an enzyme that catalyzes monoglyceride hydrolysis, and have noted inhibition of this enzyme by taurocholate and fluoride.

Thus, two competing reactions exist for monoglycerides absorbed into the intestinal cell: (*a*) esterification to higher glycerides using acyl thiolesters, or (*b*) hydrolysis to free fatty acids and glycerol. Both processes are weighted by the substrate specificities of the acid:CoA ligase and the glycerol monoester hydrolase toward production of long-chain glycerides. No significant phosphorylation of monoglycerides to lysophosphatidic acids appears to occur in the intestinal mucosa, although this does happen in the brain (119) and liver (120). Further, lysophosphatidic acid is not acylated in the gut mucosa and there is no stimulation of monoglyceride incorporation into diglycerides by ATP. The evidence against phosphorylated intermediates in production of diglycerides from monoglycerides and in favor of direct esterification has been summarized in a dissertation by Brown (121).

Intraluminal Micelle Formation

Fats ingested in various mixtures in foods are converted to a coarse emulsion in the stomach by the churning, kneading, and squirting movements which result from normal gastric motility. Phospholipids in foodstuffs probably assist in the formation of this emulsion, and some free fatty acids are liberated in the stomach. It was thought by Schønheyder and Volqvartz (122) that a gastric lipase was responsible, but Herting and Ames (123) since have suggested that regurgitation of small bowel chyme is a more likely explanation. Although droplets of fat may be seen in the gastric mucosa (124), it has been known for a long time that there is no absorption of fat from the stomach, even when the pylorus is ligated (125). The chief role of the stomach seems to be to liberate fats and phospholipids from proteins by proteolytic digestion, to churn the mixture into a coarse emulsion, and to parcel out the emulsion in a regulated fashion by squirting small portions into the duodenum where it may be mixed with bile and pancreatic juice. There appears to be a control process which slows gastric motility and emptying when fat is being absorbed. Over 50 years ago Tangl and Erdélyi (126), and von Fejér (127) had noted that the acid and pepsin secretions as well as the emptying rate of the stomach were reduced by fat in the stomach, which agreed with earlier observations by Ewald and Boas (128) and Beaumont (129). Turner (130) has observed that the amount of fatty acids in the jejunal mucosa remains fairly constant during fat absorption. This perhaps suggests a negative feedback control system regulating gastric emptying to provide just enough fat emulsion for digestion and absorption. Recently, Long (131) has found considerable inhibition of gastric acid secretion after oleic acid infusion into the proximal small gut.

As portions of the emulsified fats are squirted into the duodenum by the stomach and mixed with bile, pancreatic juice, and the chyme already present, the triglycerides become subject to hydrolysis of the outer ester bonds by pancreatic lipase. Large amounts of this enzyme, officially designated as glycerol ester hydrolase, [3.1.1.3], are released into the duodenum, and the water–oil interface of the emulsion droplets is the site of hydrolysis. Products of the cleavage are free fatty acids, 1,2-diglycerides, 2-monoglycerides, and slowly, free glycerol.

The characteristics and properties of pancreatic lipase have been reviewed by Desnuelle (132), and the specificities of lipases in general by Desnuelle and Savary (133).

Phospholipids of foods, as well as those known to be plentiful in bile (134), are principally lecithins which are attacked by another pancreatic enzyme, phospholipase A, or phosphatide acyl hydrolase, [3.1.1.4], which has been isolated and partially purified by Magee, Gallai-Hatchard, Sanders, and Thompson (135). Its specificity is in contrast to that of pancreatic lipase, in that the 2-ester is hydrolyzed, as has been reported recently by the same authors in collaboration with De Haas, Heemskerk, and Van Deenen (136). The resulting lysolecithins are powerful detergents, but their role in fat digestion and absorption has not yet been clarified.

Also in bile are the bile salts, the principal products of cholesterol breakdown (137), which occur almost exclusively conjugated through a peptide bond with glycine or taurine. Although since 1896 (138) these bile salts were known to have good solvent properties, it was shown only recently by Hofmann and Borgström (139) that conjugated bile salts in the duodenum and jejunum form complexes or micelles with fatty acids and monoglycerides, the same ternary mixture which Frazer, Schulman, and Stewart (23) had found so soluble. The micellar ratio of bile acids to fatty acids was within the physiological range of concentration of the bile acids and much more economical of them than the 8:1 ratios called for according to the idea of "choleic acid" coordination complexes described by Wieland and Sorge (223). The formation of micelles and their role in fat absorption was reviewed by Borgström (3), and more recently he has reported (140) that the diameter of pure bile salt micelles indicated by gel filtration studies is 40 A. Hofmann has reported that both 1- and 2-monoglycerides are well solubilized in dilute, micellar bile salt solutions similar to those present in human small intestinal content during fat digestion (141). In micellar solutions, long-chain 1-monoglycerides are hydrolyzed much more rapidly by pancreatic lipase than 2-monoglycerides or shorter-chain 1-monoglycerides (142). Tightness of molecular packing in the micelle may be a critical factor in determining susceptibility to attack by pancreatic lipase, although as yet the details of micellar structure are not known. In the digestion of triglycerides, 2-monoglycerides are a major product, and are actually found as such in luminal content (45, 143). The 2-monoglycerides, along with fatty acids and conjugated bile salts, form micelles which may be absorbed readily and may represent the major pathway of fat absorption (3).

Intestinal slices and even isolated brush border preparations show rapid uptake of labeled monoolein and oleic acid from micelles made to include taurodeoxycholate, as found by Johnston and Borgström (144, 110). The uptake of fatty acid and monoglyceride was much faster than from an emulsified or albumin-bound state, and appeared to be a nonenzymatic step, independent of temperature and not requiring energy. Secondarily, the fatty acids and monoglycerides from micellar solutions were found to be incorporated quickly into triglycerides by an enzymatic and energy-dependent process, as discussed previously. Hamster intestinal slices incubated in micellar solutions containing oleic acid-C^{14} and 1-monoolein-H^3 in specific activity ratio of 1.96 showed almost equal uptake of each isotope, and the lipids isolated from the tissue were mostly triglycerides, with a C^{14}/H^3 ratio of 2.03, indicating uptake of fatty acid and monoglyceride together from the micellar state (6). Micelles, made up of the major products of digestion of triglycerides by pancreatic lipase plus conjugated bile salts, therefore form under physiologic conditions to give lipid particles estimated to be only 40–100 A in diameter. These particles are less than $^1/_{100}$ the diameter of the coarse emulsion droplets delivered by the stomach, and for a given amount of lipid have over 100 times the surface area. Instead of appearing milky or turbid as the emulsions do, the micellar solutions of lipids are clear because of their very much reduced light-scattering properties.

The fact that micellar solutions of lipids are so rapidly absorbed is not evidence against particulate absorption of fat droplets from the emulsified state, which also may occur. In considering the fate of a single triglyceride droplet attacked by pancreatic lipase at its oil–water interface, it seems reasonable that a whole family of smaller droplets of triglyceride must result as the fatty acids and 2-monoglycerides are liberated and are complexed with conjugated bile salts to form micelles. The remaining tri- and diglycerides in the oil droplets, which decrease in diameter from perhaps 10,000 A down to 1000 A or less, may to some extent be absorbed in this form. When the intestinal mucosa is damaged and its surface very much reduced, as in sprue, even normally formed micelles are slowly absorbed. They may remain in the lumen where they are subject consequently for a longer time to processes of isomerization and hydrolysis of the monoglycerides. This may result in more complete hydrolysis and the formation of more free fatty acids. Other disease states which prevent pancreatic juice or bile from mixing with the emulsified fat prevent the formation of micelles and very much impair the efficiency of fat absorption.

It is of great interest that the conjugated bile salts, although essential for micelle formation, are not absorbed in the proximal small intestine (145) where the major portion of fat is absorbed (146). Lack and Weiner have reviewed their work and discussed the usefulness of this mechanism (147), which permits bile salts to remain in the lumen of the proximal small gut where fat absorption is greatest, and to be reabsorbed in the ileum after fat

