# Intracellular movement of triacylglycerols in the intestine

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**Abstract** **The intestine can vary its triacylglycerol output rate depending on differing physiological conditions. The rate-limiting step in the complex process from fatty acid and monoacylglycerol entry to triacylglycerol export is unknown but suggested to be the transport of triacylglycerol from the endoplasmic reticulum to the Golgi. The present studies were carried out to test this hypothesis. The conversion rate of absorbed fatty acid to mucosal triacylglycerol was studied in rats infused intraduodenally with trioleoylglycerol, 135 μmol/h, for 6 h followed by [$^3$H]oleate. In 30 sec, 79% of the mucosal $^3$H-labeled fatty acid was esterified to [$^3$H]triacylglycerol. The increase in the $^3$H specific activity of triacylglycerol in the endoplasmic reticulum and Golgi was studied in similarly prepared rats except that the radiolabel was [$^3$H]trioleoylglycerol. The endoplasmic reticulum triacylglycerol specific activity was always less than that of the Golgi with a steady state not reached until 60 min of [$^3$H]trioleoylglycerol infusion. The steady state of [$^3$H]triacylglycerol in the lymph was not reached until 70 min of infusion. We conclude that the data are consistent with the rate-limiting step in intestinal triacylglycerol export being the movement of triacylglycerol from the endoplasmic reticulum to the Golgi as the conversion of absorbed fatty acid to triacylglycerol is rapid and the movement of triacylglycerol from the Golgi to the lymph is rapid as well.—**Mansbach II, C. M., and P. Nevin. **Intracellular movement of triacylglycerols in the intestine. *J. Lipid Res.* 1998.** 39: **963–968.**

**Supplementary key words** intestine • lipid absorption • endoplasmic reticulum • Golgi • lymph

The human intestine processes up to 500 g of fat per day in an efficient manner (1). Rats, too, are able to efficiently absorb large amounts of lipid, up to at least 135 μmol/h, with the lipid mostly found in the proximal half of the intestine (2). During lipid absorption, amounts of fatty acids (FA), which are potentially toxic for the enterocytes, are produced during the intraluminal hydrolysis of dietary triacylglycerol (TG). The enterocyte has little control over the rate of entry of these FAs (3) and thus runs the risk of cellular injury unless rapid disposal of the FAs entering the intestinal absorptive cells is available. To reduce the cellular risk of FA injury, the intestinal cell expresses two types of fatty acid binding proteins, the hepatic form, L-FABP (4), and the intestinal form, I-FABP (5). It also has extremely active FA activation and esterification enzymes located in the endoplasmic reticulum (ER) (6) which rapidly convert the FA to the essentially bio-inert TG. The rate at which this conversion occurs has not been systematically studied *in vivo*, although prior studies using both electron microscopic observations (7) and physiological studies (8) suggest that it is rapid.

Despite this presumed rapid formation of TG, most observers have found that it takes at least 4 h of a constant intraduodenal (ID) TG infusion to reach a steady rate of TG output in the lymph (2, 9, 10) suggesting that the ability of the enterocyte to export TG into the lymph is slow compared to its rate of TG synthesis. Additional data suggest that the intestine is able to regulate its TG output. For example, physiological manipulations such as bile diversion are known to impair the ability of the intestine to transport TG into the lymph (11, 12), but including extra phosphatidylcholine (PC) in an ID TG infusion enhances this process (2). Where might this regulatory step occur? Amongst other potential sites, recent data from our laboratory have shown that the rate of movement of TG from the ER to the Golgi correlates with the output rate of TG into the lymph under the conditions of bile diversion or TG + PC infusion (13) suggesting that this is the limiting step. Although this circumstantial evidence is important, no direct data are available to further substantiate the hypothesis that this inter-organelle transport of TG is rate limiting. Therefore, one of the aims of the present studies was to more clearly delineate the rate of movement of TG through the intestinal cell during lipid absorption.

When large amounts of a specific TG (trioleoylglycerol, TO) are infused ID into rats, the mucosa contains a significant proportion of TG-FAs that are not oleate (14, 15). These endogenous FAs have been shown, in part, to come from circulating FA (15) and chylomicron remnants (16). Of considerable interest is our finding that the TG-FAs in the enterocyte that are derived from circulating FA are

Abbreviations: DG, diacylglycerol; ER, endoplasmic reticulum; FA, fatty acids; GLC, gas–liquid chromatography; TO, trioleoylglycerol; ID, intraduodenal; MG, monoacylglycerol; PC, phosphatidylcholine; TLC, thin-layer chromatography; TG, triacylglycerol.

