Intestinal Absorption of C^{14}-Palmitic Acid and C^{14}-Tripalmitin in the Rat

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DIGESTION of fats results in a mixture of fatty acids, mono-, di- and triglycerides, in the intestinal contents. However, the predominant lipid in intestinal or thoracic duct lymph is triglyceride although small amounts of free fatty acids and of phospholipids are also present. Numerous studies have been concerned with the contents of the small intestine during digestion and with the lipids found in the intestinal or thoracic duct lymph during absorption. Study of the composition of lipids in intestinal tissue during digestion and absorption has been confined largely to phospholipids and total glycerides (as the neutral fat fraction) without regard to the fractionation of the glycerides. Recently, Johnston[1] observed that *in vitro* preparations of intestine transferred C^{14}-palmitic acid from the mucosal to the serosal side. Although most of the activity found in the neutral fat fraction resided in triglycerides, a significant amount of activity was also present in the diglycerides. Skipski, Morehouse and Deuel[2] studied the absorption of a 1,3-dioleyl-2-deuteriostearyl glyceride-C^{14} and a 1-monodeuteriostearyl glyceride-C^{14} in the rat and identified in the intestinal neutral fat component a monoglyceride with an isotopic ratio very nearly equal to that of the glycerides fed.

In an effort to relate the activity of the lipids of the intestine to the composition of the lipid being absorbed we have investigated the distribution of C^{14} in the lipid fractions of the intestine and of intestinal contents at various intervals after feeding C^{14}-palmitic acid or C^{14}-tripalmitin. In some of the experiments the lipids of the liver also were fractionated and the distribution of C^{14}-fatty acids in lipid fractions was determined.

MATERIALS AND METHODS

A dose of 100 mg. of carboxyl-labeled palmitic acid or tripalmitin in 1 ml. of olive oil was administered by stomach tube to fed adult male Sprague-Dawley rats. The animals were killed by decapitation three, six or twenty-four hours later. At least two and in some cases four animals were pooled in each experiment. The contents of the small intestine were washed out carefully but thoroughly with water, after which the intestine was slit longitudinally and cut into small pieces for extraction. The lipids were extracted from intestinal samples by use of alcohol and of diethyl ether and from intestinal contents by means of diethyl ether after acidification with dilute hydrochloric acid. Purification of the lipid extracts was accomplished by extraction with petroleum ether of the residue obtained after evaporation of the original extracts to dryness under nitrogen. Livers were extracted initially with alcohol, secondly with alcohol-ether, and finally with ethyl ether in a Soxhlet apparatus for twenty-four hours. Purification of the combined alcohol-ether extracts was accomplished by evaporation to dryness under nitrogen and subsequent exhaustive extraction of the residue with boiling petroleum ether.

Phospholipids were estimated by determination of phosphorus[3] and fatty acids by titration after hydrolysis and extraction. In order to isolate fatty acids for radioactivity

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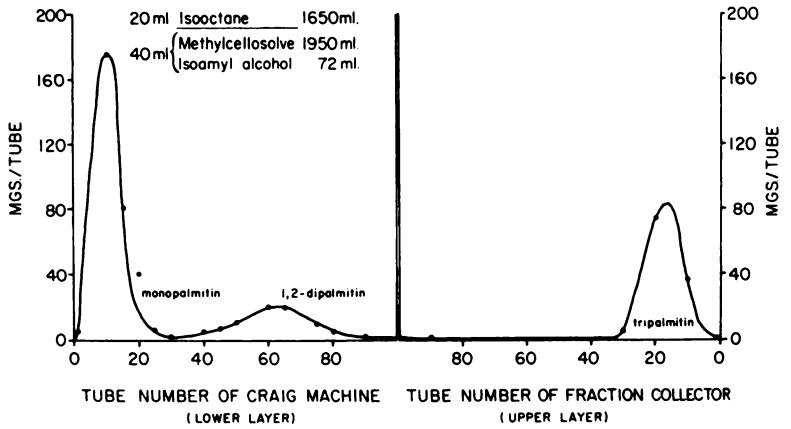

