The attack of pancreatic lipase on ingested fats, which are mainly triglycerides, has been shown by Mattson et al. (1), Borgström (2), and Savary and Desnuelle (3) to be predominantly upon the ether linkage of the two primary alcohol groups of the glycerol molecule. This intraluminal intestinal hydrolysis results in preferential formation of monoglycerides, which display a marked tendency to aggregate with the free fatty acids also produced, and with conjugated bile salts, into highly dispersed micelles before absorption (4). The major pathway of the intracellular resynthesis of chylomicron higher glycerides has been thought to be via esterification of L-α-glycerophosphate, derived from glycolysis, by fatty acyl coenzyme A derivatives of absorbed fatty acids, in a fashion similar to the mechanism demonstrated in liver by Kornberg and Pricer (5). A fatty acid thiokinase has been shown (6) to be active in the intestinal mucosal cells, which is dependent on adenosine triphosphate for activation of long chain fatty acids to the fatty acyl coenzyme A derivatives.

However, intact monoglycerides do appear to penetrate into the intestinal epithelial cells, and in part enter lymph triglycerides, as indicated by the studies of Reiser and Williams (7), and Skippe, Morehouse, and Deuel (8), in which both the glycerol and fatty acid portions of the monoglycerides used were isotopically labeled. Although some of the absorbed monoglycerides may undergo further cleaving to glycerol and free fatty acids, the possibility that direct esterification to higher glycerides may occur has been proposed recently by Clark and Hübscher (9). These workers observed that monoglycerides, especially monolein, increased the incorporation of palmitic acid-C14 into glycerides by rabbit gut mucosal mitochondria, in the presence of added ATP, CoA, and other cofactors. No inhibition of this increased incorporation was seen with polyoxyethylene sorbitan monolaurate (Tween 20), which essentially blocked the stimulation of incorporation by L-α-glycerophosphate.

To examine more closely the question of monoglyceride esterification to diglycerides, and perhaps subsequently to other glycerides, a simplified system was used to test the ability of cell fractions from rat intestinal epithelial cells to catalyze the condensation of synthetic palmityl-CoA and monoglycerides.

The object of this approach was to seek more positive evidence for the pathway of direct esterification of monoglycerides, and to avoid the need for cofactors such as ATP, detergents such as Tween 20, or consideration of multiple sequential enzymatic steps. In the present paper, evidence is presented for the existence of an enzyme system in rat jejunal mucosal cells which catalyzes the direct acylation of monoglycerides to diglycerides, as follows:

\[
\text{Monoglycerides} + \text{fatty acyl-CoA} \rightarrow \text{diglycerides} + \text{CoA}
\]

Subsequent formation of triglycerides by a similar reaction of diglycerides with another fatty acyl-CoA was also observed and net synthesis of the higher glycerides could be demonstrated. A short communication describing some of these results recently has appeared (10).

**EXPERIMENTAL PROCEDURE**

**Materials and Methods**—Female albino rats (Charles River Laboratories, Brookline, Massachusetts) weighing 200 to 250 g were fasted overnight, killed by cervical dislocation, the small gut excised and rinsed with ice-cold 0.9% NaCl, then chilled to 0°C in 0.278 M mannitol in 0.01 M Tris-maleate, pH 7.0. In a cold room at 0–4°C, the jejunal portions of each intestine were rinsed with fresh mannitol, the mucosa gently expressed on a glass plate, made up to 15 times its weight with mannitol, homogenized, and filtered through double-thickness gauze. Nuclei and cellular debris were removed by centrifugation at 1,400 \( \times g \) for 10 minutes, after which mitochondria were centrifuged at 5,900 \( \times g \) for 15 minutes, and microsomes sedimented at 105,000 \( \times g \) for 30 minutes. The pellets were washed and redissolved in 2 ml of 1.1% KCl in 0.01 M potassium phosphate (pH 7) per g of original wet jejunal mucosa. Assay of the fractions showed that the mitochondria contained high cytochrome oxidase activity as determined spectrophotometrically (11) but essentially no RNA by colorimetric measurement (12). The microsomal fraction exhibited the reverse, i.e. virtually no cytochrome oxidase activity and high RNA content.