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poorly utilized for chylomicron TG formation. Therefore, TG whose TG-FAs are from endogenous sources are present in the cell but do not readily enter the chylomicron secretory pathway. This would imply that at some point between TG synthesis and entry into the secretory pathway, this endogenously sourced TG must split from the TG which is to participate in chylomicron formation. A second aim of the present study was, therefore, to identify where this partition occurred in the cell.

## METHODS

### Animal preparation

Nonfasting Sprague Dawley rats, 250–350 g, under diethyl ether anesthesia were operated on through a right subcostal incision and a duodenal cannula (PE 50, Clay Adams, Parsippany, NJ) was placed 1 cm distal to the pylorus with its tip at the level of the entry of the common bile duct into the duodenum. A similarly sized cannula was placed in the jugular vein and plugged by a wire. The rats were placed in a restraining cage and infused with 0.15 m NaCl, 0.3 mm KCL, and 5% glucose at 3 ml/h overnight. The next day, the infusion was changed to TO, 30 mm (99% triolein, Sigma Chemical Co., St. Louis, MO), which was prepared as an emulsion by sonicating an appropriate amount of TO in 10 mm taurocholate (Sigma Chemical Co.), 0.15 m NaCl, and 10 mm Tris (hydroxymethyl)amino-methane, Tris·HCl, pH 7.0. The sonicate was infused at 4.5 ml/h for 6 h.

In experiments to determine the rate of incorporation of FA into mucosal TG, the ID TO infusion was stopped and 1 ml of air was injected through the ID cannula to cleanse the proximal intestinal lumen of the TO infusion. One ml of the TO infusate, now supplemented with [$^3$H]oleate (New England Nuclear, Boston, MA) to a final concentration of $8 \times 10^5$ dpm/ml, was immediately injected through the ID cannula to rapidly distribute the radiolabeled infusate to the proximal intestine. At the completion of the bolus injection, the [$^3$H]oleate plus TO-infusate was again allowed to flow through the ID cannula at its previous rate of 4.5 ml/h. At 0.5, 1, 2, and 5 min post the start of the [$^3$H]oleate–TO infusion (an infusion of an additional 37.5, 75, 150, or 375 μl), the experiment was terminated by injecting an overdose of pentobarbital (50 mg) through the jugular vein cannula. In 20 sec the proximal half of the intestine was removed to iced 0.15 m NaCl. The intestine was cut into two equal pieces and then opened lengthwise mucosal side up on a glass plate on ice. It was washed with 0.2% Triton X 100 to remove any [$^3$H]oleate that was adherent to the apical pole of the cells (14) and rinsed with 0.15 m NaCl to remove the Triton. The mucosa was scraped free using glass slides and homogenized after the addition of 15 ml 0.15 m NaCl using a glass–Teflon homogenizer. One ml of the mucosal homogenate was extracted of its lipid content (17) using 0.1 ml HCl to break the phases. 99% of the $^3$H dpm were in the organic phase indicating the nearly complete recovery of the radiolabel into the organic phase of the Folch extraction which was subsequently separated into its lipid components.

