

# Intestinal Metabolism of Plasma Free Fatty Acids

## INTRACELLULAR COMPARTMENTATION AND MECHANISMS OF CONTROL

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**ABSTRACT** Fatty acid metabolism in intestinal mucosa has been examined primarily in regard to lipid absorption. Since earlier studies suggested intestinal utilization of plasma free fatty acids (FFA), we investigated mucosal metabolism of plasma FFA in rats. Mucosal radioactivity (1% of administered) was maximal 2 min after i.v. [ $^{14}\text{C}$ ]palmitate. Of mucosal  $^{14}\text{C}$ , 42% was in water-soluble metabolites, including  $\text{CO}_2$  and ketoacids, 28% in phospholipids, and only 16% in triglycerides. The specific activity of mucosal triglyceride fatty acids (TGFA) was 11 times that of serum TGFA, confirming *in situ* synthesis.

Double isotope experiments showed marked differences in the metabolism of fatty acids entering mucosa simultaneously from lumen and plasma. Whereas luminal fatty acids were chiefly esterified to triglyceride, plasma FFA were preferentially oxidized and incorporated into phospholipids. Crypts did not differ from villi, indicating that intestinal metabolism of plasma FFA is related to their site of entry into epithelial cells. Mucosal metabolism of i.v. [ $^{14}\text{C}$ ]palmitate was minimally affected by glucose administration. However, intraduodenal isocaloric ethanol inhibited mucosal oxidation of FFA by 60%, and increased incorporation into triglycerides nearly twofold. During lipid absorption, mucosal uptake of plasma FFA doubled and incorporation into intestinal lymph triglycerides was increased sixfold.

These studies demonstrate an intracellular compartmentation of fatty acids in the intestinal epithelium. In contrast to absorbed luminal fatty acids, plasma FFA

in the fasting state are both an energy source and a substrate for the synthesis of tissue phospholipid. The fasting contribution of plasma FFA to mucosal and lymph triglyceride is minimal, but it increases during ethanol administration and fat absorption.

## INTRODUCTION

Studies of fatty acid metabolism in intestinal mucosa have dealt almost exclusively with luminal fatty acids, which are predominantly esterified to triglyceride. In contrast, plasma free fatty acids (FFA) undergo oxidation in many tissues, and the possibility that intestinal mucosa also utilizes plasma FFA was suggested by previous studies (1-3). Although this would imply a unique duality of fatty acid sources for the intestine (lumen and plasma), this interesting possibility has received little attention. Furthermore, to the extent that plasma FFA might be utilized by the mucosa, it is not known whether esterification or oxidation is the major pathway. Available information suggests that incorporation of plasma FFA into intestinal lymph lipoprotein triglyceride is of only minor significance in the fasting state, but it is possible that this contribution assumes greater importance under certain conditions. The present studies were designed to investigate the metabolism of plasma FFA in the intestinal mucosa, both in the fasting state and as affected by possible regulatory factors. They have been reported, in part, in abstract form (4, 5).

## METHODS

*Animals and operative procedures.* Male Sprague-Dawley rats (300-365 g) were used in all experiments, and were fed standard rat chow (Berkeley Diet Rat and Mouse Food, Feedstuff Processing Co., San Francisco, Calif.) ad lib. until operation. Under ether anesthesia a polyethylene catheter (PE 90, Clay-Adams, Inc., Parsippany, N. J.) was in-

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serted into the duodenum and secured with a purse-string suture. In some rats the mesenteric lymph duct was cannulated by a modification of the technique of Bollman, Cain, and Grindlay (6) using Silastic medical-grade tubing, 0.020 inch ID (Dow Corning Corp., Medical Products Div., Midland, Mich.) Operated rats were placed in restraining cages (7) and received intraduodenal 0.85% saline, 3.0 ml/h throughout the entire experiment, unless otherwise stated.

*Preparation of [ $^{14}\text{C}$ ]palmitic acid-rat serum complex.* About 20  $\mu\text{Ci}$ [U- $^{14}\text{C}$ ]palmitic acid (735 mCi/mmol, New England Nuclear, Boston, Mass.; radiochemically > 98% pure) in 20  $\mu\text{l}$  of methyl ethyl ketone, was neutralized with a slight excess of ethanolic KOH, slowly mixed with 2.3 ml rat serum, and diluted 1:1 with 0.85% saline. This preparation was stable at 4°C for at least 4 days, but in all cases was used within 2 days. After i.v. injection of this preparation, the half time of the initial disappearance of plasma [ $^{14}\text{C}$ ]-FFA was 1.5 min. Of the injected  $^{14}\text{C}$ , 20% was expired in  $^{14}\text{CO}_2$  by 30 min, and 38% by 3 h (unpublished observations). These findings are similar to those of other workers in regard to the metabolism of plasma FFA (8, 9) and establish the validity of the preparation employed in our studies. Approximately 1.0 ml of this preparation (0.5 ml serum) was injected per rat, representing an acute expansion of the plasma FFA pool by less than 5%.

*Preparation of lipids for intraduodenal infusion.* [9,10- $^3\text{H}$ ]palmitic acid (200 mCi/mmol, New England Nuclear) was purified radiochemically by thin-layer chromatography (TLC) to > 96%. Chemical purity (gas-liquid chromatography) was > 99%. Just before use, a tracer quantity (about 1  $\mu\text{Ci}$ ) was dissolved in 1.0 ml of 10 mM sodium taurocholate in 0.85% NaCl, pH 7.4. Mixed micelles consisted of a clear solution of 10 mM oleic acid (Calbiochem, San Diego, Calif; A grade, > 99% pure) and 5 mM glyceryl monooleate (Calbiochem; 90% pure) dissolved in 10 mM sodium taurocholate (Calbiochem; > 95% pure) in 0.85% NaCl, pH 7.0–7.5. Emulsions contained 10 times these amounts of oleic acid and glyceryl monooleate in 10 mM taurocholate, pH 7.0–7.5, and were prepared by sonication (Sonifier cell disruptor model W 185D, Heat Systems-Ultrasonics, Inc., Plainview, N. Y.).

*Experimental procedure.* The [ $^{14}\text{C}$ ]palmitic acid-rat serum complex was injected acutely into a tail vein of conscious rats. Blood was collected by decapitation at specified time intervals. The abdomen was opened immediately and the small intestine, from ligament of Treitz to cecum, was removed and flushed with 40 ml ice-cold saline. The time from decapitation to completion of the saline flush was 60–90 s. The small intestine then was divided into proximal (jejunal) and distal (ileal) halves.

