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LOCALIZATION OF FATTY ACID REESTERIFICATION IN THE BRUSH BORDER REGION OF INTESTINAL ABSORPTIVE CELLS

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SUMMARY

1. To determine whether fatty acid esterification occurs at the apical surface of intestinal absorptive cells during fat absorption, we isolated and fractionated intestinal brush borders from hamsters fed pure oleic acid and from everted intestinal sacs briefly incubated with ^{14}C -labeled oleic acid.

2. The amounts of fatty acid and triglyceride in isolated brush borders increased 3 to 5-fold after oleic acid was fed. Similarly, increased amounts of fatty acid and triglyceride were observed in the C-band fraction of disrupted brush borders. Although the C-band fraction from fasting hamsters constitutes a virtually pure preparation of microvillous membranes, this fraction was found to be contaminated with other subcellular elements when isolated from fat-fed animals.

3. When everted intestinal sacs were exposed for 30 sec to trace quantities of ^{14}C -labeled oleic acid, almost half of the radioactivity in subsequently isolated brush borders was present as triglyceride. When these brush borders were further fractionated, glyceride specific activity was found to be low in morphologically pure C-band preparations of microvillous membranes. In contrast the AB fraction, which consists of various subcellular elements including abundant smooth endoplasmic reticulum, contained glycerides with higher specific activities than those of whole brush borders or microsomes simultaneously isolated from the remainder of the cell.

4. These findings suggest that isolated whole brush borders owe their activity for glyceride synthesis, not to any component of the microvillus itself, but to the smooth endoplasmic reticulum within the rim of attached apical cytoplasm ordinarily adherent to isolated brush borders.

INTRODUCTION

During lipid absorption, small bowel mucosa synthesizes triglyceride from absorbed fatty acid and monoglyceride¹. When intestinal absorptive cells are examined with the electron microscope during fatty acid and monoglyceride absorption, oil

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droplets presumed to contain newly synthesized triglyceride are first detected in the subapical cytoplasm bounded by smooth membranes and are not seen in the microvilli or terminal web which form the brush border of the cell². Since microsomes isolated from intestinal cells synthesize triglyceride *in vitro*^{3,4}, it is generally believed that fatty acid and monoglyceride pass unchanged through the microvilli and are esterified by microsomes in the subapical cytoplasm.

This widely held view has recently been questioned. FORSTNER *et al.*⁵ demonstrated *in vitro* glyceride synthesis in purified preparations of rat brush borders. Similarly, GALLO⁶ showed that rat brush borders synthesize glycerides as actively *in vitro* as isolated microsomes. Furthermore, FORSTNER *et al.*⁷ found that when microvillous membranes are isolated from the brush borders of rats fed safflower oil, the triglyceride subsequently extracted from the microvillous membrane fraction contains increased amounts of linoleic acid. These observations raise the possibility that triglyceride may be synthesized from absorbed fatty acid at the absorptive surface, either on or within intestinal microvilli.

To examine events occurring at the surface of the intestinal cell during lipid absorption, we fed a pure fatty acid to hamsters and determined the lipid composition of subsequently isolated brush border and microvillous membrane preparations. We also exposed everted sacs of hamster small intestine to ¹⁴C-labeled oleic acid and measured radioactive glycerides present in subsequently isolated subcellular fractions. Although absorbed fatty acid was esterified in that region of intestinal cells which is isolated in standard brush border preparations, microvillous membranes lining the absorptive cell surface did not appear to account for this glyceride synthesis. The findings were consistent with the view that fatty acid esterification occurs in membranous elements associated with the rim of subapical cytoplasm adherent to isolated brush borders. During the course of these investigations it became apparent that, unlike the situation when fasting animals are used, microvillous membrane preparations obtained from fat-fed hamsters are grossly contaminated with other subcellular elements.

METHODS

Feeding experiments

Golden hamsters were fasted for 24 h and were sacrificed either in the fasting state or 2.5 h after 0.5 ml of oleic acid (Hormel Institute, Austin, Minn.) was administered via a gastric tube. The gut lumen was thoroughly flushed with the EDTA phosphate buffer used to isolate brush borders. The mucosa was lightly scraped from the entire gut length of each animal, and scrapings from 10–12 hamsters were pooled for each determination.

