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The transport and dynamic state of exogenous glycerol- and palmitic acid-labeled tripalmitin*

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SUMMARY

The radioactivities of the glycerol and fatty acid moieties of plasma, liver, and adipose tissue lipids were determined at intervals after the ingestion of glycerol- and palmitic acid-labeled tripalmitin by rats. The changes in the levels of activities of the glycerol and fatty acids, and in the ratios of those activities, demonstrated the following: Only about half of the glycerol is completely hydrolyzed from fatty acid during digestion and absorption. Plasma lecithin originating from dietary fat is synthesized extrahepatically. Plasma triglycerides are removed by the liver without change but are hydrolyzed before incorporation into adipose tissue. Liver lecithins originate from liver triglycerides. Triglycerides are metabolically more active than lecithins and the latter more active than cephalins. In the postabsorptive period, glycerol disappears from adipose tissue triglycerides more rapidly than do the fatty acids, indicating hydrolysis and renewal of glycerol. In the liver, on the other hand, glycerol and fatty acid disappear from both triglycerides and phospholipids at the same rates, indicating that they are removed as units.

In an earlier study from this laboratory, in which glycerol- and fatty acid-labeled triglycerides were used to investigate the mechanism of fat digestion (1), it was observed, though not reported, that after the deposition of the doubly labeled triglyceride in adipose tissue, the glycerol and fatty acid moieties disappeared at different rates. The present report is the result of an investigation on this phenomenon.

EXPERIMENTAL

Nineteen male rats, weighing approximately 200 g., were trained to eat a semisynthetic, fat-free ration[1] at 8:00 A.M. On the morning of the test, 200 mg. of tripalmitin, labeled in both the glycerol and fatty acid moieties, was fed with about 1 g. of the basal ration. The specific activity of the glycerol was 920,000 cpm. per mg. and that of the fatty acid was 125,000 cpm. per mg. The animals were subsequently fed the fat-free ration *ad libitum*.

Three animals were sacrificed by exsanguination through the aorta at 3, 6, 12, 24, and 72 hours after the test meal and four animals at 48 hours. The lipids were extracted from the plasma, the liver, and from the gross adipose tissue around the kidneys, testes, in the inguinal region, and under the skin. The plasma and liver lipids, about 30 mg. and 250 mg., respectively, isolated by the method of Folch *et al.* (2), were partitioned chromatographically on silicic acid columns (3).[2] The pooled adipose tissues from each animal were extracted with 10 volumes of chloroform in a Waring blendor and dried over silicic acid. The solvent was removed in a stream of nitrogen. The triglycerides from 100 mg. of the extracted adipose tissue lipid were isolated by column silicic acid chromatography (4).

The triglycerides of plasma, liver, and adipose tissues were saponified with 4 per cent alcoholic KOH. (In order to have samples of sufficient size for analyses, it was necessary to add unlabeled triglyceride as a carrier to the isolated and weighed plasma and liver triglycerides.) The fatty acids were extracted with petroleum ether (30°-60°C)[3] and dried. Twenty to 25 mg. samples were plated on "frosted" glass planchets,[4]

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[1] Nutritional Biochemical Co., Cleveland, Ohio.

[2] Because of the small amounts available, the plasma of all the rats in each group was pooled.

[3] Skellysolve F.

[4] PL-F Full-Blasted Planchets, B and Z Enterprises, Columbus, Ohio.

TABLE 1. THE RADIOACTIVITIES OF THE FATTY ACID MOIETIES OF TISSUE LIPIDS AFTER THE INGESTION OF GLYCEROL- AND FATTY ACID-LABELED TRIPALMITIN *