*Tissue preparation.* Whole mucosa was extruded from the intact intestinal segment. In some experiments, villi and crypts were prepared (10). The mucosal scrapings were homogenized in a Teflon-glass homogenizer in 3 vol of either 0.85% NaCl or in 0.28 M mannitol in 0.01 M  $\text{Na}_2\text{HPO}_4$ , pH 7.4, containing 0.05 M EDTA. In several experiments, the liver was homogenized in 5 vol of 0.85% NaCl.

Where indicated, mucosal homogenates were subsequently sonicated (Sonifier cell disruptor, model W 185D, Heat Systems-Ultrasonics, Inc.) for 625 W·s; a droplet was examined by light microscopy to determine completeness of cell disruption.

Subcellular fractions were prepared after homogenization and sonication of intestinal mucosa by the method of Rodgers, Riley, Drummey, and Isselbacher (11). The floating

fat layer was separated from the 105,000-*g* supernate by means of a tube slicer.

*Analytical methods.* Lipids were extracted from mucosal and liver homogenates, cell fractions whole serum, and defibrinated lymph by the method of Folch, Lees, and Sloane Stanley (12). Lipid classes were separated on precoated TLC plates (silica gel, 0.25 mm, EM Reagents, E. Merck, Darmstadt, West Germany), in petroleum ether: diethyl ether: acetic acid, 90:15:1.5. Plates were "washed" before use by allowing them to develop overnight in chloroform: methanol, 49:1 (13). Lipid zones, demonstrated with iodine vapors or rhodamine 6G and identified by comparison with standards, were scraped and eluted or transferred directly into counting vials. The phospholipid zone at the origin was eluted by extractions with chloroform: methanol: acetic acid: water, 25:15:4:2 (twice), followed by methanol, and methanol: acetic acid: water, 94:1:5 (recovery > 98%). Material in the combined eluates was then developed in a second TLC system, chloroform: methanol: acetic acid: water, 25:15:4:2 (14), to separate individual phospholipid classes.

Triglycerides and fatty acids were eluted from the silica gel by three extractions with chloroform: heptane: methanol, 280:210:10 (recovery > 98%). These eluates were used for determination of fatty acids (13) and of triglycerides (15).

In several experiments, water-soluble radioactivity was measured directly in the upper (water-methanol) phase of the Folch extraction system, and was consistently found to be less than the value calculated as the difference between lipid-soluble and whole homogenate radioactivity, by an amount similar to the  $^{14}\text{CO}_2$  content of the homogenate. Since the Folch extraction involves acidification of the aqueous phase,  $^{14}\text{CO}_2$  was undoubtedly lost during this procedure. Accordingly, water-soluble radioactivity was routinely taken as the difference between radioactivity in the whole homogenate and the lipid extract.

$^{14}\text{CO}_2$  in tissue was determined by acidifying homogenates with 66% citric acid in 25-ml flasks fitted with a center well containing 0.3 ml hydroxide of hyamine, and incubated in a metabolic shaker for 20 min, 37°C, 100 cpm (16). Center wells were then removed and transferred to counting vials for radioassay.

After evolution of  $^{14}\text{CO}_2$ ,  $\beta$ -ketoacids were decarboxylated with aniline citrate (16, 17) during further incubation (37°C, 2 h, 100 cpm); the evolving  $^{14}\text{CO}_2$  was collected and assayed as described above. Since the [ $^{14}\text{C}$ ]palmitate employed in these studies was uniformly labeled, and since decarboxylation of acetoacetic acid derived from this palmitate would yield only one labeled carbon atom (of the four which potentially were labeled), the measured disintegrations per minute were multiplied by four.

*Radioassays.* Homogenates, cell fractions, whole serum, and defibrinated lymph were assayed for radioactivity in Liquifluor toluene solution (New England Nuclear) containing 10% Biosolv (Beckman Instruments, Inc., Fullerton, Calif.) in a Beckman liquid scintillation system (model LS-250). For lipid-soluble extracts, Biosolv was not added. Quenching was corrected for by an automatic external standard, and results were expressed as disintegrations per minute.

*Calculation of data.* To permit comparison of results among rats injected with differing amounts of isotope, all radioactivity data were normalized to an injection of 1.0  $\mu\text{Ci}$ . Groups of three or more rats were compared by unpaired or paired *t* tests. Statistical significance of differences among more than two groups were determined by analysis of variance (18).

## RESULTS

*Uptake and metabolism of plasma FFA by intestinal mucosa.* At 2 min after i.v. administration of [ $^{14}\text{C}$ ]-palmitate,  $0.96 \pm 0.09\%$  of the radioactivity was found in the mucosa of the jejunum-ileum. Proximal and distal halves ( $0.50 \pm 0.06$  and  $0.45 \pm 0.03\%$ ) did not differ, and after 2 min, mucosal  $^{14}\text{C}$  gradually declined. Because mucosal radioactivity was greatest at 2 min, and to avoid the potentially confusing effects of subsequent secretion of mucosal [ $^{14}\text{C}$ ]lipid into intestinal lymph, or the absorption of labeled bile phospholipid, experiments were conducted at this time period, unless otherwise indicated. (In general, FFA uptake in these studies is assumed to equal total tissue radioactivity since, as noted below, contamination by plasma was insignificant.)

Distribution of [ $^{14}\text{C}$ ]palmitic acid among intestinal mucosal metabolites is shown in Table I. In both proximal and distal mucosa, incorporation into water-soluble metabolites (42.2 and 34.2%, respectively) and into phospholipids (27.6 and 35.0%) together accounted for the preponderance ( $\sim 70\%$ ) of mucosal radioactivity. Incorporation into triglyceride (16%) was minor, and only small amounts of  $^{14}\text{C}$  were present in diglyceride, cholesterol esters, and tissue FFA. Proximal and distal mucosa were similar except in regard to conversion of [ $^{14}\text{C}$ ]palmitate to water-soluble metabolites, cholesterol esters, and phospholipids, as shown. This general pattern was similar at 5 and 15 min.

Approximately 20–25% of water-soluble  $^{14}\text{C}$  were in  $^{14}\text{CO}_2$  and  $\beta$ -ketoacids (Table I), the remaining unidentified 75–80% probably representing citric acid-cycle intermediates (19). The possibility that water-soluble mucosal radioactivity was due to contamination by serum is most unlikely since admixture of 0.6 ml serum/g mucosa would have been required (the actual admixture was far less: e.g., mucosal [ $^{14}\text{C}$ ]FFA/g was only about 4% of that in serum). Thus, the water-soluble metabolites almost certainly were formed in the mucosa itself.

