Fat absorption in essential fatty acid deficiency: a model experimental approach to studies of the mechanism of fat malabsorption of unknown etiology

Susanne Bennett Clark, Thomas E. Ekkers, Akbal Singh,
John A. Balint, Peter R. Holt, and John B. Rodgers, Jr.

Gastrointestinal Division of the Medical Service, St. Luke's Hospital Center, New York 10025, and
Department of Medicine, College of Physicians and Surgeons, Columbia University, New York 10032, and
the Division of Gastroenterology, Department of Medicine, Albany Medical College of Union University,
Albany, New York 12208

Abstract Male rats were made deficient in essential fatty acids by feeding them a fat-free diet supplemented with 4% tripalmitin for 8–12 wk from the time of weaning. After feeding 0.5 ml of [^{14}C]triolein or [^{3}H]oleic acid, 72-hr stool recoveries of radioactivity were significantly greater in deficient rats than in chow-fed controls. Essential fatty acid deficiency did not reduce the absorptive capacities for triolein or for a medium-chain fat, trioctanoin, measured after 3 and 2 hr of maximal-rate duodenal infusion. In everted jejunal slices from essential fatty acid-deficient rats, uptake of micellar [^{14}C]oleic acid at 0–1°C was similar to that of controls, but the rate of incorporation of fatty acid into triglyceride after rewarming to 37°C was significantly reduced. The specific activities of the microsomal esterifying enzymes, acyl CoA:monoglyceride acyltransferase and fatty acid CoA ligase in jejunal mucosa were 30% lower in essential fatty acid-deficient rats. However, the total microsomal enzyme activity adjusted to constant weight did not differ significantly in deficient rats compared with controls. After intraduodenal perfusion of triolein, accumulation of lipid in the intestinal wall was increased in the deficient rats. Because over 90% of the absorbed mucosal lipid was present as triglyceride, essential fatty acid deficiency appears to affect the synthesis or release of chylomicron lipid from the intestine. Analysis of regions of intestine showed that this delay in transport was most marked in the midportion of the small intestine.

Supplementary key words fatty acid esterification · intestinal fat transport · chylomicron formation · intestinal enzymes

During the last few years there has been considerable interest in the detailed structure of various cellular membranes, particularly regarding the spatial and functional significance of the lipid and protein portions of the membrane. One experimental approach to a clearer understanding of this molecular interrelationship would be to alter significantly the structure of one component of the membrane and to study the functional effects of such a change. Phospholipids and cholesterol compose the major portion of the plasma membrane. The fatty acid constituents of the phospholipid in the small intestinal mucosa and the liver have been shown to consist of one saturated fatty acid molecule at position 1 and an unsaturated (usually polyunsaturated) fatty acid at position 2 of the glycerol moiety. It has been suggested that the polyunsaturated fatty acid on the phospholipid is necessary in order to fulfil the stereospecific structural requirements of the membrane, as well as to provide for molecular mobility (2).

Essential fatty acid (EFA) deficiency results in considerable alteration in the chemical structure of phospholipids, as well as other lipids, in many tissues and has been associated with a number of pathophysiological effects (3). Among the most pertinent of these has been the demonstration of increased permeability of water and toxins in the skin of rats, as well as alteration of the structure and some functions of hepatic mitochondria (4). Studies of ion transport through artificial lecithin membranes prepared from tissues of several animals suggested that sodium efflux was greater through EFA-deficient membranes than through lecithin membranes prepared from control animals (5). Impaired diffusion of methylglucose through artificial liposomes made of "EFA-deficient lecithin," as compared with liposomes of "normal" lecithin, has also been demonstrated (6).

In the gastrointestinal tract and the liver, a number of transport processes are present that may be affected by EFA deficiency, thus interfering in the normal physiologi-

Abbreviations: EFA, essential fatty acid; GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

cal functions of these organs. Previous studies have indicated that there is normal mucosal uptake of amino acid and α-methyl-D-glucoside by everted gut sacs from EFA-deficient rats but reduced transport into the serosal fluid (7). Fatty livers occur in rats fed EFA-deficient diets (8), and Fukazawa, Privett, and Takahashi (9) reported normal uptake of free fatty acid by perfused livers of EFA-deficient rats; however, exit of triglycerides into the perfusate was impaired under these circumstances. Barnes, Miller, and Burr (10), and Snipes (11) suggested that fat malabsorption was present in animals fed EFA-deficient diets.