Incubation experiments

The full length of hamster intestine was used to make an everted sac, and 10–12 such sacs were used for each experiment. Sacs were incubated for 30 sec at 37° in an oxygenated micellar solution containing 2.4 μ moles sodium taurodeoxycholate (Maybridge Ltd., Cornwall, U.K.), 0.6 μ moles oleic acid (Hormel), 0.3 μ moles 1-monoolein (Hormel), 1.0 μ moles dextrose, and 0.3 μ C of [¹⁴C]oleic acid (New England Nuclear Corp., Boston, Mass.) per ml Krebs–Ringer phosphate buffer (pH 6.4). The

lipids were found to be at least 99 % pure by thin layer chromatography and were used as supplied. Following acidification and three extractions with diethyl ether, the taurodeoxycholate was found to contain no more than 0.5 % impurity.

Immediately following incubation, the sac was rinsed several times in iced buffer and the mucosa immediately scraped with a glass slide into EDTA-phosphate buffer at 0°. Rinsing and scraping took an average time of 90 sec.

Cell fractionation

Brush borders were prepared by the method of MILLER AND CRANE.⁸ Brush borders were fractionated as described by EICHHOLZ AND CRANE⁹. With this method, disruption of brush borders with 1.0 M Tris followed by density gradient centrifugation regularly separates brush border components into bands which have been designated "A, B, C, C', and D". Whereas the material recovered from the A, B and D bands is not homogenous, both the C and C' bands yield a virtually pure preparation of microvillous membranes¹⁰. In the present experiments the brush border components were collected in 4 fractions: the material from the A and B bands was collected as a single AB fraction, while the material in the C, C' and D bands were each collected separately. After the addition of 5 vol. of water or Krebs-Ringer phosphate buffer to each fraction, the C, C' and D fractions were recovered as pellets by centrifugation at $27\,000 \times g$ for 25 min. Recovery of the AB fraction was found to be complete only after centrifugation at $105\,000 \times g$ for 30 min.

Microsomes were isolated from the supernate obtained when homogenized mucosal scrapings were centrifuged at $500 \times g$ for 10 min to remove brush borders, nuclei and other large cellular debris. This supernate was adjusted to 0.25 M with sucrose and was centrifuged at $14\,500 \times g$ for 45 min to remove mitochondria and then at $105\,000 \times g$ for 30 min to recover the microsomal fraction.

In one experiment trilinolein emulsified in 5 % lecithin was added to mucosal scrapings which were then homogenized. Cell fractionation as described above was carried out to determine whether the added triglyceride would contaminate isolated preparations of brush borders and microvillous membranes.

Morphology

Aliquots of brush border preparations from each experiment were examined with a phase microscope to assure purity. Only those preparations virtually free of undisrupted cells, nuclei, and cellular debris were utilized for chemical analysis and radioactivity measurements.

The brush border fractions for electron microscopic examination were recovered from the density gradient by centrifugation in Krebs-Ringer phosphate buffer. The resulting pellets were fixed in chrome osmium tetroxide¹¹, dehydrated, and embedded in epoxy resin¹² directly in the centrifuge tube without disruption of the pellet. Orientation was thus maintained and the pellets were carefully mounted in the microtome chuck so that the full thickness of the pellet could be sectioned. Thin sections were stained with lead citrate¹³ and the morphological appearance of each pellet was systematically studied from top to bottom using a Phillips EM 300 electron microscope.

Chemical analyses

Lipids were extracted from tissue homogenates in chloroform-methanol (2:1, by vol.) and partitioned according to FOLCH *et al.*¹⁴. Total lipid weights were determined on a Cahn electrobalance and known quantities chromatographed on ether-washed thin layers of silica gel G with a standard mixture of chromatographically pure oleic acid, cholesterol and triolein (Hormel). Non-polar lipids were separated on long plates in solvent systems of benzene-diethyl ether-ethanol-acetic acid (50:40:2:0.1, by vol.) followed by hexane-diethyl ether (94:6, by vol.) and were detected by charring with 50 % sulfuric acid. Individual amounts of lipid classes were determined in relation to standards of known weight by photodensitometric measurement as described by DOWNING¹⁵. Triplicate analyses of each sample were found to agree with an error of less than 6 % for major components (as cholesterol) and an error of less than 10 % for minor components (as diglyceride). The weight of tissue fatty acids and glycerides were calculated by assuming that the average molecular weight of the fatty acid components was 283 (oleic acid). Phosphorus in lipid extracts was determined by a modification of the procedure of MARTIN AND DOTY¹⁶ and phospholipid content was calculated by assuming the average molecular weight of phospholipid to be 775. Glycolipid content was either determined by difference or was estimated by an anthrone reaction for lipid hexose¹⁷ and the average molecular weight of glycolipid was assumed to be 846 on the basis that one molecule of glycolipid contains one molecule of hexose. Radioactivity was determined by liquid scintillation counting in a toluene-based phosphor containing 4 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis-(5-phenyloxazolyl-2)-benzene per l of toluene. Quench corrections were made by the addition of internal standard.

