Rate-Limiting Steps in Steady-State Intestinal Absorption of Trioctanoin-1-^{14}C

EFFECT OF BILIARY AND PANCREATIC FLOW DIVERSION

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ABSTRACT During continuous intraduodenal infusion of emulsified fat in rats, a steady state of intestinal absorption is achieved.

Maximal steady-state absorption of trioctanoin, a medium-chain triglyceride (MCT), by unanesthetized, restrained rats was found to be the same after total bile diversion as in controls (1560 μmoles of fatty acid per hr).

After pancreatic and bile diversion, absorption of MCT was still one-third as rapid as in controls, and mucosal uptake apparently occurred in the form of unhydrolyzed triglyceride. Returning bile to the intestinal lumen during pancreatic diversion did not increase the absorption rate.

From intestinal tissue lipid-^{14}C concentrations measured during steady-state maximal absorption it was possible to calculate turnover times for labeled lipid passing through the mucosal cells. Mucosal turnover times of about 4 min for control and bile-diverted rats, and about 20 min for animals with pancreatic diversion were obtained.

The rate-limiting step in octanoic acid absorption in control and bile-diverted rats was probably mucosal penetration. During absorption of unhydrolyzed triglyceride by pancreatic flow-diverted rats, both passage from the lumen into the mucosal cell and intracellular lipolysis were rate-controlling factors.

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INTRODUCTION

During recent years, much clinical evidence has accumulated to suggest that triglycerides of medium-chain length ($C_{6:0}$ to $C_{10:0}$, MCT) are more readily absorbed in a variety of malabsorptive conditions than long-chain dietary fats (LCT) (2–7). In particular, in patients with biliary obstruction there was appreciable absorption of MCT, and the steatorrhea of pancreatic insufficiency was also successfully reduced by feeding MCT as the sole source of fat (8).

Such therapeutic effects suggest fundamental differences in the mechanisms of absorpton of MCT and LCT. It has long been recognized that medium-chain fatty acids leave the intestinal mucosa almost exclusively by the portal route rather than via the lymphatics (9, 10). After absorption, MCT appears as free fatty acid in the portal vein indicating that intracellular reesterification of the fatty acid does not occur, in contrast to the findings during absorption of long-chain fatty acids. Recently, Greenberger, Rodgers, and Isselbacher (11) demonstrated that the intestinal mucosa could absorb small amounts of unhydrolyzed trioctanoin. However, since the capacity of the small intestine for absorption of MCT by this mechanism was not determined, its physiological significance was unknown. Valdivieso and Schwabe (12) have attempted to quantify MCT absorption in normal, bile-diverted, and pancreatic enzyme-deficient rats by monitoring the $^{14}CO_2$ in expired air after

feeding trioctanoin-1-^{14}C. However, their implicit assumption that the $^{14}CO_2$ expiration rate must be directly and exclusively related to the rate of intestinal absorption of labeled fatty acid or triglyceride is open to question. It seems likely that the collection period of only 1 hr after gastric intubation was too short to allow differences in $^{14}CO_2$ expiration to be attributable only to differences in intestinal absorption rates.

The present study aimed to assess the effect of luminal bile and of pancreatic lipase on the maximal rate of absorption of trioctanoin in vivo, and to elucidate, if possible, the rate-limiting step or steps in the absorptive sequences. The maximal steady-state intraduodenal infusion technique described by Bennett and Simmonds (13) was used. In this system, steady-state absorption of several emulsified fats by conscious, restrained rats has been shown to be limited by an intestinal controlling mechanism. The maximal absorption rate, although different for fats of differing chain length, is highly reproducible between animals for a given fat emulsion. It seemed likely that quantitative analysis of the lipid present in sequential compartments traversed during maximal steady-state absorption might indicate the rate-limiting steps in intestinal absorption under these experimental conditions.

METHODS

Preparation of animals

Male rats of Wistar strain, preoperative body weights between 240 and 280 g were prepared with indwelling duodenal cannulae in the manner described in detail previously (13). Briefly, with the animal under ether anesthesia a midventral incision was made and a fine silicone rubber cannula (O.D. approximately 0.4 mm) passed through stab wounds in the flank and in the stomach, guided through the pylorus, and anchored to the duodenal wall with a fine suture, so that the tip of the cannula lay at about the level of entry of the common bile duct. All surgery was performed 48 hr before the actual experiments. Four groups of animals with duodenal cannulae were investigated.

Group 1. Control.

Group 2. Bile diversion. Bile was diverted to the exterior via a polyethylene cannula inserted into the common bile duct above the pancreas.

Group 3. Bile plus pancreatic flow diversion. The common duct was cannulated at its point of entry into the duodenum, and the secretions diverted to the exterior via a polyethylene cannula.

Group 4. Pancreatic flow diversion only. The common duct was cannulated at the point of duodenal entry. In addition, a polyethylene-tipped, silicone rubber cannula was inserted into the bile duct above the pancreas at one end and into the duodenum via a purse string suture in the intestinal wall at the other, thus returning the bile to the small intestine while draining pancreatic secretion. Data only from animals whose cannulae were patent by inspection at laparotomy after duodenal infusion, and whose livers were grossly normal, are included in Results.