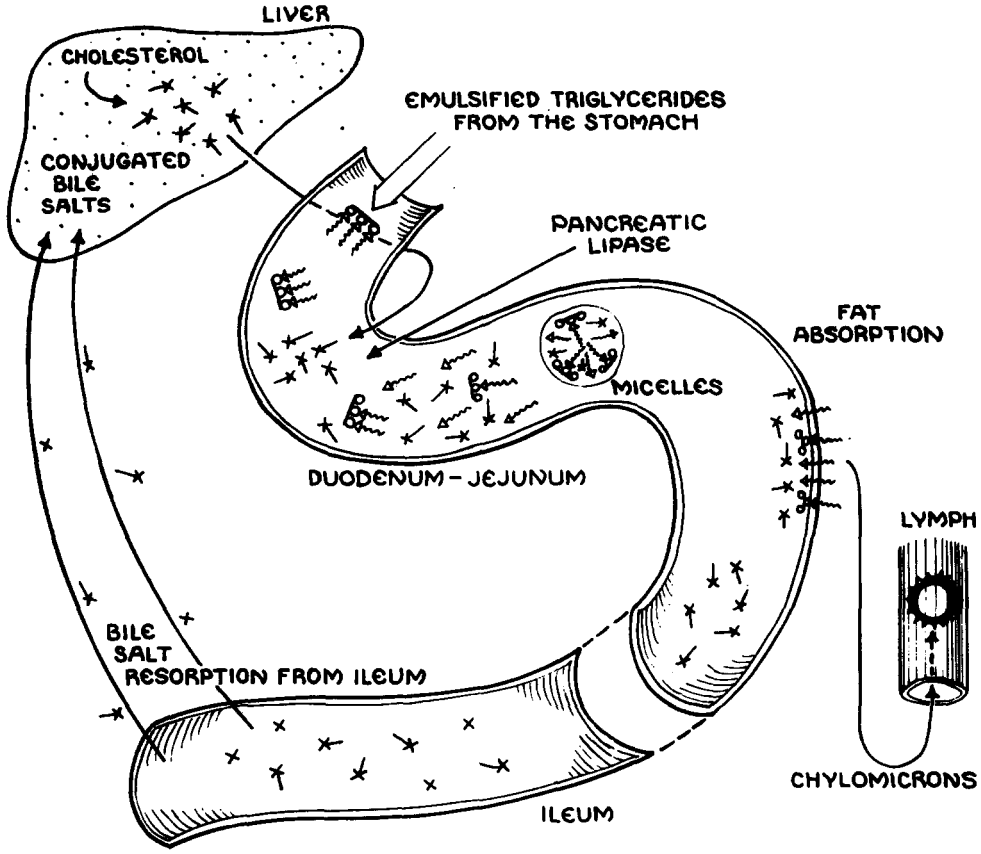

FIG. 4. A scheme of intraluminal micelle formation, fat and bile salt absorption: x–, conjugated bile salt; x, unconjugated bile salt; ◁〰, free fatty acid; OOO, free glycerol.

absorption is completed, then recirculate via the portal blood, liver, and bile to reenter the duodenum. Verzár and McDougall (16) had recognized that the amount of bile salt needed to solubilize the products of fat digestion was more than could be excreted during digestion. Lindstedt's estimates (148) of the bile salt pool size and turnover rate in man have confirmed this by means of studies using sodium taurocholate-S^{35} and sodium taurodeoxycholate-S^{35} and a nonabsorbable reference compound, polyethylene glycol (PEG). Borgström, Lundh, and Hofmann (149) found that in man net bile salt absorption is negligible in the proximal small gut but significant distally, in agreement with the findings of Baker and Searle (150) in the rat, and of Lack and Weiner (145) in rats and guinea pigs. Earlier, Frölicher (151) had recognized the significance of the intestinal site of bile salt absorption as an important part of the fat absorption process. Playoust and Isselbacher (152) have confirmed the absence in the jejunum of active transport mechanisms for bile salt absorption and in addition found them present in rat and hamster ileum. They have shown also that no hydrolysis or formation of the peptide conjugates occurs in the intestinal mucosa. Some of the points mentioned in this section are shown diagrammatically in Fig. 4.

The conjugated bile salts not only perform excellently in micelle formation, but appear to have several ancillary roles in which they facilitate steps in the absorption–digestion process. It had been noticed by Borgström (48) in 1954 that sodium taurocholate displaced the pH optimum of pancreatic lipase acting on olive oil emulsions from 8.0 to 6.0, which is into the range of pH actually encountered in the duodenum. Desnuelle (132) also observed a fourfold increase in the initial rate of hydrolysis of a triolein emulsion by pancreatic lipase at 37° upon the addition of taurocholate, a phenomenon not due to an increase in interfacial area but to a temperature-dependent enzyme activation mechanism. Another ancillary function of the conjugated bile salts, related to their micelle-producing capability, is the enhancement of dissociation of fatty acids in the micellar complex, as found by Hofmann and Borgström (142). Lowered apparent "pK'_a" values have been found for oleic acid in micelles made with monoolein and relatively high bile salt concentrations, these concentrations being comparable to those found in human intestinal content during fat

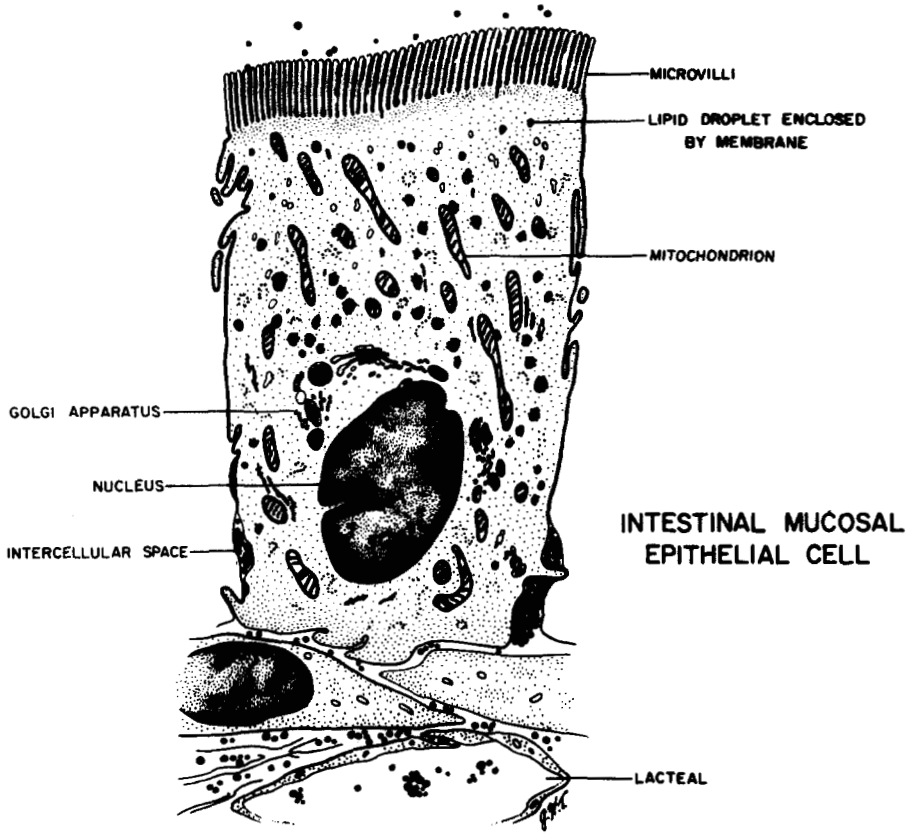

FIG. 5. Sketch of an epithelial cell during corn oil absorption, showing progression of oil droplets through the cells, from the lumen to the lacteal spaces, as observed in the electron photomicrographs of Palay and Karlin (155).

digestion (146). The importance of solubility in affecting fatty acid ionization had been recognized by Schmidt-Nielsen (153) and the lowering of the pK of long-chain quarternary amines by micellar solubilization had been noted by Weis and Hoerr (154). In addition, conjugated bile salts markedly stimulate carbohydrate incorporation into glyceride glycerol in jejunal slices, as shown by Dawson and Isselbacher (66), and Holt, Haessler, and Isselbacher (73). Also, pancreatic cholesterol esterase is both activated and protected against digestion by taurocholate (190). Another interesting property of conjugated bile salts, reported by Meyer and McEwen (224), is stimulation of rhythmic, oscillatory movements of the gut.

Pinocytosis and the Form of Absorbed Lipid

A notable contribution to the study of fat absorption was provided by Palay and Karlin (155), who, in 1959, published their beautiful electron microscope study of the intestinal villus epithelium in the rat. They had chanced to observe, as had Baker (156) and Hewitt (157), fat droplets in and between gut epithelial cells of animals which had eaten recently a fatty meal. They went on to study in detail the morphologic changes in the fine structure of these cells during absorption of corn oil. Droplets of the oil, stained with osmium tetroxide, with diameters of 500 to 1000 A, could be demonstrated in the lumen, between the microvilli of the intestinal epithelial cells, and again inside the cells where they were enclosed by thin membranes of the endoplasmic reticulum. In the deeper portions of the cytoplasm the enclosed droplets enlarged to 1100 to 2400 A in diameter. Aggregation of the droplets was observed in the cisternae of the Golgi complex, and masses of discrete droplets without surrounding membranes were visible between the basal portions of the cells. The droplets appeared to pass along intercellular spaces in the lamina propria of the villus into lacteals through fenestrations in their walls. Droplets were not seen in the microvilli, and few were in the terminal web just below the microvilli at the apex of the cells; further, droplets did not enter mitochondria, and it was exceedingly rare to find fat droplets in the endothelial cells or in the lumen of the blood capillaries of the lamina propria.