Tissue protein was estimated by the method of LOWRY *et al.*¹⁸, maltase by the method of DAHLQVIST¹⁹ and RNA by an ultraviolet absorption method described by FLECK AND BERG²⁰.

RESULTS

Morphology and purity of subcellular fractions

The general appearance and purity of brush border preparations, as judged by phase microscopy, was identical in preparations isolated from fasted and fed animals.

The C band pellets isolated from Tris-disrupted brush borders showed striking differences when pellets from fat-fed animals were compared with those from fasted animals. The C band pellets prepared both from fasted animals and everted sacs used in incubation experiments were composed almost exclusively of microvillous membranes with a membrane width of 100 to 110 Å (Fig. 1A). In sharp contrast, the C band pellets isolated from fat-fed animals were consistently heterogeneous in their composition and contained, in addition to the microvillous membranes, considerable quantities of membranous material with a membrane width of only 70 to 80 Å (Fig. 1B). The morphological features of the thinner membranous profiles appeared identical to elements of smooth surfaced endoplasmic reticulum in microsomal pellets. In addition, large quantities of finely fibrillar material, not seen in C band pellets from fasted animals, were regularly present in C band pellets from fat-fed animals. The C' bands were morphologically indistinguishable from the C bands of the same

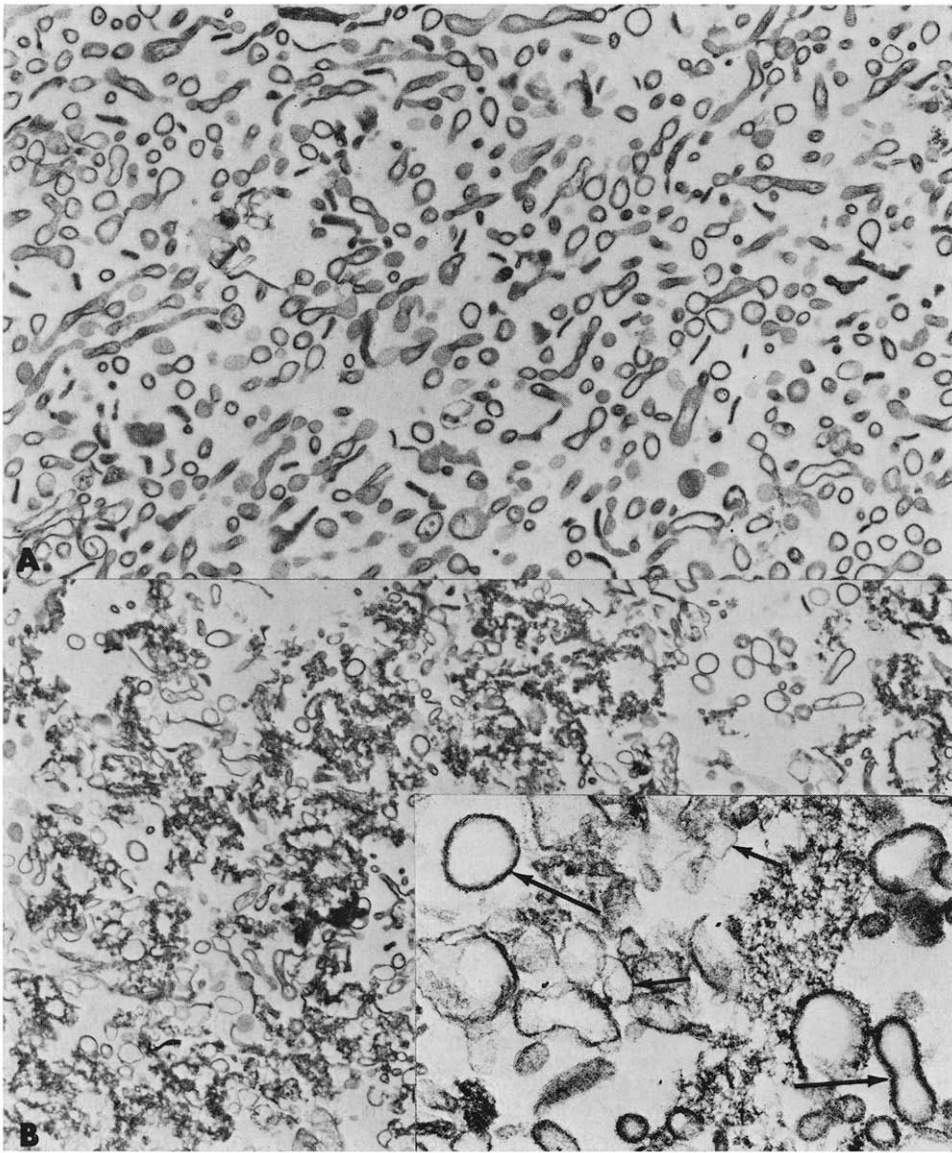


Fig. 1. Typical C-band pellets from fasted and fed animals. A. The preparation from fasted animals consists almost exclusively of microvillous membranes ($\times 12000$). B. The preparation from fed animals is heterogeneous and consists of thick membranes (long arrows), thin membranes (short arrows) and fibrillar material ($\times 12000$, insert $\times 50000$).

preparation. Maltase activity was higher in C band pellets obtained from fasted (4.8 ± 0.5 (S.D.) units) than in those obtained from fat-fed animals (3.8 ± 1.6 units).

The composition of the AB pellet which was recovered by centrifugation at $105000 \times g$ for 30 min was extremely heterogeneous. Three distinct components were regularly recognized in this fraction. First, smooth-surfaced vesicles limited by a

