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Studies on the Positional Integrity of Glyceride Fatty Acids During Digestion and Absorption in Rats

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1. Rats previously starved for 24hr. were separately given by intraduodenal injections 0·5ml. of a dispersion containing 10mg. of sodium taurocholate, with 50mg. of glycerol 1,3-dioleate 2[1-^{14}C]-palmitate, glycerol 1,2-dioleate 3[1-^{14}C]-palmitate, a mixture of [1-^{14}C]palmitic acid and triolein, or a mixture of [1-^{14}C]-palmitic acid and oleic acid. 2. At the end of 30min., the net amounts, and the radioactivity, of the neutral-lipid components recovered from the intestinal lumen and mucosa, and the position of the labelled palmitic acid in the mucosal triglycerides, were determined. 3. When glycerol 1,3-dioleate 2[1-^{14}C]-palmitate was administered, most of the labelled acid was retained in the di- and monoglycerides of the lumen; the triglycerides were the major components containing the radioactivity in the mucosa and 75–80% of the labelled acid was located at the β-position of these triglycerides. 4. When glycerol 1,2-dioleate 3[1-^{14}C]-palmitate was administered, the labelled acid was readily split off in the lumen and virtually no radioactivity could be traced in the monoglyceride fraction; in the intestinal mucosa, triglycerides were again the chief components containing most of the radioactivity, and 80–85% of the labelled acid was esterified at the outer positions of the glycerol. 5. When [1-^{14}C]palmitic acid mixed with triolein was administered, the concentrations of free fatty acids increased markedly in the intestinal lumen and mucosa, and 80–88% of the radioactivity of the mucosal triglycerides was located at the outer positions of the glycerol. 6. When [1-^{14}C]palmitic acid mixed with oleic acid was administered, the labelled acid accumulated in the lumen as well as in the cell, and it was randomly incorporated into all three positions of the mucosal triglycerides.

Although intestinal absorption of triglyceride has been extensively studied for a long time, only recently has information obtained from various directions led to a clearer understanding of the whole process (Senior, 1964). Pancreatic lipase can efficiently hydrolyse the primary ester bonds in triglyceride, so that the major products of lipolysis of dietary triglyceride in the lumen of the small intestine are 2-monoglyceride and FFA* (Mattson, Benedict, Martin & Beck, 1952; Borgström, 1953, 1954; Mattson & Beck, 1956; Savary & Desnuelle, 1956). This was further extended by the work of Hofmann & Borgström (1962), who showed that, after a meal containing fat, monoglycerides, FFA and bile salts form water-clear micellar solutions in the human small intestine. It is now generally believed that from such a micellar solution the monoglycerides and FFA enter the mucosal cell by a process of molecular diffusion (Cardell, Badenhausen & Porter, 1967), and evidence for the direct acylation of monoglyceride to triglyceride by the mucosal enzymes has been produced from several laboratories (Clark & Hübscher, 1961; Senior & Isselbacher, 1962; Brown & Johnston, 1964*a*,*b*; Ailhaud, Samuel & Desnuelle, 1963).

With regard to the quantitative aspects of lipolysis of dietary triglycerides in the intestinal lumen during absorption, Reiser, Bryson, Carr & Kuiken (1952) calculated from their results that 25–45% of the ingested triglyceride was completely hydrolysed, the remaining 55–75% being absorbed as monoglyceride to be reconverted into triglyceride in the cell. Borgström, Tryding & Westöö (1957) similarly suggested that 60% of the triglyceride was partially hydrolysed, the remaining 40% being completely hydrolysed. Later work by Savary, Constantin & Desnuelle (1961) and by Mattson & Volpenhein (1962*a*), who had observed that the positional distribution of the fatty acids in the

* Abbreviation: FFA, free fatty acids.

triglycerides in lymph of rats showed a striking resemblance to that of the dietary triglycerides, indicated that only some of the β-ester bonds of the triglycerides are hydrolysed during digestion and absorption. More precise results were obtained by Mattson & Volpenhein (1964), who showed that approx. 75% of the glycerol of the dietary triolein is absorbed as monoglyceride and is mostly reconverted as such into triglyceride. In these experiments, rats with lymph cannulae were fed with different glycerides containing label in the glycerol and in the fatty acids occupying specific positions, after which the lymph was collected over a period of 48hr., for analysis of the labelled triglycerides.