Of the [ $^{14}\text{C}$ ]lipid recovered in phospholipids, more than 50% exhibited mobility similar to that of phosphatidyl choline in a second TLC system (Methods). About 80% was recovered in this and in phosphatidyl ethanolamine ( $12.6 \pm 1.2\%$ ), phosphatidyl serine and phosphatidyl inositol ( $8.0 \pm 0.3\%$ ), and sphingomyelin ( $5.5 \pm 0.3\%$ ). The remaining 20% (similar in mobility to phosphatidyl glycerol, phosphatidic acid, and cardiolipin) was not further identified.

Hepatic uptake of plasma FFA accounted for  $17.6 \pm 1.6\%$  of the injected radioactivity at 2 min, comparable to earlier studies (20). In contrast to intestine (Fig. 1),  $^{14}\text{C}$  was mainly incorporated into triglycerides, with only a small fraction in water-soluble

TABLE I  
*Metabolism of i.v. [ $^{14}\text{C}$ ]Palmitic Acid by Intestinal Mucosa*

Mucosal radioactivity . . . . .	Proximal (5.677 $\pm$ 554 dpm/g mucosa)	Distal (5.831 $\pm$ 419 dpm/g mucosa)
H <sub>2</sub> O-soluble metabolites, % of mucosal radioactivity		
Total	42.2 $\pm$ 2.2	34.2 $\pm$ 3.0*
CO <sub>2</sub>	7.9 $\pm$ 1.0	6.2 $\pm$ 0.8*
Ketoacids	2.6 $\pm$ 0.8	0.4 $\pm$ 0.1‡
Lipids, % of mucosal radioactivity		
Total	57.8 $\pm$ 2.2	65.8 $\pm$ 3.0*
Phospholipids	27.6 $\pm$ 2.1	35.0 $\pm$ 2.1§
Diglycerides	6.0 $\pm$ 0.4	6.8 $\pm$ 0.4
Fatty acids	6.1 $\pm$ 0.7	6.6 $\pm$ 0.5
Triglycerides	16.3 $\pm$ 0.9	16.2 $\pm$ 1.0
Cholesterol esters	1.8 $\pm$ 0.2	1.2 $\pm$ 0.1‡

[ $^{14}\text{C}$ ]Palmitate was injected i.v. into fasting rats. At 2 min, rats were killed by decapitation; intestinal mucosa was prepared and analyzed for  $^{14}\text{C}$  in whole homogenate, water-soluble metabolites, and lipids (Methods). Mean  $\pm$  SE,  $n = 10$ . Significance of differences, proximal vs. distal (paired  $t$ ):

\*  $P < 0.005$ .

‡  $P < 0.05$ .

§  $P < 0.0005$ .

metabolites. Percent incorporation into phospholipids ( $30.5 \pm 4.6\%$ ) was similar to intestinal mucosa.

*Distribution of plasma [ $^{14}\text{C}$ ]FFA in subcellular fractions, crypts, and villi of intestinal mucosa.* About half (48%) of the  $^{14}\text{C}$  in proximal mucosa was recovered in the plasma membrane-nuclei fraction. Of the [ $^{14}\text{C}$ ]lipid in this fraction, more than three-quarters was in esters (predominantly phospholipids), indicating that contamination by plasma FFA was not significant. Supernate accounted for a lesser amount (28%), and still smaller quantities of  $^{14}\text{C}$  were found in the microsomal (14%) and mitochondrial (8%) fractions. Floating fat

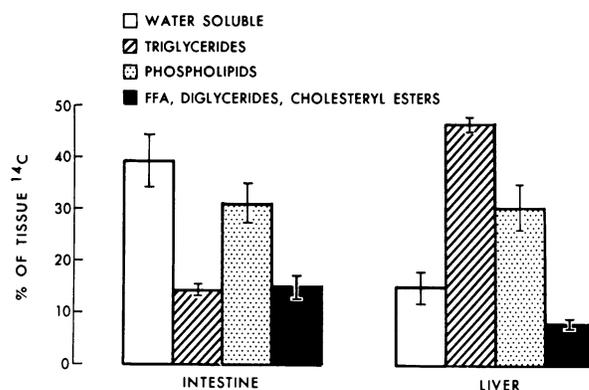


FIGURE 1 Utilization of plasma FFA by intestinal mucosa and liver of fasting rats. After an overnight fast, rats were injected i.v. with [ $^{14}\text{C}$ ]palmitate. Radioactivity in water-soluble metabolites and lipids at 2 min was determined in homogenates of liver and mucosa of proximal small intestine (Methods). Results are expressed as percentage of total tissue radioactivity. Mean  $\pm$  SE,  $n = 4$ .

TABLE II  
Uptake and Metabolism of i.v. [<sup>14</sup>C]Palmitic Acid by  
Intestinal Villi and Crypts

Mucosal radioactivity. . . . .	Villi (3,655 ± 362 dpm/g mucosa)	Crypts (5,212 ± 570 dpm/g mucosa)*
H <sub>2</sub> O-soluble metabolites, % of mucosal radioactivity		
Total	47.0 ± 5.5	49.2 ± 1.7
CO <sub>2</sub>	11.2 ± 0.8	3.8 ± 0.5*
Ketoacids	1.9 ± 0.4	0.8 ± 0.0
Lipids, % of mucosal radioactivity		
Total	53.0 ± 5.5	50.8 ± 1.7
Phospholipids	23.1 ± 2.0	26.9 ± 1.0
Diglycerides	3.4 ± 0.4	4.4 ± 0.2
Fatty acids	3.1 ± 0.2	9.6 ± 1.4‡
Triglycerides	20.9 ± 3.4	8.8 ± 1.9*
Cholesterol esters	2.5 ± 0.4	1.1 ± 0.1‡

At 2 min after i.v. injection of [<sup>14</sup>C]palmitate into fasting rats, preparations of villi and crypts from proximal intestine were analyzed for <sup>14</sup>C in water-soluble metabolites and in lipids. Mean ± SE, n = 4. Significance of differences (paired t test):

\* P < 0.005.

‡ P < 0.05.

contained only 2% of mucosal radioactivity, and was not further characterized. Proximal and distal intestine were generally similar.

Water-soluble compounds accounted for 92% of supernatant <sup>14</sup>C, whereas only 18% of microsomal <sup>14</sup>C was water soluble, consistent with the major role of the endoplasmic reticulum in fatty acid esterification. Distribution of label in lipid classes (phospholipids > triglyceride > FFA), expressed as a percentage of the total radioactivity in each cell fraction, did not differ greatly among particulate cell fractions and whole sonicate.