All animals were immediately placed in Bollman-type restraint cages. Experiments were performed 48 hr postoperatively, since it has been established that absorption may be substantially slower after 24 than after 48 hr (14). Tap water was allowed ad lib., but no food. Animals in groups 2, 3, and 4 received, in addition, 0.45% NaCl in 0.45% $NaHCO_3$ solution by continuous infusion via the duodenal cannulae, groups 2 and 4 at 0.5 ml/hr, and group 3 at 1.0 ml/hr.

The completeness of pancreatic diversion was tested in all animals from groups 3 and 4. It was assumed that trypsin and lipase were secreted together, and that absence of trypsin was evidence of removal of lipase also. The intestinal washings from all animals with pancreatic diversions were therefore tested for proteolytic activity before complete lipid analyses were carried out, by measurement of the rate of hydrolysis of a casein substrate (10 ml of a 0.1% buffered casein solution, pH 7.6, incubated at 37.5°C for 15 min with 1–10 ml of proximal intestinal washings). Animals that had less than 10% of normal proteolytic activity were deemed acceptable. In such animals more than 90% of the luminal lipid recovered at the end of the experiment was in the form of triglyceride.

Biliary and pancreatic secretion rates from all fistula animals were monitored from the time of cannulation. In particular, secretion rates immediately before and during lipid infusions were recorded. In some control animals and some with biliary or pancreatic fistulae the abdominal thoracic duct was also cannulated; lymph flow was similarly monitored, and the lymph secreted during trioctanoin infusion was collected separately.

Infusion mixture

The infusion mixture consisted of trioctanoin, Eastman Organic Chemicals, Rochester, N. Y., 25 g; lecithin (vegetable origin), Fisher Scientific Company, Pittsburgh, Pa., 2.4 g; Pluronic F-68, The Upjohn Co., Kalamazoo, Mich., 0.6 g; and Dextrose Certified Reagent, Fisher Scientific Company, 4.15 g.

Randomly labeled trioctanoin-1-^{14}C was obtained from New England Nuclear Corp., Boston, Mass. Water to make 100 ml was added, and the mixture sonicated to give a homogeneous stable emulsion. Free fatty acid and total saponifiable fat were estimated chemically using the modification of Van de Kamer's method previously described (15). Purity of the label was checked by thin-layer chromatography. Initially, more than 98% of both the label and the total saponifiable fat was present as triglyceride. This decreased to 96% after several months, the remainder consisting of free fatty acid.

The specific activities of the test meal varied in different groups of experiments. When total absorption was to be measured the specific activity was approximately 200 dpm/μmole of fatty acid, and was approximately 20,000 dpm/μmole of fatty acid when tissues and plasmas were to be analyzed.

Experimental methods

The trioctanoin emulsion was infused intraduodenally into the conscious, restrained animals. A total of 4.0 ml, containing 5.64 mmoles of saponifiable fat, was administered from a constant infusion pump in 2 hr. This infusion rate exceeded the maximal intestinal absorptive capacity for emulsified trioctanoin reported previously (15) under similar experimental conditions. In preliminary experiments with the present animal colony, it was reconfirmed that the infusion rate of 2.82 mmoles/hr corresponded with an absorption rate appearing on the plateau of the typical quadratic curve relating infusion to absorption, and thus that the absorption rate was indeed maximal under these conditions. There was no loss of intestinal contents to the cecum in 2 hr of infusion while absorption was proceeding at this steady maximal rate. At the end of the infusion, the animals were quickly removed from the cages, and the abdomens opened under ether anesthesia. 3 ml each of portal venous and aortic bloods were first drawn into heparinized syringes and centrifuged immediately at 4°C to separate the plasma. Under these conditions there was no hydrolysis of labeled trioctanoin added to control plasma.

In experiments designed to measure absorption alone, only the contents of the gastrointestinal tract were collected. Stomach, proximal and distal small intestine, and cecum were tied off individually, removed, and the entire contents washed out separately with cold isotonic NaF. The washings were made up to convenient volumes, homogenized with a syringe, and aliquots counted. Since nonlipid components were negligible, activity in the washings represented only unabsorbed lipid.

For determination of oily and micellar phases in the lumen, about 50 mg of NaF was added to undiluted intestinal contents from representative animals in each group. The contents were heated for 10 min at 70°C and centrifuged for 5 hr at 30–35°C and 100,000 *g*. There was no significant hydrolysis of labeled trioctanoin added to intestinal contents under these conditions. The volume of each phase was measured and aliquots counted. Other aliquots were diluted with acid saline, extracted with 5 volumes of chloroform-methanol (2:1), and the lipid analyzed by thin-layer chromatography.

Segments of jejunum and ileum weighing approximately 150 mg were removed between ties and their contents washed gently with isotonic NaF, pH 3.4, into graduated stoppered centrifuge tubes. The segments were slit longitudinally and rinsed briefly in nonradioactive 5% trioctanoin emulsion to dilute any adhering labeled lipid, spread mucosal side up over the inner wall of a beaker, and rinsed thoroughly with a fine jet of NaF solution from a syringe. They were then blotted lightly and weighed. This proved to be an efficient method for removal of adherent fat, for the following reasons. (*a*) No radioactivity was removed by the final wash. (*b*) An animal was infused with a mixed emulsion of trioctanoin and triolein, the intestine washed as described above, and alternate sections stained with hematoxylin and eosin or with Sudan Red. No fat adhered to the mucosal surface, but there was intense fat staining inside the undamaged mucosal cells. Simple washing with a jet of saline from a syringe, a commonly employed method, did not adequately remove lipid from between the villi, according to the above criteria. (*c*) There was no correlation between the activity in intestinal tissue segments and the concentration of label in adjacent luminal contents.