This work of Mattson & Volpenhein (1964) gave valuable information on the absorption of fats, but it was only overall information on the whole process of absorption, because no direct and separate results on the events taking place during the two phases of absorption, i.e. the digestive and the cellular phases, were provided. Attempts were made in the present work to obtain simultaneous and quantitative results on these two phases of absorption, and it is shown here that about 80% of the dietary triglyceride is absorbed as 2-monoglyceride, which is converted intact into triglyceride in the cell.

MATERIALS AND METHODS

[1-^{14}C]Palmitic acid was obtained from the Bhabha Atomic Research Centre, Trombay, India. When examined by t.l.c. (described below), it gave a single radioactive spot, corresponding to that of palmitic acid. Oleic acid (E. Merck A.-G., Darmstadt, Germany) was found to be pure by a similar t.l.c. procedure.

Synthesis of the glycerides. (*a*) Glycerol 1,3-dioleate 2[1-^{14}C]-palmitate. 1,3-Benzylideneglycerol and fatty acyl chlorides were prepared by the procedure of Mattson & Volpenhein (1962*b*). The benzylideneglycerol (2m-moles) was treated with 1m-mole of [1-^{14}C]palmitoyl chloride (30μc) in the presence of pyridine, as recommended by Mattson & Volpenhein (1962*b*). The 1,3-benzylidene-2[1-^{14}C]-palmitoylglycerol thus obtained was applied to a neutral alumina column and eluted with 2% (v/v) acetone in light petroleum (b.p. 60–80°). This step ensured complete removal of any contaminating free [1-^{14}C]palmitic acid. The pure compound thus obtained was subjected to catalytic hydrogenation by the procedure of Stimmel & King (1934), after which the 2-monopalmitin formed was crystallized from *n*-hexane. It moved as a single radioactive spot on t.l.c. plates (described below) and was completely free from acylbenzylidineglycerol. The crystallized 2-monopalmitin was acylated with excess of oleoyl chloride in the presence of pyridine. The triglyceride thus formed was finally purified by chromatography on an alumina column and elution with 4% (v/v) acetone in light petroleum (b.p. 60–80°). Its specific radioactivity was 858c.p.m./μmole of palmitic acid.

(*b*) Glycerol 1,2-dioleate 3[1-^{14}C]-palmitate. Racemic 1,2-diolein was synthesized by the method of Krabisch & Borgström (1965). The diolein (2m-moles) was treated with 1m-mole of [1-^{14}C]palmitoyl chloride (30μc) in the presence of pyridine, as described above. The triglyceride obtained was purified by chromatography on an alumina column as described above. The specific radioactivity was 960c.p.m./μmole of palmitic acid.

The two triglycerides gave single radioactive spots on t.l.c. (described below). When the positions of the labelled fatty acid in the synthetic triglycerides were examined by the method of Mattson & Volpenhein (1961), 99% of the labelled fatty acid was found to occupy the expected position in both triglycerides.

(*c*) Triolein. This was synthesized by the procedure of Mattson & Volpenhein (1962*b*) by treating glycerol with oleoyl chloride in the presence of pyridine.

Administration of the lipids to rats and subsequent analysis of tissue lipids. The lipids were given separately to the rats in the following combinations: (i) glycerol 1,3-dioleate 2[1-^{14}C]-palmitate, (ii) glycerol 1,2-dioleate 3[1-^{14}C]-palmitate, (iii) triolein mixed with [1-^{14}C]palmitic acid (specific radioactivity 960c.p.m./μmole of palmitic acid), and (iv) oleic acid mixed with [1-^{14}C]palmitic acid (specific radioactivity 960c.p.m./μmole of palmitic acid). These combinations were taken separately in a 2% (w/v) sodium taurocholate solution (pH7·5–8·0) and were dispersed by repeatedly forcing the solution through a syringe. The dispersions were prepared immediately before use, and in such a way that 1ml. contained 100mg. of the lipids. In all cases the molar ratio of palmitic acid to oleic acid was maintained at 1:2.