On theoretical grounds, the change in membrane phospholipid structure induced by EFA deficiency might be expected to affect lipid absorption by the small bowel. This could result from impaired uptake of lipid by the mucosal cell by altering membrane permeability, or from alteration of the reesterification process by changing enzyme kinetics or modification of lipid binding to the enzyme site on the microsomes, or from inhibition of the transport of resynthesized triglyceride out of the mucosal cell. The purpose of the present studies was, therefore, twofold. We wished to examine the various stages of lipid absorption in detail utilizing in vivo and in vitro techniques, and, by applying these techniques to normal and EFA-deficient animals, to define the step that is impaired as a result of EFA deficiency.

MATERIALS

Triolein for in vivo perfusions was obtained from K & K Laboratories (Plainfield, Ill.) and was washed with aqueous ethanol to remove free fatty acids. The remaining triglyceride contained the following fatty acids as measured by GLC: 18:1, 89%; 16:1, 7%; and small amounts of 14:0, 14:1, 16:0, and 18:2. Oleic acid (obtained from Applied Science Laboratories, State College, Pa.) was over 99% pure as stated by the supplier and as judged by GLC. Trioctanoin was obtained from Eastman Organic Chemicals, Rochester, N.Y. and was over 99% pure as specified by the suppliers and as confirmed by TLC and GLC. $[1-^{14}C]$Triolein and $[9,10-^3H]$oleic acid (obtained from New England Nuclear Corp., Boston, Mass.) were found to be of greater than 98% radiochemical purity by TLC. $[1-^{14}C]$Trioctanoin was obtained from Nuclear Chicago, Chicago, Ill., and was found to be of greater than 99% radiochemical purity by TLC.

Sodium taurocholate was obtained from Calbiochem, La Jolla, Calif., (grade A, >90% pure) or from Maybridge Research Chemicals, Tintagel Cornwall, England (>98% pure by TCL). Palmitic acid, palmitoyl CoA (grade II), ATP and CoA were obtained from Sigma Chemical Co., St. Louis, Mo. Bovine albumin, fraction V,

was purchased from Pentex, Kankakee, Ill., and 5,5'-dithiobis(2-nitrobenzoic acid) was obtained from Aldrich Chemical Co., Milwaukee, Wis. Monoolein, a mixture of the 1 and 2 isomers, was obtained from Hormel Institute, University of Minnesota, Austin, Minn., and was stated to be 99% pure by the supplier and confirmed by TLC and GLC.

METHODS

Weanling, male Sprague-Dawley rats were divided into control and experimental groups. The experimental group was fed a fat-free diet (12) supplemented with 4% tripalmitin by weight. The control diet consisted of the same fat-free diet but was supplemented with 4% safflower oil by weight. These two diets were obtained from Nutritional Biochemical Corp., Cleveland, Ohio. Animals were allowed these diets ad lib. for periods of approximately 8–13 wk before the various studies. All animals were housed in wire-bottom cages, two rats per cage, and they were weighed at least once weekly.

Fecal fat excretion

Fecal fat excretion was estimated after the rats had been on the special diets for 8 or 12 wk. This was carried out by determining the amount of fecal radioactivity after the oral administration of $[^{14}C]$triolein and $[^3H]$oleic acid sequentially 1 wk apart. The labeled lipids were mixed with 0.5 ml of carrier triolein or oleic acid, as indicated, and the labeled lipid was given by gavage after a 12-hr fast. Stools were collected for 72 hr after the administration of the test fat, as previously described (13).

Uptake rate of medium-chain triglycerides in vivo

Intraduodenal perfusion studies using emulsified medium-chain triglycerides were performed for 2 hr under conditions in which steady maximal and nine-tenths maximal rates of absorption were occurring (14). The absorption rate was calculated from the disappearance of ^{14}C-labeled trioctanoin from the luminal contents of the rat, as previously described in detail (14). These studies were performed at 7 and 13 wk after the initiation of experimental and control diets.

Triolein uptake and transmucosal transport capacities in vivo

A recently developed method (15), which simultaneously yields information on uptake from the intestinal lumen and transmucosal transport rates of absorbed fat in a single animal, was used. Briefly, emulsified triolein (15% w/v) labeled with $[9,10-^3H]$triolein and $[1-^{14}C]$triolein was infused intraduodenally for 2 hr at 2.33 ml/hr, followed without interruption by the same concentration of $[1-^{14}C]$triolein emulsion for 1 hr. Unabsorbed fat in the lumen was quantitatively collected by washing the intes-

tine in situ twice with 30 ml of ice-cooled 2 mM sodium taurocholate. Stomach, cecum, and colon were also washed, and the total unabsorbed radioactivity was determined. In the cold, the intestine was divided into 10 segments, which were homogenized and extracted, and the lipid radioactivity in the intestinal wall was determined (15). Aliquots of lipid extracts of intestinal contents and tissue homogenates were further analyzed by TLC (16). For any given segment, the amount of lipid ^{14}C in the intestinal wall during steady-state maximal uptake from the lumen gave a measure of the mean transmucosal transport time during the entire 3-hr infusion period; the ratio of the specific activities of the two labels was related to the uptake and transport rates during early and late phases of the infusion period. For example, if transport were to slow progressively while uptake remained constant, this would be reflected by an increase in ^{14}C relative to ^{3}H, compared with appropriate control segments, while a high ^{3}H:^{14}C ratio compared with control would imply that transport was slow already during the earlier stages of the infusion.