Analytical procedures

Intestinal tissue segments were homogenized in acid NaF solution, with a Potter-Elvehjem homogenizer. The samples of luminal contents washed from these segments with acid NaF were made up to 2 ml with acid saline. 1 ml aliquots of portal and aortic plasmas, or smaller volumes made up to 1 ml with isotonic saline, were acidified with 5 N HCl. 1 ml aliquots of biliary or pancreatic secretions collected during trioctanoin infusion from fistula rats were similarly acidified. All samples were then shaken vigorously for 2 min with 5 volumes of chloroform-methanol (2:1) in stoppered, graduated centrifuge tubes, and the solvent layers separated by centrifugation. Addition of carriers before extraction was found to be unnecessary, since less than 10% of the fatty acid was left in the methanol fraction. The volumes of the aqueous methanol, protein-containing interface, and chloroform fractions were recorded, methanol and chloroform layers were separated, and the radioactivity in aliquots of each of these was determined. Where necessary, chloroform extracts were first washed once with chloroform–saturated acid saline; the washings contained negligible activity, and were discarded. Chloroform extracts were stored at −20°C until further analyses by thin-layer chromatography.

Reextraction of the interface in a series of representative samples revealed that less than 10% of the total label was bound to precipitates, and its composition did not differ from that removed by the initial extraction. A second extraction was, therefore, not routinely carried out.

Separation of lipids by thin-layer chromatography

The lipid classes were separated by silicic acid thin-layer chromatography, using two systems. All solvents were of reagent grade, and were used without further purification. (*a*) Anasyl B[1]–$CaSO_4$ (50:50), 0.5 mm thick plates, developed in ethyl acetate–acetone–acetic acid–heptane (22:10:4.5:163.5). This system permitted good separation into phospholipids, monoglycerides and fatty acids usually not easily separated, diglycerides, triglycerides, and cholesterol esters. Since long-chain and medium-chain lipids of the same class ran substantially together in this system, spots were made visible by addition of 10 μg of nonradioactive oleic acid, 10 μg of diolein, and 20 μg of

[1] Analabs, Inc., Hamden, Conn.

triolein to each sample, and exposure of the plate to iodine vapor. The spots were marked, the plates allowed to stand at room temperature until the iodine had disappeared (1–2 hr), and the marked areas scraped into counting vials for determination of radioactivity. Recovery of standard octanoic acid–^{14}C and trioctanoin-^{14}C from the plates was better than 95%. (*b*) Boric acid–Anasyl B plates were spotted and developed in chloroform-acetone (96 : 4), according to the method of Thomas, Scharoun, and Ralston (16). In this system monoglycerides can be readily separated from fatty acids. A control strip containing 25 μg of nonradioactive octanoic acid, 25 μg of mixed $C_{8:0}$ and $C_{10:0}$ monoglycerides, and 50 μg of trioctanoin was run on each plate. This strip was sprayed with 10% ethanolic phosphomolybdic acid and the plate heated for 8 min at 110°C to make the standards visible. Longer heating led to significant losses of standard labeled medium-chain triglyceride, but not of lower glycerides or fatty acid. The plates were marked according to the standards, and the required areas scraped into counting bottles. Agreement between the two systems was excellent.

Plasma $^{14}CO_2$ and ketones-^{14}C

1 aliquot of plasma was used for both determinations. 100–300 μl in 2 ml of isotonic neutral saline were placed in the outer compartment of small (25 ml) center-well flasks containing 1 ml of Hyamine hydroxide[2] in the center well. The flasks were capped tightly with rubber stoppers, and 0.3 ml of 50% citric acid was injected through the caps into the outer compartments. The flasks were then shaken gently for 30–60 min at 37°C, after which the Hyamine containing the released CO_2 was completely transferred into counting bottles for $^{14}CO_2$ estimation. Octanoic acid–^{14}C distilled into the center well in only negligible amounts under these conditions.

Fresh 1 ml aliquots of Hyamine hydroxide were then placed in the center wells, the flasks recapped, and aniline citrate (1 ml of 1 : 1 aniline in 50% citric acid) injected into the outer compartment. By this means, acetoacetate is split so that the carboxyl carbon is released as CO_2, the rest of the molecule remaining behind as acetone (17, 18). The flasks were shaken for 90 min at 37°C, and the Hyamine was again washed out and counted. In suitable control flasks, negligible amounts of acetone-^{14}C distilled into the center well during this procedure.

Since recoveries of $^{14}CO_2$ from acetoacetate were negligible, no further attempts were made to estimate also the label on the 1-carbon atom of acetoacetate.