The general procedure followed in this laboratory to study the intestinal absorption of various lipids has been to offer the test material mixed in a small amount of diet to rats that were previously starved for 24hr. This procedure was considered unsatisfactory in the present experiments, because only small quantities of the labelled material were available and also because there were wide variations in the rate of emptying of the stomach under these conditions. An alternative procedure suitable for this purpose consisted of intraduodenal injection of 0·5ml. of a dispersion containing 50mg. of the test lipid and 10mg. of sodium taurocholate into rats previously starved for 24hr. In preliminary experiments where a triolein dispersion was injected, it was found that at the end of 30min. 30% of the fat could be recovered in the intestine contents, while at the same time the amount of mucosal triglyceride showed a marked increase. Therefore in all the experiments reported here 30min. was chosen as the optimum time of absorption.

As a routine, normal albino rats of the strain of this Institute, weighing 120–130g. and starved for 24hr., were given 0·5ml. of a dispersion containing 50mg. of the test material by intraduodenal injection, while under light ether anaesthesia, and the incision made was closed with suture clips. At 30min. after the injection they were killed by heart puncture under light ether anaesthesia and the whole small intestine was immediately removed into beakers maintained at 0°. The intestinal contents were speedily washed out with 6 × 10ml. of ice-cold 0·9% (w/v) NaCl, after which the mucosa was scraped off with the blunt end of a scalpel.

The extraction of the lipids and their separation into individual components have been described in detail by Raghavan & Ganguly (1967). Briefly, they were as follows: after extraction with chloroform–methanol (2:1, v/v), the

total lipids were applied to silicic acid (Mallinckrodt, 100–200 mesh) columns and the neutral lipids were eluted with 200ml. of diethyl ether. The eluted neutral lipids were then resolved into monoglycerides, 1,2-diglycerides, 1,3-diglycerides, FFA and triglycerides on t.l.c. plates made of silica gel (National Chemical Laboratory, Poona, India)–Plaster of Paris, 36:4 (w/w), with the solvent system *n*-hexane–diethyl ether–acetic acid (30:6:0·5, by vol.). Subsequent identification, elution and determination of the lipid components were as described by Raghavan & Ganguly (1967). The counting of the radioactivity was as described by Raghavan, Juneja, Murthy & Ganguly (1965); the efficiency of counting was 5%.

Positional analysis of the labelled fatty acid in the mucosal triglycerides. The mucosal triglycerides were isolated in the purified state by preparative t.l.c. with the solvent system described above, and the position occupied by the labelled fatty acid was determined by the action of pancreatic lipase. To the purified triglycerides isolated from each rat were added 10mg. of an acetone-dried powder prepared from goat pancreas, 1ml. of 1M-tris–HCl buffer, pH 8·0, 0·1ml. of 22% (w/v) $CaCl_2$ solution and 0·25ml. of 0·1% (w/v) sodium taurocholate solution. The incubations were carried out at 40° for 15min., after which the reactions were stopped by the addition of 0·5ml. of 6M-HCl. The digestion mixtures were extracted with diethyl ether, and the lipids were separated into individual components by t.l.c. as described above, after which the amounts of the lipid components and the radioactivity of the fractions were determined. The amount of the labelled palmitic acid present at the β-position of the triglyceride was calculated by the method of Mattson & Volpenhein (1961).

RESULTS

Fate of glycerol 1,3-*dioleate* 2[1-^{14}C]-*palmitate injected intraduodenally into the rat* (*Expt.* 1). Table 1 shows that the injected labelled triglyceride was partially hydrolysed in the lumen of the small intestine to diglyceride, monoglyceride and FFA. It was calculated that, of the total radioactivity recovered in the three components, diglyceride, monoglyceride and FFA, 51% was present in the diglyceride fraction, 35% in the monoglyceride fraction and 14% in the FFA fraction. Thus the fatty acids esterified at the primary hydroxyl groups are removed by hydrolysis stepwise, giving rise to 1,2-diglyceride and 2-monoglyceride, the labelled fatty acid at the position 2 being removed only to a limited extent. This point was further substantiated when the molar percentage of the label in each of the three components was examined, because Table 1 shows that, although almost all the mono- and di-glycerides were found to be labelled, only 9% of the total FFA was labelled.

The triglycerides were the main components of the neutral lipids of the intestinal mucosa, and most of the radioactivity of the mucosa was also recovered in this fraction. Although small amounts of labelled mono- and di-glycerides were found in the cell, no appreciable amounts of the labelled fatty acid were present there in the unesterified state.