Isomeric monoglycerides, 1- and 2-monopalmitin, and 1- and 2-monoollein (generously supplied by Dr. F. H. Mattson) were dissolved in a small amount of diethyl ether, homogenized gently in 10% bovine serum albumin solution, 1 ml per mg of monoglyceride, and the ether evaporated at 35–40°C, leaving a clear, stable dispersion of the monoglyceride. The L-α-glycerophosphate, a gift of Dr. E. Baer, was used as the diodium salt.
Monopalmitin labeled in the glycerol portion was synthesized from glycerol-1-(3)-C⁴ (Nuclear-Chicago Corporation), which was converted to dl-isopropylidene-glycerol-C⁴ by exhaustive reflux with anhydrous aceton and petroleum ether, using p-toluene sulfonic acid as a catalyst. Water was trapped in a Barret receiver beneath the refluxing solvents, as described by Newman and Renoll (13). Redistilled palmityl chloride was used in dry quinoline to esterify the isopropylidene-glycerol, according to the procedure of Baer and Fischer (14). The product was twice recrystallized from diethyl ether and purified on a silica acid column. The resulting dl-glycerol-1-(3)-C¹⁴-1-palmitate moved as a single monoglyceride spot in several solvent systems on silica acid thin layer plates, melted at 71-72°C, and had a specific activity of 101,000 c.p.m. per mg.

Palmityl-CoA was prepared from palmityl chloride and CoA in 50% aqueous tetrahydrofuran (freshly redistilled over LiAlH₄ and used at once to avoid peroxides) by the method of Scelbert (15), was purified and made up to 0.002 M solution in 0.01 M phosphate buffer, pH 5. Palmityl-C¹⁴-CoA was synthesized enzymatically from palmitate-1-C¹⁴ (Volk Radiochemical Company, Skokie, Illinois), by a modification of the procedure of Kornberg and Prier (5), similarly purified and made up, and had a specific activity of 450,000 c.p.m. per pmol.

The incubations were carried out at 38°C, and were terminated by addition of 3.2 n perchloric acid or methanol, and chilling to 0°C, after which lipids were extracted by the technique of Folch, Lees, and Sloane Stanley (16). The solvents containing the extracted lipids were removed under a stream of N₂ at 40°C, the lipids redissolved in diethyl ether, and passed through silicic acid columns to absorb the phospholipids. The silicic acid (BioRad Laboratories, Richmond, California; 325 mesh) had a specific activity of 101,000 c.p.m. per mg. Each incubation mixture contained 0.2 pmoles (90,000 c.p.m.) of palmityl-1-C¹⁴-CoA, 0.1 g of bovine serum albumin, 90 pmoles of KCl, 200 pmoles of NaCl, 30 pmoles of potassium phosphate, 3 pmoles each of MgCl₂ and CaCl₂, plus additions, in a total volume of 2.2 ml at pH 7. After 30 minutes at 38°C, each flask was chilled to 0°C, and to each was added 0.8 ml of 3.2 N HClO₄. Lipids were extracted and fractionated as described in text.

<table>
<thead>
<tr>
<th>Subcellular particles added</th>
<th>Glycercide fraction isolated</th>
<th>Labeled palmitate incorporated in glycercides after acceptor added:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L-α-Glycerophosphate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5 pmol)</td>
</tr>
<tr>
<td>None</td>
<td>Tri-*</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Di-</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Mono-</td>
<td>0.4</td>
</tr>
<tr>
<td>Mitochondria (0.7 mg protein)</td>
<td>Tri-</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Di-</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Mono-</td>
<td>2.5</td>
</tr>
<tr>
<td>Microsomes (0.7 mg protein)</td>
<td>Tri-</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>Di-</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>Mono-</td>
<td>2.0</td>
</tr>
<tr>
<td>Both (1.4 mg protein)</td>
<td>Tri-</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td>Di-</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>Mono-</td>
<td>2.5</td>
</tr>
</tbody>
</table>