Estimation of medium- and long-chain fatty acids–^{14}C in intestinal tissue

It was found that small amounts of medium- and long-chain fatty acids could be effectively separated by evaporation under controlled conditions because of their differing volatilites. 250-μl aliquots of chloroform extracts of intestinal tissue were saponified with 2 ml of 15% ethanolic KOH, acidified with 5 N HCl, and 1 ml of H_2O was added. The fatty acids were completely extracted with petroleum ether, bp 40–70°C (6 × 1 ml), the solvent removed at room temperature, and the fatty acid residues evaporated in counting bottles under a controlled stream of nitrogen at 40°C for 1 hr. The final residues were taken up in 1 ml of petroleum ether and counted. Since under identical conditions there was no loss of an equimolar quantity of palmitic acid–^{14}C (560 μg), while a similar amount of octanoic acid–^{14}C (700 μg) had completely evaporated, it was assumed that if any activity remained in the evaporated tissue extracts it must have been present in the tissue as long-chain fatty acid or triglyceride.

Counting methods

^{14}C radioactivity was counted in a Packard Tri-Carb liquid scintillation counter, model 314E. Hyamine solutions were counted with internal standards in 12 ml of toluene a scintillator (19). All other samples were counted in 15 ml of Bray's solution (20) with internal standards (IS) when necessary to correct for quenching.

Intestinal contents (in duplicate)	washings 1 ml; IS micellar and oil phases 10 μl
Plasmas	25 μl
Biliary or pancreatic secretions	50 to 100 μl
Aqueous methanol extracts	1 ml; IS
Chloroform extracts	0.1–1.0 ml dried to 25–50 μl under N_2 in presence of 50 μl of carrier trioctanoin
Petroleum ether extracts	1 ml
Silicic acid	slurried in 1 ml hexane; allowed to settle before counting

Calculations

(*a*) Luminal absorption rate in μmoles of fatty acid per hour =

$$\frac{\text{Total } \mu\text{moles of octanoic acid–}^{14}\text{C infused as triglyceride} - \text{total recovered from gastrointestinal tract}}{\text{time (2 hr)}}.$$

(*b*) "Turnover Times." Time taken for an average molecule of lipid–^{14}C to transverse intestinal tissue (min) =

$$\frac{\text{Wet weight of total intestinal tissue (g)} \times \text{mean tissue lipid–}^{14}\text{C concn. } (\mu\text{moles of fatty acid–}^{14}\text{C/g})}{\text{absorption rate } (\mu\text{moles of fatty acid–}^{14}\text{C/min})}.$$

(*c*) All statistical evaluations were done by analyses of variance (21).

[2] *p*-(diisobutyl-cresoxyethoxyethyl) dimethylbenzylammoniumhydroxide, Packard Instrument Company, Inc., Downers Grove, Ill.

TABLE I

Steady-State Maximal Intestinal Absorption of Trioctanoin-1-^{14}C

Group	Luminal abs. rate*	Estimated portal transport*‡	Intest. tissue lipid-^{14}C concn.§	"Turnover time"
	μmoles FA/hr	*μmoles FA/hr*	*μmoles FA/g wet wt*	*min*
Control	1570 ± 57(11)	900 ± 179(7)	20(5)	~4
Bile diverted	1560 ± 15(9) (NS)	1116 ± 84(7)	18(3) (NS)	~3.5
Panc. + bile diverted	510 ± 63(6) (<0.01)	396 ± 226(6)	27(4) (NS)	~16
Panc. diverted (bile returned)	290 ± 87(3) (<0.001)	560 ± 87(5)	21(5) (NS)	~21

Infusion rate, 2820 μmoles of fatty acid (FA)/hr.

* Mean ± SE. Number of animals in groups appear in parentheses. Levels of significance of difference from controls are shown. NS, not significant.

‡ Assume portal blood flow 600 ml/hr, hematocrit 40%. Portal transport calculated from difference between portal and aortic lipid-^{14}C concentrations in Table IV.

§ Mean of 2 or 3 segments of both jejunum and ileum from each animal.

‖ Assume total wet wt of small intestine 5 g. Calculations of "turnover times" of lipid-^{14}C from luminal absorption rates.

RESULTS

Steady-state maximal absorption of trioctanoin. In general, during steady-state intestinal absorption the rate of uptake of lipid by the mucosa must equal the rate of removal from the mucosa. In the present system, lymphatic transport during infusion of trioctanoin was shown to be less than 1% of the amount absorbed in representative control animals, and was less than 0.25% of the absorbed lipid in an animal with pancreatic and bile diversion. The major transport route of medium-chain lipid after absorption was therefore the portal vein. In Table I the similarity between the luminal absorption rate and the estimated rate of portal transport is apparent in all four experimental groups. Thus the conditions for a steady state of transmucosal movement are satisfied. Since absorption is also maximal for these experimental conditions (15 and footnote 3) and since more than 85% of the ^{14}C activity was present in the mucosal layer during the steady state, the calculated turnover times for labeled lipid passing through intestinal tissue approximately represent the maximum average rates of passage of lipid through the mucosa. The four experimental groups of animals appear to fall into two subdivisions. Turnover times in control and bile-diverted animals are similar, but are clearly different from those in both groups of animals with pancreatic diversion. These differences in turnover times are due entirely to variations in maximal absorption rates. Intestinal tissue lipid-^{14}C concentrations are similar in all groups.