At the junction of the microvilli with the main portion of the cells, between the bases of the microvilli furrows penetrating into the terminal web were seen in profile,

some with small vesicles about 600 A in diameter. These vesicles usually appeared empty, especially in the fasting animal, but sometimes carried a small fat droplet, constituting possible evidence of pinocytosis by the intermicrovillus plasma membrane. In the rat absorbing fat considerably more vesicles were demonstrable. Inside the cell the membranes enclosing the round, dense lipid droplets ranged from circular to elliptical or even tubular, and in some areas were studded with ribosomal granules, suggesting that the lipid droplets were moving through an interconnecting labyrinthine complex of tubular membranes, the endoplasmic reticulum described by Palade (158). This pleomorphic network, at least intermittently, provides cavities continuous with the extracellular space and with all parts of the cell, even to the nuclear envelope as shown by the demonstration of fat droplets in the latter site by Palay (159). Demonstration of this three-dimensional network, changing with time to give it a fourth dimension, is difficult indeed using the instantaneous thin two-dimensional tissue section, and calls for considerable art by the electron microscopist. These observations by Palay demonstrated a physiologic function of the endoplasmic reticulum in providing channels for materials from outside cells to any part of the cell without leaving the system, nor even necessarily crossing a membrane surface. Fig. 5 summarizes in a sketch many of these ideas of intracellular movement of lipid droplets, as adapted from the electron microscopic photographs.

Earlier claims that the striations of the microvillar border disappeared or shortened (37) were not borne out. Palay and Karlin did not confirm the report by Sjöstrand and Zetterqvist that the double-contoured surface structure of the microvillus during absorption changed into a single electron-dense layer (160), although the latter authors were reporting on changes during absorption of protein as well as of fat. Weiss (161) had been unable to find any signs of particulate fat absorption in the striated border and had concluded that fat was absorbed in soluble form, then concentrated in the region of the Golgi apparatus as lipid droplets. Newborn rats and mice had been shown by Clark (162) to be able to absorb intact proteins by pinocytosis occurring at the surface of the small intestinal epithelial cells, although he recognized that this capacity was soon lost and did not persist into adult life.

Although it seemed clear that pinocytosis of fat droplets could be demonstrated, it was by no means argued by Palay and Karlin that this mechanism explained entirely the facts of fat absorption, nor did it resolve the ancient conflict over particulate versus soluble absorption which they reviewed and discussed so well (155). They recognized that pinocytotic activity never appeared great enough to explain the rapid absorption of large amounts of fat known to occur and that fat particles were very infrequently demonstrable in the terminal web. They commented on the failure of the rather primitive mechanism of pinocytosis to explain the specificity and selectivity of fat absorption for long-chain fatty acids and glycerides, and against sterols. By assuming that myriads of vesicles formed rapidly, that fat droplets could move quickly across the terminal web zone, and that the movement ceased when the apical portion of the cell had become to a degree loaded with fat droplets, they calculated that pinocytosis could possibly be the mechanism of fat absorption. However, they concluded that it was not possible to evaluate the relative importance of particulate and soluble absorption of fat, and that perhaps both proceeded concurrently.

On the other hand, certain limitations of the electron microscopic technique have given rise to some doubts about pinocytosis, and biochemical studies have tended to weigh more heavily on the side of some form of molecular or soluble absorption. Fatty acids in the lumen have been known since 1880 (13) to be converted to lymph triglycerides, during their passage through the cell; even the most exquisite electron microphotographs have not been able to distinguish in a fat droplet the molecular form of its contents, whether triglyceride, lower glyceride, or free fatty acid. Further, particles as small as the micelles described by Hofmann and Borgström (139) do not stain well, tend to become indistinguishable from nondescript, electron-dense particles which may be seen even in fasting animals, and may be overlooked in contrast to the very prominent, osmiophilic oil droplets of the much larger size suggested as candidates for pinocytotic transfer. Further, improvements in techniques have made it possible for Sjöstrand to state (163) that the microvillus plasma membrane is morphologically different from the membranes of the apical vesicles surrounding the absorbed lipid droplets. The latter are described as smooth-surfaced, geometrically symmetric, and about 70 A thick compared to the asymmetric, thicker (95 to 100 A) membranes of the microvilli, so that it seemed unlikely that one was derived from the other. It is his opinion that the membranes around fat droplets inside the cells could not be pinocytotic vesicles. These different interpretations remain to be resolved.

Nevertheless, pinocytotic vesicles have been seen, whether or not they indicate this mechanism as the predominant one in fat absorption. Ashworth, Stembridge, and Sanders (164) confirmed the findings of Palay and Karlin concerning triglyceride absorption in the animal, and Strauss (165) has demonstrated the same in everted hamster gut sacs in vitro. The hydraulic biopsy tube developed by Flick, Quinton, and Rubin (166) has been used to study fat absorption in the normal human jejunum by Ladman, Padykula, and Strauss (167) as well as by Phelps, Rubin, and Luft (168). The last group con-

cluded that pinocytosis could not explain fat absorption, mainly because of paucity of demonstrable fat particles in the microvillar-terminal web area at various times after corn oil administration to normal volunteers. Ashworth and Johnston (169) also concluded that the pinocytotic mechanism seemed quantitatively insufficient to account for the known facts of fat absorption but agreed that the answer was indeterminate. Their studies of the absorption of oleic acid-C^{14} in rats indicated that luminal droplets of diameters down to 100 A were still composed mainly of free fatty acid, that the process of intracellular droplet movement appeared similar to that found for corn oil triglycerides, and that some of the osmiophilic droplets in the cells probably contained labeled triglycerides, since the C^{14}-labeled fatty acid entered the chyle mainly as triglyceride.

Nevertheless, particulate absorption by the intestine could be demonstrated although Juhlin (170) had found that synthetic, indigestible particles such as ionized methyl methacrylate particles containing fluorescent dyes were not absorbed by the gut mucosa. However, Sanders and Ashworth (171) were able to show uptake of latex particles over 2000 A in diameter and to find them in the liver. Resin particles (172) and dye particles (173) may also be taken up by the intestinal mucosa. Triglycerides resistant to hydrolysis by pancreatic lipase because of steric hindrance (174, 175) caused by methyl groups in the 2′-position of the fatty acyl portion are absorbed to some extent if fed with olive oil (176, 177). Also, paraffin oils are not only absorbed, as shown by Channon and Collinson (178), but are extensively metabolized after absorption by ω-oxidation, as Stetten demonstrated in 1943 (179). Even when liquid paraffin is given directly into the upper small gut of fasted rats, considerable absorption occurs if it is supplied as an emulsion of particles less than 5000 A in diameter, as found by Frazer, Schulman, and Stewart (23), although not all investigators agree to this. There has been no study in which any direct openings between cells have been found, and in fact the intracellular space is especially closed off in the region just below the free luminal surface (155, 161).

Pinocytosis, or simple engulfment of liquid droplets by membrane enclosure, is a primitive mechanism which is most impressive in organisms such as some of the lower metazoa (180), and Lewis has observed macrophages in tissue culture to imbibe one-third of their total cell volume of fluid in an hour (181). The work of pinocytosis involved in new membrane formation has been estimated by Parsons (182). This apparently simple mechanism may even achieve some degree of specificity, as indicated by the studies of Marshall, Schumaker, and Brandt (183) on *Chaos chaos*. However, it is difficult to be persuaded that the well-ordered adaptation of the luminal surface of the luminal surface of the mammalian intestinal epithelial cells into hundreds of microvilli per cell has evolved to use only the basal intermicrovillus portion for invagination. Development of the microvilli as specialized adaptations of the cell surface has been studied in the duodenal cells of the chick embryo by Overton and Shoup (184). The microvilli, which have been carefully counted and measured by Brown (185), increase the surface area of the epithelial cells by a factor of about 20, and are not especially motile nor well suited to large scale pinocytotic activity compared to the protean surface of the hydra. A concept that complements the idea of adaptation toward maximal surface area for absorption, therefore, has many attractive features. Dispersion of the products of fat digestion into micellar form and penetration of the major portion of the surface of the microvilli by some physicochemical mechanism is such a concept, as was the idea of lipid solubility which Hogben (186) put forth. The idea of membrane flow from microvilli to endoplasmic reticular vesicles, as suggested by Bennett (187), is subject to challenge on the basis of the recent observations of Sjöstrand (163).

The questions concerning the form in which lipids actually penetrate the gut mucosal cells and the quantitative significance of pinocytosis remain unsolved; they represent most intriguing and critical problems yet to be pursued in the field of fat absorption.

Possibly both pinocytosis and a more nearly molecular penetration from the micellar state may occur during fat absorption. Biochemical evidence tends to favor the latter as perhaps the more efficient and normally predominant mode of entry of fat, but in the case of particles or unhydrolyzed droplets of oil, pinocytosis may be the only way by which the substance can cross the membrane of the microvillus. In Fig. 6 a sketch of the alternatives is presented. Morphologic observations, even at the highest possible resolution, have not settled this question.