* Tri-, di-, and mono- = triglyceride, diglyceride, and monoglyceride, respectively.

| Activity of microsomes in stimulating this incorporation was much greater than that of mitochondria; and no further increase in labeling of glycerides appeared when both mitochondria and microsomes were added. The supernatant fractions appeared to be relatively inactive. A small but definite amount of incorporation of fatty acid into tri- and diglycerides occurred when no acceptors were added to the subcellular fractions, though to be due to endogenous acceptors in the fractions themselves. Most striking was the much greater incorporation of the labeled palmitate into glycerides when monopalmitin and monolein were added, compared to that seen with L-α-glycerophosphate, which has been considered the "natural" acceptor. The finding of small amounts of labeled monoglycerides was taken to indicate some degree of breakdown of newly formed diglycerides or possibly an exchange of fatty acid chains.

The impressively rapid labeling of diglycerides, when monopalmitin and palmityl-1-C¹⁴-CoA were incubated with rat gut...
of labeled free palmitate probably reflected both the hydrolysis of glycerides and decylation of the palmitoyl-CoA. Upon inspection of Fig. 1, it may be seen that the labeled triglycerides could not be the product but not the precursor of the diglycerides. As in the preceding experiments, again small amounts of labeled monoglycerides were noted and only negligible amounts of labeled phospholipids were detected. The rate of breakdown of the monoglyceride fraction was quite rapid, and that of the diglycerides considerably greater than the triglycerides. The most acceptable explanation for the curves observed was that the monopalmitin was being directly acylated to dipalmitin, which then either accumulated, was further esterified to tripalmitin or was hydrolyzed to monopalmitin. Although the data in Table I and Fig. 1 were strongly suggestive of the direct esterification of the monoglyceride to diglyceride, the possibility of transesterification without over-all synthesis could not entirely be excluded.

**Synthesis of Glycerides from Glycerol-labeled Monopalmitin and Unlabeled Palmitoyl-CoA**—To demonstrate that the monopalmitin could be esterified directly to higher glycerides, experiments were carried out using synthetic monopalmitin labeled in its glycerol moiety. Any appearance of this label in di- and triglycerides, occurring only upon the addition of palmitoyl-CoA, could not be explained by transesterification. The data given in Table II clearly indicate that rat gut microsomes catalyze the direct condensation of palmitoyl-CoA with monopalmitin-C<sup>14</sup>. This microsomal enzyme will be referred to as monoglyceride acylase. With boiled microsomes, the monopalmitin remained unchanged, whereas in the complete system about 40% was converted to the higher glycerides. It may be noted that the addition of ATP was not necessary for this process nor did it provide any further stimulation. The recovery of the label was essentially complete, and no phospholipid-C<sup>14</sup> compounds were found after incubations under these conditions.

In contrast to the marked synthesis observed in the complete system, when palmitoyl-CoA was omitted, the monopalmitin was completely hydrolyzed in 30 minutes to free glycerol-C<sup>14</sup>, indicating the presence of an active monoglyceride lipase. To

### Table II

**Formation of labeled glycerides from monopalmitin-C<sup>14</sup> and palmitoyl-CoA by rat intestinal epithelial microsomes**

<table>
<thead>
<tr>
<th>Incubation system</th>
<th>Labeled product isolated</th>
<th>Free glycerol</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glycerides</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tri-</td>
<td>Di-</td>
<td>Mono-</td>
</tr>
<tr>
<td>Complete ..........</td>
<td>0.200</td>
<td>0.213</td>
<td>0.007</td>
</tr>
<tr>
<td>Complete, plus 10 μmoles of ATP</td>
<td>0.203</td>
<td>0.210</td>
<td>0.007</td>
</tr>
<tr>
<td>Complete, minus palmitoyl-CoA</td>
<td>0.001</td>
<td>0.001</td>
<td>0.005</td>
</tr>
<tr>
<td>Boiled microsomes</td>
<td>0.002</td>
<td>0.006</td>
<td>0.304</td>
</tr>
</tbody>
</table>