Total esterified <sup>14</sup>C in all cell fractions was about 10% less than in whole sonicate; conversely, [<sup>14</sup>C]FFA

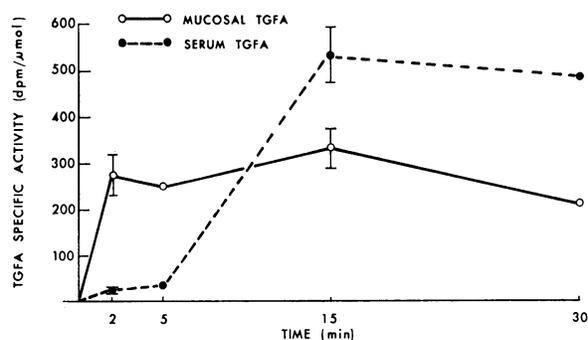


FIGURE 2 Specific activity of triglyceride-fatty acids (TGFA) in intestinal mucosa and serum after i.v. [<sup>14</sup>C]-palmitic acid. After an overnight fast, rats received i.v. [<sup>14</sup>C]-palmitate, and specific activities of mucosal and serum TGFA were determined at the indicated times. Mean ± SE: 2 min, n = 10 (P < 0.0002, paired t); 15 min, n = 3; 5 and 30 min, n = 2.

in the combined cell fractions exceeded the sonicate by a comparable amount. These discrepancies most likely reflect the action of mucosal lipases (21) during homogenization and centrifugation. Although its effect on overall lipid recoveries was not of major significance, this phenomenon precludes meaningful interpretation of mucosal FFA specific activities.

Metabolism of plasma FFA also was compared in crypts and villi. Uptake per gram tissue was somewhat greater in crypts (Table II). Percent conversion to CO<sub>2</sub>, triglycerides, and cholesterol esters was greater in villi, while [<sup>14</sup>C]FFA was greater in crypts. Possibly the crypt preparation was contaminated by plasma [<sup>14</sup>C]-palmitate, but the relatively small absolute difference in [<sup>14</sup>C]FFA between the fractions was small compared to the difference in uptake.

Of the total mucosal radioactivity, 21% or less was recovered in triglycerides (Tables I and II), raising the possibility that this small amount reflected contamination by plasma (hepatogenous) triglycerides rather than *in situ* synthesis. As shown in Fig. 2, however, the specific activity of mucosa triglyceride fatty acids (TGFA)<sup>1</sup> at 2 and 5 min was much greater than that of serum TGFA, thereby excluding any significant contribution by plasma [<sup>14</sup>C]triglyceride, and establishing that plasma FFA are incorporated into triglycerides by intestinal mucosa.

Since triglyceride synthesis takes place in the endoplasmic reticulum (22), it was expected that the specific activity of microsomal TGFA would exceed that of the whole homogenates. However, specific activities of microsomal and sonicate TGFA (263 ± 61 and 299 ± 50 dpm/μmol, respectively) did not differ. This finding suggests that there is rapid equilibration of newly synthesized triglyceride within the cell, and/or that microsomal TGFA specific activity is diminished by the admixture of unlabeled fatty acid absorbed from the intestinal lumen.

*Compartmentation of fatty acids in intestinal mucosa.* Fatty acids which enter intestinal mucosa from the lumen are mainly esterified to triglyceride, only a small fraction being oxidized. In contrast, plasma FFA are incorporated into mucosal triglyceride only to a minor extent (Table I). This finding suggested compartmentation of mucosal fatty acids.

To investigate this question further, tracer quantities of [<sup>3</sup>H]palmitate in 1.0 ml 10 mM taurocholate were administered intraduodenally to fasting rats, simultaneously with i.v. administration of [<sup>14</sup>C]palmitate. Incorporation of each label into mucosal metabolites was measured at 2 min, by which time 41% of luminal [<sup>3</sup>H]palmitate had been absorbed.

<sup>1</sup> Abbreviations used in this paper: FABP, fatty acid-binding protein; TGFA, triglyceride fatty acids.

A striking difference in the mucosal metabolism of the palmitate administered by these two routes was observed (Fig. 3). As expected, more than 60% of the absorbed luminal ( $^3\text{H}$ ) fatty acid had been incorporated into triglyceride, and only 17% was recovered in water-soluble metabolites. By contrast, triglyceride accounted for only 21% of the i.v. [ $^{14}\text{C}$ ]palmitic acid radioactivity, whereas 44% were found in water-soluble metabolites. The marked differences in metabolism of fatty acids simultaneously administered by different routes strongly supports the concept that there is compartmentation of fatty acids in the intestinal mucosa. That this compartmentation does not simply reflect the anatomical and functional heterogeneity of the mucosa (e.g., the differences between villi and crypts) was established by experiments in which double isotope administration [ $^3\text{H}$ ]palmitate intraduodenally; [ $^{14}\text{C}$ ]palmitate i.v.) was combined with differential mucosal scraping, permitting comparison of villi and crypts. As shown in Fig. 4, mucosal radioactivity derived from luminal palmitate was greater in villi than in crypts (consistent with the role of villi in fat absorption), and that from i.v. palmitate was slightly greater in crypts (as in Table II). Despite this, in both villi and crypts the major fate of Plasma FFA was oxidation to water-soluble metabolites, whereas this fate accounted for only a minor fraction of luminal fatty acids ( $P < 0.005$ ).

These findings strongly suggest that the observed compartmentation of fatty acids is intracellular. In other words, the metabolism of fatty acid in the epithelial cell appears to be determined by its site of entry into the cell. The basis for this compartmentation remains to be elucidated, but may be related in some manner to the structural polarity of the cell (see Discussion).

*Incorporation of plasma FFA into intestinal lymph lipids.* To determine whether mucosal lipids synthesized from plasma FFA were incorporated into intestinal lipoproteins and secreted into lymph, rats with intestinal lymph fistulas were studied. After an overnight fast, [ $^{14}\text{C}$ ]palmitate was administered i.v., and intestinal lymph and blood were collected at intervals up to 2 h; in some experiments, proximal mucosa was studied at 10, 20, and 30 min. Lymph flow averaged  $2.7 \pm 0.2$  ml/h, and lymph TGFA concentration was  $6.28 \pm 0.68$   $\mu\text{mol/ml}$ . Results are shown in Fig. 5.