Fate of glycerol 1,2-*dioleate* 3[1-^{14}C]-*palmitate* (*Expt.* 2). Table 2 shows that, in contrast with the results obtained in Expt. 1, no radioactivity could be detected in the monoglyceride fraction, although considerable amounts of the label were present in the diglyceride and FFA fractions of the lipids isolated from the intestinal lumen. These results therefore confirmed the conclusions drawn in the previous experiment that in the intestinal lumen

Table 1. *Appearance of* [1-^{14}C]*palmitic acid in the FFA and glycerides of the intestine of rats at* 30*min. after an intraduodenal injection of* 0·5*ml. of a dispersion containing* 10*mg. of sodium taurocholate and* 50*mg. of glycerol* 1,3-*dioleate* 2[1-^{14}C]-*palmitate* (*specific radioactivity* 858*c.p.m./μmole of palmitic acid*)

The treatment of the animals was as described in the Materials and Methods section. The amount of the labelled species in each of the lipid components was calculated by dividing the radioactivity (c.p.m.) of the component by the specific radioactivity (c.p.m./μmole of the fatty acid) of palmitic acid in the administered material. The values are averages of three separate experiments and were corrected to the nearest decimal place mentioned. The range for each value is given in parenthesis. Abbreviations: MG, monoglyceride; DG, diglyceride; TG, triglyceride.

	Intestine contents				Mucosa			
Lipid component	Total amount (μmoles)	Radioactivity (c.p.m.)	Amount of labelled component (μmoles)	Molar percentage of labelled component	Total amount (μmoles)	Radioactivity (c.p.m.)	Amount of labelled component (μmoles)	Molar percentage of labelled component
MG	3·9 (3·3–4·8)	2547 (2140–3100)	3·0 (2·5–3·6)	77·0 (72–84)	4·2 (3·6–4·9)	950 (850–1000)	1·1 (1·0–1·2)	27·0 (24–33)
DG	4·4 (4·0–5·0)	3803 (3430–4200)	4·4 (4·0–4·9)	100·0 (98–102)	5·4 (5·0–5·7)	657 (500–800)	0·8 (0·6–0·9)	14·4 (10·8–18·0)
FFA	13·0 (10·0–16·0)	1033 (700–1400)	1·2 (0·8–1·6)	9·0 (7·5–11·3)	11·0 (10·0–12·0)	200 (0–400)	0·2 (0·0–0·5)	2·0 (0·0–4·0)
TG	6·2 (5·5–6·5)	4697 (4290–5000)	5·5 (5·0–5·8)	87·0 (85·8–89·1)	20·0 (16·0–25·0)	10400 (8100–13500)	12·1 (9·4–15·7)	60·0 (51·0–69·0)

the primary ester bonds are readily hydrolysed by the pancreatic lipase. Table 2 also shows that, of the two possible diglycerides, glycerol 2-oleate 3[1-^{14}C]-palmitate and glycerol 1,2-dioleate, the former species constituted 36% of the total diglycerides. Palmitic acid was 38% of the total FFA.

The triglycerides were again found to be the major constituents of the neutral lipids of the intestinal mucosa, and also contained most of the radioactivity recovered from the cell. The rest of the radioactivity was present in the diglyceride and FFA fractions, with none in the monoglyceride fraction. The respective molar percentages of the labelled diglyceride and FFA were 8% and 7·7%, and the labelled triglyceride constituted almost half of the total triglycerides. Clearly, therefore, the resynthesis of triglyceride in the intestinal mucosa is a very rapid process.

Fate of [1-^{14}C]*palmitic acid injected with triolein* (*Expt.* 3). When a mixture of [1-^{14}C]palmitic acid and triolein was injected, large amounts of FFA accumulated in the intestinal contents, and, as expected, most of the radioactivity was recovered from the FFA fraction (Table 3). At the end of 30 min. the molar percentage of the labelled palmitic acid was only 23% of the total FFA in the lumen. However, smaller amounts, i.e. 13–14% of the total labelled fatty acid present in the lumen, were esterified in the form of mono-, di- and tri-glyceride, presumably by a lipase-catalysed exchange reaction.