mucosal cell microsomes may be seen in Fig. 1. About 30% of the labeled palmitate was found in diglycerides after 30 minutes of incubation, after which the synthetic process appeared to diminish and lipolysis to predominate. The progressive increase of labeled free palmitate probably reflected both the hydrolysis of glycerides and decylation of the palmitoyl-CoA. Upon inspection of Fig. 1, it may be seen that the labeled triglycerides could not be the product but not the precursor of the diglycerides. As in the preceding experiments, again small amounts of labeled monoglycerides were noted and only negligible amounts of labeled phospholipids were detected. The rate of breakdown of the monoglyceride fraction was quite rapid, and that of the diglycerides considerably greater than the triglycerides. The most acceptable explanation for the curves observed was that the monopalmitin was being directly acylated to dipalmitin, which then either accumulated, was further esterified to tripalmitin or was hydrolyzed to monopalmitin. Although the data in Table I and Fig. 1 were strongly suggestive of the direct esterification of the monoglyceride to diglyceride, the possibility of transesterification without over-all synthesis could not entirely be excluded.

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In contrast to the marked synthesis observed in the complete system, when palmitoyl-CoA was omitted, the monopalmitin was completely hydrolyzed in 30 minutes to free glycerol-C<sup>14</sup>, indicating the presence of an active monoglyceride lipase. To

### Table III

**Net synthesis of higher glycerides from monopalmitin and palmitoyl-CoA by rat gut mucosal cell microsomes**

<table>
<thead>
<tr>
<th>Incubation system</th>
<th>Net change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total ester bonds</td>
</tr>
<tr>
<td></td>
<td>μg</td>
</tr>
<tr>
<td>Complete ..........</td>
<td>+0.72</td>
</tr>
<tr>
<td>Complete, plus 10 μmoles of ATP</td>
<td>0.64</td>
</tr>
<tr>
<td>No palmitoyl-CoA; 4 μmoles each of palmitate and CoA</td>
<td>−0.81</td>
</tr>
<tr>
<td>Boiled microsomes</td>
<td>0.00</td>
</tr>
</tbody>
</table>
date, it has not been possible to separate the microsomal lipase from the monoglyceride acylase. The presence in the microsomes of those two enzymes is noteworthy, especially since it has already been shown (6) that the microsomes possess an active long chain fatty acid thioquinase which can, with CoA and ATP, activate fatty acids to the acyl-CoA derivatives.

Net Synthesis of Ester Bonds—Chemical assay of ester bonds by alkaline hydroxylamination was carried out to provide additional proof of net glyceride synthesis by nonisotopic means. It may be seen in Table III that confirming evidence of the overall synthesis of higher glycerides from monopalmitin and palmitoyl-CoA was obtained. In these experiments again the addition of ATP provided no further stimulation. Free palmitic acid and CoA could not substitute for palmitoyl-CoA in producing net synthesis of glycerides; in fact, significant loss of ester bonds occurred, indicating hydrolysis. Noteworthy also was the conversion of ATP provided no further stimulation. Free palmitic acid and CoA could not substitute for palmitoyl-CoA in producing net synthesis of glycerides; in fact, significant loss of ester bonds occurred, indicating hydrolysis. Noteworthy also was the conversion of 50% of the monopalmitin to higher glycerides, in only 5 minutes of incubation.

Effect of Other Acceptors—As observed by Mattson et al. (1), the hydrolysis of triglycerides by pancreatic lipase results predominantly in formation of 2-monoglycerides, due to cleaving of the primary alcohol ester bonds. Although the 2-monoglycerides thus produced spontaneously isomerize into 1-monoglycerides, it was decided to test also the effects of the 2-monoglyceride isomers in this system. As shown in Table IV, use of 2-monopalmitin and 2-monoolein as acceptors for the palmityl-1-C¹⁴-CoA led to results similar to those found with the 1 isomers. About 30 to 50% of the label from palmityl-1-C¹⁴-CoA was incorporated into diglycerides, when the various monoglycerides were added. However, the addition of monoglycerides did not result in increased labeling of triglycerides, cholesterol esters, or phosphatidic acids.