In vitro studies of kinetics of fatty acid uptake and esterification

Everted rings of jejunum were used to compare the rates of uptake and esterification of oleic acid in the control and EFA-deficient animals. In one set of experiments, the tissue was incubated in micellar sodium taurocholate–[^{14}C]oleic acid media of differing concentrations at 37°C, and the total ^{14}C-labeled lipid in the tissue was determined as previously described (14) in order to compare the rates of fatty acid uptake. In other studies, [^{14}C]oleic acid was absorbed from a micellar medium at a temperature of 0–1°C, which prevented esterification of the absorbed fatty acid. The rate of subsequent conversion of this absorbed [^{14}C]oleic acid to ^{14}C-labeled glycerides at 37°C was measured during postincubation periods of 15–180 sec. Tissues were homogenized and extracted, and the ^{14}C-labeled lipid classes were quantified by TLC and scintillation counting as previously described (16).

Measurement of lipid reesterifying enzymatic activities in jejunal microsomes

Microsomal acyl CoA:monoglyceride acyltransferase (MG-acyltransferase) and fatty acid CoA ligase (FA-CoA ligase) activities were measured in nonfasted rats after 10 wk on the dietary regimens. Animals were killed by a blow on the head, the mucosa from the jejunum was scraped off and homogenized in 0.28 M mannitol with 50 mM EDTA in 0.01 M Na$_2$HPO$_4$ buffer, pH 7.4, and microsomes were prepared by centrifugation as previously described in detail (17). Microsomal preparations were used immediately. MG-acyltransferase activity was measured by continuous-recording spectrophotometry employing 5,5′-dithiobis(2-nitrobenzoic acid) to measure the ap-

TABLE 1. Fatty acid patterns of lecithins of bile and intestinal mucosa of normal and EFA-deficient rats at 10–12 wk

	16:0[a]	18:0	18:1	18:2	20:3	20:4
	mole %					
Bile						
Control	42.5[b]	6.0	6.4	37.1	0	7.0
EFAD[c]	47.6	6.4	35.1	3.8	6.1	1.8
Intestinal mucosa						
Control	23.4	21.5	9.4	25.8	0	18.9
EFAD	27.9	8.0	51.3	1.9	3.3	4.3
Mucosal microsomes						
Control	32.4	20.9	7.5	14.4	0	13.7
EFAD	25.9	15.2	30.1	2.4	6.4	4.1

Total lipids of each tissue were extracted as described, transmethylated using boron trifluoride, and analyzed in an F & M 400 gas chromatograph.

[a] Number of carbon atoms:number of double bonds.
[b] Mean values from three rats.
[c] EFAD, essential fatty acid-deficient.

pearance of liberated CoA, since palmitoyl CoA reacts with monoolein forming diglyceride and free CoA (17). FA-CoA ligase was assayed spectrophotometrically using the hydroxamate-trapping method as previously modified (17, 18). Microsomal protein was determined by the method of Lowry et al. (19).

Specific enzymatic activities were expressed as nanomoles of product formed per minute per milligram of microsomal protein. Total enzymatic activities of the jejunal samples were calculated by multiplying specific activity by the amount of microsomal protein recovered from the entire jejunal sample. To correct for differences in body weights of control and experimental animals, a factor previously shown to affect the total jejunal enzymatic activity (20), values for total enzymatic activities were expressed per 100 grams of body weight.

RESULTS

Both control and EFA-deficient rats were started on their respective diets at weaning (60 g). Rats fed the EFA-deficient diet showed significantly retarded growth rates by 4 wk, although weight gain continued without plateauing throughout the experimental period. By 8 wk, the experimental EFA-deficient animals showed the features of deficiency in the form of scaly paws and dry fur. Examinations of the fatty acid composition of lecithins extracted from bile, intestinal mucosa, and the lecithin fractions of jejunal microsomes confirmed the presence of EFA deficiency as judged by the significant reduction of linoleic and arachidonic acid content and the increase in the content of eicosatrienoic acid after 10–12 wk (Table 1). Partially purified membranes of intestinal mucosal cells showed the same distribution of fatty acids as extracts from the whole mucosa,[1] indicating that there was no se-

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[1] Rodgers, J. B. Unpublished observations.

