# METABOLISM OF GLYCEROLIPIDES: A COMPARISON OF LECITHIN AND TRIGLYCERIDE SYNTHESIS*

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Enzymatic synthesis of glycerolipides involves the formation of ester bonds between glycerol and fatty acids or phosphoric acid. The similarity among the structures of the different glycerolipides speaks in favor of common metabolic pathways and interchange of major parts of their molecules. Recently Kennedy and coworkers (1, 2) have shown that preparations of rat liver mitochondria can synthesize both lecithins and triglycerides from added diglyceride mixtures. Consequently, intact tissues may possibly utilize diglycerides as the common intermediates in the synthesis of these glycerolipides. If this were the case, a predictable relationship should be produced between the patterns of radioactive isotope incorporation in triglycerides and phosphoglycerides.

Accordingly, radiocarbon-labeled precursors were incubated with sliced tissues and the lipides were isolated in order to compare the amounts of isotope in each type of lipide. The results of these experiments suggest that the diglyceride unit in the phospholipides is metabolically different from that in the triglycerides.

## EXPERIMENTAL

Lung tissue, chosen for these experiments because of its high rate of lipide synthesis, was removed from rats and placed in ice-cold, oxygenated Krebs-Ringer-bicarbonate buffer, at pH 7.4, containing glucose. The tissue was sliced by hand and incubated in 4 ml. of the buffer at 38° in an atmosphere of 95 per cent $O_2$-5 per cent $CO_2$ in a shaking incubator. Micromolar quantities of radioactive glycerol and acetate containing 2 to 4 $\mu$c. were added to the various containers. After 4 hours the tissue was homogenized twice in 10 ml. portions of methanol and the lipides were further extracted from the dehydrated tissue with chloroform-methanol (2:1). The combined extracts were then purified according to Folch *et al.* (3) to remove water-soluble contaminants. The washing was repeated to

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remove traces of radioactive precursors. The total lipide extract was then evaporated to dryness, suspended in ether, and chromatographed on silicic acid columns.

The columns were routinely prepared by suspending 5 gm. of silicic acid (Mallinckrodt, 100-200) in ether in a 1 × 30 cm. glass tube, and the adsorbent was packed by tapping the column while the solvent drained. The lipides were applied to the column, which was then developed with

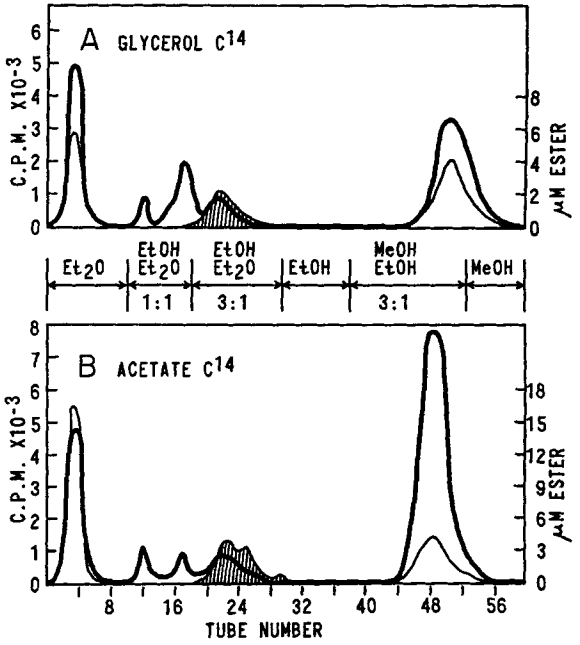



FIG. 1. The heavy line indicates the amount of radioactivity and the thin line shows the amount of ester in micromoles. The hatched area shows the results of amino nitrogen analyses (5).

ether. After the neutral fats were removed in the ether, gradient elution was begun with ethanol-ether (1:1) flowing into a constant volume mixing chamber containing 100 ml. of ether. The solvent entering the mixing chamber was replaced at intervals with solvents of increasing polarity to maintain a continually increasing gradient, as shown in Fig. 1.