Hours	Plasma †			Liver			Adipose
	TG ‡ *cpm./mg.*	Le ‡ *cpm./mg.*	Ce ‡ *cpm./mg.*	TG ‡ *cpm./mg.*	Le ‡ *cpm./mg.*	Ce ‡ *cpm./mg.*	TG ‡ *cpm./mg.*
3				495	462	270	4
				718	827	640	11
				1348	2047	1519	12
Average	2211	1105	569	854	1112	810	9
6				3715	3088	2513	588
				1649	1821	1337	247
				4144	2454	2685	411
Average	3931	2549	801	3169	2454	2178	415
12				2849	3137	2051	261
				1600	1558	1374	121
				1818	2336	1927	217
Average	152 §	2157	158 §	2089	2344	1784	200
24				1711	2385	2500	250
				1110	1565	1796	173
				2884	3377	2434	637
Average	2122	2871	1016	1902	2442	2243	353
48				77	700	729	196
				922	1340	1328	362
				919	1250	1323	455
				345	493	567	182
Average	352	591	227	566	946	987	299
72				219	257	321	333
				159	202	241	263
				233	279	290	385
Average	146	197	232	204	246	284	327

* Palmitic acid activity 125,000 cpm./mg.; glycerol activity 920,000 cpm./mg.; 200 mg. ingested in 1 g. of feed.

† Plasma of 3 animals pooled for analyses.

‡ TG = triglycerides; Le = lecithin; Ce = cephalins.

§ Unexplained dilution by unlabeled compound.

counted in a gas-flow counter to at least 5,000 counts, corrected for background and self-absorption, and their specific activities (cpm. per mg.) calculated. The glycerol was isolated from the saponification liquor as the tribenzoate derivative (5, 6). Infinitely thin platings of the derivative were made on "frosted" glass planchets, and the time for 5,000 counts determined with a gas-flow counter.

The liver and the plasma cephalins were saponified with 4 per cent alcoholic KOH, the fatty acids isolated and plated in the same manner as the triglyceride fatty acids. The plasma cephalins were diluted with unlabeled cephalin[5] to make the sample size suitable for analysis. Their glycerol activities were calculated from those of their methylene bismethone derivatives, prepared as previously described (7) from their glycerophosphate fractions.

The plasma and liver lecithin plus sphingomyelin fractions were differentially saponified by the method

[5] Isolated by silicic acid chromatography from rat liver lipids.

of Thannhauser *et al.* (8) in 0.25 N NaOH at 37°C for 6 days. The plasma lecithins were diluted with unlabeled lecithin[5] to make the sample size suitable for analysis. After decomposition of the soaps at a mild acid pH (methyl red), the mixtures were chilled and filtered through Celite pads on cold sintered glass funnels. The aqueous solutions contained the glycerophosphate of the lecithin. The C^{14} activities of the glycerophosphate were determined in the same manner as for the glycerophosphate of the cephalin fractions. The fatty acids and sphingomyelin were removed from the Celite pads with chloroform. The chloroform extracts were dried over anhydrous Na_2SO_4 and filtered. The dried and filtered chloroform solutions were slurried with 1 g. silicic acid in order to remove the sphingomyelin. The isolated fatty acids were dried and plated in the same manner as the fatty acids of the triglycerides and cephalins.

RESULTS AND DISCUSSION

Tables 1 and 2 present the relative specific activities of the fatty acid and glycerol moieties of liver and plasma cephalin, lecithin, and triglyceride, and of adipose tissue triglycerides.

Since the test fat was fed in a single meal, preceded and followed by the ingestion of a fat-free ration, the changes in the levels of the dietary fat into tissue lipids may be divided into two phases. The first phase is that during which the exogenous fat was incorporated into tissue lipids. This may be called the "absorption phase." The second phase is that during which the exogenous fat underwent changes in the tissues. The changes during the latter phase are a measure of the dynamic state of tissue lipids, and may be designated as the "postabsorption phase."

The changes in the activities of both fatty acids and glycerol in tissue lipids with respect to time show, with a few questionable exceptions, that the maximum levels of incorporation of dietary fatty acids and glycerol occurred at the sixth hour. This represents the absorption phase. The levels of activities of both fatty acid and glycerol in plasma and liver show that in the earlier hours after doubly labeled triglyceride ingestion, the exogenous fat is incorporated to the greatest extent into the triglycerides, less in the lecithins, and least in the cephalins. After the forty-eighth hour these relationships were reversed. These data lead to the conclusion that triglycerides are more dynamic, or "turn over" more rapidly, than lecithins, and that the cephalins are the most sluggish, though by no means inert. As will be discussed in more detail below, the data also indicate that the triglycerides are the precursors of lecithins. This may, at first sight, appear to be contrary to the mechanism of the syntheses of triglycerides and phospholipids as reported by Kennedy and Weiss (9, 10). The latter work, however, was on lipids synthesized *de novo*, while the present study describes the synthesis of lipids from exogenous triglycerides.