TABLE 2. Fecal excretion of orally administered
isotopically labeled triglyceride and fatty acid

| | 8 wk[a] | 12 wk[a] | |
	[14C]Triolein	[14C]Triolein	[3H]Oleic Acid
		% of dose	
Control	5.4 ± 2.1 (4)[b]	7.8 ± 2.2 (10)	13.7 ± 2.7 (12)
EFAD[c]	12.2 ± 3.5 (4)	16.5 ± 3.8 (9)	20.7 ± 2.7 (15)
P	NS[d]	<0.01	<0.01

[14C]Triolein was administered by gastric tube in 0.5 ml of olive oil, and [3H]oleic acid was given in 0.5 ml of carrier oleic acid. Stools were collected for 72 hr and radioactivity was determined as described in Methods.

[a] Duration of dietary period.
[b] Means ± SEM, number of rats in parentheses.
[c] EFAD, essential fatty acid-deficient diet.
[d] NS, not significant.

lectivity in the distribution of fatty acids as a result of the deficiency state.

1. Measurement of steatorrhea (fecal excretion after oral feeding)

When [14C]triolein was administered by gavage 8 wk after initiation of the diets, the mean fecal excretion of 14C was greater in the EFA-deficient group (12% of the dose) than in the normals (5.4% of the dose), although the difference was not significant (Table 2). By 12 wk, significant steatorrhea was evident in the experimental group after oral administration of both [14C]triolein and [3H]-oleic acid to the same animals (Table 2) studied sequentially 1 wk apart.

2. Measurement of small intestinal factors in digestion and mucosal uptake in vivo

In order to evaluate the capacity of the small intestine for fat digestion and absorption, the system was stressed by using conditions that ensure maximal absorption. Since the rate of gastric emptying after fat feeding is variable and, in the rat, does not exceed the absorptive capacity of the small intestine, it was therefore necessary to perfuse lipid directly into the duodenum (21, 22).

A. *Absorption of [14C]triolein and [3H]triolein.* Recoveries of unabsorbed, labeled lipid from the small intestinal lumen, cecum, and stomach in the EFA-deficient

animals were identical with those found in control animals during steady-state perfusion. Thus, EFA deficiency failed to impair the absorptive capacity for triolein (Table 3). Indeed, the data from this group of studies also indicated that intraluminal digestion was unimpaired in EFA deficiency, because the extent of lipolysis (as determined by the percentage of hydrolyzed [14C]- and [3H]triglyceride in luminal contents) was close to that of the controls (Table 3).

B. *Absorption of [14C]trioctanoin.* Measurement of absorptive capacity for trioctanoin was used as an indicator of mucosal membrane absorptive integrity, because luminal bile, intramucosal esterification, and chylomicron formation are not required for medium-chain triglyceride absorption (14). Maximal-rate steady-state absorption was measured in EFA-deficient and normal control animals. Controls whose fat-free diet was supplemented with safflower oil absorbed at average rates of 1766 μeq of octanoic acid/hr at maximal trioctanoin perfusion rate and 1520 μeq/hr at nine-tenths maximal perfusion rate (Table 4). These values are similar to those previously reported for rats fed regular pelleted chow (14). Animals fed EFA-deficient diets for 7 and 13 wk had absorptive rates that did not differ from those of the control animals at either perfusion rate (Table 4).

3. In vitro measurement of fatty acid uptake and esterification

Data presented heretofore suggested that neither the intraluminal events nor, probably, mucosal uptake were responsible for the fat malabsorption found in intact EFA-deficient animals after test lipid challenge. The kinetics of fatty acid uptake and the capacity of the intramucosal esterifying system were, therefore, studied under controlled conditions in vitro.

A. *Kinetic measurement in vitro.* The uptake and esterification of [14C]oleic acid in vitro using everted jejunal rings were measured. It should be noted that monoglyceride was absent from the incubation medium in these experiments. As shown in Fig. 1, initial uptake rates of

TABLE 3. Absorption rate and composition of luminal contents during maximal triolein infusion[a]

	Absorption Rate[b]	Gastric Contents	Intestinal Contents							
			14C-labeled Lipid				3H-labeled Lipid		
	μeq 14C-labeled FA/hr	% of 14C-labeled lipid infused	μeq 14C-labeled FA recovered	%			μeq 3H-labeled FA recovered	%		
				FA	MG	TG		FA	MG	TG
Control (4)[c]	570 ± 35[d]	10 ± 3	1341 ± 105	47 ± 1	19 ± 0.5	19 ± 1	635 ± 43	44 ± 1	17 ± 1	19 ± 1
EFAD (3)	595 ± 25	11 ± 4	1263 ± 75	43 ± 2	18 ± 0.2	23 ± 2	547 ± 47	41 ± 1	16 ± 1	21 ± 2

Duplicate aliquots of diluted intestinal contents from each animal were extracted and analyzed by TLC as described in Methods. EFAD, essential fatty acid-deficient; FA, fatty acid; MG, monoglyceride; TG, triglyceride.