Consistent with the findings at 2 and 5 min in rats without lymph fistulas (Fig. 2), specific activity of mucosal TGFA at 10 min in fistula rats was significantly higher than in serum (Fig. 5). Labeled triglyceride, which first appeared in intestinal lymph after 10 min, increased in specific activity between 20 and 30 min. Although the data do not conclusively establish a precursor-product relationship between mu-

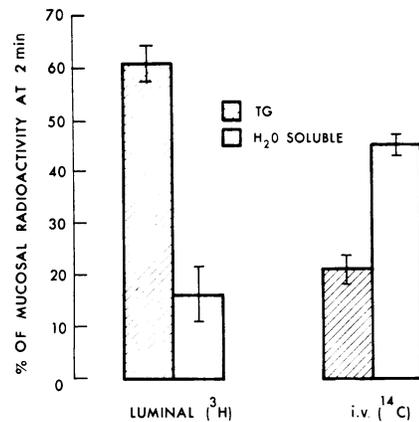


FIGURE 3 Intestinal metabolism of palmitic acid: effect of route of administration. After an overnight fast, rats received, simultaneously, [ $^{14}\text{C}$ ]palmitate i.v. and [ $^3\text{H}$ ]palmitate intraduodenally. Radioactivity of triglycerides (TG) and water-soluble metabolites at 2 min was determined and expressed as a percentage of the total mucosal radioactivity for each isotope. Mean  $\pm$  SE,  $n = 4$ . Significance of differences (paired  $t$ ) in triglycerides and water-soluble metabolites, respectively (i.v. vs. intraluminal), were  $P < 0.001$  and  $< 0.05$ .

cosal and lymph triglyceride, available evidence indicates that plasma very low density lipoproteins (theoretical alternative "precursors") do not enter intestinal lymph (23, 24).

Of the lipid-extractable radioactivity in lymph during the 20–30 min interval, only  $10.0 \pm 2.8\%$  was present in phospholipids, whereas triglyceride accounted for  $68.5 \pm 5.2\%$ , despite the fact that in the mucosa incorpora-

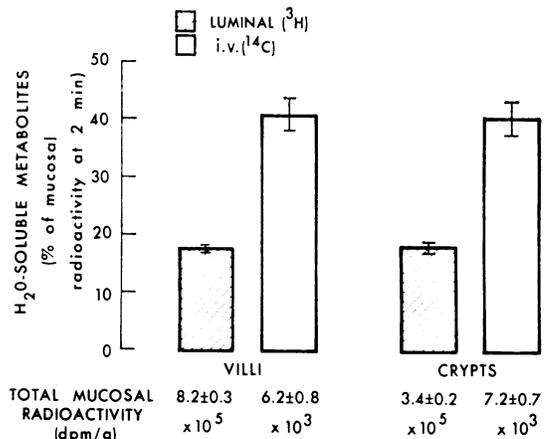


FIGURE 4 Intestinal metabolism of luminal and i.v. palmitic acid: villi vs. crypts. After an overnight fast, rats received simultaneous i.v. and intraduodenal [ $^{14}\text{C}$ ]- and [ $^3\text{H}$ ]palmitate (as in Fig. 3). Radioactivity in homogenates and water-soluble metabolites of villi and crypts at 2 min was determined and expressed as a percentage of the total mucosal radioactivity for each isotope. Mean  $\pm$  SE,  $n = 4$ .

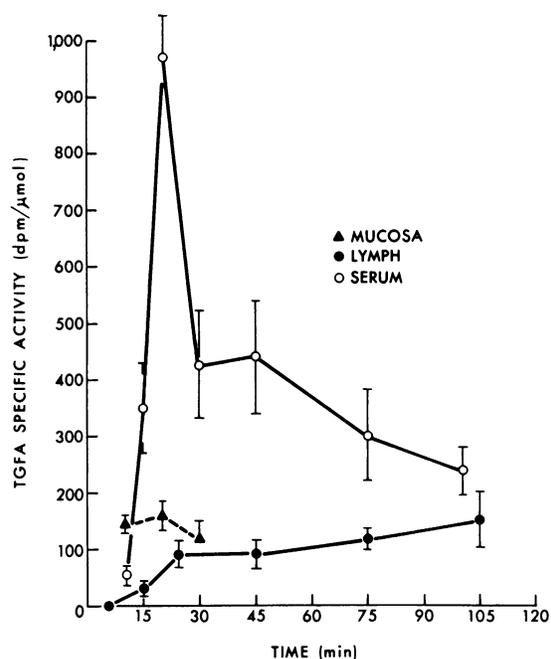


FIGURE 5 Specific activity of triglyceride fatty acids (TGFA) in intestinal mucosa, lymph, and serum after i.v. [ $^{14}\text{C}$ ]palmitate. After an overnight fast, lymph fistula rats received i.v. [ $^{14}\text{C}$ ]palmitate. Intestinal lymph was collected for 10-min periods until 30 min, and for 30-min periods to 2 h. In experiments ended at 10, 20, and 30 min, mucosal homogenates were prepared and blood was collected after decapitation; in more prolonged experiments, blood was collected from the tail vein at the midpoint of the indicated 30-min lymph collection periods. TGFA specific activity was determined as in Methods. Mean  $\pm$  SE,  $n \geq 3$ . Values for lymph are plotted at the midpoint of the collection period.

tion of plasma FFA into phospholipid greatly exceeded that into triglyceride (Table I).

*Effect of glucose and ethanol on the intestinal metabolism of plasma FFA.* Rats received a constant

intraduodenal infusion of glucose (20% wt/vol in isotonic saline) over 15 h (40 kcal/rat), resulting in a 25% fall in serum FFA concentration (Table III). Since tissue uptake of plasma FFA is proportional to FFA concentration (25, 26), this suggests that mucosal uptake of plasma FFA was diminished during glucose administration, possibly contributing to the observed decrease in mucosal FFA concentration (Table III). (Mucosal FFA concentrations may be spuriously increased by about 10% due to action of mucosal lipases during tissue preparation, but it is unlikely that the significant differences among the three groups can be attributed to this.) Although a somewhat lower percentage of plasma FFA entering the mucosa under these conditions was oxidized, metabolism was generally similar to that in fasting controls (Table IV).

In another group of animals, ethanol (0.33 g/kg/h) was substituted isocalorically for a portion ( $< 1/3$ ) of the glucose, and the two substances were infused simultaneously over 15 h. The rate at which ethanol was infused in these experiments was similar to its maximal rate of metabolism (27). These animals exhibited no evidence of intoxication, and could not be distinguished on inspection from glucose-infused controls.