In other experiments, β-glycerophosphate was used as an acceptor, but yielded no increase in glyceride incorporation of the fatty acid label.

Table IV
Effect of monoglyceride structure on microsomal incorporation of palmityl-1-C¹⁴-CoA.

The incubation systems each contained 0.01 pmole of palmityl-1-C¹⁴-CoA (450,000 c.p.m. per pmole), 0.1 g of bovine serum albumin, 2.0 mg of microsomal protein in a total volume of 2 ml of Krebs-Ringer-phosphate buffer (pH 7.4). If added, 3 µmoles of the monoglycerides were dispersed in the albumin solution. Incubations at 38° were stopped after 10 minutes by addition of 0.5 ml of 3.2 N HClOd, and lipids were processed as described in the text. Incubations were carried out at 38° for 15 minutes, then stopped by addition of 12 ml of ice-cold methanol, after which lipids were extracted, separated, and assayed as described in Table III.

Comparison with Rabbit Intestinal Cell Activity—In the presence of the microsomal fraction of rabbit gut mucosal cells, palmityl-1-C¹⁴-CoA was incorporated into glycerides, primarily diglycerides, when monopalmitin was the added acceptor. Lesser activity was observed in mitochondria than in microsomes, and the supernatant fractions were inactive. Attempts to solubilize the condensing enzyme system from the microsomes were made using Trimeresurus flavoviridis venom which has been successful with some other microsomal enzymes (18). However, the activity remained in the fraction precipitable at 105,000 × g for 30 minutes and no active soluble preparation was obtained.

Lack of Effect of Free Glycerol as Acceptor—Experiments were carried out to determine whether glycerol 2 H² or glycerol 1(3) C¹⁴ could be directly esterified with palmityl-CoA by microsomal or mitochondrial enzymes of the rat intestinal mucosal cells, under conditions similar to those employed with the monoglycerides. Only trace amounts of labeled glycerides could be detected, which represented less than 0.008% of the glycerol present (5 µmoles), and which were no greater than in the absence of the subcellular fractions. Furthermore, no esterification of the glycerol by palmityl-CoA could be stimulated by addition of free fatty acids or conjugated bile salts to the medium, although Saunders and Dawson (19) have been able to obtain increased incorporation of glycerol-C¹⁴ into lipids of everted intestinal segments by such additions.

Comparison with Liver Microsomes—In view of the findings of Weiss, Kennedy, and Kiyasu (20) of a diglyceride-acylating enzyme in chicken liver microsomes, experiments were carried out with rat liver microsomes, derived from 0.25 M sucrose homogenates, to search for a monoglyceride acylase. Using monopalmitin-C¹⁴ and palmityl-CoA, under conditions listed in Table V, it can be seen that no diglyceride synthesis occurred. These results contrasted sharply with those obtained with the intestinal
cell microsomes. However, it is interesting to note that the liver microsomes were even more active than the gut microsomes in cleaving the monopalmitin C\textsuperscript{14} to free palmitate and glycerol C\textsuperscript{14}.

**DISCUSSION**

In keeping with the general principle of separate pathways for biosynthetic and degradative processes within the intestinal epithelium, the enzymatic reassembly of higher glycerides from free fatty acids and 3-carbon acceptor polyalcohols is an energy-requiring sequence of reactions distinct from lipolysis (21). Although some reversibility of lipase reactions has been shown to occur intraluminally (22), and perhaps is possible within the cell, no efficient net synthesis of higher glycerides can be shown to occur by this means. As is true for most synthetic mechanisms thus far elucidated, the energy is derived from ATP, through the intermediate formation of activated fatty acid thiesters. No evidence has been obtained to indicate that free glycerol can be directly phosphorylated to L-\alpha-glycerophosphate in the intestinal mucosa, although it can be incorporated to a considerable extent into triglycerides under suitable circumstances, as recently reported by Saunders and Dawson (19), and Buchs and Favarger (23). The mechanism for the incorporation of free glycerol into higher glycerides, if not by direct acylation or phosphorylation, remains obscure, although some evidence suggests re-entry of the glycerol into the carbohydrate pool.