[a] Constant intraduodenal triolein infusion (1017 μeq of fatty acid/hr) for 3 hr; first 2 hr, 3H- and 14C-labeled triolein, final hour, 14C-labeled triolein only.

[b] Absorption rate = (μeq of 14C-labeled fatty acid infused in 3 hr minus μeq of 14C-labeled fatty acid recovered in stomach, small intestine, and cecum) divided by 3.

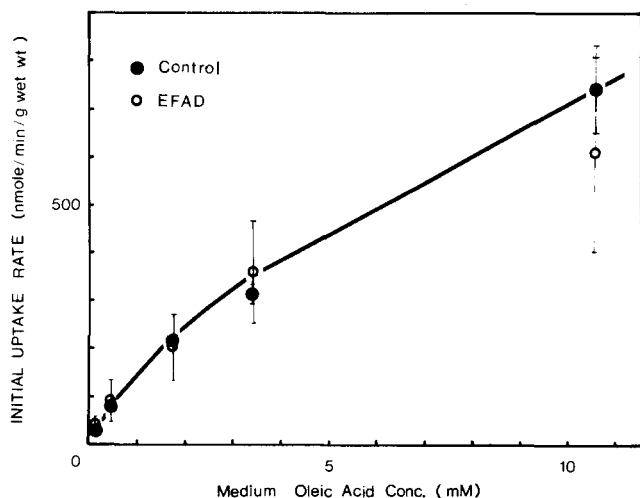[c] Number of animals per group.

[d] Means ± SEM.

Fig. 1. Effect of oleic acid concentration on initial uptake by jejunal everted slices. Incubations at 37°C for 5 min in pH 6.8 buffer containing micellar [1-^{14}C]oleic acid. Sodium taurocholate concentration was 10 mM for fatty acid concentrations up to 1.73 mM, 30 mM for 3.39 mM fatty acid, and 60 mM for 10.06 mM fatty acid. Each data point is from three slices. Means ± SEM. ●, control (three); ○, EFA-deficient (three).

Fig. 2. Oleic acid incorporation into triglyceride by jejunal everted slices at 37°C after preincubation in the cold. Preincubations were for 15 min at 0–1°C in 0.39 mM [1-^{14}C]oleic acid, 10 mM sodium taurocholate and pH 6.8 buffer. During this period the slices took up 226 ± 7 (SEM) (control) and 233 ± 7 (EFAD) nmoles of labeled fatty acid, of which <4% was triglyceride before warming (i.e., at zero time in the figure). ●, control (four); ○, EFA-deficient (five).

[^{14}C]oleic acid, at concentrations of fatty acid ranging from 0.062 to 10.06 mM, were similar in normal and EFA-deficient jejunal rings incubated at 37°C.

Uptakes from 0.39 mM micellar oleic acid for 15 min at 0–1°C were also unchanged in EFA-deficient rings (226 ± 7 [SEM] vs. 233 ± 7 nmoles FA/g wet wt in control and EFA-deficient tissues, respectively). However, the capacity of the jejunum to convert the absorbed oleic acid to triglycerides at 37°C was clearly reduced in EFA deficiency (Fig. 2). Both the initial rate of esterification and the maximal concentration of triglyceride achieved were significantly reduced compared with normal controls, despite identical initial fatty acid ^{14}C concentrations in the tissues (Fig. 2). Initial fatty acid incorporation rates under these conditions, determined graphically, were 103 and 63 nmoles/g wet wt/min for control and EFA-deficient slices, respectively, with corresponding rate constants of 53 and 25. Control rings incorporated 120 ± 7 nmoles of fatty acid into triglyceride after 3 min at 37°C, while only 89 ± 4 nmoles was found in rings from EFA-deficient animals.