Relative to fasting (saline-infused) controls, there was a 40% fall in serum FFA concentration during the ethanol-glucose infusion (Table III), suggesting that net mucosal uptake of plasma FFA was diminished correspondingly. Despite the low infusion rate of ethanol, it resulted in a significantly increased triglyceride concentration in serum and, to a lesser extent, in mucosa (Table III). The most striking effect of the ethanol-glucose infusion, however, was a 60–65% decrease in the conversion of i.v. [ $^{14}\text{C}$ ]palmitate to water-soluble (oxidative) metabolites, and a near doubling of its incorporation into triglyceride, relative to both sa-

TABLE III  
*Effect of Glucose and Ethanol on FFA and Triglycerides in Serum and Intestinal Mucosa*

	FFA		Triglyceride	
	Serum	Mucosa	Serum	Mucosa
	$\mu\text{mol/ml}$	$\mu\text{mol/g}$	$\mu\text{mol/ml}$	$\mu\text{mol/g}$
Saline	$0.51 \pm 0.02$	$1.57 \pm 0.21$	$0.30 \pm 0.02$	$0.84 \pm 0.07$
Glucose	$0.38 \pm 0.03$	$0.87 \pm 0.10$	$0.38 \pm 0.04$	$1.05 \pm 0.13$
Ethanol-glucose	$0.30 \pm 0.06$	$1.08 \pm 0.19$	$0.63 \pm 0.08$	$1.26 \pm 0.11$
Analysis of variance	F = 10.63 $P < 0.01$	F = 4.38 $P < 0.05$	F = 18.54 $P < 0.01$	F = 4.55 $P < 0.05$

Rats received intraduodenal infusions, over 15 h, of 0.85% saline, glucose (2 g/kg/h), or ethanol (0.33 g/kg/h) combined with glucose (1.42 g/kg/h). Serum and mucosal concentrations of FFA and triglycerides were determined. Mean  $\pm$  SE,  $n \geq 7$ , compared by one way analysis of variance for unequal sample sizes.

TABLE IV  
Effect of Glucose and Ethanol on Uptake and Metabolism of i.v. [<sup>14</sup>C]Palmitic Acid by Intestinal Mucosa

	Saline	Glucose	Ethanol and glucose	Analysis of variance	
				F	P
Mucosal radioactivity, <i>dpm/g mucosa</i>	5,677±554	4,516±447	6,220±408	2.13	NS
H <sub>2</sub> O-soluble metabolites, % of mucosal radioactivity	42.2±2.2	35.9±3.3	14.7±5.4	15.87	<0.01
Lipids, % of mucosal radioactivity					
Phospholipids	27.6±2.1	35.7±3.1	40.3±7.1	3.56	<0.05
Diglycerides	6.0±0.4	7.8±0.8	10.9±1.0	11.19	<0.01
Fatty acids	6.1±0.7	4.4±0.4	3.7±0.6	3.30	NS
Triglycerides	16.3±0.9	14.5±1.2	27.0±4.9	9.31	<0.01
Cholesterol esters	1.8±0.2	1.7±0.2	3.4±0.9	4.88	<0.05

After overnight infusions of saline (*n* = 10), glucose (*n* = 7), or ethanol-glucose (*n* = 4) as in Table V, rats were injected i.v. with [<sup>14</sup>C]palmitate; 2 min later radioactivity in mucosal metabolites was determined (Methods). Mean±SE, compared by one way analysis of variance for unequal sample sizes.

line and glucose-infused rats (Table IV). A slight increase in the incorporation of plasma FFA into triglyceride by distal mucosa (not shown) was statistically insignificant, suggesting that the effect of ethanol was localized to its site of absorption. Incorporation of isotope into other esterified lipids was also increased somewhat. Differences in total mucosal radioactivity among the three groups (Table IV) may reflect changes in mucosal blood flow, with corresponding effects on FFA uptake.

Glucose and ethanol-glucose infusions did not affect hepatic FFA uptake. Similar to intestinal mucosa, however, ethanol-glucose infusion was associated with a decrease in the conversion of plasma FFA to water-soluble metabolites (15.1±3.1 and 13.8±2.2% of total hepatic radioactivity in fasting and glucose-infused, respectively; 3.9±1.7% in ethanol infused), with corresponding increases in esterification.

*Effect of fat absorption on intestinal metabolism of plasma FFA and their incorporation into lymph lipids.*

TABLE V  
Effect of Intraduodenal Taurocholate and Lipid on Uptake and Metabolism of i.v. [<sup>14</sup>C]Palmitic Acid by Intestinal Mucosa

	Saline 0.85%	Taurocholate 10 mM	Lipid in 10 mM taurocholate		Analysis of variance	F	P
			Micelles (8.2 mg total lipid)	Emulsion (82.0 mg total lipid)			
Mucosal radioactivity, <i>dpm/g mucosa</i>	4,671±368	5,208±687	6,188±810	10,398±623	19.13	<0.01	
H <sub>2</sub> O-soluble metabolites, % of mucosal radioactivity	51.2±2.3	57.2±2.3	46.3±3.8	42.6±3.4	4.23	<0.05	
Lipids, % of mucosal radioactivity							
Phospholipids	22.9±0.4	19.1±0.6	24.3±1.2	24.0±2.1	3.31	NS	
Diglycerides	3.5±0.2	3.3±0.3	3.8±0.3	4.4±0.3	3.82	<0.05	
Fatty acids	5.0±1.0	6.0±0.6	6.3±1.4	4.8±0.5	0.60	NS	
Triglycerides	15.7±1.6	13.1±2.3	18.6±3.8	23.9±2.6	2.79	NS	
Cholesterol esters	1.7±0.2	1.4±0.1	0.7±0.0	0.3±0.0	36.25	<0.01	
Serum FFA, <i>μmol/ml</i>	0.49±0.07	0.52±0.07	0.66±0.05	0.47±0.07	1.90	NS	

After an overnight fast, rats received 30 min intraduodenal infusions of saline (*n* = 3), 10 mM taurocholate (*n* = 4), mixed micelles in 10 mM taurocholate (*n* = 4), or lipid emulsion in 10 mM taurocholate (*n* = 4), followed by i.v. [<sup>14</sup>C]palmitate. Serum FFA concentration and radioactivity in mucosal metabolites were measured at 2 min (Methods). Mean±SE, compared by one way analysis of variance for unequal sample sizes.