Table 2. *Appearance of* [1-^{14}C]*palmitic acid in the FFA and glycerides of the intestine of rats at* 30 *min. after an intraduodenal injection of* 0·5 *ml. of a dispersion containing* 10 *mg. of sodium taurocholate and* 50 *mg. of glycerol* 1,2-*dioleate* 3[1-^{14}C]-*palmitate* (*specific radioactivity* 960 *c.p.m./μmole of palmitic acid*)

Treatment of animals, calculation of results and abbreviations were as given for Table 1.

	Intestine contents				Mucosa			
Lipid component	Total amount (μmoles)	Radioactivity (c.p.m.)	Amount of labelled component (μmoles)	Molar percentage of labelled component	Total amount (μmoles)	Radioactivity (c.p.m.)	Amount of labelled component (μmoles)	Molar percentage of the labelled component
MG	3·7 (3·0–4·0)	—	—	—	3·9 (3·5–4·2)	—	—	—
DG	3·3 (3·0–4·0)	1133 (1000–1200)	1·2 (1·2–1·2)	36·0 (30–40)	3·9 (3·2–4·5)	313 (240–450)	0·33 (0·25–0·47)	8·0 (6·0–10·0)
FFA	11·0 (10·0–12·0)	4166 (3500–4800)	4·3 (3·6–5·0)	38·0 (36·0–41·7)	11·0 (8·0–12·0)	813 (400–1200)	0·85 (0·4–1·2)	7·7 (5·5–10·0)
TG	6·0 (5·0–7·0)	5347 (4400–6240)	5·6 (4·6–6·5)	92·7 (92·1–93·3)	20·0 (16·0–25·0)	8903 (8160–9750)	9·3 (8·5–10·2)	46·5 (40·5–53·1)

Table 3. *Appearance of* [1-^{14}C]*palmitic acid in the FFA and glycerides of the intestine of rats at* 30 *min. after an intraduodenal injection of* 0·5 *ml. of a dispersion containing* 10 *mg. of sodium taurocholate and* 50 *mg. of a mixture of* [1-^{14}C]*palmitic acid and triolein* (*specific radioactivity* 960 *c.p.m./μmole of palmitic acid*)

Treatment of animals, calculation of results and abbreviations were as given for Table 1.

	Intestine contents				Mucosa			
Lipid component	Total amount (μmoles)	Radioactivity (c.p.m.)	Amount of labelled component (μmoles)	Molar percentage of labelled component	Total amount (μmoles)	Radio activity (c.p.m.)	Amount of labelled component (μmoles)	Molar percentage of labelled component
MG	4·3 (4·0–4·5)	280 (240–320)	0·29 (0·25–0·30)	6·7 (5·5–8·3)	4·5 (3·9–5·0)	—	—	—
DG	4·1 (3·3–5·0)	343 (280–400)	0·36 (0·3–0·4)	8·8 (7·2–10·4)	5·2 (5·0–5·3)	366 (300–400)	0·38 (0·3–0·4)	7·3 (6·2–8·0)
FFA	30·0 (28·0–32·0)	6566 (6400–6900)	6·8 (6·7–7·2)	22·7 (20·8–24·0)	26·0 24·0–28·0)	2033 (1900–2200)	2·1 (2·0–2·3)	8·2 (7·0–9·5)
TG	5·2 (4·6–6·0)	433 (400–500)	0·45 (0·4–0·5)	8·7 (8·4–8·9)	17·7 (11·0–22·0)	5830 (4290–6600)	6·1 (4·5–6·9)	35·4 (31·2–40·5)

Table 4. *Appearance of* [1-^{14}C]*palmitic acid in the FFA and glycerides of the intestine of rats at* 30*min. after an intraduodenal injection of* 0·5*ml. of a dispersion containing* 10*mg. of sodium taurocholate and* 50*mg. of a mixture of* [1-^{14}C]*palmitic acid and oleic acid (specific radioactivity,* 960*c.p.m./μmole of palmitic acid)*

Treatment of animals, calculation of results and abbreviations were as given for Table 1.

