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The intestinal absorption of fatty acid: A biochemical and electron microscopic study*

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SUMMARY

Following the administration of 2 ml of C^{14}-labeled oleic acid to rats by stomach tube, osmiophilic droplets and particles, from 10 to 300 mμ in diameter, were demonstrated in the intestinal lumen. The smaller droplets of fatty acid were frequently found between the microvilli of intestinal epithelial cells and were of the same size range as has been proposed for lipid micelles (10 mμ). The radiochemical examination of the lumen contents revealed that the activity was still present in free fatty acids. Larger osmiophilic droplets, averaging 150 mμ in diameter, were found in cytoplasmic vesicles within the epithelial cells.

These findings suggest that electron microscopic visualization of lipid droplets cannot distinguish between fatty acid and mono-, di-, and triglycerides. Therefore, conclusions on the nature of osmiophilic lipid droplets in intestinal absorption must be based upon a correlation with biochemical findings.

Chemical and radiochemical findings in the absorption of fatty acid indicate that the fatty acid enters the chyle primarily as triglyceride, that the triglyceride content of the intestinal wall is increased, and that there is a significant transfer of the C^{14} label from fatty acid to triglyceride at some stage during absorption. These observations mean that at least some of the osmiophilic droplets seen with electron microscopy in the intestinal epithelial cells in fatty acid absorption must be triglycerides. The relationship of these findings to the synthesis of triglyceride from fatty acids during internalization and transcellular passage is discussed.

Although some of the aspects of lipid absorption have recently been clarified by biochemical and electron microscopic studies, several facets of this problem remain unsolved. In some respects, the biochemical and electron microscopic findings appear to conflict. The acme of these conflicting observations is expressed in the two prevalent concepts of the mechanism of internalization of lipid in the absorptive epithelial cells. The work of Baker (1), Hewitt (2), and Palay and Karlin (3), based upon morphological studies of the process of lipid absorption, have stressed the uptake of lipid droplets by pinocytosis. Biochemical studies (4), on the other hand, have tended to favor the molecular uptake of fatty acid and the lower glycerides. More recently (5), it has also been proposed that lipid is taken up as micelles during intestinal absorption, presumably by pinocytosis.

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It is well established that some degree of hydrolysis of triglyceride occurs (6) in the intestinal lumen prior to absorption, and that, in the intestinal epithelial cell, triglyceride (7) and phospholipid (8) are synthesized. This has been substantiated by more recent biochemical studies of intestinal cell homogenates (9, 10), of everted intestine sac (11), and of thoracic duct lymph (12) during fat absorption. On the other hand, electron microscopic studies give no indication of such a transformation of the absorbed product. Instead, lipid droplets visible in the cytoplasmic vesicles resemble one another at all levels in the cells, and they resemble droplets of lipid found in the intestinal lumen and in the lymphatics of the intestinal wall.

In order to obtain a closer correlation between the morphological and chemical findings, it was proposed to carry out a combined electron microscopic and biochemical study of the intestinal absorption of fatty acid, the hydrolytic product of triglyceride cleavage. The resulting evidence was compared with that obtained when triglyceride was administered.

The direct intestinal absorption of fatty acid has been amply demonstrated in both the everted intestinal sac (11) and in the intact animal (12). Previous studies have shown that administered fatty acid is almost completely converted to triglyceride, in which form it appears in the chyle of the intact animal (13). Fatty acid has also been found to be largely converted to triglyceride in the serosal fluid of the everted sac (11, 14).

METHOD

Adult male rats of the Sprague-Dawley strain, weighing about 250 g, were used. In the first group of six animals, fasted for 24 hr, 2 ml of oleic acid was administered by stomach tube under light ether anesthesia. Two fasting control animals were also lightly anesthetized, but did not receive oleic acid. Small blocks of tissue were obtained from the upper, middle, and lower jejunum for histological and electron microscopic study at 30, 60, and 180 min after administration of the oleic acid. Blood was obtained from the aorta of each of these animals for determinations of triglyceride (15), phospholipid (16), cholesterol (17), fatty acid (18), and total lipids.

In the second group, ten animals were fasted 24 hr and then given 2 ml of C^{14}-labeled oleic acid in order to follow isotopically the metabolic fate of the fatty acid. Six of these were studied at 30 min and four at 60 min following the administration of fatty acid. Gut wall samples for radiochemical analyses were obtained from the same areas from which samples were taken for electron microscopy. Lumen contents were also analyzed in some experiments. Extracts of the gut wall segments and of the lumen contents were fractionated, and different lipid components containing activity were identified.