FIG. 1. Countercurrent distribution of a synthetic mixture of 1-monopalmitin, 1,2-dipalmitin and tripalmitin. After 100 transfers had been applied the 100 upper layers were collected in a fraction collector.

determinations various classes of lipids were separated as follows:

Free fatty acids were isolated by alkaline extraction and phospholipids by precipitation with acetone. The glycerides remaining in the extract were separated from each other by countercurrent distribution in an automatic 100-tube Craig apparatus. The solvent systems used were either heptane:methyl cellosolve-isoamyl alcohol[4] or isooctane:methyl cellosolve-isoamyl alcohol. After 100 transfers had been applied the upper layers were collected in a fraction collector. The relative amount of material in upper and lower layers was established by analyses of weight, of radioactivity or of both. When weight analysis was used for location of fractions, correction for free cholesterol was necessary in tubes 30 to 60. The triglyceride peak was found to contain also the esterified cholesterol fraction. Therefore, when liver samples were analyzed the triglyceride peak was fractionated further by use of a silicic acid column according to the method of Fillerup and Mead.[5] The contents of tubes containing a particular fraction were pooled and, after extraction, were hydrolyzed with potassium hydroxide. After acidification the resulting fatty acids were extracted, titrated, and determinations of radioactivity made on barium carbonate obtained by wet combustion of samples and precipitation of carbon dioxide. Counting was done in a windowless counter and corrections were applied for self-absorption. Later samples were counted as fatty acids in a Packard Tri-Carb Liquid Scintillation Spectrometer using diphenyloxazole as primary phosphor, P-bis [2-(5-phenyloxazolyl)]-benzene (POPOP) as secondary phosphor and toluene as solvent. All samples were checked for quenching. Glycerol analyses were done by the method of Lambert and Neish.[6]

RESULTS

The countercurrent distribution patterns of known glycerides* were determined and are shown in Figure 1. Mono- and diglycerides remained in the lower layer. Triglycerides, having high partition coefficients, moved with the advancing upper layer and were collected in the fraction collector, usually in tubes 0 to 35.

A typical countercurrent distribution pattern of glycerides of the small intestine is shown in Figure 2. In this particular sample the rats were killed six hours after feeding C^{14}-palmitic acid. Separations were accomplished in 200 transfers in both isooctane-methyl cellosolve and heptane-methyl cellosolve. The fractions were located by determination of radioactivity of an aliquot from a number of tubes but analyses of weight usually gave

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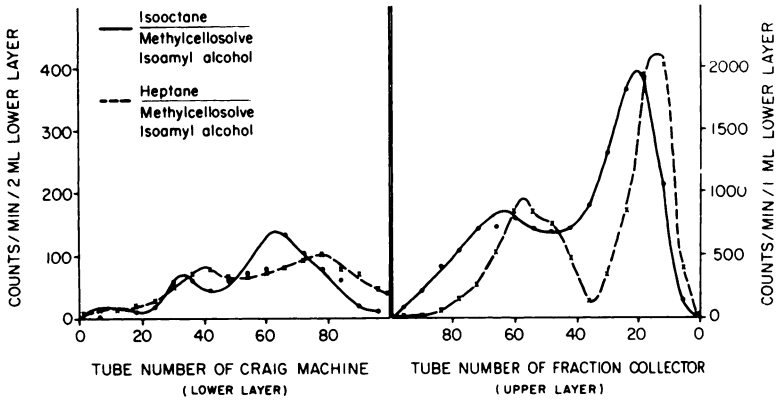

FIG. 2. Countercurrent distribution of glycerides of the small intestine six hours after C^{14}-palmitic acid administration. After 100 transfers had been applied the 100 upper layers were collected in a fraction collector. Analysis was done by radioactivity determination but analysis of weight usually gave similar patterns.