Intracellular Transport and Transformation

Little is really known about the details of how material is moved from one part of the intestinal epithelial cell to another, and what chemical or physical changes may take place at various sites. A substance may be transferred spatially from one side of a membrane to another, from cytoplasm into endoplasmic reticular channels or into mitochondria; it may perhaps be removed just as effectively by a chemical change to another compound, or by a physical change such as an alteration in its solubility or molecular configuration. It appears likely that all these phenomena may be used to facilitate movement of fatty materials in cells. Mention has been made already of the marked increase in water solubility that occurs when a fatty acid is transformed into its CoA thiolester derivative. When the activated fatty acids and mono-

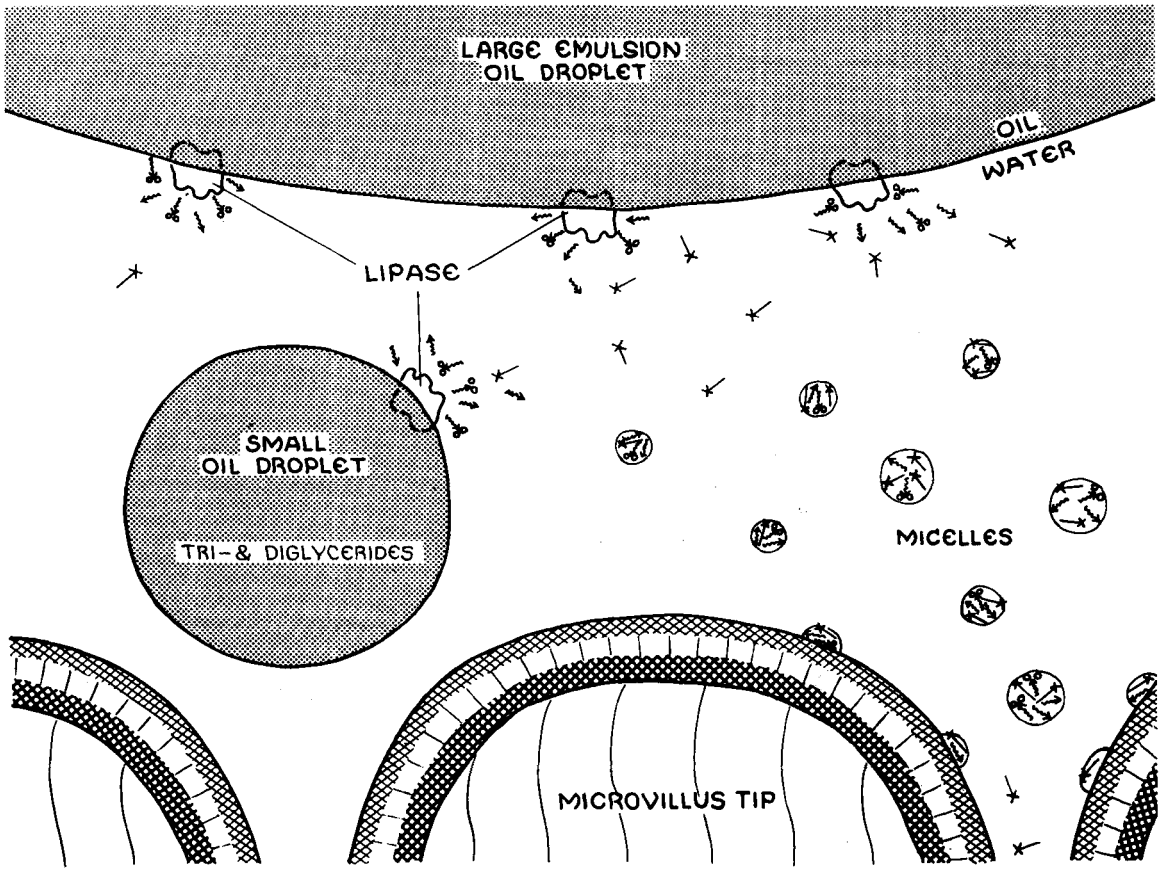

FIG. 6. Microvillus' eye view of fat absorption: the comparative "appearance" of oil droplets vs. micelles. Symbols as in Fig. 4.

glycerides condense to form di- and triglycerides the reverse happens; i.e., water solubility decreases sharply. At some point during the passage of the fat through the cells this does occur, since ingested fatty acids are converted to triglycerides before they enter the lymph. Since fat droplets in the cells are enclosed in membranes of the endoplasmic reticulum throughout the entire period of their journey from the subterminal web area to lateral ejection into the intercellular space, it may well be that for much if not all of their existence as droplets visible by electron microscopy they are made up of newly synthesized or engulfed higher glycerides. The protein content of cell sap is high enough to make possible excellent solutions or dispersions of monoglycerides even in vitro.[1] Certainly fatty acyl CoA compounds may exist as molecules in solution at cell sap pH values. Thus both of these substrates for fat synthesis are capable of being present in the cell sap or in membranes, and in this form would not show as fat droplets. The exact specificity of staining by osmium tetroxide is not entirely understood, so that the failure to demonstrate cytoplasmic darkening due to staining of unsaturated fatty acids (155) cannot yet be taken as persuasive evidence against the possible presence of such fatty acyl thiolesters or monoglycerides in molecular form rather than as droplets.

The close association of the fat droplets with the endoplasmic reticulum throughout their passage through the cells is circumstantial evidence that this network of channels is the principal means of moving fat from lumen to intercellular spaces. The labyrinth of interconnecting channels is continuous with the lumen when pinocytosis is occurring and with the extracellular space when droplets are ejected without their membranous enclosure. It was this that led Palay and Karlin to mention (155) that the entire system of endoplasmic reticular space might be regarded as extracellular, at least at times.

The finding that the cell fraction that showed the highest fatty acid:CoA ligase specific activity (86) was that derived principally from the membranes of the endoplasmic reticulum (188) was therefore quite exciting. This fraction, microsomes in the parlance of the biochemist, was obtained by high speed differential ultracentrifugation of neutral, isotonic, mannitol homogenates of rat jejunal epithelial cells. As may be appreciated easily from

[1] Senior, J. R. Unpublished observations.

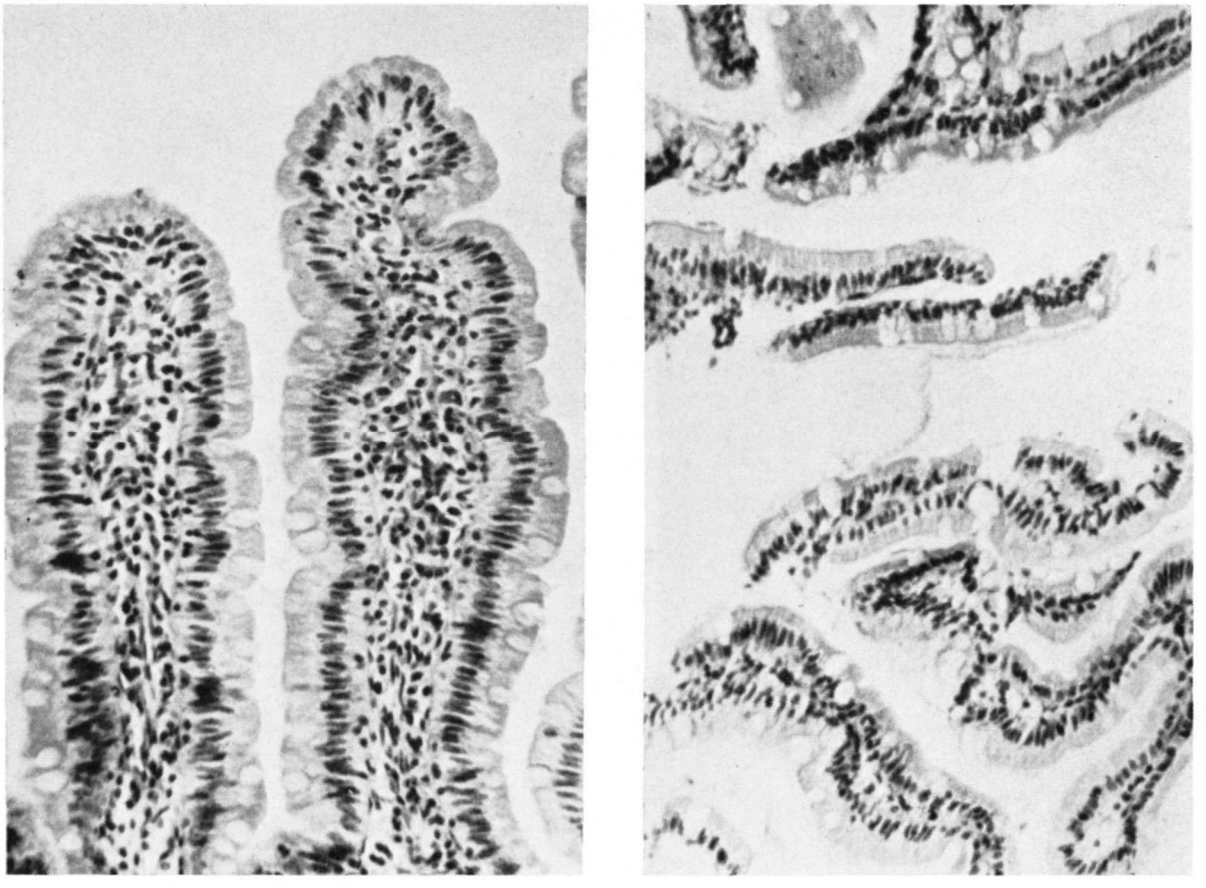

FIG. 7. Photomicrograph of the villus of rat jejunum and the epithelial cell preparation (right) used in obtaining subcellular fractions. Note the single layer of epithelial cells on the lamina propria of the villus core, and the ease with which these cells may be stripped off with very little admixture with other types of cells. The epithelial cells are derived from the sides and tips of the villi, and are fully developed, mature cells.