The radioactivities of the plasma and liver lecithin fatty acids were very similar at every period, which might mislead one to suspect the liver as the sole origin of the plasma phospholipid. However, the plasma lecithin glycerol activity was higher than that of the liver, at least during the first 24 hours. This apparent contradiction may be resolved by the concept that during, or shortly after, fat absorption plasma lecithin does not arise in the liver, but is formed mainly in the mucosa from exogenous fat. The low values of the labeled glycerol to fatty acid ratio in liver lecithin as compared to liver triglycerides, show that liver lecithin, on the other hand, arises mainly from triglycerides, as has been postulated by Harper *et al.* (11) as well as Stein and Shapiro (12).

The above discussion shows the danger of drawing conclusions with respect to precursor-product relationships of complex molecules, such as phospholipids, by following only one moiety. Furthermore, the dietary state of the animal and the experimental conditions must be taken into account. The injection of labeled P^{32} or C^{14}-acetate into a fasting animal, rather than an animal absorbing fat, may well result in quite different relationships between the levels of the label in lipids of various organs. Even greater differences should be expected if labeled lipids are being absorbed. For example, in studies with parenterally administered P^{32} (13, 14) and C^{14}-palmitic acid (15) in fasted liverless dogs, and C^{14}-acetate in pigs (4), the levels of labeled lipids indicated the hepatic origin of plasma phospholipid. On the other hand, oral administration of P^{32} resulted in a higher specific activity in the intestine and blood plasma than in the liver (16).[6] More recently, Zilversmit and Bollman, using P^{32} (17), found that the feeding of cream increased the amount of plasma phospholipid and concluded that after the feeding of fats, the intestine may contribute appreciably to plasma phosphatide.

The dissimilarity between glycerol activities of the plasma and liver cephalin, and the latter's lower levels,

[6] In a review of his work (*Phosphorus Metabolism*, Baltimore, Johns Hopkins Press, 1952, vol. 2, p. 223) Artom appears to have interpreted his data somewhat differently, concluding that labeled phosphorus in the liver and intestinal phospholipid reach about the same levels.

TABLE 2. THE RADIOACTIVITIES OF THE GLYCEROL MOIETIES OF TISSUE LIPIDS AFTER THE INGESTION OF GLYCEROL- AND FATTY ACID-LABELED TRIPALMITIN *

Hours	Plasma †			Liver			Adipose
	TG ‡ *cpm./mg.*	Le ‡ *cpm./mg.*	Ce ‡ *cpm./mg.*	TG ‡ *cpm./mg.*	Le ‡ *cpm./mg.*	Ce ‡ *cpm./mg.*	TG ‡ *cpm./mg.*
3				2224	459	347	0
				3217	923	638	0
				3882	2069	924	0
Average	8641	2833	1397	3107	1150	636	0
6				5343	1592	884	340
				1537	854	489	138
				5374	2550	1637	252
Average	12,400	4925	2364	4084	1665	1003	243
12				2886	2768	1569	259
				1449	1344	655	105
				1986	1655	1092	187
Average	247 §	4397	167 §	2107	1919	1105	183
24				1671	1578	1244	187
				1365	1183	822	130
				3325	2204	1639	245
Average	2395	3393	935	2120	1655	1235	187
48				51	559	781	186
				564	766	855	175
				310	553	674	102
				161	278	319	61
Average	95	512	162	272	539	657	131
72				77	183	227	81
				170	115	137	67
				84	135	151	102
Average	281	175	175	110	144	172	83

* Palmitic acid activity 125,000 cpm./mg.; glycerol activity 920,000 cpm./mg.; 200 mg. ingested in 1 g. of feed.

† Plasma of 3 animals pooled for analyses.

‡ TG = triglycerides; Le = lecithin; Ce = cephalins.