similar patterns (after corrections for free cholesterol in tubes 30 to 60 of lower layer). In these solvent systems cholesterol esters travel in the triglyceride fraction. However, no significant amount of esterified cholesterol was observed in this fraction either from samples of intestine or of intestinal contents. The glycerides of this six-hour intestinal extract were divided into the following fractions: (1) monoglycerides—very small amount of material between tubes 0 and 20 to 25 of lower layer with a fatty acid-glycerol ratio of about 1; (2) diglycerides in tubes 30 to 95 (lower layer) and having a fatty acid-glycerol ratio of about 2; (3) triglycerides found in tubes 0 to 35 of the upper layers with fatty acid-glycerol ratio of approximately 3; and (4) material contained in tubes 40 to 90 (fraction "X") of the upper layer with variable fatty acid-glycerol ratios. Infrared analysis of this material has shown the presence of OH groups of diglycerides but fatty acid-glycerol ratios have varied usually between two and three. Phosphorus has not been detected chemically. This fraction has also been obtained when glycerides from liver lipids have been separated by countercurrent distribution. Only one peak has been obtained in countercurrent extractions of this lipid with as many as 800 transfers applied. However, because of the variable fatty acid-glycerol ratios this fraction may be a mixture of diglycerides and triglycerides. Further work to elucidate its exact composition is now in progress.

TABLE I

Total C^{14} Activity of Lipid Fractions of Intestinal Contents and of Intestine After Feeding C^{14}-Tripalmitin

Lipid Fractions	Intestinal Contents*			Intestine†		
	3 hr.	6 hr.	24 hr.	3 hr.	6 hr.	24 hr.
Free fatty acids	41	62	0	9	16	23
Phospholipid	0	0	0	12	15	16
Monoglyceride	9	2	8	1	0	<1
Diglyceride	9	10	0	4	6	4
"X"	8	5	86	11	10	32
Triglyceride	33	21	6	63	53	25

* Total activity expressed as per cent of total counts in lipids of intestinal contents.

† Total activity expressed as per cent of total counts in lipids of intestine.

The total C^{14} activity present in various lipid classes of intestine and of intestinal contents at three, six and twenty-four hours after feeding C^{14}-tripalmitin is shown in Table I. The results are expressed as per cent of total counts present in intestine and intestinal contents, respectively. Extensive hydrolysis occurred as evidenced by the high activity present at three and six hours in the free fatty acid fraction of intestinal contents. The balance of the activity was largely in tri-

glycerides. At twenty-four hours almost all the activity was present in the unidentified fraction designated "X." In the intestine itself C^{14}-activity accumulated rapidly in triglycerides. Activity was found in free fatty acids even at twenty-four hours but there was almost no C^{14}-activity present as monoglyceride. Although the intestinal contents of these rats did not contain phospholipid, a significant amount of the C^{14}-fatty acids in the intestinal tissue was found associated with phospholipids at all time periods studied.

When C^{14}-palmitic acid was fed instead of tripalmitin, the total activity in the intestinal contents was predominantly in the free fatty acids at both three and six hours after feeding (Table II). However, a considerable amount of C^{14} was found in the triglyceride fraction at these times. In the intestine, initially, the activity was associated largely with triglycerides but at six and twenty-four hours this fraction contained decreasing amounts of C^{14}. Phospholipid fatty acids showed a progressive increase in C^{14} activity from three to twenty-four hours.

The total C^{14} activities of the fatty acids of hepatic lipids of rats killed three hours after feeding C^{14}-palmitic acid or C^{14}-tripalmitin are summarized in Table III. Similar distribution patterns were obtained with the two labeled substrates. Most of the activity was found in the phospholipid fraction with the triglyceride containing almost all of the balance. Diglycerides had about 2 per cent of the total activity and monoglycerides only 0.3 to 0.5 per cent of the total. Fraction "X" also was present in the liver lipids and contained about 4 per cent of the labeled fatty acids. Only 0.7 to 1.5 per cent of the total activity of liver lipids was due to labeled fatty acids esterified with cholesterol.

Although the total activity of liver lipids was predominantly in the phospholipid fatty acids the specific activity of the triglyceride fatty acids was greater than that of the phospholipid fatty acids in both experiments.