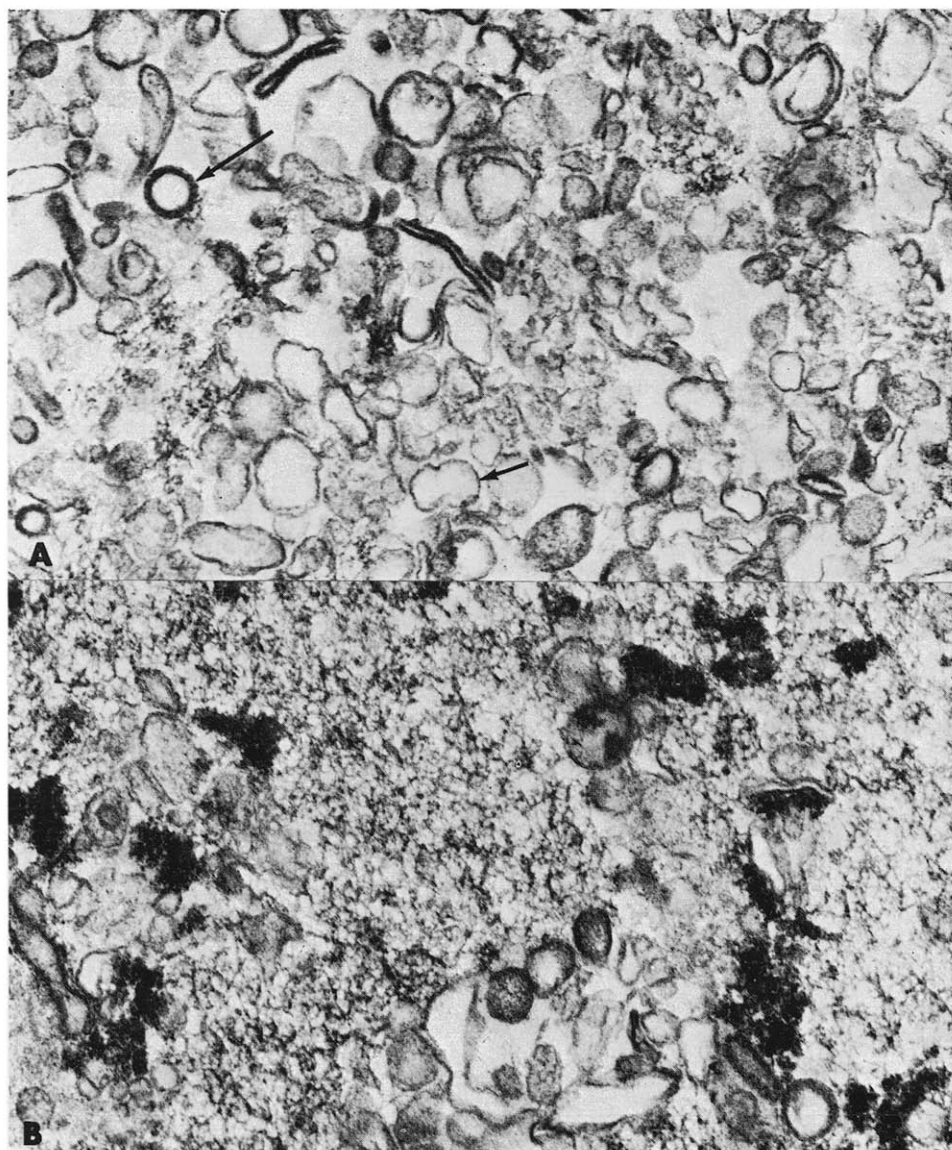


Fig. 2. Appearance of a typical AB pellet. A. The lower portion of the pellet consists largely of membranes. Thin membranes (short arrow) are most abundant but some thick membranes (long arrow) are also present ($\times 50\,000$). B. The upper portion of the pellet consists largely of fibrillar material although some membranes are also present ($\times 50\,000$).

70 to 80 Å membrane were the most abundant structure in this fraction (Fig. 2A). These were thought to be elements of smooth-surface endoplasmic reticulum. Secondly, fine fibrillar material of unknown nature was also abundant, especially in the upper half of the pellet (Fig. 2B). Thirdly, membranous profiles composed of membranes approximately 100 to 110 Å wide were regularly seen in AB pellets especially near the bottom of the pellet (Fig. 2A). These were identical in appearance to the micro-

villous membranes seen in pure form in the C or C' band from brush borders from fasted animals. AB band pellets from fasted and fed animals appeared identical except that pellets from fed animals occasionally contained profiles which resembled lipid droplets. In comparison to the maltase activity of the C band pellet containing pure microvillous membranes, the maltase activity of the AB fraction was markedly reduced (4.8 ± 0.5 units *vs.* 0.4 ± 0.1 units).

The microsomal pellets consisted almost exclusively of smooth-surfaced and ribosome-studded elements of endoplasmic reticulum. The RNA content of the microsomal pellets was 7.7 ± 2.8 μ g RNA-P/ml per mg protein compared to only 3.5 ± 2.5 in the AB pellets.