§ Unexplained dilution by unlabeled compound.

may be explained by the hypothesis that cephalin has a slower turnover rate than lecithin. (The irregularities in the response of the "cephalin" fraction are probably due to the complex nature of this fraction.) This was also demonstrated by the fact that both the glycerol and fatty acids of liver cephalin reached their maximum levels 12 hours or more after lecithin, and dropped off more slowly. Similar conclusions were reached by a study of differences in the activities and polyunsaturated fatty acid composition of cephalin in tissue lipids of pigs injected with labeled acetate (4).

Because of possible differences in the degrees of biological dilution, comparisons of the activities of the fatty acids or glycerol in lipids or tissues are undependable for some relationships, especially those of precursors and products. This difficulty may be circumvented by consideration of the ratios of the relative activities of the glycerol moieties to those of the fatty acids, a device which has been employed previously (7). Thus if this ratio in the ingested fat is considered as unity, any changes in the ratio are an indication of hydrolysis and resynthesis, and of the

TABLE 3. RELATIVE AMOUNTS OF GLYCEROL AS COMPARED TO FATTY ACIDS REMAINING IN TISSUE LIPIDS AFTER THE INGESTION OF GLYCEROL- AND FATTY ACID-LABELED TRIPALMITIN *

Hours	Plasma †			Liver			Adipose
	TG ‡	Le ‡	Ce ‡	TG ‡	Le ‡	Ce ‡	TG ‡
3				0.61	0.13	0.17	0
				0.61	0.15	0.14	0
				0.31	0.14	0.08	0
Average	0.53	0.35	0.33	0.51	0.14	0.13	0
6				0.19	0.07	0.05	0.08
				0.13	0.06	0.05	0.08
				0.18	0.14	0.08	0.08
Average	0.43	0.26	0.40	0.17	0.09	0.06	0.08
12				0.14	0.12	0.10	0.13
				0.12	0.12	0.06	0.12
				0.15	0.10	0.08	0.12
Average	0.22	0.28	0.14	0.14	0.11	0.08	0.12
24				0.13	0.09	0.07	0.10
				0.17	0.10	0.06	0.10
				0.16	0.09	0.09	0.05
Average	0.15	0.16	0.12	0.15	0.09	0.07	0.09
48				0.09	0.11	0.15	0.13
				0.08	0.08	0.09	0.07
				0.05	0.06	0.07	0.03
				0.06	0.08	0.08	0.05
Average	0.04	0.12	0.10	0.07	0.08	0.09	0.07
72				0.05	0.10	0.10	0.03
				0.14	0.08	0.07	0.04
				0.05	0.07	0.07	0.04
Average	0.26	0.12	0.10	0.08	0.08	0.08	0.03

* To allow for a much higher loss of glycerol as compared to fatty acid, the tripalmitin fed contained glycerol with a specific activity 7.4 times higher than the fatty acid. The relative loss of glycerol with respect to fatty acid is easier to understand if the relationship between the two is considered to be unity. Therefore the values in this table are equal to the expression: $\frac{\text{cpm./mg. glycerol}}{\text{cpm./mg. fatty acid} \times 7.4}$.

† Plasma of 3 animals pooled for analyses.

‡ TG = triglycerides; Le = lecithin; Ce = cephalins.

relative rates of disappearance of the two moieties. The differences in the ratios between the lipids may be used as a measure of product-precursor relations.

These ratios are given in Table 3. The values given here are not the actual ratios of glycerol to fatty acid activities but represent the changes from the dietary triglyceride. Thus the ratio of 0.5 in the liver triglycerides at the 3-hour period means that it incorporated only half as much of the labeled dietary glycerol as of fatty acid.