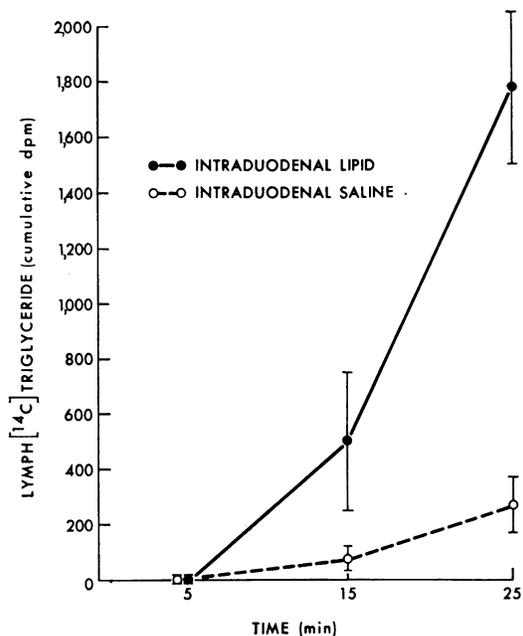


FIGURE 6 Effect of fat absorption on incorporation of plasma FFA into intestinal lymph triglyceride. After an overnight fast, lymph fistula rats received 30-min intraduodenal infusions of saline ( $n = 4$ ) or of an emulsion containing 82 mg lipid in 10 mM taurocholate ( $n = 3$ ) (Methods). [ $^{14}\text{C}$ ]Palmitate was then administered i.v., and lymph was collected in 10-min periods up to 30 min. Lymph triglycerides were isolated and assayed for radioactivity. Mean  $\pm$  SE of cumulative  $^{14}\text{C}$  in triglycerides are plotted at the midpoint of collection periods. The difference during the second collection period (10–20 min) falls just short of statistical significance; for the 20–30 min period,  $P < 0.002$ .

After an overnight fast, rats received intraduodenal infusions of lipid in 10 mM taurocholate during the 30 min period preceding i.v. [ $^{14}\text{C}$ ]palmitate. As shown in Table V, administration of 10 mM taurocholate alone (or, not shown, 30 mM taurocholate) caused insignificant changes in mucosal uptake and metabolism of plasma FFA, compared to saline controls. Infusion of 10 mM oleic acid and 5 mM glyceryl monooleate (8.2 mg total lipid) in 10 mM taurocholate caused only a slight increase in uptake and percent esterification, compared to taurocholate alone. However, an emulsion containing 10 times this amount of oleic acid and glyceryl monooleate (82 mg total lipid) in 10 mM taurocholate approximately doubled the total mucosal uptake of i.v. [ $^{14}\text{C}$ ]palmitate, and caused a relative decrease in oxidation and increase in incorporation into triglyceride. (The increase in mucosal FFA uptake in the absence of a rise in plasma FFA concentration most likely is due to the increased intestinal blood flow associated with fat absorption (28, 29)). Thus, incorpora-

tion of i.v. [ $^{14}\text{C}$ ]palmitate into mucosal triglyceride was increased nearly fourfold during fat absorption, compared with the taurocholate-infused rats. Mucosal oxidation of plasma FFA (despite its relative decrease), increased absolutely, as did incorporation into phospholipids and diglycerides. The decreased percent incorporation of plasma [ $^{14}\text{C}$ ]palmitate into cholesterol esters may reflect preferential esterification of cholesterol with absorbed oleic acid (30). Serum triglyceride concentrations (0.23–0.37 mM) did not differ significantly among the groups, indicating that the absorbed fat had not entered plasma to any important extent.

Intestinal metabolism of plasma FFA also was studied under conditions designed to diminish uptake of luminal lipid. In these experiments, cholestyramine (0.45 g/kg; Questran Mead Johnson Labs., Evansville, Ind.) was infused intraduodenally during the 30 min preceding i.v. [ $^{14}\text{C}$ ]palmitate administration. No effect on uptake or metabolism of plasma FFA by intestinal mucosa was observed (unpublished observations).

The enhanced incorporation of plasma FFA into mucosal triglyceride during the absorption of intraluminal fat suggested the possibility that plasma FFA might contribute to lymph triglycerides to a greater extent under these conditions than in the fasting state. To explore this possibility, rats with intestinal lymph fistulas received an intraduodenal infusion of a lipid emulsion (82 mg) in 10 mM taurocholate over 30 min as in Table V; controls received saline. Immediately at the completion of this infusion, [ $^3\text{H}$ ]palmitate was administered intraduodenally, simultaneously with the i.v. administration of [ $^{14}\text{C}$ ]palmitate. Intestinal lymph was then collected for three 10-min periods.

At 30 min after the administration of isotope, mucosal  $^{14}\text{C}$  (from plasma FFA) was significantly higher in lipid-infused animals than in controls ( $7,168 \pm 998$  vs.  $4,631 \pm 328$  dpm/g mucosa,  $P < 0.05$ ). Furthermore, mucosal compartmentation of luminal and plasma fatty acids persisted at this later time period. Thus, of the radioactivity remaining in the mucosa at 30 min, the  $^{14}\text{C}$  (i.v.) was predominantly in phospholipids (40.3%) and water-soluble metabolites (37.8%), whereas most of the  $^3\text{H}$  (luminal) was in triglyceride (54.7%).

The effect of fat absorption on the lymphatic output of i.v. [ $^{14}\text{C}$ ]palmitate as triglyceride is shown in Fig. 6. The results show that fat absorption is associated not only with increased mucosal uptake of plasma FFA and incorporation into triglyceride (Table V), but also with a marked increase in the mucosal secretion of lymph triglyceride derived from plasma FFA. A less significant increase in lymphatic output of [ $^{14}\text{C}$ ]phospholipid also occurred (unpublished observations).

## DISCUSSION

The present studies show that, in addition to absorbing luminal fatty acid, the intestinal mucosa also utilizes plasma FFA. This function is not unexpected, since it is shared by many tissues in the body, but the finding that fatty acids derived from the two sources (lumen and plasma) are metabolized so differently by the same cell population is somewhat surprising and, to our knowledge, unique. Thus, although there appear to be two FFA pools within the adipocyte (31), this amounts to a separation of fatty acids entering this cell type from those which are exiting, presumably via a common portal. Teleologically, it is understandable that these two distinct adipocyte functions (fatty acid uptake and release) should be separated, since they are responsive to divergent regulatory factors (plasma FFA concentration and adipocyte hormone-sensitive lipase, respectively), but the mechanism for this separation is not known.