Feeding experiments

Table I indicates the lipid composition of homogenized intestinal mucosa, whole brush borders, and the C band fraction obtained from fasted hamsters and from animals fed pure oleic acid. When animals were sacrificed 2.5 h after fatty acid administration, the percentage of whole mucosal lipid present as fatty acid and triglyceride increased more than 3-fold. Following feeding, triglyceride alone comprised almost 33 % of the total lipid. Both in brush border and C band preparations there was a similar 3-fold increase in the fatty acid and triglyceride fractions after animals were fed fatty acid. However, the percentages of fatty acid and triglyceride present after feeding in brush borders and C bands were considerably smaller than in the whole mucosa, and the more polar phospholipids and glycolipids continued to be the preponderant lipid classes.

Following feeding, total lipid to protein ratios (mg/g) increased in brush borders from 430 ± 50 to 520 ± 70 and in C band preparations from 870 ± 150 to 1070 ± 130 but these changes were not significant. Individual lipid amounts per g of protein are shown in Table II. Whereas the amounts of phospholipid, glycolipid, cholesterol and diglyceride did not change with feeding, there was a 3.5 to 5-fold increase in the quantities of fatty acid and triglyceride in these tissue fractions.

TABLE I

THE PERCENTAGE OF THE TOTAL LIPID EXTRACTED FROM WHOLE MUCOSA, BRUSH BORDERS, AND THE C BAND IN FASTED AND FATTY ACID-FED HAMSTERS

The numbers in parenthesis represent the number of preparations analyzed. All values are shown as the mean \pm S.D.