Segments of jejunum were obtained from the four animals of the preceding group, killed after 60 min, for quantitative determination of triglyceride and fatty acid. An additional four animals were studied 3 hr after oleic acid administration, and two fasting animals were similarly studied as controls.

In all cases, the jejunal segments were first carefully rinsed with saline to remove as much of the unabsorbed oleic acid as possible. Specimens were also obtained from each of the 24 animals of the preceding groups for histological and electron microscopic study.

Isolation and separation of the individual lipid components for radioactive assay were carried out on these jejunal segments by extraction with choloroform-methanol 2:1 followed by thin-layer chromatography (19) and liquid scintillation counting (20). This resulted in the distinct separation of fatty acids and mono-, di-, and triglycerides. Fatty acids and triglyceride fractions were also quantitatively determined on the extracts.

Tissue for electron microscopy was fixed in 1% osmium tetroxide, buffered to pH 7.2 with veronal. It was dehydrated in graded alcohols and embedded in butyl-methyl methacrylate. Sections were studied with an RCA EMU-3 microscope. Histologic sections were obtained from carbowax embedded tissue and were stained for fat, using Sudan black B.

RESULTS

Fat stains of the jejunal mucosa studied with light microscopy disclosed a faint but definite increase in diffuse staining of the cytoplasm of epithelial cells in the tips of the villi. Distinct droplets could not, however, be recognized with this method.

In all experiments, electron microscopy regularly showed evidence of lipid absorption, consisting of variable-sized droplets of osmiophilic material within cytoplasmic vesicles of the absorptive cells (Figs. 1, 2, and 3). As others (21) have observed during uptake of triglyceride by the intestinal epithelial cell, those cells in the tips of the intestinal villi participated most actively in this visible phase of absorption. The epithelial cells at the base of the villi and those in the crypts of Lieberkühn were always free from lipid droplets. In fasting animals, except for rare small dense particles about 10–50 mμ in size, the cytoplasmic vesicles were empty.

After the administration of fatty acid, the intestinal lumen contained many minute, rounded, osmium-stained droplets, forming a continuous spectrum of sizes from 10 to 300 mμ (Fig. 4). The smaller ones (diameter 10–40 mμ) were frequently observed in the spaces between the microvilli (Fig. 5). The smallest particles in the lumen became indistinguishable from nondescript, electron dense particles, which may be seen in the intestinal lumen contents in the fasting animal. Comparison of the intestinal epithelial cells of fasting animals with those of animals absorbing fatty acid revealed no difference in the number or appearance of minute infoldings of the intermicrovillous membrane. The infoldings averaged one per 20 microvilli in fasting and absorbing states and could be traced into and across the terminal web as extremely narrow channels when seen in longitudinal or oblique section. They appeared as minute round or oval vesicles about 70 mμ in diameter when they were transversely sectioned (Fig. 6). The cytoplasm immediately beneath the terminal web contained numerous larger vesicles. These were of

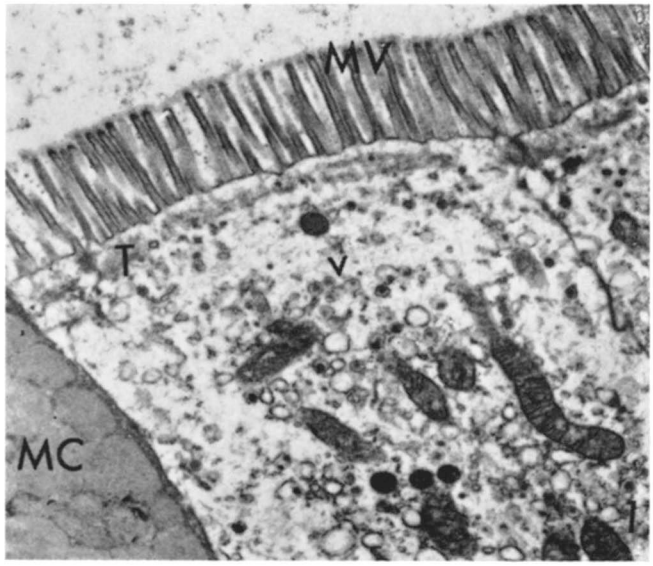

FIG. 1. Electron micrograph of intestinal epithelium 30 min after fatty acid administration. Portions of two absorptive epithelial cells and one mucous cell (MC) are shown. In the cytoplasm beneath the terminal web (T) are seen vesicles (V), many of which contain small osmiophilic droplets about 100 mμ in diameter. Barely visible between the microvilli (MV) are minute, slightly dense particles of 15–30 mμ size. Magnification × 10,000, reduced 50%.