B. *Measurement of microsomal esterifying enzymes.* Since oleic acid absorbed into the jejunum in EFA-deficient animals did not appear to be esterified as rapidly as in normal animals, a possible defect in microsomal esterifying activity was sought. In jejunal microsomal samples from nonfasted animals that had been maintained on the dietary regimen for 10 wk, the specific activities of MG-acyltransferase and FA-CoA ligase were significantly reduced in EFA-deficient animals (Table 5). However, total activity of these enzymes corrected for animal weights (per 100 g body wt) was not significantly reduced in EFA-deficient animals.
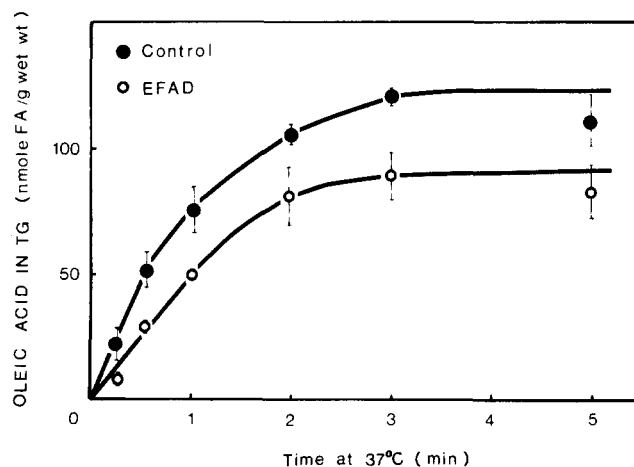
4. Measurement of fat transport through the intestine in vivo

Mucosal esterifying capacity was assessed in vivo following intraduodenal perfusion of ^{14}C-labeled and ^{3}H-labeled triglyceride by measuring the chemical composition of absorbed fat present in the intestinal mucosa under the experimental conditions used. More than 90% of total labeled test fat present in the small intestinal mucosa at the end of the perfusion was triglyceride in all studies, independent of the concentration of fat present in the mucosa.

The amount of ^{14}C-labeled lipid recovered from the wall of the whole small intestine after 3 hr of maximal rate infusion was significantly higher in EFA-deficient animals (938 ± 47 μeq FA/g wet wt; three animals) than in controls (707 ± 20 μeq FA/g wet wt; four animals; P <0.005) (Table 6). Analysis of intestinal tissue after subdivision into 10 segments revealed the characteristic peak concentration of ^{14}C-labeled lipid in the form of triglyceride (>90%) in segments 5, 6, and 7 in both groups of animals (15). The peak concentration was significantly higher (P <0.05) in EFA-deficient animals (Fig. 3). Since

TABLE 4. Absorption rate of [^{14}C]trioctanoin

	Weeks	Infusion Rate	Absorption Rate
		μeq FA/hr [a]	μeq FA/hr
Control (2)[b]	7	2510	1762, 1771[c]
EFAD[d] (3)	7	2510	1884 ± 77[e]
Control (3)	13	1675	1520 ± 53
EFAD (6)	13	1675	1445 ± 45
EFAD (2)	13	2510	1925, 1513

[a] FA, fatty acid.
[b] Number of animals.
[c] Individual values.
[d] EFAD, essential fatty acid-deficient diet.
[e] Means ± SEM.

TABLE 5. Lipid reesterifying enzyme activities in jejunal microsomes of nonfasted rats

| | Acyl CoA : Monoglyceride Acyltransferase | | Fatty Acid CoA Ligase | |
	Specific Activity [a]	Total Activity [b]	Specific Activity	Total Activity
	per 100-g rat		per 100-g rat	
Control (11)[c]	131 ± 6 [d]	820 ± 52	101 ± 7	632 ± 76
EFAD[e] (8)	100 ± 6	676 ± 50	72 ± 4	494 ± 45
P	<0.01	<0.1, >0.05	<0.01	<0.1, >0.05

[a] Nanomoles of product formed/min/mg microsomal protein.

[b] Nanomoles of product formed/min/jejunal sample was calculated by multiplying the specific activity by the total microsomal protein recovered in the sample. Since the average weight of control animals (291 g) was more than that of EFA-deficient animals (240 g), greater total jejunal enzyme activities in controls were anticipated. To correct for differences in body weight between the two groups, total enzyme activity was expressed per 100 g of body weight.

[c] Number of animals studied.

[d] Means ± SEM.

[e] EFAD, essential fatty acid-deficient.

Fig. 3. Intestinal tissue ^{14}C-labeled lipid concentration after maximal rate [^{14}C]triolein perfusion in vivo. Emulsified [^{14}C]triolein was perfused intraduodenally for 3 hr at 1017 μeq ^{14}C-labeled FA/hr. The whole intestine was washed and divided into 10 segments of equal weight. Means ± SEM. ——, control (four); - - -, EFA-deficient (three).

total uptake rates from the lumen did not differ (Table 3), these observations suggested that the mean transport time of lipid passing through the intestinal mucosa was longer in EFA deficiency. Total ^{3}H-labeled lipid in the small intestinal wall, indicative of lipid absorbed during an earlier phase of the infusion period, was also increased in the EFA-deficient rats (664 ± 71 [SEM] vs. 531 ± 19 μeq FA; 0.05 < P <0.1). Excess ^{14}C-labeled lipid, indicative of uptake during the later part of the infusion, was similarly higher in EFA deficiency (234 ± 28 vs. 183 ± 25; 0.05 < P <0.1). This lipid was also present as triglyceride. Although these latter differences do not reach statistical significance, they indicate that slow turnover of lipid in the mucosa of EFA-deficient rats was occurring throughout the infusion period.