COMMENTS

Countercurrent distribution technic has been shown to be an effective method for the separation of mono-, di- and triglycerides.[4] In the present study this technic has been combined with other more common chemical separations to fractionate lipids of intestine, intestinal contents and liver during and following the period of absorption. In addition to the three general groups of glycerides (mono-, di- and triglycerides) one other well defined peak was obtained in all samples subjected to countercurrent distribution This lipid group migrated at a slightly slower rate than the

TABLE II

Total C^{14} Activity of Lipid Fractions of Intestinal Contents and of Intestine After Feeding C^{14}-Palmitic Acid

Lipid Fractions	Intestinal Contents*		Intestine†		
	3 hr.	6 hr.	3 hr.	6 hr.	24 hr.
Free fatty acids	73	88	8	17	4
Phospholipid	0	0	17	36	74
Monoglyceride	<1	1	<1	<1	—
Diglyceride	7	1	4	2	3
"X"	1	4	9	24	4
Triglyceride	18	6	62	21	15

* Total activity expressed as per cent of total counts in lipids of intestinal contents. Not enough material was present in twenty-four-hour samples to permit lipid fractionation.

† Total activity expressed as per cent of total counts in lipids of intestine.

TABLE III

Radioactivity of Fatty Acids Derived from Liver Lipid Fractions of Rats Fed C^{14}-Palmitic Acid or C^{14}-Tripalmitin Three Hours Prior to Sacrifice

Derivation of Fatty Acids	Total C^{14} Activity (% of total activity in total fatty acids of liver)	
	Rats Fed C^{14}-Palmitic Acid	Rats Fed C^{14}-Tripalmitin
Phospholipids	57	50
Monoglycerides	0.3	0.5
Diglycerides	2	2
Triglycerides	37	42
Cholesterol esters	0.7	1.5
Fraction "X"	3	4

triglycerides and faster than the diglycerides. Because of the presence of the OH radical shown by infrared analysis and because fatty acid-glycerol ratios usually were in the region of 2:1 to 3:1 it is assumed that this fraction is a mixture of di- and triglycerides. In some samples the specific activity of the fatty acids obtained by hydrolysis of this material was similar to that of the free fatty acids, but alkaline extraction did not remove the activity. Because ethyl esters of fatty acids have a partition coefficient similar to the material in this fraction the possibility that fatty acids might esterify with ethyl alcohol during the process of extraction was tested. Non-radioactive intestines were extracted by the usual procedure in the presence of C^{14}-ethyl alcohol. The glycerides were then fractionated and fraction "X" tested for C^{14} activity. A negligible count was obtained. Experiments being conducted presently will establish whether or not fed oleic acid-1-C^{14} results in accumulation of C^{14} activity in this fraction.

The results of the C^{14}-tripalmitin feeding experiment indicate that extensive hydrolysis of triglycerides occurred in the intestine of the rat. The accumulation of C^{14}-triglycerides in the intestine three hours after feeding indicates the rapid synthesis of triglycerides from lower glycerides or fatty acids or the absorption of triglycerides as such or both. Exact interpretation of the data is complicated by the added factor of passage of the absorbed lipids into the lymph. Twenty-four hours after feeding C^{14}-tripalmitin, when very little material was found in intestinal contents, considerable C^{14} activity was still present in the intestine in several lipids, including free fatty acids, phospholipids, triglycerides, and fraction "X." When C^{14}-palmitic acid was fed, a much larger percentage of the activity accumulated in the phospholipids by the end of the twenty-four–hour period. During this time the activity in the triglycerides decreased in about the same proportion. It is apparent from these data that synthesis of phospholipids occurs to a significant extent when large amounts of free fatty acids are entering the intestine. According to the findings of Weiss and Kennedy[7] the diglycerides form a common intermediate for triglycerides and for phospholipids in liver tissue. In our experiments much more C^{14} activity was found in diglycerides than in monoglycerides of the intestine. Absorption experiments performed by Johnston[1] revealed that of the labeled glyceride fatty acids in intestinal wall 11 per cent were diglycerides. No activity was found in the monoglyceride fraction. Skipski, Morehouse and Deuel[2] found a significant amount of monoglyceride in intestinal tissue of rats fed a labeled triglyceride or a labeled monoglyceride. Their data indicated that the monoglyceride (1-monodeuteriostearyl glyceride-C^{14}) was absorbed as a unit. It is known that the intestinal lymph contains largely triglycerides with minor amounts of phospholipid and free fatty acids but does not contain mono- or diglyceride.[8–10] Data obtained in our experiments at three, six and twenty-four hours indicate that absorbed fatty acids are present in intestine at these times not only in the form of triglycerides and phospholipids but also diglycerides and to a very slight extent monoglycerides. Since mono- and diglycerides are not found in lymph, these amounts found in the intestinal wall must be converted completely into triglycerides or into phospholipids before leaving the intestine.