Experiments were also performed to determine the specific activity of $^3$H-TG in the ER and Golgi as a function of the time of $^3$H-TO ID infusion. For these experiments, rats were prepared with an ID cannula, a jugular vein cannula, and one in the main mesenteric lymph duct (15). The rats were placed in a restraining cage after the operation and infused overnight as in the previous experiment. The next day, the rats received a sonified TO infusion, 135 μmol TO/h, for 6 h to establish a mass steady state in the mucosa of the proximal intestine (2, 14). After 6 h of TO infusion, the infusate was stopped. One ml of air was injected ID to cleanse the proximal intestine of non-radiolabeled infusate which was immediately followed by injecting ID 1 ml of the 30 mm TO infusate now supplemented with a tracer amount of ([$^3$H]oleoyl) TO (166,667 dpm/μmol). After completion of the bolus injections, the $^3$H-TO infusate was allowed to flow ID at 4.5 ml/h. Five to 70 min later, the experiment was terminated and the proximal half of the intestine was removed to iced 0.15 m NaCl. The mucosa was scraped free and homogenized and a purified ER and Golgi fraction was prepared (13). In brief, the homogenate was centrifuged for 140,000 g·min in a Sorvall centrifuge (DuPont Instruments, Wilmington, DE) and the supernatant was centrifuged for $9 \times 10^6$ *g*·min in a 60 Ti rotor in an L8-M centrifuge (Beckman Instruments, Fullerton, CA) to produce an ER and Golgi pellet. The pellet was collected, gently suspended in buffer A (0.25 m sucrose, 30 mm HEPES, 2.5 mm Mg acetate, 30 mm KCl, pH 7.2) and the density of the suspension was adjusted to 1.22 m sucrose as controlled by an Abbe refractometer. Three ml of this suspension was overlaid with 2.6-ml portions of 1.15 m, 0.86 m, and 0.25 m sucrose in buffer A as suggested by Howell, Ito, and Palade (18). The discontinuous gradient was centrifuged in a SW 41 rotor for $14.4 \times 10^6$ *g*·min in the L8-M centrifuge at 4°C. The membranes at the 0.86/0.25 and 0.86/1.15 m sucrose interfaces were collected using a Pasteur pipette and were combined as the Golgi fraction. The Golgi had a 27-fold increase in galactosyl transferase activity as compared to the whole homogenate and were contaminated 34% by ER as suggested by NADPH-cytochrome C reductase activity present in the Golgi (**Table 1**), although this is likely to be an overestimate because cytochrome C reductase activity is normally present in the Golgi (18). The ER was found in the 1.22 m sucrose layer and the pellet. These were combined as the ER which had a 3.6-fold increase in the specific activity of NADPH-cytochrome C reductase as compared to the whole homogenate and was contaminated 11% by Golgi as suggested by galactosyl transferase activity present in the ER (Table 1). Purified ER was prepared by recentrifuging the ER through the discontinuous sucrose gradient. The lipids in the purified ER and Golgi were extracted (17) and the TG was isolated by differential organic extraction (19). This method was found to be comparable to that of TG separated by thin-layer chromatography (TLC) (13). The $^3$H dpm was determined by evaporating a sample of the extract and determining its radioactive content in a Packard model 1500 liquid scintillation analyzer (Packard Instrument Co., Downers Grove, IL). The mass of TG was determined by gas–liquid chromatography (GLC) as previously described (20).

A final set of experiments was designed to determine the rate at which ID infused $^3$H-TO appeared in the lymph. For these experiments, rats were given an ID and main mesenteric lymph duct cannula (2) and placed in a restraining cage overnight while being infused with saline, KCl, and glucose as before. The next morning, the infusate was changed to TO, 135 μmol/h, which was sonified in 10 mm TC as previously described. The in-

TABLE 1.

| Organelle | Galactosyl Transferase[a] | Cytochrome C Reductase[b] |
|---|---|---|
| | *nmol/mg prot/min* | |
| Whole homogenate | 0.054[c] | 52[c] |
| ER | 0.17 | 188 |
| Golgi | 1.48 | 64 |

[a] UDP-N-acetyl-d-glucosamine-α-galactosyl transferase.
[b] NADPH cytochrome C reductase.
[c] The data are the average of n = 3.

fusate was given for 6 h to establish a mass steady state rate of TG output into the lymph (2) and then altered to include $^3$H-TO (135 μmol/h, 10 × $10^6$ dpm/h) which was constantly infused over the next 80 min. Lymph was collected every 5 min on ice and its radioactivity was determined without separation as >90% of the dpm are in chylomicron-TG under these conditions.

### Enzyme assays and chemicals

NADPH cytochrome C reductase activity, which is associated with the ER, was determined as previously performed (21) as was the Golgi associated UDP-N-acetylglucosamine-α-galactosyl transferase (13, 22). Protein was measured by dye binding (Bio-Rad). All chemicals were purchased from Sigma Chemicals unless otherwise noted.

### Statistical analysis

Significant differences between two means were tested by Student's *t* test using the non-paired, two tailed method. Where more than two means were compared, ANOVA was used with post-Bonferroni corrections (Instat, GraphPad, San Diego, CA) in combination with Student's non-paired, two-tailed *t* test. $P < 0.05$ was taken as a significant difference between means.