	<i>Mucosa</i>		<i>Brush borders</i>		<i>C band</i>	
	<i>Fasted</i> (5)	<i>Fed</i> (4)	<i>Fasted</i> (4)	<i>Fed</i> (4)	<i>Fasted</i> (6)	<i>Fed</i> (4)
Phospholipids	52.0 ± 7.2	36.1 ± 5.2	48.6 ± 5.4	41.5 ± 6.0	36.6 ± 4.5	31.4 ± 4.9
Glycolipids	26.5^*	14.4^*	32.3^*	27.8^*	33.4 ± 1.4	29.0 ± 3.1
Cholesterol	7.7 ± 1.1	5.0 ± 0.7	13.0 ± 2.5	9.7 ± 1.6	13.4 ± 1.6	11.5 ± 1.9
Cholesterol esters	1.0 ± 0.7	0.3 ± 0.3	not detected	not detected	not detected	not detected
Fatty acids	3.3 ± 0.9	10.9 ± 6.6	1.8 ± 0.1	8.9 ± 5.6	2.7 ± 0.7	7.2 ± 2.3
Diglycerides	0.6 ± 0.4	0.7 ± 0.2	1.5 ± 0.9	1.9 ± 0.3	2.3 ± 1.0	2.3 ± 0.4
Triglycerides	8.9 ± 2.7	32.6 ± 9.6	2.8 ± 0.5	10.2 ± 2.3	1.5 ± 1.2	5.9 ± 2.2

* Determined by difference.

TABLE II

LIPID CONTENT IN mg/g TISSUE PROTEIN OF BRUSH BORDERS AND C BAND FRACTIONS FROM FASTED AND FATTY ACID-FED HAMSTERS

All values are shown as the mean \pm S.D.

	<i>Brush borders</i>		<i>C band</i>	
	<i>Fasted</i>	<i>Fed</i>	<i>Fasted</i>	<i>Fed</i>
Phospholipids	211 \pm 33	213 \pm 7	317 \pm 38	331 \pm 32
Glycolipids	153 \pm 23*	138 \pm 48*	284 \pm 58	328 \pm 55
Cholesterol	52 \pm 7	50 \pm 8	115 \pm 10	122 \pm 18
Fatty acids	8 \pm 1	45 \pm 25**	23 \pm 5	76 \pm 27**
Diglycerides	6 \pm 4	10 \pm 1	20 \pm 7	25 \pm 7
Triglycerides	13 \pm 12	54 \pm 18**	12 \pm 9	63 \pm 26**

* Determined by difference.

** $P < 0.05$.

TABLE III

DISTRIBUTION OF RADIOACTIVITY IN BRUSH BORDERS AND COMPONENTS OF BRUSH BORDERS AFTER 30 SEC OF INCUBATION OF GUT SACS IN MICELLAR LIPID CONTAINING [^{14}C]OLEIC ACID

	<i>Percentage disint./min \pm S.D.</i>				
	<i>Fatty acid</i>	<i>Phospholipid</i>	<i>Monoglyceride</i>	<i>Diglyceride</i>	<i>Triglyceride</i>
Whole brush borders	23.4 \pm 1.9	8.8 \pm 1.4	1.3 \pm 1.1	18.6 \pm 1.4	48.0 \pm 4.0
A + B band*	19.4 \pm 3.1	10.5 \pm 1.0	0.1 \pm 0	17.3 \pm 2.1	52.7 \pm 2.1
C band	41.6 \pm 3.2	8.4 \pm 0.8	0.8 \pm 0.1	15.6 \pm 0.3	33.6 \pm 3.4
D band	39.6 \pm 6.8	9.1 \pm 0.8	2.0 \pm 2.1	14.7 \pm 2.5	38.0 \pm 6.4

* Fractions identified in METHODS section.

When trilinolein was added to mucosal scrapings from fasted animals, the amount of triglyceride present in subsequently prepared C band fractions did not increase. Thus it is unlikely that the increase in C band triglyceride observed after feeding was merely the result of contamination of the C band preparation by triglyceride released from the rest of the cell during the isolation procedure.

Incubation experiments

Everted sacs of hamster intestine were incubated for 30 sec in micellar solutions containing trace quantities of [^{14}C]oleic acid. Table III summarizes the distribution of radioactivity in subsequently prepared brush borders as well as in the various subcellular components isolated after disruption of these brush borders. After incubation with labeled fatty acid, approximately half the total radioactivity recovered from whole brush borders was present as triglyceride. Of the brush border components, only the AB band contained a comparably high proportion of radioactivity in the triglyceride fraction. In contrast, the percentage of radioactivity recovered as triglyceride was lowest in the C band and was nearly 20 % less than that found in either whole brush borders or the AB band.