The results show that bile exerts no marked effect at any stage of trioctanoin absorption, either in the presence or the absence of luminal pancreatic lipase. Whereas absorption of trioctanoin in bile-diverted animals was no less than control absorption rates, after diversion of both biliary and pancreatic secretions the maximum absorption rate was depressed to about one-third of the control rate ($P < 0.01$). Returning bile in the absence of pancreatic juice caused no increase in absorption; in fact, a further decrease was observed ($P < 0.05$).

Examination of the distribution of lipid-^{14}C between luminal fatty acid and glycerides and between aqueous and oil phases showed that in bile-diverted as well as control animals the majority of the label was in the watery phase, and was present predominantly as hydrolyzed fat (Table II). In animals with pancreatic diversion more than 99% of the lipid was recovered from the oil phase, which occupied about 40% of the volume, and almost all the lipid was in the form of triglyceride. The total lipid present in the aqueous phase in this group of animals, both as fatty acid and triglyceride, was negligible. It seemed likely, therefore, that trioctanoin was absorbed by pan-

[3] Bennett Clark, S. Unpublished observations.

TABLE II

Percentage Distribution of Lipid-^{14}C in Intestinal Contents

Group		Volume	Total counts	O	FA	MG	DG	TG
Control	Whole conts. (4)	100	100	0–1	46–87	6–7	0–30	13–24
	Aqueous phase (3)	90–95	78–90	2–5	54–62	30–34	0*	6–9
	Oil phase (3)	tr.	10–23	0	58–62	13–17	2–5	15–25
Bile diverted	Whole conts. (4)	100	100	1–2	44–68	7–8	3–19	26–42
	Aqueous phase (3)	90–95		7–12	52–57	19–33	0*	6–16
	Oil phase (3)	tr.						
Panc. + bile diverted	Whole conts. (5)	100	100	0–3	3–10	0–2	4–10*	83–91
	Aqueous phase (1)	30	<1	5	53	3	4	34
	Oil phase (1)	40	>99	0	6		2	90
Panc. diverted, (bile returned)	Whole conts. (5)	100	100	0–1	1–11	0	1–6	80–98
Infusion mixture				2	6		2	90

Numbers of animals in groups appear in parentheses. Ranges of levels within groups are indicated throughout.
* Apparent discrepancy in DG probably due to incomplete inactivation of pancreatic lipase before centrifugation to separate phases.

creatic flow-diverted animals as the triglyceride, from an oil phase. Bulk uptake of unhydrolyzed trioctanoin was substantiated by the distribution of lipid classes found in intestinal tissue segments (Table III).

It was noteworthy that the total labeled lipid concentrations in the jejunal segments of all groups were statistically the same, despite differences in absorption rates. The same was true for ileal segments. Nor was there a significant difference between jejunal and ileal lipid levels in any one group. Specifically, there was no appreciable

TABLE III

Steady-State Distribution of ^{14}C from Octanoate-^{14}C in Intestinal Tissue Fractions

Group		Total labeled lipid*	TG	FA	Methanol-soluble ^{14}C
Jejunum					
Control	(5)	20.4	3.9	15.1	0.90
Bile diverted	(3)	24.1 (NS)	6.5 (NS)	15.3 (NS)	2.09
Panc. + bile diverted	(5)	43.5‡(NS)	33.8 (<0.05)	5.2 (<0.05)	0.68
Panc. diverted, (bile returned)	(5)	25.5 (NS)	21.2	3.3	0.61
Ileum					
Control	(5)	19.2	3.3	14.9	1.26
Bile diverted	(3)	12.6 (NS)	3.4 (NS)	8.1 (NS)	2.17
Panc. + bile diverted	(5)	31.0‡(NS)	24.5 (<0.05)	3.9 (<0.05)	0.66
Panc. diverted, (bile returned)	(5)	10.3 (NS)	9.2	0.9	0.55

All numbers represent group mean μmoles of fatty acid-^{14}C per g wet wt tissue. Number of animals in each group appear in parentheses. Tested levels of significance of difference from controls are shown.
* MG, DG, phospholipid, and cholesterol esters all negligible.
‡ This group contained one anomolous animal with very high tissue lipid levels, which was not included in Table I.

effect of bile in ileal tissues of groups 1 and 4, where high ileal tissue bile salt levels should have occurred. However, the triglyceride level in pancreatic- plus bile-diverted animals was significantly higher than in controls, both in jejunum and ileum ($P < 0.05$ for each). Control and bile-diverted animals had very little labeled triglyceride in the intestinal tissue and much fatty acid, whereas the reverse was true for pancreatic- and doubly-diverted groups.

More than 95% of the tissue lipid activity in fatty acid and triglyceride fractions in all groups was shown, by evaporation as described, to be in medium- or short-chain fatty acid. The concentration of labeled material in the methanol fraction was in all cases very low, suggesting either that the mucosa does not metabolize much octanoate during absorption, or that metabolites diffuse readily from the tissue.