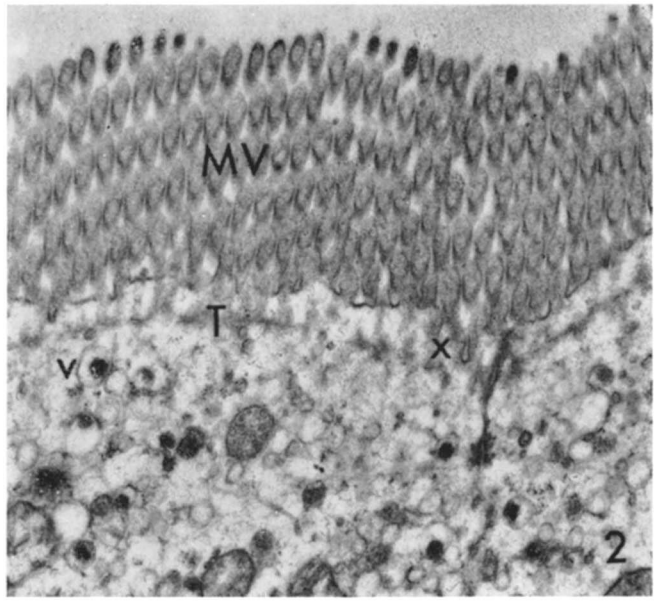

FIG. 2. Electron micrograph of portions of two absorptive epithelial cells during fatty acid absorption. The microvilli (MV) are cut slightly tangentially. A few pinocytotic membrane inclusions are present (X). Lipid droplets, measuring up to 100 mμ in size, are present in vesicles (V) of the cytoplasm beneath the terminal web (T). Magnification × 18,000.

fairly uniform size, averaging about 250 mμ in diameter. Continuity could occasionally be seen between these larger vesicles and the smaller membrane extensions in the terminal web. The larger cytoplasmic vesicles were as numerous in fasting animals as in those actively absorbing fatty acid. In both instances, the membranes of the vesicles near the terminal web were smooth; deeper in the cell, ribosomes were present on the exterior of similar appearing vesicles. The vesicles in the fasting animal contained only rare and very small osmiophilic particles. During the process of fatty acid absorption, however, the majority of the cytoplasmic vesicles contained distinct droplets of the osmiophilic material. These resembled the osmiophilic droplets seen in rats absorbing corn oil (3, 22), except that, during fatty acid absorption, the droplets tended to be smaller and more uniform in size. The droplets were nearly uniform in size in different portions of the cell, averaging about 150 mμ.

Clusters of osmiophilic lipid droplets were also observed in the intercellular spaces (Fig. 7). These spaces were often dilated and were only partly filled with the lipid droplets. Similarly, lipid particles partially filled lake-like areas between the absorptive epithelial cells and the adjacent basement membrane. The interstices of the lamina propria were also widened and contained numerous lipid droplets. Many of the lymphatic channels of the lamina propria contained similar osmiophilic droplets measuring 100–300 mμ (Fig. 8).

Determinations of serum lipids revealed an increase in total lipid and triglyceride at 1 and 3 hr after the administration of oleic acid by stomach tube (Table 1).