DISCUSSION

Intestinal absorption of dietary long-chain triglyceride involves intraluminal digestion, micelle formation, absorption into the mucosal cell, reesterification to triglyceride, and transport into the submucosal lymphatics. We have studied various stages of the absorption process in normal and EFA-deficient rats. The presence of EFA deficiency in the experimental animals was demonstrated by the pres-
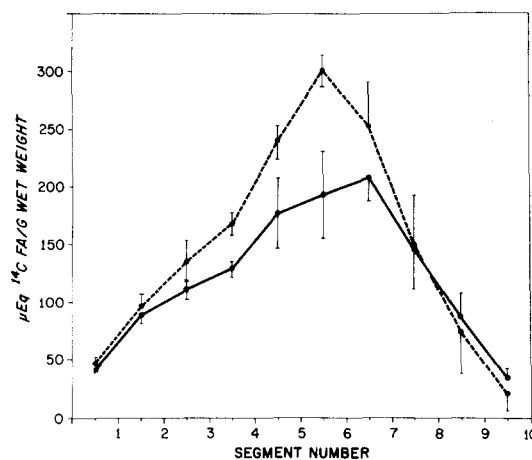
ence of scaly paws, dry, brittle fur, and growth retardation, as well as by the typical changes in fatty acid composition of the lecithins of bile, intestinal mucosa, and mucosal microsomes (Table 1).

The presence of steatorrhea in the EFA-deficient animals (Table 2) was demonstrated by increased fecal excretion of both [^{14}C]triolein and [^{3}H]oleic acid. It is theoretically conceivable that labeled oleic acid fed either as free fatty acid or as triglyceride might have exchanged with endogenous membrane lipid to a greater extent in EFA-deficient animals than in controls, since membrane oleic acid was more abundant in EFA deficiency (Table 1). Such exchange might have resulted in an overestimate of steatorrhea, since excreted lipid was not quantified chemically. However, the degree of fat malabsorption demonstrated in the present studies by fecal loss of radioactive lipid label is in close agreement with that reported in EFA-deficient mice and rats by Snipes (11), who used a quantitative measurement of fecal lipids. That the steatorrhea documented in EFA-deficient rats was not due to maldigestion is indicated by the fact that it was present after administration of both triolein and oleic acid (Table 2). This conclusion is further supported by the data pre-

TABLE 6. Intestinal tissue labeled lipid after maximal triolein infusion

| | Labeled Lipid in Small Intestinal Wall | | | Intestinal Weight | Animal Weight |
	Total ^{14}C	Total ^{3}H	Excess ^{14}C		
	μeq of labeled fatty acid			g	g
Control (4)[a]	707 ± 20 [b]	531 ± 19	183 ± 25	5.90 ± 0.38	316 ± 12
EFAD[c] (3)	938 ± 47	664 ± 71	234 ± 28	6.33 ± 0.14	286 ± 22
P	<0.005	<0.1, >0.05	<0.1, >0.05	NS[d]	NS[d]

[a] Number of animals.

[b] Means ± SEM.

[c] EFAD, essential fatty acid-deficient.

[d] NS, not significant; P > 0.30.

sented in Table 3, which shows that a similar proportion of intraluminal lipid was present as free fatty acid and monoglyceride in normal and EFA-deficient animals after maximal rate infusion of labeled triolein.

Studies were, therefore, performed to evaluate mucosal uptake of lipid in vivo and in vitro. Since trioctanoin absorption has been shown to be unaffected by bile diversion, and since octanoic acid does not undergo reesterification in the mucosa or incorporation into chylomicrons (14), its absorption is primarily dependent upon diffusion through the mucosal cell membrane. Absorption of trioctanoin was shown to be normal in EFA-deficient rats, as shown in Table 4, thus indicating no change in membrane permeability to a lipid probe molecule. This conclusion was further substantiated by the demonstration of normal maximal mucosal uptake of triolein in vivo (Table 3).