^{14}C in portal and aortic plasmas. In order to calculate rates of portal transport of absorbed lipid from the intestine in the present system, we subtracted the steady-state arterial lipid-^{14}C concentration from the portal concentration (see Table I). It was assumed that aortic plasma was identical with that in the mesenteric arterioles. Total lipid-^{14}C levels were always higher in portal than arterial plasma, and the difference was found to be almost entirely in the free fatty acid fraction of the total lipid-^{14}C (Table IV). The concentrations of triglyceride-^{14}C were similar in portal and aortic plasmas. There was thus no evidence for significant portal transport of unhydrolyzed trioctanoin from the intestine in these experiments. During cellulose acetate electrophoresis of representative plasmas, the fatty acid–^{14}C was found to be partly bound to albumin and partly not bound, presumably in true solution.

The distribution of the ^{14}C label between lipid and methanol soluble fractions in plasmas of all groups, and within lipid classes for portal plasma is shown in Table IV. Again, biliary diversion produced no change from controls, and no difference was demonstrated between the two groups with pancreatic diversion. Methanol-soluble ^{14}C constituted a major fraction of the total plasma activity in both portal and aortic plasmas from all groups, indicating that extensive metabolism of octanoate had occurred. Additional data obtained from analysis of segments of liver suggested that most of these metabolic products were of hepatic origin.[4]

Plasma $^{14}CO_2$ levels in all animals ranged between 0.1 and 0.8 μmole/ml of plasma. All experi-

[4] Bennett Clark, S., and P. R. Holt. Hepatic metabolism of ^{14}C-octanoate during steady state liver perfusion in unanaesthetized rats. Manuscript submitted for publication.

TABLE IV

Octanoate-^{14}C Incorporated in Portal and Aortic Plasma Fractions

	Total lipid-^{14}C	O	FA + MG*	TG	Methanol-^{14}C
Portal plasma					
Control	5.4 (7)	1.1	3.5	0.6(3)	3.4 (7)
Bile diverted	6.1‡(7)	0.9	4.6‡	0.8(3)	2.4‡(7)
Panc. + bile diverted	1.4 (6)	0	1.4	0.1(6)	0.4(6)
Panc. flow diverted (bile returned)	1.8 (5)	0.1	1.7	0.1(5)	0.3 (5)
Aortic plasma					
Control	2.9 (7)	1.0	1.8	0.3(3)	3.0 (7)
Bile diverted	3.0‡(7)	0.8	2.5‡	0.4(3)	2.4‡(7)
Panc. + bile diverted	0.3 (6)				0.4 (6)
Panc. flow diverted (bile returned)	0.2 (5)				0.3 (5)

All values expressed as mean μmoles of fatty acid–^{14}C incorporated per ml of plasma. Numbers of animals in groups appear in parentheses.

* MG negligible where measured in selected samples.

‡ These values are not significantly different from corresponding control values.

mental groups showed much higher concentrations than controls, possibly due to a somewhat excessive postoperative bicarbonate infusion which may have resulted in a mild alkalosis. Arterial $^{14}CO_2$ levels were in general lower than portal levels in animals from all groups. Both portal and arterial acetoacetate-^{14}C concentrations were extremely low (0.01–0.04 μmole of $^{14}CO_2$ per ml of plasma), with no apparent difference between groups. These observations also suggest that there was little metabolism of octanoate by the intestinal mucosa, except perhaps some conversion to CO_2.

Biliary and pancreatic secretion during trioctanoin infusion. During the 2 hr of intraduodenal infusion of trioctanoin-^{14}C, between 1 and 2 ml of bile containing some radioactivity was excreted by bile fistula rats. The label was distributed approximately equally between chloroform and methanol soluble fractions. In three rats, the total labeled lipid excreted in 2 hr was equivalent to 12.2, 5.8, and 5.6 μmoles of fatty acid–^{14}C respectively, of which 50–60% was in phospholipid-^{14}C. In animals with pancreatic and bile diversion the total label excreted was much less, reflecting the lower absorption rate. Since the total excreted was relatively small in all fistula animals, recycling of label through the small intestine in control animals due to excretion in the bile was ignored in the calculation of maximal absorption rates.

During infusion, secretion of bile by animals with bile fistulae increased between 14 and 230% over the preinfusion secretion rate. The mean increase for six animals was 67.5% above the basal level. In these animals about half the luminal fat was in the form of free fatty acid. In animals with no appreciable luminal fatty acid, the combined biliary and pancreatic secretions did not change significantly (mean decrease of 1.6%, ranging from an increase of 25% to a decrease of 64% in 11 animals). In six animals with only pancreatic flow diverted there was also no significant change (mean increase of 5%). Thus in the absence of luminal free fatty acid there was no net change in either biliary or pancreatic flow. However as a stimulator of bile secretion, luminal octanoic acid appeared to be equally as potent as long-chain fatty acids.

DISCUSSION

Earlier studies have shown that the small intestine has a limited capacity for absorbing fat, and that the maximum absorption rate decreases with increasing chain length (15). Preliminary experiments in the present investigation established that the maximum absorptive capacity for emulsified trioctanoin, a typical medium-chain triglyceride, was 1560 μmoles/hr in normal, unanesthetized rats with duodenal cannulae. It seemed that if the steady-state maximal absorption of trioctanoin were affected by biliary or pancreatic diversion, it should be possible to determine not only the quantitative importance of such effects, but possibly also at which stages in the absorptive sequence these effects were predominant.