Table IV compares the specific activities of the lipid classes in various brush

TABLE IV

RELATIVE SPECIFIC ACTIVITIES* OF BRUSH BORDER COMPONENTS AND MICROSOMES AFTER 30 SEC INCUBATION OF EVERTED SACS IN MICELLAR LIPID CONTAINING [^{14}C]OLEIC ACID

Values expressed as means and ranges for 2** to 3 experiments.

	<i>Fatty acid</i>	<i>Phospholipid</i>	<i>Diglyceride</i>	<i>Triglyceride</i>
Brush borders	1.00	1.00	1.00	1.00
A and B band	0.88 (0.61–1.14)**	1.75 (1.50–2.00)**	6.14 (3.50–8.78)**	1.72 (1.42–2.01)**
C band	0.61 (0.45–0.76)**	0.61 (0.50–0.72)	0.29 (0.25–0.33)**	0.66 (0.57–0.76)
D band	0.73 (0.58–0.91)	0.88 (0.69–1.19)	0.80 (0.41–1.37)	0.79 (0.73–0.84)
Microsomes	0.93 (0.45–1.63)	1.43 (1.25–1.62)	1.98 (0.76–2.62)	1.36 (1.16–1.57)

* Relative specific activity determined as the ratio of specific activity (disint./min per μg lipid) of each lipid class to the brush border specific activity of the corresponding experiment.

border components and in microsomes with the specific activities of lipids extracted from whole brush borders. When everted intestinal sacs were exposed to [^{14}C]oleic acid for 30 sec, the specific activities of phospholipid, diglyceride and triglyceride extracted from the AB pellet and from microsomes were all distinctly higher than the specific activities of lipids extracted from whole brush borders. Furthermore, glycerides extracted from the AB pellet regularly had higher specific activities than glycerides from simultaneously isolated microsomes. Of particular note are the relative diglyceride activities at this early period of intestinal uptake and reesterification of fatty acid: the diglycerides of the AB fraction were 6-fold higher than whole brush borders and 3-fold higher than microsomes. In contrast, the specific activities of all glyceride fractions extracted from the relatively pure preparations of microvillous membranes found in C band material were distinctly lower than those observed in whole brush borders or microsomes.

DISCUSSION

During fat absorption *in vivo*^{21,22} or micellar lipid absorption *in vitro*²³ the presence of oil droplets within smooth membranes within the endoplasmic reticulum in the apical cytoplasm constitutes the earliest discernible evidence of triglyceride accumulation within the absorptive cell. At no stage of absorption are oil droplets visible by electron microscopy within the microvilli of the brush border or within the plasma membrane which lines the microvilli^{21–23}. Furthermore, when triglyceride synthesis is prevented by incubating intestinal tissue at 0°, no oil droplet formation can be observed²¹. Combined radioautography and lipid analysis indicate, however, that at 0° fatty acid does enter the intestinal cell². These findings suggest that: (1) fatty acid normally gains entrance to the intestinal cell by diffusion and (2) triglyceride is resynthesized within the cell only below the region of the brush border, within the smooth membranes of endoplasmic reticulum.

The assessment of higher glyceride synthesis by morphologic criteria alone has obvious limitations. It is possible that when first synthesized in the intestinal cell, triglyceride may not immediately aggregate to form droplets but might be solubilized in membranes. Furthermore, the earliest droplets formed could be microemulsion

droplets less than 100 Å in diameter and not readily resolved by electron microscopy. In addition, recent reports from three laboratories²⁴⁻²⁶ describe losses of 15-30 % of tissue lipid when intestinal mucosa is processed for electron microscopy. Although one reviewer²⁷ has suggested that the amounts of lipid lost are minimal, it is entirely possible that whatever lipid may be lost comes preferentially from the brush border surface of the cell.