Fig. 7, the epithelial cell monolayer quite readily separates from the villus core with light pressure, yielding a cell preparation of most acceptable homogeneity. Some mucus cells are present, interspersed with the epithelial cells, but brief fasting (overnight) seems to decrease their secretory activity.[1] Perhaps because each tissue has different characteristics of viscosity and density, as well as different water and polymer content, the differential centrifugation conditions which had been worked out in detail for other tissues such as liver did not give good results when applied to homogenates of intestinal mucosal cells. As shown in Fig. 8, however, eventually it was feasible to obtain reasonably clean mitochondrial, microsomal, and cell sap fractions (99).

Not only was the fatty acid:CoA ligase specific activity highest in the microsomal fraction, but the monoglyceride and L-glycerol-3-phosphate acyl transferases were also most active in this fraction (99), as was the glycerol monoester hydrolase (113). Mitochondria seemed generously endowed with these enzymes also, except for somewhat lower specific activities of the acyl transferases. The finding of these enzyme activities in the same membranes which morphologically appeared to enclose the fat droplets seems more than fortuitous. Since the membranes of the endoplasmic reticulum, mitochondria, and other membranes are composed mainly of lipid and protein, the concept that fat synthesis might be occurring within the membranes or while substrates were crossing the membranes is most attractive. Attempts to purify the fatty acid:CoA ligase and the acyl transferases by dissociating the enzyme protein from its lipid matrix with bile salts or detergent resulted in loss of activity (86, 99). Recently, Pope and Tidwell (118) have reported some success in purification of the glycerol monoester hydrolase from microsomes. Earlier, some investigators had associated the glyceride synthetic function with mitochondria (94) or cell sap (108) fractions, but more recently these workers have concluded that the microsomal fraction is predominant in the several steps of fat synthesis (189). Localization of fatty acid:CoA ligase also in intestinal cell microsomal fractions was confirmed by Ailhaud, Samuel, and Desnuelle (88).

Thus, both morphologically and biochemically the transport and synthesis of fat in the intestinal mucosa are related to the endoplasmic reticulum, including the Golgi apparatus. More precise correlation of structure and function of intracellular organelles awaits exploitation of some of the newer techniques now becoming available. Some extremely interesting mechanisms of control and regulation are related to the process of fat synthesis that occurs in the intestinal mucosal cells. Already mentioned has been the ability of conjugated bile salts to stimulate intestinal mucosal carbohydrate utilization, and especially incorporation of carbon from glucose (66) and glycerol (73) into lipids. Whether this may be brought about by conjugated bile salts facilitating the entry of fatty acids into the cell, or by some more direct effect of bile salts on enzymes of the carbohydrate sequences, has not yet been established. It has been postulated recently by Vahouny, Weersing, and Treadwell that taurocholate forms a complex with the pancreatic enzyme cholesterol esterase, and that this complex formation not only is necessary to enzyme activity but also protects the enzyme against proteolytic degradation by trypsin or chymotrypsin (190). Another control mechanism which is of considerable importance is the slowing of gastric motility and secretion (131) and perhaps of pinocytosis (155) when the intestinal cell endoplasmic reticulum is full of particulate fat. Since the feeding of fat inhibits motility in totally denervated and transplanted gastric pouches, as shown by Farrell and Ivy in 1936 (191), there is substantial reason to believe that a hormonal substance, termed enterogastrone by Kosaka and Lim (192), may be released by the intestinal mucosal cells during fat absorption. Its intracellular site of elaboration, pathway of excretion, and chemical nature are still unknown. Verzár and McDougall in their monograph (16) discussed the observation that iodoacetate blocks fat absorption, not by delaying passage of fatty acids into cells but by inhibiting synthesis of triglycerides from fatty acids. Verzár and Laszt (193) had noted that phlorizin does the same, although Frazer (29) has questioned these observations on the basis that mucosal damage was produced. This dissociation of uptake of fatty acids from glyceride synthesis is consistent with recent observations of Johnston and Borgström (110, 144) concerning rapid, nonenzymatic, and nonenergy-requiring uptake of fatty acid and monoglyceride from the micellar state.

Chylomicron Formation and Release

Even after triglycerides have been synthesized, complete chylomicron formation requires increments of phospholipid, protein, and possibly esterified or free cholesterol. All these are found in chylomicrons (194, 195). The protein content of chylomicrons has been measured to be as low as 0.2 to 0.5% by Bragdon (196) and less than 0.3% by Peterson (197), yet the contribution of the relatively

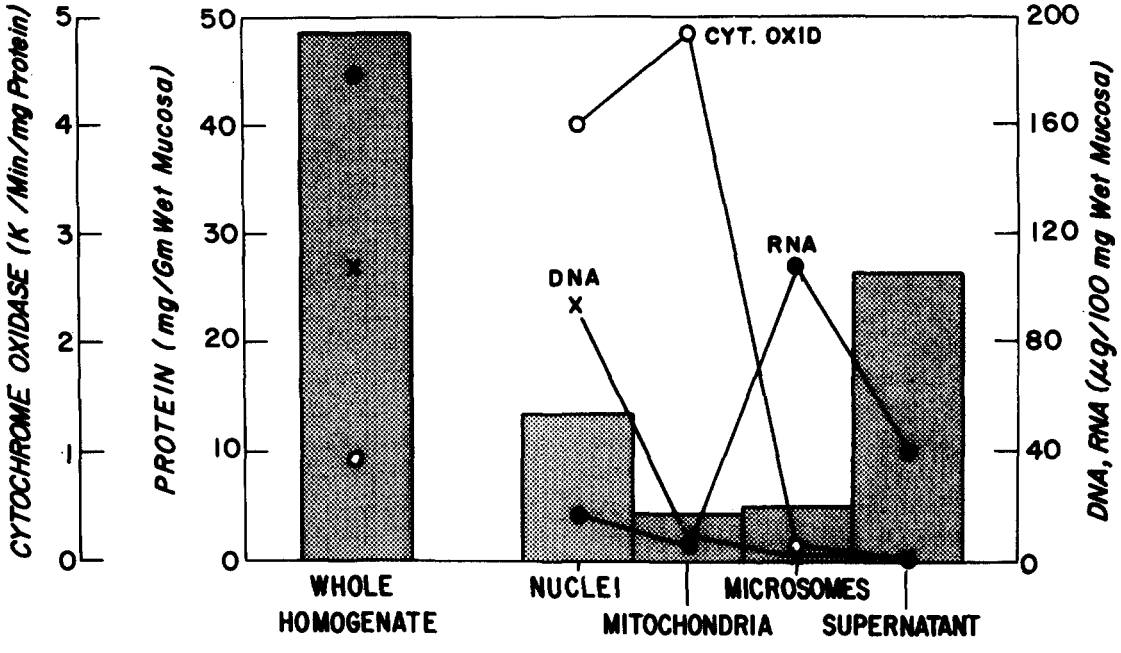

FIG. 8. Subcellular fractions obtained by homogenization of epithelial cells in neutral isotonic mannitol and separated by differential centrifugation. The nuclear fraction is badly mixed, but the mitochondria show most of the cytochrome oxidase activity and very little RNA, while the microsomal fraction shows the reverse, indicating fairly good separation.

small protein moiety is essential to chylomicron formation, as most dramatically demonstrated by Sabesin, Drummey, Budz, and Isselbacher (198). After giving a moderate, nonlethal dose of puromycin to rats, they were able to show blockade of intestinal lipid transport, in which there was a histologic appearance of fat accumulation in jejunal epithelial cells but not in lymphatics, associated with decreases in serum triglyceride levels to only one-tenth of normal after corn oil administration. This chemical and histologic picture resembles that of the rare human disease, hereditary β-lipoprotein deficiency or acanthocytosis (199), in which a primary defect in ability to synthesize the protein moiety of low density lipoproteins is thought to be responsible for the disturbances observed. Since puromycin is known to inhibit amino acid incorporation into protein (200), perhaps by displacing peptide chains from ribosomes where proteins are assembled (201), it was of fundamental interest that puromycin very much reduced incorporation of leucine-C^{14} into chylomicron protein, as reported by Isselbacher and Budz (202) and also by Hatch, Hagopian, Rubenstein, and Canellos (203). Other inhibitors of protein synthesis such as ethionine and acetoxycyclohexamide also produce similar changes. Despite the inability of these cells to make and export chylomicrons if protein synthesis was inhibited, no evident cell damage could be seen even on electron microscopy. Moreover, there was no depression of glucose transport and palmitate-C^{14} continued to be incorporated into triglycerides.