## RESULTS

We first wished to determine how rapidly fatty acids entering the intestinal cell from the lumen are esterified to TG. In contrast to prior studies (3, 7), we used rats that had been previously infused ID with large amounts of TO until they reached a mass steady state in the intestinal mucosa (21) to more closely mimic the mid-point of digestion of a normal lipid-containing meal. After 6 h of ID TO infusion, [$^3$H]oleate was added so that the rate of its esterification could be observed. Despite the large amount of lipid entering from the intestinal lumen, as can be seen in **Fig. 1**, 79 ± 1% of the mucosal radioactivity was incorporated into mucosal TG in 0.5 min from the start of the radiolabeled infusion. In a separate set of experiments (mean of n = 2), at 0.5 min of infusion, 25% of the infused dpm were recovered from the intestinal mucosa, 81% of the mucosal dpm were in TG, 13% in FA, 4% in diacylglycerol (DG), 1% in monoacylglycerol (MG), and 1% in phospholipids. At later times of [$^3$H]oleate infusion (Fig. 1), the percentage of oleate incorporated into TG was constant at an average of 89%. These data suggest rapid and nearly complete conversion of absorbed oleate into TG.

We next wished to determine how quickly ID infused [$^3$H]TO was exported from the enterocytes into the mesenteric lymph when the radiolabel was given during the course of an established, constant lipid infusion. Under our conditions, as shown in **Fig. 2**, almost no radiolabel was present in the lymph until 25 min after the start of the $^3$H-TO infusion. This was followed by a rapid increase in radiolabel in the lymph that did not reach a steady state until 70 min of infusion. These data were not due to a slow synthetic rate of TG by the enterocytes (Fig. 1) but rather to a slow delivery of synthesized TG to the lymph. The data indicate that the lipid pool subserving chylomicron formation is turned over at a rate slower than would

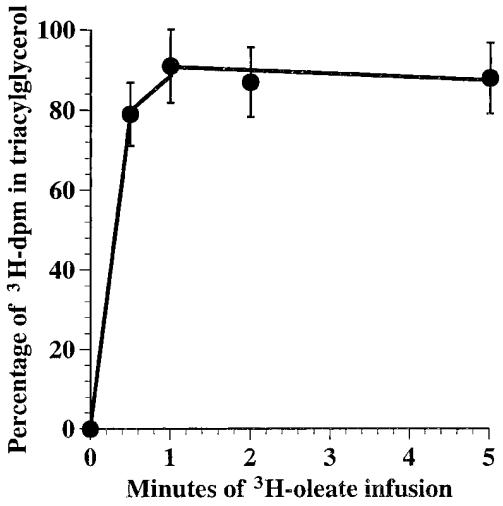


**Fig. 1.** The percentage of $^3$H dpm that were in TG in the intestinal mucosa at the indicated times after the infusion of [$^3$H]oleate into the duodenum. Rats were infused intraduodenally for 6 h with TO, 135 μmol/h. At $t_0$, the TO infusion was supplemented with [$^3$H]oleate. At the indicated times afterward, the mucosa was harvested and the percentage of dpm that was in TG was determined. The data suggest that the incorporation of absorbed oleate into mucosal TG is rapid. The data are the mean ± SEM; n = 4.

be suggested by the rapid appearance time of radiolabeled FA delivered to the fasting intestinal lumen and collected in the lymph. Data similar to Fig. 2 were obtained when the ID TO infusion was supplemented with [$^3$H]oleate instead of $^3$H-TO (n = 2, data not shown).

Because the conversion of absorbed FA into TG was rapid and its delivery to the lymph comparatively slow, we considered whether the delay could be explained by a

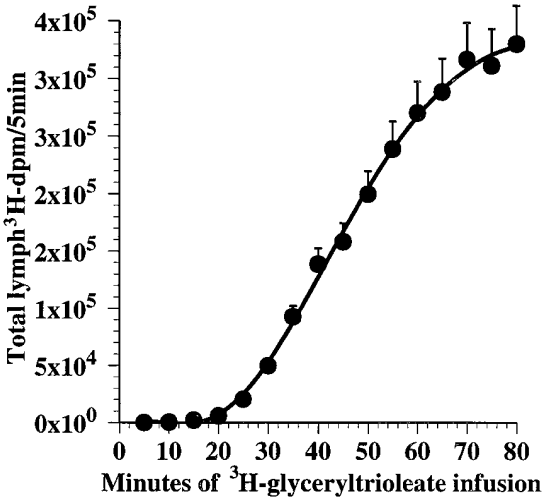


**Fig. 2.** The increase in total lymph radioactivity as a function of the time of intraduodenal $^3$H-TO infusion. Rats were infused with TO, 135 μmol/h, for 6 h. At $t_0$ the infusion was supplemented with $^3$H-TO (75,000 dpm/μmol). Lymph was collected every 5 min and its radioactivity was determined. More than 90% of the dpm are in chylomicron TG. The data suggest that a steady state of $^3$H-TG lymphatic output is reached slowly. The data are the mean ± SEM; n = 4.