When C^{14}-palmitic acid was fed there was considerably more activity in the phospholipids of the intestine than when C^{14}-tripalmitin was used. At six and twenty-four hours after feeding there was more C^{14} activity in the phospholipid fatty acid fraction than in the triglyceride fatty acid fraction. These results indicate that a considerable portion of free fatty acids entering the intestinal cell can be converted to phospholipids. The specific activity of the phospholipid fatty acids in those samples was higher than that of triglyceride fatty acids. In studies of absorption of oleic and palmitic acids in man Blomstrand and Ahrens[10] found more labeled absorbed acids in neutral fats than in phospholipids in the lymph. The possibility exists that even though labeled absorbed fatty acids were incorporated into intestinal phospholipids in considerable quantities in our studies, transfer to lymph was slow in comparison to transfer of triglycerides.

The finding of extensive labeling of triglyc-

eride and diglycerides in intestinal contents after feeding free C^{14}-palmitic acid indicates considerable incorporation of free palmitic acid into glyceride fatty acids within the intestinal lumen. These data confirm findings reported by Borgstrom.[11] Johnston[1] found labeled glycerides on the mucosal side of intestinal strips when incubated with C^{14}-palmitic acid accessible to mucosal side. It was suggested that this was due to sloughing of mucosal cells during incubation, but mention was also made of the possibility of absorption of fatty acids into the intestinal wall with synthesis to glycerides and secretion in part into the mucosal compartment. The exact mechanism responsible for the appearance of C^{14} in glycerides of intestinal contents in our experiments with C^{14}-palmitic acid cannot be ascertained from our data.

In the three-hour absorption experiments hepatic lipids were fractionated and C^{14} activity was found not only in the usually reported fractions such as triglycerides, phospholipids and cholesterol esters but also in monoglycerides and diglycerides. The origin of the latter two lipid classes is not known, but it is of interest that Mead and Fillerup[12] have found as much as 20 per cent of newly ingested fatty acids present in plasma as mono- and diglycerides.

SUMMARY

C^{14}-palmitic acid or C^{14}-tripalmitin has been fed to rats and distribution of the C^{14} in various lipids of intestine, of intestinal contents and of liver determined. Mono-, di- and triglyceride as well as free fatty acid fractions of intestinal contents were found to contain C^{14} activity during the period of absorption. Most of the activity, however, resided in free fatty acids and triglycerides. At this time the triglycerides of the intestine had the predominant labeling and the mono- and diglycerides had the least.

When free palmitic acid was fed much more of the activity was found in the free fatty acid fraction of intestinal contents but considerable activity was also found in diglycerides and triglycerides. Initially, triglycerides of the intestine had the predominant labeling but as absorption proceeded this activity decreased with a concomitant increase in labeling of the phospholipid fraction. Monoglycerides had insignificant C^{14} activity at all times.

Total C^{14} activity measured three hours after feeding either C^{14}-palmitic acid or C^{14}-tripalmitin was greater in phospholipids than in triglycerides of hepatic lipids. However, the specific activity of the triglyceride fatty acids was higher than that of phospholipid fatty acids. Small but significant amounts of C^{14} were also found in mono- and diglycerides as well as cholesterol esters of hepatic lipids.

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