In order to delineate better the mucosal uptake of fatty acid, and to separate this process from that of reesterification, in vitro studies were undertaken. At 37°C, uptake of $[^{14}C]$oleic acid was identical in control and EFA-deficient animals (Fig. 1). Incubation at 0–1°C, which reversibly inhibits esterification, confirmed this observation. However, when these rings were washed and further incubated at 37°C, a significant decrease in esterification was demonstrated in tissues from EFA-deficient rats (Fig. 2). The rates of esterification in both normal and EFA-deficient tissues were lower than can be obtained in tissues incubated at 37°C in higher concentrations of micellar oleic acid and, therefore, these studies do not allow comparisons of V_{max} for oleic acid esterification. Nevertheless, because in both normal and experimental preparations the substrate $[^{14}C]$oleic acid was present in excess while the initial rates differed markedly, these data indicate impaired esterifying capacity via the glycerol phosphate pathway in EFA deficiency. Further evidence of impaired esterifying capacity was obtained by direct measurement of FA-CoA ligase and MG-acyltransferase in microsomes. The specific activity of both these enzymes was significantly reduced in EFA-deficient rats (Table 5). This finding is in agreement with those reported for membrane-bound 5′-nucleotidase in livers from EFA-deficient animals (23). It is theoretically possible that these differences could be due to changes in the physical properties of membranes secondary to changes in the fatty acid composition of their component lipids. This seems unlikely in view of the observations of van Golde and van Deenen (24) that force–area curves of lecithins from EFA-deficient rat liver were similar to those from normal animals. Furthermore, the temperature dependence of initial swelling rates of lecithin liposomes changes strikingly only with great changes in the degree of unsaturation of the component fatty acids, i.e., two- or threefold changes (25). In the present studies, only a 1.3-fold change in unsaturation was produced by the EFA deficiency.

Although the specific activities of the mucosal lipid reesterifying enzymes were significantly reduced in EFA-deficient rats, it appears unlikely for several reasons that this abnormality was of major significance in the pathogenesis of the steatorrhea observed in these animals. First of all, reductions in total microsomal enzyme activities in the experimental rats, when corrected for total body weights, were not statistically significant when compared with the controls. This was due to somewhat greater recoveries of jejunal microsomal protein per unit of body weight in the experimental group. Also, after in vivo intestinal perfusion with triolein, more than 90% of mucosal lipid was in the form of triglyceride in both groups. These two observations thus suggest normal esterification via the monoglyceride pathway in EFA deficiency. The results presented in Tables 3 and 6 indicate that uptake and esterification of oleic acid in vivo was normal in EFA-deficient animals but that removal of triglyceride from the mucosa into the lymphatics was impaired.

In order to complete the studies on the mechanisms that may be responsible for impairment of normal fat absorption, it would be useful to measure the appearance of lipid in lymph chylomicrons under control and experimental conditions. The best methods presently available necessitate an intraduodenal infusion rate of no more than about 150 μeq of triglyceride fatty acid/hr, a rate that is one-eighth of that used in the majority of the studies presented. At this low lipid infusion rate, only the upper small intestine is involved in active fat absorption.[2] Studies are presently in progress in one of our laboratories to measure the formation of intramucosal and lymphatic fat particles during intraduodenal perfusion of larger quantities of test fat.

To summarize the experimental data presented, our studies suggest that the fat malabsorption that occurs in EFA-deficient rats is not due to alteration of intraluminal digestive functions, nor to changes in mucosal membrane uptake of fat. Reduction of mucosal lipid esterifying capacity, demonstrated by in vitro slice experiments and microsomal enzyme assays, might contribute to the lipid malabsorption observed in EFA-deficient rats. However, the magnitude of these changes does not appear to be sufficient to account for the degree of steatorrhea observed. It is more likely that the delay in removal of newly synthesized triglyceride from the mucosa is the major factor. There are at least two possible explanations for these data: The assembly of chylomicrons within the mucosa is impaired and/or the exit of intact chylomicrons from the cell and their transport into lymphatics is altered. In EFA deficiency it is possible that changes occur in the availability of appropriate phospholipids for chylomicron formation, or that the membranes of the endoplasmic reticulum or Golgi apparatus (where chylomicron formation and as-

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[2] Bennett Clark, S., and P. R. Holt. Unpublished observations.

sembly is thought to occur) may be affected. The binding of lipids by intracellular structures has been shown to be altered by changing lipid composition, perhaps by altering hydrophobic bonds between phospholipid and structural protein (26). Studies on possible changes in "prechylomicron" or chylomicron formation and transport into lymphatics in essential fatty acid deficiency are presently underway. It is noteworthy that both in gut and in liver the exit step of transport appears to be disturbed in EFA deficiency (7, 8) and that in the liver fat accumulation occurs (8). Studies by one of us (27) indicate that this is true for phospholipid and bile salt transport by liver into bile. Thus current evidence suggests that the defective transport of large molecules (sugars, amino acids, lipids, and bile salts) in EFA deficiency is due to abnormalities of the exit step, and not of uptake. ▪

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