slow equilibration of the radiolabel in the ER, Golgi or neither. To answer this question we prepared purified ER and Golgi from rats that had been infused with TO for 6 h to ensure mass steady state conditions followed by supplementation of the TO infusion with $^3$H-TO. We then measured the specific activity of TG in both organelles as a function of time. The interesting results of this experiment are shown in **Fig. 3**. As noted in the figure, the specific activity curve of the purified ER attained steady state levels after 60 min of infusion which was similar to that of the Golgi. Of particular importance, however, is that at the steady state, the ER had a specific activity that was only 40% of the $^3$H-TO infused ID. The Golgi, by contrast, developed a steady state specific activity that was significantly greater, 67% of the input $^3$H-TO specific activity. These data should be compared to chylomicron TG specific activity as a percentage of infusate specific activity under similar $^3$H-TO infusion conditions that have been shown to be 75% (23) and 77% (14). The specific activity of Golgi-TG as a fraction of infusate-TG is likely to be an underestimate as there is contamination of the Golgi by the ER despite the fact that the specific activities of the ER and Golgi marker enzymes (Table 1) compare favorably with that recently obtained from liver (24). The actual amount of ER in the Golgi cannot be accurately calculated due to the known activity of cytochrome C reductase in the Golgi (18).

The Golgi had a significantly greater specific activity than the ER at all the time points tested after radiolabel infusion (Fig. 3, $P < 0.05$). Figure 3 also shows that the rate of increase in specific activity in both the ER and Golgi fractions was a linear function of the time of $^3$H-TO infusion for the first 35 min of infusion. The rate of increase was 64% greater for the Golgi than the ER, indicating that a subset of TG in the ER that was more heavily radiolabeled than the entire ER-TG pool was preferentially transported to the Golgi.

Because the specific activity of the ER was less than that of the Golgi during all time periods, it would be expected that the TG-FA composition of the ER would differ from that of the Golgi. As shown in **Fig. 4**, oleate comprised the vast majority of the TG-FA in the Golgi (81%) but was a lesser percentage ($P = <0.0001$) in the ER (54%). By contrast, the other major TG-FAs were all a greater percentage of the total TG-FA in the ER than in the Golgi. The percentage of TG-oleate in the Golgi is close to that previously found in chylomicrons (20) suggesting that the TG in the Golgi eventually exits the cell in this lipoprotein.

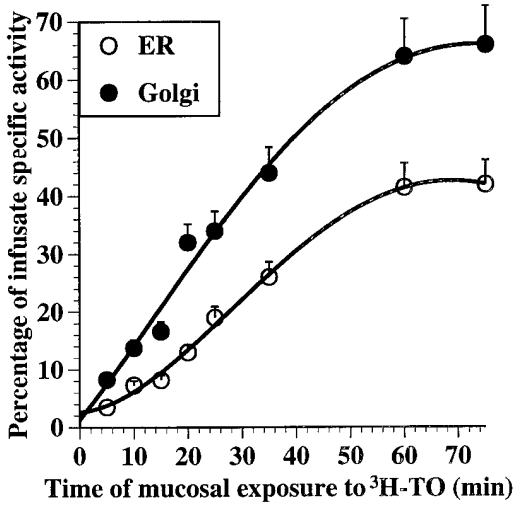


**Fig. 3.** The percentage of infusate specific activity in the ER and Golgi as a function of the time of $^3$H-TO infusion. Rats were infused intraduodenally with TO, 135 μmol/h, for 6 h which was supplemented at $t_0$ with $^3$H-TO. At the indicated times, the mucosa was harvested and the ER and Golgi fractions were isolated as described in Methods. The lipids from the organelles were extracted and the TG specific activity (dpm/μmol) was determined. The organelle specific activity was compared to that of the infusate. The data are the mean ± SEM; n = 5. The Golgi had a greater specific activity than the ER at all time points ($P < 0.05$). At mass steady state, after 6 h of TO infusion, the ER contained 29 ± 3 nmol TG and the Golgi contains 63 ± 8 nmol TG. The specific activity of the infusate was 27,450 ± 2,000 dpm/μmol TO. The equations for the linear regression lines of the data from $t_0$ to t = 35 min are: ER y = 0.766X − 1.21, $r^2 = 0.9762$. Golgi y = 1.26X + 1.75, $r^2 = 0.9521$. Chylomicron TG specific activity as a percentage of infusate specific activity under similar $^3$H-TO infusion conditions have been shown to be 75% (23) and 77% (14).