Similarly inhibition of lipoprotein synthesis by the liver, resulting in fatty liver and decrease in serum lipids had been reported by Robinson and Harris after administering ethionine to rats (204) and by Robinson and Seakins after puromycin injection into rats (205). Both liver and intestinal mucosa seem to be sites of synthesis of low density lipoproteins of more than one type. These include not only the β-lipoproteins (199) and the even lower density class of lipoproteins more commonly called chylomicrons, but also possibly other members of the lipoprotein family, as indicated by the findings of Havel and Goldfien (206) in hepatectomized dogs. Chylomicrons, named by Gage (207), were recognized by him (208) as the main transport form of fat into the venous blood via the lymph. Rodbell and Frederickson (209) studied the *N*-terminal amino acids of the chylomicron protein of thoracic duct lymph, and with Ono (210) the labeling of high density lipoproteins associated with chylomicrons after feeding C^{14}-amino acids. They concluded that the protein moieties were similar. However, the possibility of protein adsorption to the surface of the chylomicrons has been raised, and there has been doubt concerning the interpretation of these findings (211). Peterson found that repeated washing of chylomicrons did not remove traces of serum proteins from the fat particles, as detected by highly specific immunological methods, even when the protein content of the chylomicrons has been reduced to less than 1% (197). Recently, Bierman, Gordis, and Hamlin have separated two types of "chylomicrons" from postprandial human serum using differential polyvinylpyrrolidone flocculation. The first type they think is of intestinal origin and the other slightly more dense particles they believe are made secondarily in the liver (212).

Borgström had shown in 1952 (213) that the lymph triglycerides were made up almost completely from fatty acids of fat being absorbed, while the phospholipids of lymph contained fatty acids derived more from endogenous sources. This has been confirmed repeatedly, although Wood, Imaichi, Knowles, Michaels, and Kinsell (214) have reported some decrease in the relative proportion of short-chain fatty acids in lymph triglycerides of normal men fed a large butter meal, explained by the tendency of shorter fatty acids to enter the portal blood. Some degree of preservation of dietary glyceride structure may even be found in the "secondary particles" or so-called liver chylomicrons, as reported by Gordis (215). These studies have emphasized the need for a separate designation for the fat particles synthesized in liver versus those of gut origin. Perhaps it would be best to reserve the term chylomicron for the fat particle of intestinal mucosal origin, as originally defined (205), and use some other term for fat particles of similar density originating in the liver, although no general agreement has been reached on this point.

Since phospholipids are also needed in chylomicron formation, and are synthesized in the intestinal mucosa (34), it would be of great interest to find a situation in which inhibition of phospholipid synthesis caused a block in fat absorption, as was suggested in the choline deficiency studies of Sagrott (216). The previous finding of Johnston and Bearden that phosphatidic acid specific activity increased during fatty acid absorption by hamster gut sacs (89) has been shown recently by Gurr, Pover, Hawthorne, and Frazer (217) not to hold in vivo for rats fed olive oil. They found that the specific activity of gut phospholipids in rats injected intravenously with orthophosphate-P^{32} during absorption of olive oil rose only very slightly, and the whole increase was due to phosphatidyl choline rather than phosphatidic acid. This evidence confirmed the work of Zilversmit, Chaikoff, and Entenman (35) and supports the idea that the major pathway of triglyceride synthesis during fat absorption is via the direct acylation of monoglycerider rather than of glycerophosphate.

The site of chylomicron completion in the intestinal cell is not yet known. If the morphologic observations may be trusted, it would appear that it may be at some point in the luminal half of the cell within the endoplas-

mic reticulum, or at least, before ejection of chylomicrons into the intercellular spaces. In the puromycin-fed rats (198) and acanthocytotic patients (199) release of fat from the apical half of the cells appears blocked and the triglycerides accumulate there. Protein synthesis had been associated with ribosomal particles (201, 218), which are present in the gut cells and which may be seen attached to membranes of the endoplasmic reticulum, the so called "rough vesicles" or α-cytomembranes. It may be speculated that protein synthesis in these rough vesicles could provide the protein wrapping necessary for exporting the chylomicrons from the gut cells. However, the basal portion of the gut epithelial cells seems to have most of the ribonucleoprotein-studded membranes, and the alternative suggestion has been made by Sjöstrand (219) that large fat globules may be released into the lateral intercellular spaces and there broken up into fairly uniform smaller globules of 1000–2000 A diameter. This he suggested might be facilitated by release of protein from the basal portions of the cells, which could coat the fat droplets and complete the formation of the chylomicrons. Passage of the formed chylomicrons into the lacteals has been reported to occur through open intercellular junctions (155, 219) or fenestrations (164) in the lacteal walls. In studying this aspect of fat absorption more closely, Casley-Smith (220) observed chylomicrons of diameter 1000 A to 1 μ and smaller fat particles designated "lipoproteins" of diameter 100–1000 A, passing through valve-like open junctions between overlapping cells of the lacteals but no fenestrations were seen. He proposed that these oblique openings through the lymphatic endothelial intercellular junctions could act as inlet valves to prevent reflux of chylomicrons when the smooth muscle of the villus contracts. As a result of improvement in techniques the lipoprotein particles appeared to stain very well, although the difficulties of knowing to what structure osmium tetroxide fixes have been discussed by Hayes, Lindgren, and Gofman (221). Pinter and Zilversmit (222) have reported the determination of canine lymph chylomicron diameters using sucrose density gradient techniques, and found median values of about 2000–3000 A, which is in good agreement with the electron microscopic data.

Structural Determinants of Lipid Products

In considering glycerides, three types of structural determinants may be listed: (*a*) fatty acid chain length and degree of saturation, (*b*) position in the glyceride molecule into which the fatty acid may be incorporated, and (*c*) stereospecificity of the completed molecule of di- or triglyceride or phospholipid. Considerable amounts of data have accumulated concerning the first of these, and recently some important observations have been made with regard to the second, but relatively little investigation has yet been made of how important steric factors may be in controlling or directing glyceride synthesis.[2]

Evidence has been cited (41, 44, 214) in support of the idea that shorter-chain fatty acids show a lesser tendency to be absorbed as glycerides compared to the long-chain fatty acids, but are absorbed without further change other than perhaps to their sodium salts and enter the portal blood. Marked differences in the rate of hydrolysis of triglycerides by pancreatic lipase were noted to be a function of the chain lengths of the esterified fatty acids, as reviewed by Desnuelle and Savary (133). Reaction rates depend also on the conditions employed and on the physical form of dispersion of the substrate. Rapid hydrolysis of short-chain 2-monoglycerides by pancreatic lipase has been observed, and thought to be due to very fast spontaneous isomerization to 1- or 3-positions (226). Fernandes, van de Kamer, and Weijers (227) have found that patients with steatorrhea due to celiac disease, cystic fibrosis of the pancreas, or surgically shortened small intestine can absorb shorter-chain and unsaturated fatty acids fed as triglycerides better than the ordinary, relatively saturated, long-chain triglycerides common in human foods. These clinical findings have been confirmed, and are consistent with the concept that shorter-chain triglycerides are more easily split in the intestinal lumen or in the mucosal cells, and the resulting free fatty acids are more readily transported via the portal blood.

Inside the cells, the shorter-chain fatty acids are less readily activated to CoA thiolesters, a function of the specificity of the ligase (86, 87), and the short-chain monoglycerides are more rapidly hydrolyzed by the glycerol monoester hydrolase (113, 117). Even triglycerides of shorter-chain fatty acids seem to be split by an ester hydrolase of the gut mucosal cells, as shown by Playoust and Isselbacher (228). All these effects tend to produce a preferential incorporation of long-chain fatty acids into the lymph triglycerides, while the shorter-chain acids are delivered via the portal blood to the liver where they predominantly are oxidized to 2-carbon fragments (229). Peterson (230) found a discrimination against myristic acid compared to oleic acid in lymph chylomicron triglycerides when a mixture of their triglycerides was fed to a patient with chyluria. Larger amounts of short-chain fatty acids produce a rise in blood glucose concentration, according to Ash, Pennington, and Reid (231), possibly by decreasing tissue uptake of glucose, although increased liver release of glucose was not excluded.

[2] In order to avoid confusion, the glyceride glycerol carbons are numbered here in the same fashion as has been used conventionally for the triose phosphates (see Fig. 3). Fatty acyl carbon atoms will be numbered as primes from the carboxyl end. With respect to stereospecificity of glycerol derivatives, there is much to recommend use of the rules described by Hirschmann (225), in which case D- or L- prefixes become redundant.

Saturation of the fatty acyl chain also governs rates of incorporation into various lysolecithins, as shown by Lands and Hart (232). Unsaturated chains are mainly esterified at the 2-position, while saturated chains are directed toward the 1-position by enzymes in the rat liver microsomes, although other tissues show other specificities. During absorption of mixtures of fatty acids, preferential incorporation of oleate into cholesterol esters (233), of stearate and linoleate into lecithins (234), and of palmitate into sphingomyelin (235) has been observed. It has been pointed out that lymph chylomicron triglycerides show much less specificity and reflect closely the fatty acid composition of the luminal fat (213, 236). Lecithins in chylomicrons were found by Whyte, Goodman, and Karmen (237) to show also the marked tendency for unsaturated fatty acids to be esterified in the 2-position, saturated fatty acids in the 1-position, in harmony with what Lands and Hart had found for liver tissue (232).