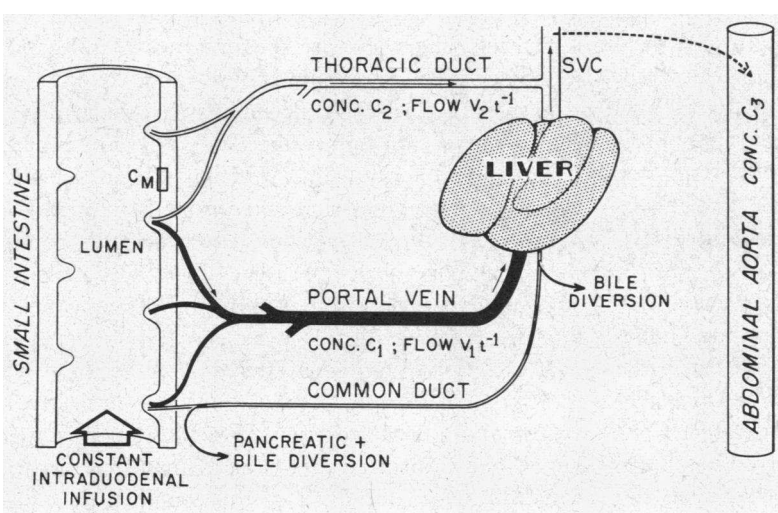

FIGURE 1 Experimental design for determination of mucosal turnover times in vivo. C_M = steady-state intestinal tissue lipid-^{14}C concentration. More than 85% was present in the mucosa. C_1, C_2, C_3 = concentrations of lipid-^{14}C in portal plasma, thoracic duct lymph, and aortic plasma, respectively. $V_1 t^{-1}$, portal plasma flow rate. SVC, superior vena cava.

During steady-state intestinal absorption,

(1) luminal absorption rate = portal venous transport rate + lymphatic transport rate = $(C_1 - C_3) \cdot v_1 t^{-1}$ since here $C_2 \simeq 0$.

(2) C_M is constant.

It is theoretically possible to determine rate-limiting steps during maximal steady-state transport through the intestinal wall, since the average time taken for a single absorbed molecule to traverse the mucosa can be calculated from the rate of maximal absorption and the constant mucosal concentration of the absorbed material during the steady absorptive state. This is indicated in Fig. 1. The same calculations can be extended to the turnover rate through any subcompartments in the mucosal tissue, such as, in the present experiments, the mucosal levels of free fatty acid or triglyceride during steady-state absorption. When steady-state intestinal tissue concentrations or absorption rates differ between different experimental groups, turnover times through subsidiary compartments will also differ. Hence possible rate-limiting steps concerned with such processes as micelle formation, mucosal cell uptake and metabolism, and transport out of the mucosa may be revealed.

In the intestinal contents of control animals, the distribution of label between lipid classes during steady-state maximal absorption of trioctanoin was and Borgström for absorption of dietary fat by generally similar to that described by Hofmann normal humans (22). The triglyceride level was somewhat higher than after gastric feeding; however, our data suggest that the maximum rate of pancreatic lipolysis had not been exceeded in control animals since the mean quantity of unhydrolyzed triglyceride recovered from the lumen after duodenal infusion was equivalent only to the amount of lipid infused during the final 10 min of the experiment. Most likely this lipid would not yet have been adequately mixed with intestinal contents when the small intestine was washed out. The monoglyceride concentration was always low, perhaps not unexpectedly since monoactanoin is known to be more completely hydrolyzed to free fatty acid by pancreatic lipase (23) than long-chain monoglyceride (24–26). Absorption of trioctanoin by normal animals therefore seems to occur to a major extent as the free fatty acid.

Effect of biliary diversion. Within the limits of the present experiments no effect of bile on the absorption, mucosal transport, or intestinal metabolism of octanoate could be demonstrated. Control and bile-diverted animals behaved similarly in all respects. This is in direct contrast with the results obtained by Valdivieso and Schwabe (12) who reported a significantly decreased "absorption" in terms of $^{14}CO_2$ output in the absence of bile. These authors did not feed pure medium-chain triglyceride, however, but an emulsified 10% solution of trioctanoin in a long-chain triglyceride, olive oil. Possibly direct instillation of such an oily mixture into the duodenum in the absence of bile would have led to reduced pancreatic lipolysis during the 1st hr after instillation in bile-diverted animals, because of inadequate emulsification. In the present experiments, when pure, emulsified trioctanoin was continuously infused intraduodenally, the average rate of uptake during 2 hr of steady-state absorption from the lumen was identical in bile-diverted animals and controls. Biliary diversion somewhat reduced the extent of luminal lipolysis (35% luminal triglyceride after bile diversion compared with 18% in controls), but this was obviously not a rate-limiting factor in the absorption of trioctanoin. As in control animals, little monoglyceride was present. During absorption of long-chain fat, monoglyceride appears to be an essential component in the formation of a micellar solution, whose function is probably merely that of a vehicle to transport insoluble fatty acid through the watery phase into close proximity with the cell membrane, which the lipid-soluble molecules might then penetrate directly. The water solubility of octanoic acid, however, which may be of the order of 6–15 mmoles/liter (27, 28) is perhaps sufficient to allow its transport through the watery intestinal contents in true solution. Sodium salts of octanoic acid would have a very high solubility. Micelle formation, and therefore the presence of bile and monoglyceride, would be unnecessary. From the results of the present experiments it would appear that turnover through a micellar phase containing bile salt, monoglyceride, and fatty acid is not essential for medium-chain fat absorption, although it probably proceeds to some extent by this route if all components are present. The rate-limiting step appears to be the passage of octanoic acid into the mucosal cell.