## DISCUSSION

The studies reported here show that FAs that penetrate the apical membrane of the enterocyte are rapidly converted to TG. This is accomplished despite the number of steps required: desorption from the cytosolic face of the

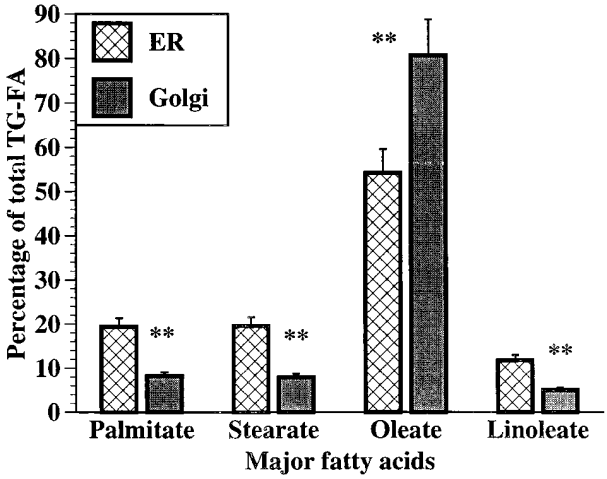


**Fig. 4.** The four major FAs present in the ER and Golgi fractions are shown as a percentage of the total TG-FA from rats that had been infused with $^3$H-TO for varying lengths of time, up to 80 min, after a 6 h intraduodenal TO infusion, 135 μmol/h. The rats used in Fig. 3 were studied with respect to their TG-FA composition in the ER and Golgi fractions. The data shown are the mean ± SEM; n = 30. Differences between means were evaluated by ANOVA and were shown to be significant, $P < 0.0001$, as were the individual differences between means, $P < 0.0001$. The Golgi had a greater percentage of TG-FA that was oleate as compared to the ER and had a lesser percentage composition of the other FAs shown.

apical membrane of the enterocytes, diffusion to the ER, the site of TG synthesis (6), and synthesis to TG by the complex lipid synthetic enzymes in the intestine (6, 25, 26). The likely transport vehicles for FA movement from the apical membrane to the ER are the two fatty acid binding proteins (L-FABP and I-FABP) known to be expressed in the intestine (4, 5). However, without the ability to rapidly re-esterify FA to TG or to bind FA by especially L-FABP (27), the enterocyte would be exposed to potentially toxic amounts of FA as its ability to control FA entry is limited (3). For example, in the present studies, 270 μmol of FA equivalents and 135 μmol *sn*-2-monoacylglycerol were delivered each hour to the intestine as a result of lipolysis. If it is assumed that one half of the intestinal mucosa removed by scraping are enterocytes (≈1 g), it would mean that the intestinal cells would be exposed to very high concentrations of FA unless rapid re-esterification occurred.

The [$^3$H]oleate infused ID was very quickly (within 0.5 min) incorporated into TG despite the large amounts of TO that had been infused for the prior 6 h. Assuming that [$^3$H]oleate absorption accurately reflects TO absorption rates, 7.5 μmol would be expected to be absorbed into the mucosa in 0.5 min. (30 μmol injected × 0.25). Of this, 79% would be in TG in the mucosa, or 5.9 μmol TG. This rapid incorporation of FA into complex lipids supports prior work in Caco-2 cells (28) and rat intestine (7) although in neither case was there prior exposure to lipid.

After the prolonged ID infusion of TO, the 80 min infusion of [$^3$H]TO produced only a very slow increase in ER-TG specific activity, indicating that the newly synthesized TG entered a large TG pool already present in the ER as shown in electron micrographs (29). The rapid conversion of absorbed [$^3$H]oleate to TG indicates that the reason for the slow increment in ER-TG radioactivity was not due to slow TG synthesis but rather suggests the slow turnover of the resident ER-TG pool compared to the rate of $^3$H-TG entry. Indeed, the ER must process the entire large mass of TG delivered to the intestine, 135 μmol of TO each hour. Even more TG must pass through this system as oleate is not the only FA present in mucosal TG (15).