Rather little has been learned yet concerning the relative effect of chain unsaturation on triglyceride formation. Brown and Johnston (101) showed that palmitoyl CoA is directed especially to primary hydroxyl groups of monoglycerides by the intestinal acyl transferase system, and that no stereospecificity appears to exist for 1-monoglycerides with respect to their rates of esterification. However, Savary and Desnuelle (238) as well as Mattson and Volpenhein (239) have shown that the fatty acids of vegetable triglycerides are not randomly distributed but tend to have unsaturated 18-carbon acids esterified at the 2-position while the saturated long-chain fatty acids are bound in the 1- and 3-positions. Animal fats have not in general shown such specific distribution patterns (240), except for the concentration of linoleate at the 2-position and of palmitate there in pigs and related animals (240*a*). Upon absorption of plant triglycerides the fatty acids are not randomized in the lymph chylomicron triglycerides, which reflect very closely not only the fatty acid composition but also the predominant unsaturation of acyl groups at the 2-position, as demonstrated by Savary, Constantin, and Desnuelle (241).

Karnovsky and Wolff (242) could not find evidence that pancreatic lipase produces an enantiomorphic diglyceride isomer, and steric specificity has not been well demonstrated in triglyceride formation, although Weiss, Kennedy, and Kiyasu (104) did find that D-2,3-diolein was considerably better for triglyceride formation than the L-enantiomer and very much better for lecithin synthesis, using chicken liver microsomal enzymes. Recently, optically active natural plant triglycerides have been studied by Maier and Holman (243), who demonstrated the presence of single enantiomers, asymmetric about the 2-carbon of the glycerol moiety of triglycerides, in seed oils of two species of Chinese tallow trees. The mechanism of formation of such optically active plant triglycerides has not been worked out as yet.

Net Effects of the Over-all Mucosal Process

Triglycerides represent a useful form of lipid which is hydrophobic, chemically unreactive, of relatively low density, and composed of a great many hydrogen atoms per molecule. These characteristics make triglycerides a most convenient storage form of energy and of long-chain fatty acids for the mobile mammalian species. Our diets include triglycerides stored in tissues of animal food sources. We move the unsynthesized chylomicron triglycerides in our lymph and blood and redeposit the fats as triglycerides in the adipose depots. Wonderful adaptations illustrating the value of this mechanism are apparent in the migrating birds which store large weights of triglycerides in preparation for their long over-water flights to and from Central or South America (244), and in the desert beasts, the camels which carry their energy and their hydrogen in their humps, using the only available ingredient in their harsh environment, atmospheric oxygen, to utilize the stored energy and to make water. Yet triglycerides, the preferred storage and shipping form of fat, seem to be hard to move across membranes; efficiency may require breakdown into a more reactive and relatively more polar form, for absorption as well as for deposition in adipose tissue. What is the cost of this apparently unproductive triglyceride hydrolysis, resynthesis, hydrolysis, resynthesis? It may be calculated (245) that oxidation of a molecule of stearic acid to CO_2 and water, coupled with oxidative phosphorylation, can produce 148 molecules of ATP from ADP, at a cost of breaking down only one ATP to AMP (equivalent to two ATP to ADP) to convert free stearic acid to the reactive thiolester derivative. One cycle of triglyceride hydrolysis and resynthesis costs less than 0.7% of the energy which may be usefully trapped chemically at an efficiency of just under 40% when the fatty acids are oxidized. Hydrolysis of triglycerides thus permits a very great increase in the ease of their transfer from one aqueous compartment into another at the very modest price of their resynthesis. An additional dividend is the opportunity to synthesize preferred forms of lipid products, selecting appropriate fatty acids and placing them in suitable configurations.

Thus the major intestinal process involved in fat absorption is the conversion of food triglycerides to lymph triglycerides, at low energy cost, and without alteration in the structure of the fatty acids absorbed. The monoglyceride pathway of diglyceride formation, quantitatively much more important in the gut mucosa than in any other tissue studied thus far, and perhaps the more important mode of higher glyceride resynthesis, represents a further adaptation towards efficiency of the overall process. Not only is one hydrolytic and reactivation

step avoided by forming monoglycerides instead of free glycerol, but the favorable physical properties of the amphipathic monoglycerides are used to facilitate fatty acid entry.

Even greater relative molecular hydrogen content exists in the paraffins, which can be absorbed to some extent (178), although the rates of absorption are slow. As was shown by Stetten (179), ω-oxidation occurs during the metabolism of these compounds, and they subsequently are handled as their corresponding fatty acids. Long-chain alkyl alcohols, such as are found in nut oils and spermaceti as waxes, and in fish liver oils as glycerol ethers, may also be absorbed fairly well (178). Munk and Rosenstein (246) fed cetyl palmitate, as spermaceti, to a patient with a lymph fistula and recovered only triglycerides of palmitic acid in the lymph. Stetten and Schoenheimer (247) used deuterated cetyl alcohol to prove its absorption by the rat, and its conversion to palmitate in the gut mucosa, as was confirmed by Blomstrand and Rumpf (248). In subsequent studies Bergström and Blomstrand (249) demonstrated absorption of the glycerol ether of cetyl alcohol (chimyl alcohol) as well as esterification of its free hydroxyl groups, although splitting of the chimyl alcohol predominated.

The over-all process of triglyceride absorption, involving hydrolysis and reesterification, does require energy, even if 100 g of dietary triglycerides are completely absorbed and converted to merely the same amount of chylomicron triglycerides. The action of pancreatic lipase is not simply reversed, even though pancreatic lipase in vitro can catalyze the recombination of fatty acids and glycerol. This was shown long ago by Pottevin (250) and by Artom and Reale (251) although most workers feel this is not significant in vivo. Fatty acids in which the 2-position is doubly methylated are activated and incorporated into lymph triglycerides (175) even though pancreatic lipase will not cleave such sterically hindered ester bonds, but the positional specificity of their incorporation has not been studied. In studies in which attempts were made to inhibit lipase by fluoride or Tweens, formation of glycerides could be shown to proceed (84), and the dependence of effective incorporation of fatty acids into glycerides upon ATP and CoA further suggested an energy-requiring process. Since the source of this energy probably is glycolysis or oxidative phosphorylation, inhibition of fat absorption in animals treated with phlorizin or iodoacetate (193) is also in agreement, although the work needs confirmation. It is clear that one of the important tasks of the intestinal mucosa is fat absorption and in this process glyceride synthesis is involved. Synthesis of fatty acids de novo does not appear to be a major function of this tissue, although not many data are available on this point other than those of Coniglio and Cate (252). On the other hand, oxidation of fatty acids is not very active either in comparison with liver, muscle, and other tissues, as reported by Jedeiken and Weinhouse (82).

Since two pathways of diglyceride formation have been demonstrated to occur in the gut mucosa, the glycerophosphate–phosphatidic acid pathway and that via the direct esterification of monoglycerides, the question arises as to which is quantitatively more important under various circumstances. Some evidence has been adduced that the level of glycerophosphate regulates the rate of liver triglyceride synthesis (253). However, this does not appear to be the case in the gut mucosa, where the alternative process of monoglyceride acylation appears to be so active. In fact, addition of monoglycerides to the medium in which fatty acids and glucose or glycerol are being synthesized into higher glycerides very markedly reduces the incorporation of the carbohydrate into the glyceride glycerol.[3] Inhibition of phosphatidate breakdown by Tween 20 fails to slow glyceride formation from monoglycerides and fatty acids, as shown by Clark and Hübscher (94). However, the physiological importance of one pathway versus another cannot be predicted from results obtained by in vitro techniques.

The observations of Savary, Constantin, and Desnuelle (241), and those of Mattson and Volpenhein (254) in the intact animal are of great importance, in demonstrating preservation of the 2-ester bond of fed triglycerides in the chylomicron triglycerides. The latter authors were able to show that saturated fatty acyl groups in the 2-position were especially resistant to hydrolysis or isomerization, and that this considerably enhanced their rates of absorption compared to those of the free acids. Further very recent work by Mattson and Volpenhein (255) using lymph duct-cannulated rats, has added very convincing quantitative data to indicate that approximately 75% of the glycerol of dietary triolein may be absorbed as monoglyceride, most of which is reesterified to triglycerides in the gut mucosa. In analysis of their data on the positions of labeled oleic acid or glycerol in lymph triglycerides Mattson and Volpenhein made a number of assumptions: that all 1-monoglycerides absorbed are hydrolyzed and none esterified, that no fatty acids were used in phospholipid or other ester syntheses, that all glycerol liberated in the lumen is not reused but all liberated from 1-monoglycerides in the cell is reacylated, that all esterification of free hydroxyl groups is random and not dictated by chain or other structural characteristics. The magnitudes of these individual steps are unknown and perhaps these assumptions are not entirely justified by available evidence. However, even in sum, rejection of the assumptions would not alter the over-all conclusions

[3] Holt, P. R., H. A. Haessler, and K. J. Isselbacher. The effect of fatty acid and monoglyceride absorption on glucose-C^{14} metabolism by intestinal mucosa. In preparation.