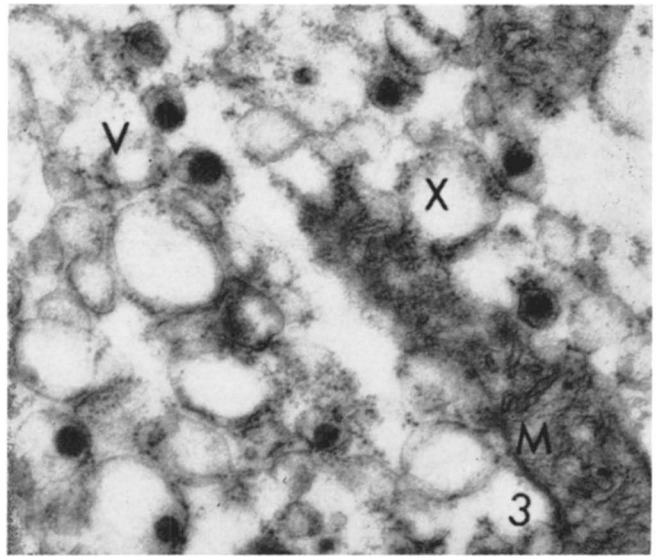

FIG. 3. Higher magnification of portion of epithelial cell during fatty acid absorption. The cytoplasmic vesicles (V) average about 250 mμ in diameter, and they contain lipid droplets about 100 mμ in diam. Ribosomes are present on the outer surface of some of the vesicles (X). Mitochondria (M) are also observed. Magnification × 40,000.

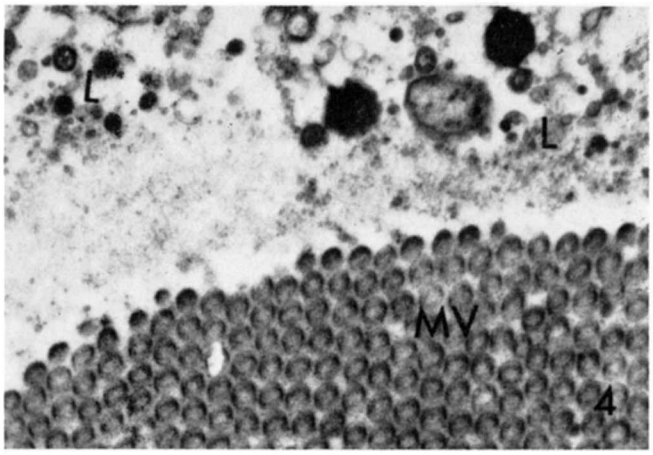

FIG. 4. Electron micrograph of portion of lumen and adjacent tips of microvilli (MV) of small intestinal epithelium 30 min after fatty acid administration. Some of the lumen contents are present, containing lipid droplets (L) and particles measuring 10–300 mμ. None of the lipid droplets are seen between the microvilli in this area. Magnification × 25,000.

One hour after the administration of fatty acid, the triglyceride content of the bowel wall was increased over that of normal controls, and the fatty acid content was also increased. At 3 hr, these had returned to near normal levels (Table 2).

In Table 3 is shown the distribution of the label among the fatty acid and glyceride fractions isolated from segments of the intestinal wall and lumen contents at 30 and 60 min after the administration of C^{14}-oleic acid. A significant portion of the label was present in triglyceride obtained from the intestinal wall at the two time periods studied, being greater at 30 than 60 min. No conclusive evidence of significant conversion of the labeled fatty acid into glyceride was found in the intestinal lumen contents under these conditions, as is shown by the major portion of the radioactivity remaining in the fatty acid fraction. In addition, no appreciable activity was found in the monoglyceride

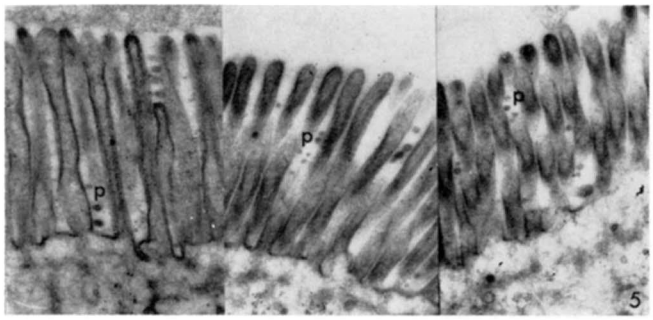

FIG. 5. Composite of three electron micrographs of intestinal epithelium during fatty acid absorption. Several small moderately dense particles (P) about 10–40 mμ size are seen between the microvilli. These are believed to represent micelles of fatty acid. Magnification × 40,000.