	Intestine contents				Mucosa			
Lipid component	Total amount (μmoles)	Radioactivity (c.p.m.)	Amount of labelled component (μmoles)	Molar percentage of labelled component	Total amount (μmoles)	Radioactivity (c.p.m.)	Amount of labelled component (μmoles)	Molar percentage of labelled component
MG	2·5 (2·0–3·0)	233 (200–300)	0·24 (0·2–0·3)	9·6 (8·3–10·4)	3·8 (3·0–4·5)	—	—	—
DG	2·2 (1·2–3·3)	607 (400–920)	0·63 (0·4–0·9)	28·6 (26·0–34·8)	3·5 (3·1–4·0)	493 (400–600)	0·51 (0·42–0·62)	14·6 (12·4–17·8)
FFA	36·0 (30·0–42·0)	8820 (7500–9600)	9·2 (7·8–10·0)	25·6 (23·8–27·1)	26·0 (24·0–28·0)	5266 (4800–6000)	5·5 (5·0–6·2)	21·1 (18·6–24·0)
TG	3·5	776 (730–800)	0·81 (0·76–0·83)	23·1 (21·6–23·7)	14·7 (12·0–16·0)	2633 (1800–3300)	2·7 (1·9–3·4)	18·3 (15·6–21·6)

Table 5. *Relative incorporation of* [1-^{14}C]*palmitic acid into the β-position of the mucosal triglyceride in the four types of experiments described in the text and in Tables* 1–4

Values of three experiments for each type are presented separately.

	[1-^{14}C]Palmitic acid remaining in β-position (%)		
Type of compound used Run no.	I	II	III
Glycerol 1,3-dioleate 2[1-^{14}C]-palmitate (Expt. 1)	80	79	75
Glycerol 1,2-dioleate 3[1-^{14}C]-palmitate (Expt. 2)	16	15	20
[1-^{14}C]Palmitic acid + triolein (Expt. 3)	16	12	20
[1-^{14}C]Palmitic acid + unlabelled free oleic acid (Expt. 4)	38	32	35

Table 3 also shows that the net amounts of FFA were markedly higher in the intestinal mucosa, compared with the corresponding values obtained in the two previous experiments. However, although the molar percentage of the exogenous free palmitic acid was about 8% of the total FFA of the cell, 35% of the total triglyceride of the cell was labelled. This indicates that during absorption palmitic acid is rapidly incorporated into the triglyceride of the cell.

Fate of [1-^{14}C]*palmitic acid injected with free oleic acid (Expt.* 4). Table 4 shows that the administered FFA were present in the intestinal lumen mostly in the unesterified state, with small amounts of the labelled acid being incorporated into the mono-, di- and tri-glycerides. In the lumen the ratio of the labelled palmitic acid to other FFA was about 1:2·9, which was slightly lower than the original ratio of 1:2 of the injected material.

In the intestinal mucosa the amounts of the FFA increased significantly, as was observed in Expt. 3. However, whereas in Expt. 3 the bulk of the radioactivity was incorporated into the triglycerides, in this case a major portion of it remained in the unesterified stage. Also, whereas in the earlier experiments the molar percentages of the labelled triglycerides were always much higher, compared with diglycerides, monoglycerides and FFA, in the present experiment they were close to one another, thereby indicating poor esterification of the labelled fatty acid to give triglyceride.

Positional incorporation of the labelled palmitic acid into the triglyceride of the intestinal mucosa. Table 5 shows that, when the labelled fatty acid was introduced in the β-position of the administered triglyceride, 75–80% of the total radioactivity of the mucosal triglyceride still occupied the same position. In contrast, when the labelled acid was in the α-position of the injected triglyceride, or when it was given as free acid mixed with triolein, about 20% of the total radioactivity of the mucosal triglyceride was located in the β-position. However, when only the FFA were injected (without the exogenous glycerol backbone; Expt. 4), the labelled

palmitic acid was randomly incorporated into all three positions of the triglyceride of the mucosal cell.

DISCUSSION

As discussed above, our present knowledge about the absorption of triglycerides in the living animal has been based mainly on the analysis of the triglycerides of the lymph of rats collected over a long period after oral administration of triglyceride of known structure to rats with lymph cannulae. Such experiments yielded only information about the overall process, and did not produce direct evidence about the events taking place during the digestive and cellular phases of absorption; attempts were made in the present work to obtain such information.