Despite the recognized difficulties and pitfalls of intracellular localization of enzyme activities, our data consistently indicate that the microsomal fraction of the intestinal epithelial cell has the highest specific activity and total capacity in catalyzing both biosynthetic and degradative processes within the intestinal epithelium, and other tissues. In our experiments, neutral isotonic mannitol solutions were used to make homogenates of gut mucosa because of the quite active sucrase located in the brush border portions of the epithelial cells as shown by Miller and Crane (28). Since the specific gravity of isotonic mannitol is 1.016, compared to 1.034 for 0.25 M sucrose, one might expect the sedimentation of the microsomal fragments in a given time and gravitational field to be more complete. It has been observed before by Palade and Siekevitz (26) that the microsomes can be obtained relatively free of either mitochondria or supernatant fraction, whereas the reverse is not true: both mitochondrial and supernatant fractions are appreciably contaminated with microsomes. Therefore, the differences in observed localization of these enzymes may well be due to differences in technique, and to the physicochemical conditions existing during centrifugation.

It is of some interest that cells which synthesize large amounts of lipid for export, such as the Meibomian sebaceous gland cells of the rat, have been shown in the electron microscopic studies of Palay (24) to have a striking content of smooth endoplasmic reticular membranes associated with the newly formed lipid droplets. The association of the endoplasmic reticulum of the rat gut epithelial cell with fat droplets during intestinal absorption has been beautifully shown by Palay and Karlin (25), although these studies in fine structure morphology were not suited to demonstrate chemical changes in the lipids during the absorptive process. Palade and Siekevitz (26) have found that fragments from the rough- and smooth-surfaced endoplasmic reticular network from liver cell homogenates make up the fraction referred to as microsomes.

Our studies of the microsomal fraction derived from isolated rat intestinal epithelial cells have indicated the presence of several enzymes concerned with the sequential steps in fat absorption: a long chain fatty acid thiolase, a monoglyceride acylase, as well as a monoglyceride acylase and diglyceride acylase. Although we have found considerable activity of each of these enzymes in mitochondria, the specific activity has been consistently lower than in microsomes; even lower, almost negligible specific activity of these several enzymes has been found in the postmicrosomal supernatant fractions of both rat and rabbit intestinal cells. This intracellular localization of the monoglyceride-acylating enzyme differs from that found by Hubscher (9, 27), who has reported stimulation of the incorporation of palmitate-C\textsuperscript{14} into diglycerides upon addition of monoglycerides in the presence of either mitochondria or soluble fractions. Those fractions were derived from 0.25 M sucrose homogenates of intestinal epithelium, and other tissues. In our experiments, neutral isotonic mannitol solutions were used to make homogenates of gut mucosa because of the quite active sucrase located in the brush border portions of the epithelial cells as shown by Miller and Crane (28). Since the specific gravity of isotonic mannitol is 1.016, compared to 1.034 for 0.25 M sucrose, one might expect the sedimentation of the microsomal fragments in a given time and gravitational field to be more complete. It has been observed before by Palade and Siekevitz (26) that the microsomes can be obtained relatively free of either mitochondria or supernatant fraction, whereas the reverse is not true: both mitochondrial and supernatant fractions are appreciably contaminated with microsomes. Therefore, the differences in observed localization of these enzymes may well be due to differences in technique, and to the physicochemical conditions existing during centrifugation.
tion. However, it is of interest that in both liver (20) and adipose tissue (29) diglyceride acylase activity has been found in particular fractions derived from homogenates of these tissues.