In the intestine, our studies show that fatty acids which enter mucosa from plasma are destined, for the most part, to serve the structural and caloric requirements of the epithelium itself, whereas those absorbed from the lumen serve the organism as a whole. As with the adipocyte, compartmentation can be understood on teleological grounds but its mechanism is obscure. Possibly, structural factors play a role in this regard. In the intestinal epithelium, mitochondria are dispersed throughout the absorptive cell, in contrast to the endoplasmic reticulum which is relatively concentrated in the apical and supranuclear portions (32). Accordingly, at the basal pole of the cell, i.e., the region at which FFA must certainly enter from plasma, there is a relative preponderance of mitochondria. This structural polarization of the epithelial cell could account for an increased probability that fatty acids entering the basal aspect of the cell would be oxidized. Similarly, the apical preponderance of endoplasmic reticulum could explain the predominant esterification of fatty acids which enter from the lumen. This tentative scheme is based on the assumption that the metabolic fate of a given fatty acid molecule is determined by the enzymatic properties of the particular cell organelle with which it associates. Thus, fatty acid activation to its acyl-CoA thioester in the endoplasmic reticulum (22, 33) would be followed by transacylation and glycerolipid synthesis, whereas activation at the mitochondrial surface (34) would favor acylcarnitine synthesis and intramitochondrial oxidation. This assumption requires only that long-chain fatty acyl thioesters, once formed in endoplasmic reticulum or mitochondria, not equilibrate in a common intracellular pool. In fact, compartmentation of long-chain acyl-CoA is implicit in the observation that luminal and plasma fatty acids undergo differing

metabolic fates in the absorptive cell, despite the fact that they are converted to this common intermediate.

An alternative explanation for the observed compartmentation is that the greater mass of luminal fatty acid in some manner "preempts" the triglyceride pathways. This explanation is unlikely, however, since our studies show that neither increased nor decreased luminal fatty acid influx significantly affected the probability that plasma FFA would undergo oxidation in the mucosa.

An additional possibility is that compartmentation is related to cytoplasmic fatty acid-binding protein (FABP). Available evidence suggests that in the intestine, FABP participates in the intracellular transport and metabolism of absorbed fatty acid (35-37). Although it is likely that FABP also participates in the utilization of plasma FFA, its possible role in fatty acid compartmentation requires further study.

The present studies show that plasma FFA are an energy source for the intestinal mucosa. However, although i.v. palmitate was oxidized to a relatively greater extent than luminal palmitate, this does not necessarily indicate that plasma FFA are quantitatively more important in this regard. Thus, calculating from the data of Baker and Schotz (9), net plasma FFA flux in the fasting rat is 432  $\mu\text{mol/h}/300\text{ g}$ . Our findings show that about 1% of this amount, or 4.3  $\mu\text{mol/h}$ , enters the mucosa, of which about 45%, or 1.9  $\mu\text{mol/h}$ , are oxidized. (Since mucosal uptake was determined at 2 min, or slightly more than one half-life of plasma FFA, it is likely that steady-state uptake is somewhat greater than the calculated values.)

For endogenous luminal fatty acids, on the other hand, flux through the mucosa equals or exceeds fatty acid output in lymph lipids, since the fasting contribution from other sources may be regarded as negligible (2, 23, 38). This output is approximately 17.0  $\mu\text{mol/h}/300\text{ g}$  (see above, and 2, 23, 39), or about five times the mucosal uptake of plasma FFA. Since about 16% of absorbed endogenous fatty acids are oxidized (Fig. 3), or 2.7  $\mu\text{mol/h}$ , endogenous luminal fatty acids provide about 58% of the fatty acid which is oxidized by whole mucosa. Presumably, oxidation of luminal fatty acid is more important in the villi, whereas crypt cells are more dependent on plasma FFA.

Glucose had little effect on intestinal fatty acid metabolism. The finding that plasma FFA are converted to ketone bodies in intestinal mucosa suggests that the intestine, along with other extrahepatic tissues, including kidney (40, 41) and forearm muscle (42), may contribute to circulating ketones, but this contribution is probably minor.

Plasma FFA were incorporated into mucosal phospholipids to a significant extent. Of these, a relatively

small quantity was secreted into intestinal lymph, suggesting that they served a structural role primarily. The  $\alpha$ -glycerophosphate pathway (43) almost certainly is involved, since Johnston, Paultauf, Schiller, and Schultz (44) showed that, in intestinal microsomes, only diglycerides formed via the  $\alpha$ -glycerophosphate pathway could be incorporated into phospholipid.

In general, these studies confirm earlier evidence that plasma FFA are incorporated into lymph lipoproteins (1), but the quantitative significance of this contribution appears to be small. Although incorporation of plasma FFA into mucosal triglycerides was not affected by glucose, it was significantly increased in two other experimental circumstances, i.e., ethanol administration and absorption of exogenous lipid.

In these studies, the concentration of ethanol in the intraduodenal infusion was only 3.4% wt/vol, and hemorrhagic mucosal erosions seen with higher concentrations (45) did not occur. The effect of ethanol on intestinal metabolism of plasma FFA resembled that in liver, in that esterification was increased at the expense of oxidation. Since mucosal oxidation of ethanol proceeds via the alcohol dehydrogenase pathway (46, 47), the mechanism for this effect presumably is similar to that in the hepatocyte, and reflects changes in mucosal NADH/NAD ratio. In contrast to previous studies (3), mucosal triglyceride concentrations were only minimally elevated after ethanol, probably reflecting different schedules of administration. Our present findings support the concept that ethanol inhibition of mucosal oxidation of FFA (luminal and plasma) contributed to the observed increase in mucosal and lymph triglycerides (3).

The intraduodenal infusion of 82 mg of lipid (approximately 15–20% of average daily dietary intake) essentially doubled the mucosal uptake of plasma FFA and its relative incorporation into triglycerides, and caused a slight relative decrease in fatty acid oxidation. Since plasma FFA levels remained unchanged, the increased uptake probably reflected increased mucosal blood flow (28). As a result, absolute mucosal metabolism of plasma FFA increased significantly during fat absorption and its output in lymph triglyceride was increased sixfold. Intraduodenal taurocholate alone had no significant effect. We explored the possibility that increased mucosal FFA uptake during fat absorption might be caused by cholecystokinin (1.5 Ivy U/kg rat, i.v.), a hormone which is released during fat absorption and which causes increased mesenteric blood flow (29), or glucagon (3 or 30  $\mu$ g/kg), which increases in plasma during fat absorption (48). However, the results of these experiments were inconclusive (unpublished observations).

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