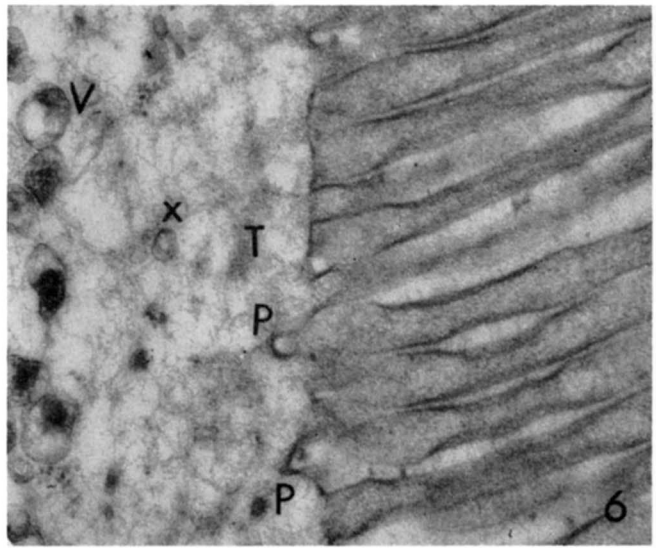

FIG. 6. Electron micrograph of portion of intestinal epithelial cells during fatty acid absorption At (P) are seen pinocytotic cell membrane inclusions; in the terminal web (T), several minute membrane profiles about 50–70 mμ in diameter (X) are seen. Larger cytoplasmic vesicles (V) are also present at deeper levels in the cell, some of which contain lipid droplets. Magnification × 40,000.

fraction isolated from either the intestinal wall or lumen contents.

DISCUSSION

These observations confirm the biochemical studies already cited, which indicate the direct intestinal absorption of fatty acid, its conversion to triglyceride in the intestinal epithelium, and the release of triglyceride chylomicrons into the intestinal lymphatics. The amount of transformation of fatty acid to triglyceride by the intestinal epithelium may not be entirely reflected by our studies, because any residual fatty acid on the surface of the mucosal epithelium would decrease the apparent transfer to glycerides. Although the intestinal mucosa was rinsed before extraction, it is likely that the fine layer of mucus on the surface, which is present in the rat, retained some of the un-

TABLE 1. AVERAGE SERUM LIPID VALUES AFTER ADMINISTRATION OF 2 ML OF OLEIC ACID BY STOMACH TUBE

Time After Administration of Fatty Acid	No. of Animals	Total Serum Lipid	Serum Triglyceride
		mg/100 nl	*mg/100 nl*
Fasting 24 hr	2	313	62
30 min	2	358	113
1 hr	2	460	248
3 hr	2	440	212

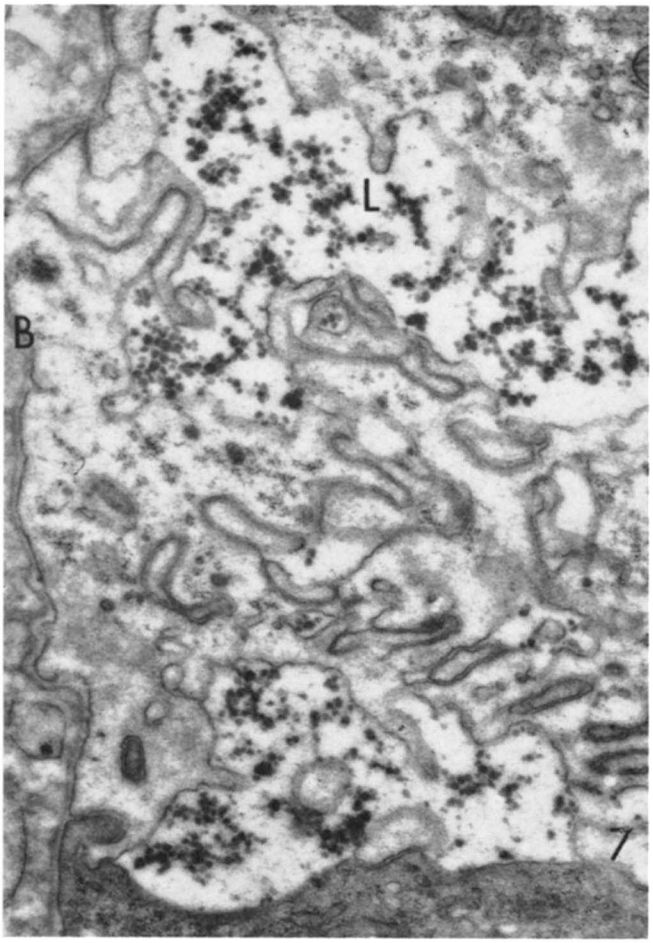

FIG. 7. Electron micrograph of basal portions of intestinal epithelial cells during fatty acid absorption, showing intercellular lake-like spaces (L) filled with osmiophilic droplets measuring up to 100 mμ. The basement membrane (B) is shown. Magnification × 18,000.