Absorption of unhydrolyzed triglyceride by pancreatic flow–diverted animals. When both pancreatic and biliary secretions were absent from the lumen, the absorption rate of emulsified trioctanoin was still about one-third of the control rate, and

the labeled lipid inside the intestinal mucosa was largely in the form of medium-chain triglyceride. The unhydrolyzed medium-chain triglyceride in the mucosa was most likely taken up intact. Although the crucial experiment of infusing glycerol-labeled trioctanoin was not performed, studies on intestinal mucosal metabolism of trioctanoin in vitro have repeatedly shown that this lipid is readily hydrolyzed and poorly esterified by mucosal enzymes (29 and footnote 5). The maximum rate of uptake of triglyceride was about 500 μmoles/hr expressed as fatty acid (1), which is as rapidly as the long-chain triglyceride, triolein, can be absorbed by undiverted control animals.[3] Significant absorption of triolein from fine watery dispersions has been demonstrated in vitro in hamster intestinal slices (30), although its uptake was appreciably slower than that of unhydrolyzed trioctanoin in vivo in the rat. In the present experiments recovery of the luminal triglyceride-^{14}C exclusively from the oily layer after high speed centrifugation suggests that absorption took place under these conditions from the oil phase and not from the watery phase, although the possibility of rapid turnover through the aqueous layer cannot be entirely ruled out. It is possible that a small amount of unhydrolyzed trioctanoin might have been absorbed also by rats with bile diversion only, since the percentage of labeled lipid present as triglyceride in the intestinal lumen was higher in these animals than in controls, whereas the absorption rates and intestinal tissue fatty acid concentrations were the same as those of controls. Whether some triglyceride absorption occurred also in control animals could not be demonstrated in the present experiments, although in the light of the over-all findings it seems reasonable that this would have taken place to a very minor extent.

Inside the mucosa, triglyceride was hydrolyzed before passing into the portal blood as free fatty acid. This result is at variance with those obtained from the acute experiments of Greenberger et al. who, using irrigated loops of intestine (11), found that unhydrolyzed triglyceride apparently entered the portal vein, but it agrees with an earlier report by Playoust and Isselbacher (29) in which trioctanoin taken up by rat mucosa was hydrolyzed intracellularly, and only the fatty acid passed into the portal system. In the present experiments all the absorbed lipid could be accounted for as free fatty acid in the portal blood if the flow was assumed to be about 10 ml/min (31). Thus during bulk absorption of trioctanoin intramucosal lipolysis before portal transport was virtually complete.

It would appear at first that the rate-limiting step in the uptake of unhydrolyzed triglyceride was the actual passage through the mucosal membrane, since the total labeled lipid in intestinal segments from animals with pancreatic flow diverted was the same as that of controls, despite the difference in the amount of unabsorbed lipid in the lumen. However, since the rate of uptake was one-third or less of the control rate, and since this slower absorption was reflected in the one-to-three ratio of the portal plasma lipid concentrations of these same groups, their mucosal lipid levels should have been in this ratio also if there were no slow, intramucosal reaction intervening. Since the experimental results did not show this mucosal lipid ratio, it is reasonable to conclude that the intracellular lipolysis of trioctanoin is a contributing rate-limiting factor during the absorption of unhydrolyzed triglyceride. This is reflected by the longer turnover times for lipid-^{14}C passing through the mucosal cells after pancreatic diversion when compared with control or bile-diverted animals. Probably both a slower initial penetration and the need for intracellular lipolysis are contributory rate-controlling factors in trioctanoin absorption. Lipid turnover times have been estimated by electron microscopy and radioautography in studies of safflower oil absorption by normal rats (32). It was suggested that only 5 min was required for the hydrolyzed products to be reesterified and transported from the mucosal cell. If these results are physiologically representative, the intramucosal lipolysis of medium-chain triglyceride would appear to be a relatively inefficient process, slower than the reesterification of long-chain fatty acid. Since the free fatty acid levels in intestinal tissue of pancreatic flow–diverted rats were always low, transport of octanoic acid from the cell into the portal vein must have been a relatively rapid process, and not rate-limiting during intestinal absorption.

In conclusion, in the unanesthetized rat absorbing trioctanoin maximally under steady-state con-

[5] Brause, B., M. Tajjudin, S. Bennett Clark, and P. R. Holt. Unpublished observations.

ditions, it has been possible to deduce rate-limiting steps in the intestinal transport of a medium-chain fat in vivo, by quantifying the perfused material and its products in sequentially traversed compartments. With suitably designed controls, maximal rate perfusion techniques could have wide application in the determination of rate-limiting steps in a variety of transport processes in vivo. Application of the present procedures to the investigation of hepatic processes during trioctanoin absorption will be described in a subsequent paper.[4]

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