The microsomal fraction of the rat intestinal epithelial cells thus appears from our studies to contain the highest concentration of fatty acid thiokinase (6) and monoglyceride acylase, as well as a monoglyceride lipase. Since some triacylglycerol formation occurred in these experiments, and the major product of lipid synthesis in the intestinal mucosa or slice (21) is triglyceride, the presence in the microsomes of a diglyceride acylase similar to that described in other tissues (20, 29) is suggested. Because the membrane structures of the endoplasmic reticulum constitute the major portion of the endoplasmic reticulum (26), it appears that the enzymes needed for lipid resynthesis are associated with the endoplasmic reticulum. This agrees with the observations by electron microscopy of rat intestinal epithelial cells (25), made at various times during corn oil absorption, in which the lipid droplets appeared to be enclosed by membranes of the endoplasmic reticulum during their passage through the cells. Therefore, the endoplasmic reticulum or the microsomal fraction of the intestinal mucosal cells seems to be instrumental in carrying out the synthesis as well as the transport of lipid.

The failure of ATP to stimulate further the rapid and efficient condensation of fatty acyl-CoA and monoglyceride indicates that the requirement for this cofactor is at the first step in the process, namely, that of fatty acid activation. Although Hokin and Hokin (30) reported the phosphorylation of monoglycerides to lysophosphatic acid by brain microsomes, in our experiments no significant labeling of phospholipids was detected at any time, either with or without added ATP.

The quantitative role of the monoglyceride direct esterification pathway in lipid absorption remains to be demonstrated. As is apparent from the scheme shown in Fig. 2, clarification is also needed concerning the mechanism for the incorporation of free glycerol into the glycerides, as well as the manner whereby the lipid products of digestion penetrate across the brush border into the intestinal cell.

**SUMMARY**

Epithelial cells of rat and rabbit gut mucosa have been shown to contain an enzyme system, referred to as monoglyceride acylase, which catalyzes the direct condensation of palmitoyl coenzyme A and monoglycerides to diglycerides as follows:

\[
\text{Monoglycerides} + \text{palmitoyl coenzyme A} \rightarrow \\
\text{diglycerides} + \text{coenzyme A}
\]

The enzyme system was found to be heat labile, and the highest specific activity was present in the microsomal fraction of manitol homogenates of the mucosal cells. The reaction did not require adenosine triphosphate, nor were phosphorylated intermediates detected. An active monoglyceride lipase was also demonstrated in the microsomal fraction.

Since the microsomes of the rat intestinal epithelial cells also contain a long chain fatty acid thiokinase which activates fatty acids to fatty acyl coenzyme A derivatives, the enzymes necessary for the resynthesis of higher glycerides from free fatty acids are found together in the microsomal fraction of the gut mucosal cells. This correlates well with the electron microscopic observations of rat intestinal epithelial cells during fat absorption, in which lipid droplets were found to be surrounded by membrane structures of the endoplasmic reticulum. These membrane structures presumably give rise to the microsomal fraction by ultracentrifugation. It is suggested that the microsomes derived from the endoplasmic reticulum play an essential role in the intracellular resynthesis and transport of lipids in the intestinal epithelium.

**Acknowledgment**—The authors are grateful to Miss Dorothy Busz for her skillful technical assistance.

**REFERENCES**

1. **Mattson, F. H., Benedict, J. H., Martin, J. B., and Beck, L. W., J. Nutrition, 45, 336 (1952).**
3. **Savary, P., and Dussfeld, P., Biochim. et Biophys. Acta, 21, 349 (1956).**
8. **Skipski, V. P., Morehouse, M. G., and Deuel, H. J., Jr., Arch. Biochem. Biophys., 41, 93 (1959).**
9. **Clark, B., and Hübischer, G., Biochim. et Biophys. Acta, 46, 479 (1961).**
11. **Smith, L., Arch. Biochem., 60, 285 (1954).**
15. **Seubert, W., in H. A. Lardy (Editor), Biochemical Preparations, Vol. 7, John Wiley & Sons, New York, 1960, p. 80.**
17. **Snyder, F., and Stephens, N., Biochem. et Biophys. Acta, 34, 244 (1959).**
Direct Esterification of Monoglycerides with Palmityl Coenzyme A by Intestinal Epithelial Subcellular Fractions
John R. Senior and Kurt J. Isselbacher

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