Even in the early stages of absorption of the test meal (Fig. 1), half as much of the labeled dietary glycerol as of fatty acids was incorporated into plasma triglycerides. This confirms the results of an earlier study on digestion (1) in which it was shown that up to 40 per cent of ingested glycerides are completely

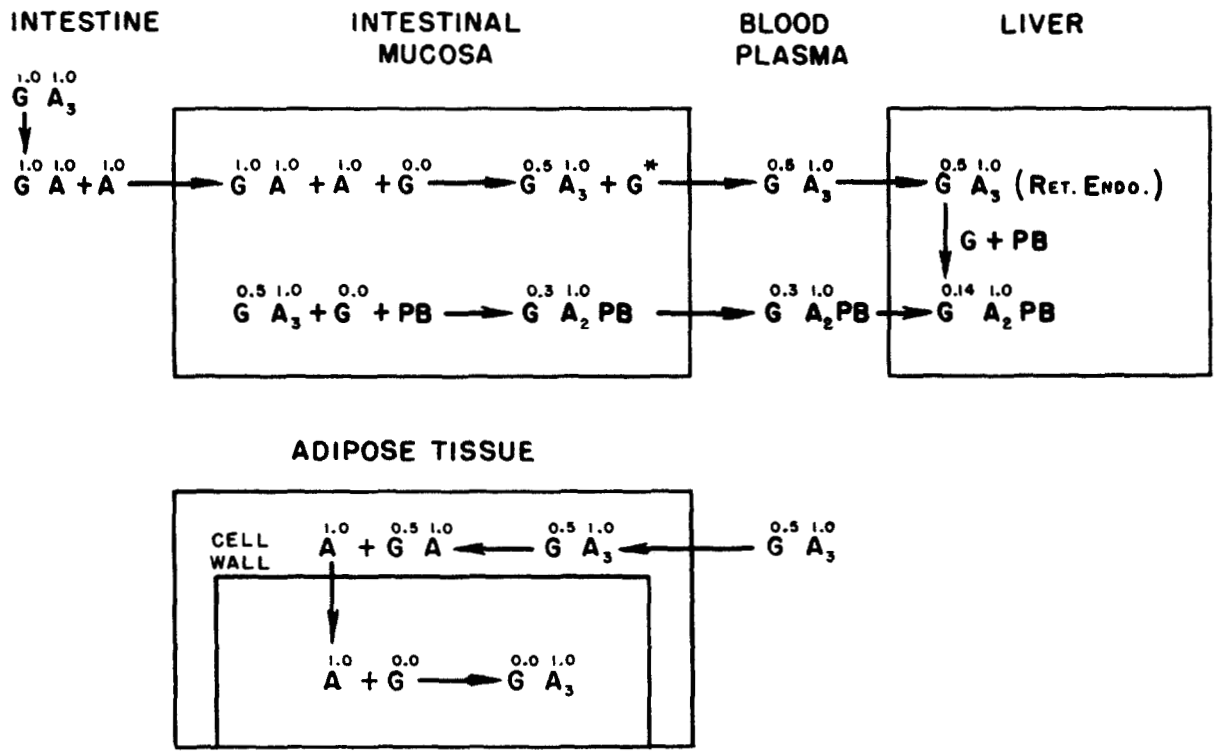


FIG. 1. Mechanism of absorption and transport of fat during the first 3 hours after ingestion. Superscripts represent activity of labeled glycerol as compared to labeled fatty acid. G = glycerol; GA_3 = triglyceride; GA = monoglyceride; GA_2PB = lecithin and cephalin; PB = phosphorylated choline or ethanolamine.

hydrolyzed during the digestion and absorption processes, and that the freed dietary glycerol is replaced by endogenous glycerol during glyceride resynthesis.

The relatively greater replacement of dietary glycerol in plasma phospholipids than in plasma triglycerides at the third hour (a ratio of 0.35 as compared to 0.53, Table 3 and Fig. 1) is a clear indication that the phospholipids are the product of more synthetic action than the triglyceride. Actually, the change in the ratio from 0.53 to 0.35 can be almost exactly accounted for by the stoichiometric reaction 2 GA_3 + GPB → 3 GA_2PB,[7] in which there is a one-third replacement of the glycerol. One can thus conclude that the triglyceride is the precursor of the phospholipid, probably in the mucosa, as has been postulated previously (7).