Measurements of enzymatic activity in isolated fractions of the intestinal cell tend to support morphologic evidence concerning the site of triglyceride synthesis. Isolated microsomal preparations are more active than other intracellular organelles in activating fatty acid to the acyl derivative²⁸ and synthesizing the higher glycerides^{3, 4, 29}. Nevertheless, FORSTNER and associates⁵ reported that purified preparations of brush borders were capable of incorporating fatty acid into higher glycerides *in vitro* and that the activity of one enzyme involved in triglyceride synthesis (diglyceride acylase) was consistently greater in brush border fractions than in microsomal fractions. Furthermore, GALLO⁶ demonstrated that the brush border and microsomal fractions could each convert glycerol monoether to diglyceride and triglyceride analogues in the presence of fatty acid-CoA. Since synthetic activity was approximately equal in both tissue fractions and since cross-contamination, as assessed by RNA and invertase measurements, was not great, GALLO concluded that brush borders and microsomes constituted two separate sites for glyceride synthesis.

When one compares the enzymatic activities of several tissue fractions *in vitro*, problems of interpretation may arise because the tissue fractions examined may not be of comparable purity or because the *in vitro* assay conditions may not appropriately simulate the *in vivo* environment of the whole cell. We therefore chose to examine fatty acid esterification in the intact mucosal cell. Absorptive cells were fractionated: (1) after pure fatty acid was fed to hamsters and (2) after incubation of everted sacs of hamster intestine with radioactive fatty acid for only 30 sec.

When we fed chromatographically pure fatty acid to hamsters, the amount of triglyceride present in subsequently isolated intestinal brush borders increased substantially. Fractionation of these brush borders yielded C band preparations with a comparably increased triglyceride content. Since our experience³⁰ and that of others³¹ indicated that C band material constituted a virtually pure preparation of microvillous membranes and since the addition of exogenous triglyceride to mucosal homogenates failed to alter the lipid composition of subsequently prepared C band fractions, we initially interpreted our results to indicate that microvillous membranes synthesized triglyceride from absorbed fatty acid³². Subsequent morphologic studies demonstrated that microvillous membrane (C and C' band) preparations from intestinal mucosa which contained an increased quantity of absorbed lipid, unlike preparations from fasting animals, were extensively contaminated with other subcellular elements. The contaminating elements included endoplasmic reticulum, presumably from the rim of apical cytoplasm adherent to isolated brush borders. It is not at all clear why the presence of increased amounts of cellular lipid should alter the homogeneity of the C band fraction prepared from the disrupted brush borders. The brush border preparations themselves were indistinguishable by phase microscopy from brush borders isolated from fasting animals. In any event, our feeding experiments confirmed previous demonstrations^{5, 6} of glyceride synthesis in whole brush borders isolated from intestinal cells, but failed to distinguish whether

microvillous membranes constituted an active site of triglyceride formation during fatty acid absorption.

To avoid the impure microvillous membrane preparations obtained when the lipid content of intestinal mucosal cells was increased, we performed experiments with everted intestinal sacs briefly exposed to only trace quantities of [^{14}C]oleic acid. The lipid content of brush borders prepared under these conditions was not increased, and fractionation of these brush borders yielded a C band fraction which was a virtually pure preparation of microvillous membranes. After exposure of intestinal mucosa to ^{14}C -labeled fatty acid for only 30 sec, greater than 75 % of the radioactivity of the brush borders was recovered as higher glycerides (Table III). When these brush borders were further fractionated, however, the specific activities of glycerides present in the microvillous membrane (C band fraction) were distinctly lower than those of glycerides obtained from whole brush borders (Table IV). It therefore seemed likely that the microvillous membrane accounted for not more than a small fraction of the total glyceride synthesis observed in whole brush borders.

On the other hand, components of the brush border which separate predominantly into the AB band appear to represent a major site for conversion of fatty acid to glycerides. AB material regularly contained labeled glycerides with considerably higher specific activities than did either whole brush borders or microsomes isolated from whole intestinal mucosa. The AB band contains diverse subcellular elements including vesicular microvillous membranes as well as other membranous and fibrillar material. Even highly purified brush border fractions contain a firmly attached rim of the cell's apical cytoplasm^{30, 31, 33}. The appearance of much of the AB pellet suggests that it may, in part, be derived from this apical cytoplasm. It seems reasonable, therefore, to speculate that whole brush border fractions owe their activity for glyceride synthesis to a population of smooth membranes at the very apex of the cell which early in the absorptive process appear to synthesize triglyceride more actively than microsomes isolated by conventional sedimentation techniques from the whole intestinal cell.

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