Despite the 80-min $^3$H-TO infusion, the ER-TG specific activity never was greater than 40% of the $^3$H-TO being infused ID. Because a radiolabel steady state was reached, the data can be interpreted as indicating that the ER-TG contained TG-FA, the majority of whose acyl groups were of endogenous origin. These data are consistent with our previous finding that the TG specific activity in the enterocyte, as a whole, is similarly low by comparison to the radiolabeled TO infused ID (14). The data presented here are the first indication of the intracellular level at which endogenous TG is present in the cell. As the ER-TG has a reduced specific activity compared to the Golgi-TG at the radiolabel steady state, the data further suggest that diet-derived TG is preferentially transported from the ER to the Golgi and that TG-FA from endogenous sources preferentially desorbs from the ER to enter the intracellular TG storage pool. Because the TG that appears in the lymph has a specific activity that is greater than the specific activity of the ER-TG (14), it is further suggested that only the TG that is to be secreted in chylomicrons gains entry to the ER lumen to enter the secretory pathway. These data support previous information from the intestine in which two pools of TG were identified, only one of which subserved chylomicron formation (14), and in liver in which two pools of TG were found, one slow and one quickly turning over, both of which contribute to VLDL synthesis (30).

The TG in the Golgi increases in specific activity more slowly than might be expected from the lymphatic appearance time data of ID injected [$^3$H]oleate (8). This finding is consistent, however, with the prolonged time (70 min) for lymph TG to reach a radiolabel steady state output in our experiments, an observation that supports similar findings by Tso, Pitts, and Granger (31) (hydrated mucosa) using a smaller input rate of TG. From the present data, it would appear that the reason for the slow increase in Golgi specific activity is that the newly synthesized and translocated $^3$H-TG in the ER lumen is slow to equilibrate with the large mass of non-radiolabeled TG already present. In this event, TG exiting the ER for the Golgi would be of slowly increasing radioactivity. This may be a function of the load of TG presented to the intestine and might not be as apparent if a lesser load had been given.

The data raise the question as to how TG that is to enter the secretory pathway can be segregated from TG that does not. One possibility is that this is due to differing acyl CoA synthetases in the enterocyte. Although this has not been demonstrated in rat intestine, there are at least four (32) or five (33) CoA synthetases that have been described in yeast which perform separate functions. It is possible, therefore, that activated dietary FA (FA-CoA) are delivered to the TG-synthetase enzyme complex (6) for translocation across the ER membrane as pre-chylomicron-TG separately from FA-CoA preferentially from endogenous sources that are targeted to glycerophosphate acyltransferase for delivery to the storage pool. In support of this hypothesis is the considerable amount of data that shows that the TG-FA exiting the cell in chylomicrons closely reflects that of dietary TG-FA (14, 34–36) despite the fact that the intestinal mucosa contains large amounts of endogenous lipid during active fat absorption (14, 37). The differences in the fate of TG from varying sources is most clearly shown by the fact that during TG-mass steady state conditions in the mucosa induced by ID TO infusions, if the radiolabeled lipid probe enters the intestinal cell from the lumen, the specific activity of mucosal TG is approximately one half that of the infused TO, yet the chylomicron TG specific activity approximates that of the infusate (14). By contrast, if the FA-probe enters from the basolateral membrane, the mucosal TG specific activity is ≈2.5-fold that of chylomicron-TG (15). Therefore, the route of delivery of the FA to the TG- esterifying enzyme influences the ultimate distribution of the TG that is synthesized from it.

The present data can also be used to more clearly identify the rate-limiting step in the movement of FA from the intestinal lumen to the lymph. The first step in the process, the absorption of FA and its re-synthesis to TG is not

limiting as lumenal FA conversion to intracellular TG occurs so quickly (Fig. 1). The time taken for TG in the Golgi to reach steady state (60 min) (Fig. 3) is similar to that seen for the appearance of radiolabeled TG in the lymph (70 min) (Fig. 2). This would suggest that the transit of TG from the Golgi to the lymph is rapid. The current data, therefore, support our previous conclusion (13) that the likely rate-limiting step in TG export by intestinal mucosal cells resides between the ER and the Golgi.

This study was supported by National Institutes of Health Grant DK-38760 and in part by Veterans Administration Medical Research Funds.

*Manuscript received 2 May 1997 and in revised form 24 December 1997.*

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