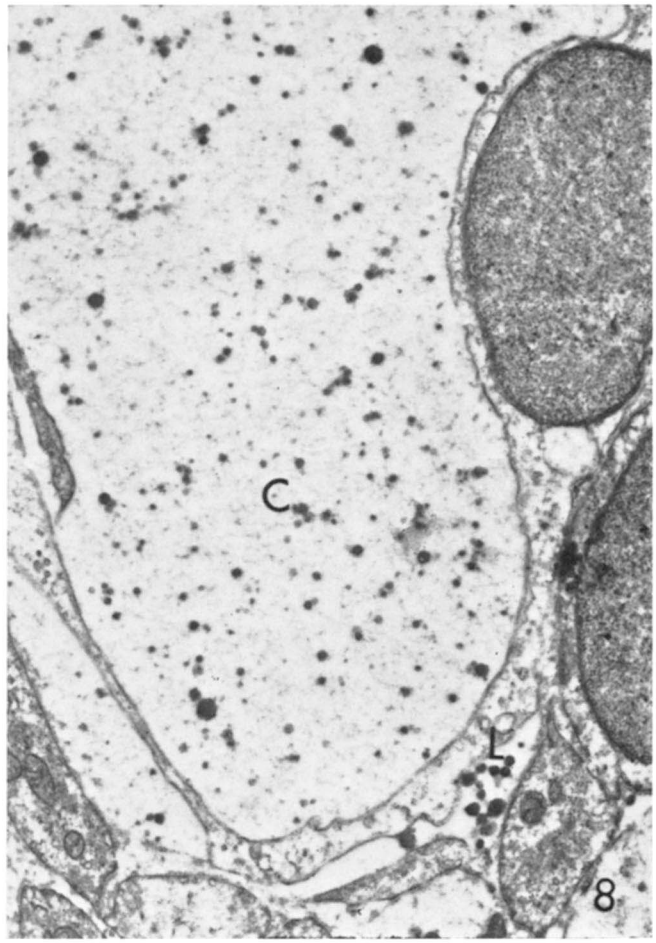

FIG. 8. Electron micrograph of lamina propria at 1 hr after administration of fatty acid. Lipid droplets (L) are present in the interstitial spaces, and small chylomicrons (C) measuring up to 200 mμ are seen in the lumen of a lymphatic channel. Magnification × 14,000.

absorbed fatty acids. In spite of this, the demonstration of about 40% of the isotope label in glyceride fractions after 30 min and about 30% at 1 hr, in addition to very low radioactivity in glycerides in the lumen contents, indicates that fatty acid was being converted to glyceride in the intestinal epithelial cells. The rise in serum triglyceride at 1 and 3 hr after the administration of oleic acid conforms with previous observations (13).

While it is possible to demonstrate with electron microscopy the uptake of osmiophilic lipid by the intestinal epithelium and the presence of lipid droplets in the cytoplasmic vesicles of the intestinal epithelium during fatty acid absorption, it is evident that the chemical nature of the osmiophilic droplets within the cytoplasmic vesicles is not revealed by this method of study. The intestinal lumen in these experiments contained fatty acid in emulsified and possibly in micellar form, which was visible as numerous round osmiophilic droplets. The droplets were indistinguishable from triglyceride droplets seen in the intestinal lumen during the absorption of corn oil (3, 22), and they appear to be identical with lipid droplets in the lymphatic vessels, which also are known to be mainly triglyceride. It is evident that, at some stage between the uptake of fatty acid by the intestine epithelium and the delivery of triglyceride chylomicrons to the lymphatics, conversion of fatty acid to triglycerides takes place. At what level in the cell, or at what stage in the transport of the absorbed fatty acid, this process occurs cannot be determined by electron microscopy. It could occur at the time the fatty acid particle or micelle is internalized at the intermicrovillous space, either by pinocytosis or transport across an intact cell membrane. The unit membrane here is known to be endowed with a high concentration of ATP-utilizing enzyme systems (23) and other phosphorylytic enzymes (24, 25). These could contribute