It was shown above that, when glycerol 1,3-dioleate 2[1-^{14}C]-palmitate was administered, almost all the di- and mono-glycerides isolated from the lumen of the small intestine retained the label, which is in conformity with the currently accepted mechanism of action of pancreatic lipase, i.e. stepwise hydrolysis of the triglyceride to 1,2-diglyceride and then to 2-monoglyceride. The present work also showed that the β-ester bond of the triglyceride is quite resistant to lipase action in the intestinal lumen: only 9% of the FFA of the lumen was labelled. It was calculated on the basis of the labelled FFA and mono- and di-glycerides isolated from the lumen that, at 30min., of all the glycerides hydrolysed only 14% was completely hydrolysed. The α-ester bonds of the triglycerides are readily attacked in the lumen, as shown in the experiments where the α-position of the administered triglyceride was labelled. In these experiments practically no radioactivity could be located in the monoglyceride fraction isolated from the lumen, whereas considerable radioactivity was found in the FFA fraction.

When glycerol 1,3-dioleate 2[1-^{14}C]-palmitate was administered, at 30min. triglyceride was the main component of the neutral lipids isolated from the mucosal cell, and 27% of the monoglyceride was monopalmitin. There was little radioactivity in the FFA fraction of the cell and most of the labelled fatty acid of the mucosal triglyceride remained esterified at the β-position. Thus the β-monopalmitin formed in the lumen was absorbed by the mucosa and rapidly converted into triglyceride in the cell, without any appreciable hydrolysis. The results of Expt. 2 can similarly be interpreted to imply the formation of unlabelled β-mono-olein in the lumen and its absorption and rapid conversion into triglyceride inside the cell. Skipski, Morehouse & Deuel (1959) demonstrated that 2-monoglyceride is absorbed by rats intact, and, by an approach similar to ours, Paris & Clement (1968) independently concluded that 2-monoglyceride is absorbed into rat intestinal mucosa and readily acylated intact to triglyceride. The results of Expt. 2 further show that, although the labelled palmitic acid was absorbed as the free acid, at the given time it constituted only about 8% of the FFA of the cell, whereas 46% of the triglyceride was labelled. It therefore appears that the exogenous FFA are more readily available for triglyceride synthesis in the mucosa, whereas the endogenous FFA are probably present in some other compartments of the cell.

During digestion the fatty acids at the α-positions of the dietary triglycerides are split off and during re-esterification in the cell these positions are again occupied by the absorbed fatty acids, as further demonstrated in Expt. 3. In these experiments, where free labelled palmitic acid was given with unlabelled triolein, the triglyceride isolated from the mucosal cells contained the labelled fatty acid in the α-positions. However, it is known that the monoglyceride pathway is not the only pathway for triglyceride synthesis in the intestinal mucosa; the L-3-glycerophosphate pathway has also been shown to operate in these cells, and the results of Expt. 4 demonstrate the existence of such a pathway. Thus in these experiments, where only FFA were administered (without any supply of exogenous 2-monoglyceride), the synthesis of triglyceride in the cell had to depend on the limited availability of endogenous glycerol precursors, and this was shown by the accumulation of [1-^{14}C]palmitic acid, as FFA, in the cell, with only poor conversion into triglyceride. Even more compelling evidence was the fact that the labelled acid was randomly incorporated into all the three positions of glycerol, in contrast with the positions it occupied in Expts. 1, 2 and 3, which is only possible if the intracellular re-esterification takes place through the L-3-glycerophosphate pathway.

In Expts. 1 and 2, where only triglycerides were administered, there was no significant change in the amounts of FFA in the intestinal mucosa, whereas increasing amounts of triglycerides were found there. However, in Expt. 3, where free palmitic acid was added to the triolein, larger amounts of FFA appeared in the lumen as well as in the cell; this accumulation of FFA in the cell was obviously due to the availability of inadequate amounts of 2-monoglyceride for re-esterification. These FFA of the lumen were mainly unlabelled fatty acids, and this can readily be explained, because palmitic acid was administered in the unesterified state, so that it was rapidly absorbed, whereas the absorption of oleic acid had to await prior hydrolysis of the triolein. In contrast, in Expt. 4, where only FFA were given, there was a large accumulation inside the cell of FFA, including palmitic

acid. Here the accumulation of FFA was obviously due to the scarcity of the glycerol acceptor, which had to come from endogenous sources. Therefore it appears that the 2-monoglyceride pathway is the natural pathway, more efficient than the L-3-glycerophosphate pathway for the resynthesis of triglycerides in the mucosal cell, during absorption of triglyceride.

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