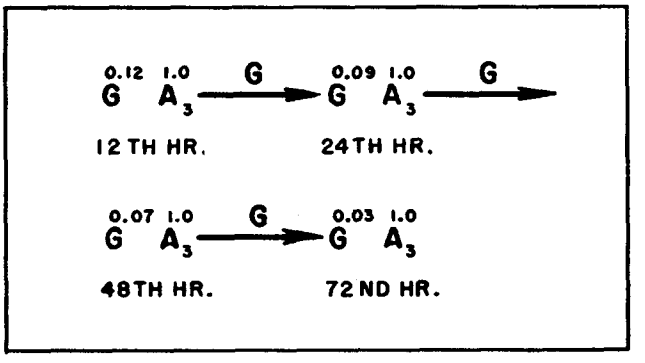
The relative amounts of active glycerol in the plasma and liver triglycerides are almost identical at the third hour, as shown by the ratios in Table 3, demonstrating retention of unmodified plasma triglyceride by liver reticulo-endothelium.

[7] G = glycerol; A = fatty acid; P = phosphate; B = nitrogenous base.

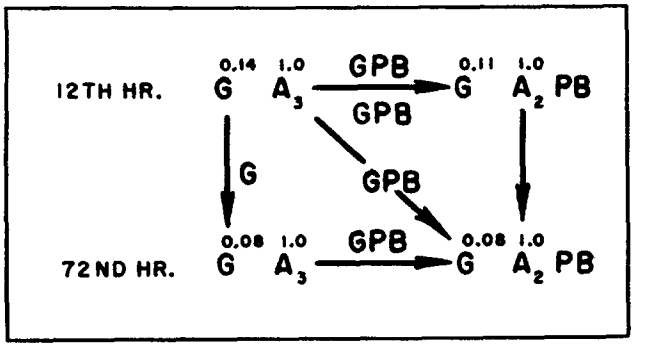
The much greater replacement of labeled glycerol in liver than in plasma phospholipids (both lecithin and cephalin) shows that liver phospholipids are not simply unmodified plasma phospholipids. In this respect they differ from the triglycerides, which are the same in both tissues. This again demonstrates that liver phospholipids must be a product of liver triglycerides. The degree of glycerol dilution from liver triglycerides to liver phospholipids (from 0.51 to 0.14) at the 3-hour period is greater than can be accounted for by the simple stoichiometric relationship outlined above for the plasma phospholipids. This may be interpreted to indicate a dynamic state of liver phospholipids in which they are rapidly being "turned over" with the labeled glycerol being replaced by unlabeled.

During the first 3 hours no measurable amount of labeled glycerol entered the fat depots (Table 2), although small amounts of fatty acids did (Table 1). During the second 6-hour period, glycerol actually entered the adipose tissue at a faster rate than the fatty acid, increasing the ratio to 0.12 at the twelfth hour, the end of the "absorption period."

A possible interpretation of this increase in the



ADIPOSE TISSUE



LIVER

FIG. 2. The dynamic state of tissue lipids during the postabsorptive state. Superscripts represent activity of labeled glycerol as compared to labeled fatty acid. G = glycerol; GA_3 = triglyceride; GA = monoglyceride; GA_2PB = lecithin and cephalin; PB = phosphorylated choline or ethanolamine.

glycerol:fatty acid ratio in the adipose tissue is that the plasma fat may be hydrolyzed to fatty acid and monoglyceride at, or in, the adipose tissue cell wall. The fatty acid may then enter the cell body more rapidly than the monoglyceride, and be rapidly resynthesized into triglyceride with ubiquitous unlabeled glycerophosphate. Thus the adipose tissue triglyceride would be labeled in the fatty acid only. Monoglyceride may enter more slowly, and, for that reason, toward the end of the absorptive period, would enter in relatively larger amounts than the fatty acid. This would cause a gradual increase in the labeled glycerol to labeled fatty acid ratio, as was obtained.

During the postabsorptive period (Fig. 2) the adipose tissue triglycerides maintained the largest percentage of their labeled fat of any lipid. Although the adipose tissue had the least loss of labeled fatty acid and glycerol, it should be noted that it was only in this tissue that the ratio of active glycerol to fatty acid changed significantly during the postabsorptive period up to 72 hours. It thus appears that triglycerides and phospholipids disappear from blood and liver as units, but that in adipose tissue, hydrolysis and resynthesis are part of the dynamic picture, with new glycerol replacing old during the process.

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