TABLE 2. AMOUNTS OF FATTY ACID AND TRIGLYCERIDE RECOVERED FROM RINSED WHOLE GUT HOMOGENATES AFTER ADMINISTRATION OF 2 ML OF OLEIC ACID BY STOMACH TUBE

Time After Administration of Oleic Acid	No. of Animals	Fatty Acid		Triglyceride	
		Average	Range	Average	Range
		mg/100 g		*mg/100 g*	
Fasting controls	2	458	447–470	187	183–191
1 hr	4	1,557	1,247–1,846	653	374–925
3 hr	4	796	699–991	138	92–208

to biosynthetic mechanisms. On the other hand, the morphologic observations reported here on fatty acid absorption are also compatible with the synthesis of triglyceride and phospholipid within the cytoplasmic vesicles, while they are in transit across the intestinal epithelial cell.

The size distribution of the lipid droplets within the intestinal lumen are of interest in regard to Hofmann and Borgström's (5) studies on the nature of lipid within the intestinal lumen. These investigators showed that, during the digestion and absorption of triglycerides, the resulting monoglycerides and fatty acids, in the presence of bile salts, are reduced to a micellar solution in the intestinal lumen. They proposed that it is in this form, as micelles approximately 100 Å in size, that cellular uptake of lipid may occur. Our observations with electron microscopy lend support to this proposal in that they show lipid droplets in the lumen up to 3,000 Å diam, with a regular gradation downward in size to particles of about 100 Å. These smallest particles are actually below the limit of certain identification since other nondescript particles of similar size can be seen in the intestinal lumen. Nevertheless, the appearance of such minute particles between the microvilli during fatty acid absorption suggests that they may be the lipid micelles, which are in position for internalization.

The question of the mechanism of internalization of absorbed lipid has been a difficult one to solve and still remains unanswered. Although lipid droplets of small size have been seen in the lumen and in the cytoplasmic vesicles in the cytoplasm below the terminal web, the actual process of pinocytotic uptake of droplets of similar size probably has not been observed. The demonstration of the ability of the intestinal epithelial cell to take up various particles (26–28), even as large as 2,600 Å, has not resolved this problem, because the degree to which the intestinal absorptive cells normally carry out this type of pinocytotic uptake has not been shown.

Membrane infoldings from the intermicrovillous spaces have been described by several observers (3, 26–28), but these have always been noted to be rather sparse. The degree to which this process contributes to the internalization of lipid material may therefore be questioned. Study of numerous micrographs of intestinal epithelium of the rat in the fasting state and during fatty acid absorption in our material has revealed that the necks of pinocytotic membrane inclusions can be seen on the average of about one for every 20 microvilli in both fasting and absorbing animals. This could indicate either (a) that pinocytotic inclusions are unrelated to absorption, (b) that they continue to form, even during fasting, or (c) that the rate of formation of pinocytosis inclusions varies in the fasting and absorptive states. The calculations of Palay and Karlin (3), and some we have made based on the frequency and size of pinocytotic membrane inclusions in the intestinal samples of our material, suggest that this process could contribute significantly and quantitatively to lipid internalization. But this would require a certain rate of formation of pinocytosis vesicles and trans-

TABLE 3. PERCENTAGE DISTRIBUTION OF C^{14} RECOVERED IN DIFFERENT LIPID COMPONENTS IN INTESTINE AFTER ADMINISTRATION OF LABELED OLEIC ACID

Time After Administration of C^{14}-Labeled Oleic Acid	Source of Material Analyzed	No. of Animals	Percentage Distribution of Recovered C^{14} in Lipid Fractions			
			Fatty Acid	Monoglyceride	Diglyceride	Triglyceride
30 min	Lumen contents	6	86	0	4	3
	Entire gut wall	6	58	4	7	30
60 min	Lumen contents	2	76	0	3	10
	Entire gut wall	4	56	4	4	21

cellular movement, and this remains indeterminate. The final answer to this problem is plainly yet to be determined.