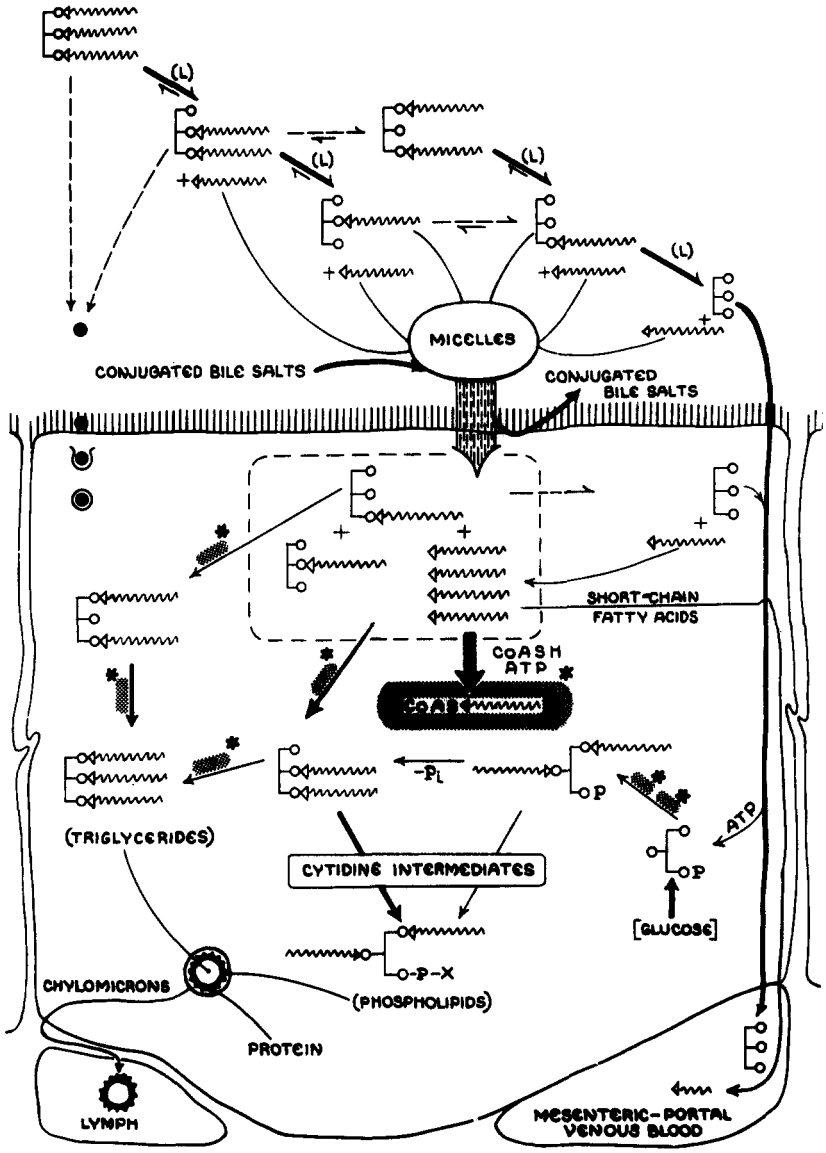

FIG. 9. A scheme of chemical events occurring during the digestion and absorption of triglycerides. Especially evident are (*a*) partial but extensive lipolysis of the triglycerides in the lumen, (*b*) micelle absorption, (*c*) intracellular resynthesis of triglycerides, phospholipids, and chylomicrons, and (*d*) partition of relatively water-soluble materials into the portal blood. In this scheme there is something in agreement with each of the classic theories although none of them describes all events. Symbols as in Fig. 4.

reached by these investigators. Thus, in the intact, unanesthetized animal, an experimental situation about as close to "physiologic" as possible, it appears that during fat absorption the major pathway of di- and triglyceride synthesis is by way of direct monoglyceride esterification. This work complements beautifully the studies on the intraluminal phase of fat digestion in normal, intact humans done by Hofmann and Borgström (256), which demonstrate that fat digestion leads to the absorption of lipids predominantly in the form of 2-monoglycerides and fatty acids in the micellar state.

THE PRESENT POSITION

Steps in the Absorption and Digestion of Fat

The most important dietary lipid constituent is triglyceride, nearly all of which is normally absorbed when reasonable quantities are ingested, i.e., not more than 120–150 g per day for an adult human being. Division of the food triglyceride into a coarse emulsion occurs principally in the stomach by its mechanical squirting and churning movements, admixture with phospholipids and other chyme components. With ejection of portions of the

fat emulsion into the duodenum, mixing with bile and pancreatic juice occurs, and the emulsion droplets are attacked by the hydrolytic action of pancreatic lipase, facilitated by conjugated bile salts. As the triglyceride primary ester bonds are cleaved, free fatty acids and 2-monoglycerides are formed as principal products, and these combine with conjugated bile salts to form a micro-emulsion or micellar solution. The emulsion droplets decrease in size as a result of the hydrolysis of material in them, and partial isomerization of 2-ester bonds to 1- or 3-ester bonds occurs. Some free glycerol may be produced intraluminally, depending on the fatty acid esterified at the 2-position, more in the case of unsaturated fatty acids and very much more with short-chain acids. Hydrolysis of triglycerides is extensive but not complete in the intestinal lumen normally. The glycerol formed is water-soluble, is quickly absorbed by passive transport, and mostly enters the mesenteric venous blood although a small fraction of it may be phosphorylated by enzymes in the intestinal cell cytoplasm. Short-chain fatty acids released in the lumen or in the cytoplasm are relatively more water-soluble also, and tend to be transported into the mesenteric portal blood without being activated and incorporated into triglycerides. In Fig. 9 these points are diagrammatically shown above and to the right of the schematic cell.

Micelle formation occurs when bile salts are present at concentrations greater than critical, and the 2-monoglycerides formed by pancreatic lipase readily combine with the bile salts to form mixed micelles. Fatty acids also enter into the micellar state. The exact mode of their penetration into or through the microvilli of the gut epithelial cells is still unknown, but the conjugated bile salts which are so important in forming micelles are not absorbed across the mucosa with the fatty acids and monoglycerides. Conjugated bile salts reenter the lumen and eventually are absorbed in the distal small bowel, then recirculate via liver and bile.

Within the cell the fatty acids are converted to water-soluble, chemically reactive thiolester derivatives using energy from ATP breakdown to AMP, the reaction being catalyzed by enzymes principally localized in membrane structures. The monoglycerides again are important as acceptors of fatty acyl chains on their free hydroxyl groups, although if activated fatty acids are not available, monoglycerides may be split by ester hydrolases also located in the same membrane structures. The direct esterification of monoglycerides appears to be the major pathway to diglyceride formation during fat absorption. In addition glycerol-3-phosphate derived from glucose metabolism, or to a minor extent from phosphorylation of free glycerol, may be esterified to phosphatidic acid from which diglycerides may be formed alternatively. From diglycerides the major products are triglycerides, although the phospholipids also synthesized are important in chylomicron formation. Before the completed chylomicrons can be exported effectively from the intestinal cells, however, a small moiety of protein is formed on or adsorbed to their surfaces. As the finished chylomicrons are made, they are released from the cell by reverse pinocytosis into the lateral intercellular spaces, from which they may be seen to move between cells into lacteals. From these lacteals they are collected into lymphatic channels and finally are distributed throughout the body via the venous and arterial blood systems. The intracellular events are also summarized in Fig. 9, as they may be thought to take place according to present evidence.

Problems to be Resolved

Although great strides have been made toward understanding the details in the fat digestion–absorption process, a number of problems remain. These unsolved questions are not all corners of minor ignorance waiting to be "cleaned up," but in several cases represent major conceptual difficulties. It has been encouraging that laboratories around the world independently have been able to reconcile different interpretations of data and come gradually to agreement on the points which have emerged in the past 16 years of work cited.

Still to be clarified are at least the following problems, solutions of which will undoubtedly lead to further future questions:

1. How are the molecules arranged in the micelles?
2. By what mechanism do micelles penetrate the microvilli?
3. What is the significance, functionally and quantitatively, of pinocytosis?
4. Exactly where within the cells do the various activation and synthetic reactions take place?
5. What determines the relative importance of each of the two pathways of diglyceride synthesis?
6. To what extent do structural and steric characteristics play a role in directing specific synthetic or transport processes?
7. How are chylomicrons constructed, with respect to the triglyceride, cholesterol, phospholipid, and protein components?
8. What relationship exists between chylomicron protein moieties and those of β- or other lipoproteins?
9. How are the intracellular movements of lipid coordinated and controlled?
10. What is the nature of the presumed hormonal control substance from the intestinal mucosa which appears to inhibit gastric function, where is it synthesized, and in response to what mechanism?

In most of these questions basic problems of molecular structure, exact intracellular localization, and integrated

control systems have been raised. It may not be fruitful to continue attacking these riddles with present techniques, many of which are static methods or which use only parts of the whole intestinal tissue. What does happen in the intact animal may be a far cry from what can be demonstrated to happen under such experimental conditions. Boldness and imagination hopefully will provide tools to solve some of the persisting mysteries related to mammalian fat absorption.

For very great encouragement and guidance I am indebted to Dr. Kurt J. Isselbacher, in whose laboratories have been worked out most of our contributions to this field. I am grateful also to Dr. Fred Mattson, Dr. J. M. Johnston, and Dr. Alan Hofmann for materials and much helpful comment. During the preparation of this review I have been pleased to have the partial support of Research Grant AM-06377 from the United States Public Health Service.

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