If it is assumed that fatty acid is taken into the cells by means of cell membrane infolding, the question of the mechanism of triglyceride synthesis and of membrane elaboration around osmiophilic droplets deeper in the cytoplasm might be considered. The continual formation of transport vesicles would seem to require a plentiful supply of cytomembranes according to the proposal of Bennett (29). As fatty acid is internalized and then transported deeper into the cell, it is apparently converted to triglyceride since the radiochemical studies show the labeled fatty acid to be present in triglyceride of the intestinal wall. This presumably occurs through the phosphatidic acid pathway (30, 31). Such a biochemical mechanism would also provide intermediates for the biosynthesis of phospholipid, which seems to be manifested by the abundant elaboration of visible vesicular membranes known to be composed in part of phospholipid. Simultaneously, as in the scheme proposed by Kennedy, the fatty acid may be converted, within the newly formed phospholipid membrane, into the di- and triglycerides and ultimately almost entirely into the latter.

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REFERENCES

1. Baker, J. R. *Quart. J. Microscop. Sci.* **84**: 73, 1942.
2. Hewitt, W. *Quart. J. Microscop. Sci.* **95**: 153, 1954.
3. Palay, S. L., and L. J. Karlin. *J. Biophys. Biochem. Cytol.* **5**: 373, 1959.
4. Hogben, C. A. M. *Ann. Rev. Physiol.* **22**: 381, 1960.
5. Hofmann, A. F., and B. Borgström. *Federation Proc.* **21**: 43, 1962.
6. Reiser, R., M. J. Bryson, M. J. Carr, and K. A. Kuiken. *J. Biol. Chem.* **194**: 131, 1952.
7. Blankenhorn, D. H., and E. H. Ahrens, Jr. *J. Biol. Chem.* **212**: 69, 1955.
8. Schmidt-Neilson, K. *Acta Physiol. Scand.* **12**: Supp. 37, 1, 1946.
9. Clark, B., and G. Hübscher. *Biochem. Biophys. Acta* **46**: 479, 1961.
10. Dawson, A. M., and K. J. Isselbacher. *J. Clin. Invest.* **39**: 150, 1960.
11. Johnston, J. M. *J. Biol. Chem.* **234**: 1065, 1959.
12. Bergstrom, S., B. Borgström, A. Carlsten, and M. Rottenberg. *Acta Chem. Scand.* **4**: 1142, 1950.
13. Blomstrand, R., and E. H. Ahrens, Jr. *J. Biol. Chem.* **233**: 321, 1958.
14. Tidwell, H. C., and J. M. Johnston. *Arch. Biochem. Biophys.* **89**: 79, 1960.
15. Van Handel, E., and D. B. Zilversmit. *J. Lab. Clin. Med.* **50**: 152, 1957.
16. Fiske, C. H., and Y. Subarrow. *J. Biol. Chem.* **66**: 375, 1925.
17. Sperry, W. M., and M. Webb. *J. Biol. Chem.* **187**: 97, 1950.
18. Dole, V. P. *J. Clin. Invest.* **35**: 150, 1956.
19. Mangold, H. K. *J. Am. Oil Chemists' Soc.* **38**: 708, 1961.
20. Brown, J. L., and J. M. Johnston. *J. Lipid Res.* **3**: 480, 1962.
21. Padykula, H. A., E. W. Strauss, A. J. Ladman, and F. H. Gardner. *Gastroenterology* **40**: 735, 1961.
22. Ashworth, C. T., V. A. Stembridge, and E. Sanders. *Am. J. Physiol.* **198**: 1326, 1960.
23. Ashworth, C. T., F. J. Luibel, and S. C. Stewart. *J. Cell Biol.* **17**: 1, 1963.
24. Brandes, D., H. Zetterqvist, and H. Sheldon. *Nature* **177**: 382, 1956.
25. Holt, S. J., and R. M. Hicks. *J. Biophys. Biochem. Cytol.* **11**: 47, 1961.
26. Clark, S. L., Jr. *J. Biophys. Biochem. Cytol.* **5**: 41, 1959.
27. Barrnett, R. *Exptl. Cell Res.*, Suppl. 7: 65, 1959.
28. Sanders, E., and C. T. Ashworth. *Exptl. Cell Res.* **22**: 137, 1963.
29. Bennett, H. S. *J. Biophys. Biochem. Cytol.* Suppl. 2: 99, 1952.
30. Kennedy, E. P. *Ann. Rev. Biochem.* **26**: 119, 1957.
31. Clark, B., and G. Hübscher. *Nature* **185**: 35, 1960.