Ester concentrations were measured by a modification of the procedure of Goddu *et al.* (4). Equal volumes of 4 per cent sodium hydroxide and 4 per cent hydroxylamine hydrochloride (w/v) in 95 per cent ethanol were mixed and the sodium chloride was removed by filtration. 2 ml. of the clear filtrate were added to a dry lipide sample in a screw cap culture tube. The tubes were shaken, heated at 65° for 5 minutes, and cooled. 5 ml. of

ferric perchlorate solution (4 ml. of stock ferric perchlorate (4) and 2.5 ml. of 70 per cent perchloric acid, diluted to 100 ml. with cold absolute ethanol) were added as the tubes cooled, and absorption was measured at 530 mμ after 15 to 20 minutes. The optical density is proportional to concentration up to an optical density of 2.0 and 15 μeq. of ester per sample. The radioactivity in the lipides was measured directly from small aliquots of the sample having no significant self-absorption.

Fig. 1, *A*, shows the distribution of lipides in an extract that contained about 4 mg. of triglyceride and 10 mg. of phospholipide. In this case glycerol-$C^{14}$ was supplied to the tissue, and one can see that the relative heights of the ester and radioactivity peaks are similar for the triglycerides and the phospholipides.

The specific activities are 2100 and 2400 c.p.m. per μmole of lipide. In contrast, Fig. 1, *B*, shows the pattern obtained when acetate-$C^{14}$ was the labeling agent. Here the phospholipides have a relatively greater amount of radioactivity than the triglycerides, and the specific activities are 3680 and 660, respectively.

In some experiments glycerol-$C^{14}$ and acetate-$C^{14}$ were added to the same vessel and the isolated lipide fraction was saponified in order to determine the isotope ratio. A portion of the lipide was hydrolyzed for 24 to 36 hours at 38° in 2 ml. of N potassium hydroxide. The resulting opalescent solution was acidified with 0.2 ml. of concentrated hydrochloric acid and the fatty acids were extracted with three 3 ml. portions of chloroform. A similar treatment of singly labeled incubations showed that the conversion of glycerol into the fatty acids and acetate into glycerol was negligible, agreeing with the findings of others (6). The amount of radioactivity in the non-saponifiable phospholipides was also found to be negligible.

## DISCUSSION

The amount of radioactive glycerol in a lipide in all likelihood provides a measure of the extent of new synthesis. Obviously, glycerol must be incorporated by the formation of three new ester linkages, thereby ruling out an exchange mechanism. On the other hand, acetate may donate its carbons to the lipide by known exchange reactions with labeled fatty acids (7) as well as by the new synthesis of lipide from diglycerides labeled in the fatty acid moiety.

In a simplified picture of lipide synthesis, acetate carbon (as fatty acids) and glycerol are combined in a diglyceride which can then form either phospholipide or triglyceride. This diglyceride would receive a certain amount of radioactivity from acetate and glycerol, respectively. If the diglyceride is the sole precursor of the phospholipide, these compounds should have the same ratio of incorporated acetate-$C^{14}$ to incorporated

glycerol-$C^{14}$. Triglycerides might be expected to have relatively more radioactivity from acetate, since a third fatty acid is added to the diglyceride unit. Therefore, the amount of acetate-$C^{14}$ in the immediate precursor of the triglyceride would probably be about two-thirds that in the final product, while the glycerol-$C^{14}$ would be the same in both compounds. This scheme is presented below, where the symbols $C^{14}Ac$ and $C^{14}Gl$

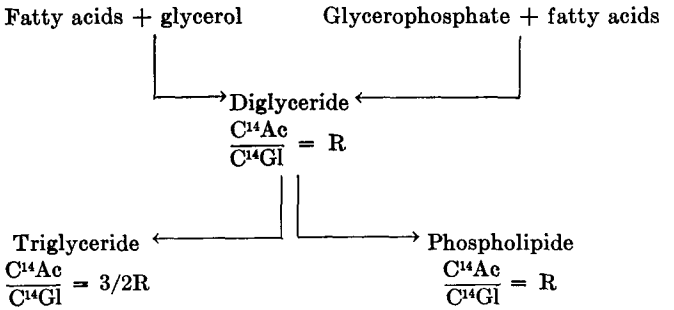
TABLE I

*Ratios of Radioactivities from Acetate and Glycerol*

The ratio R describes the relative activities of fatty acids and glycerol calculated for the diglyceride unit of the lipide.

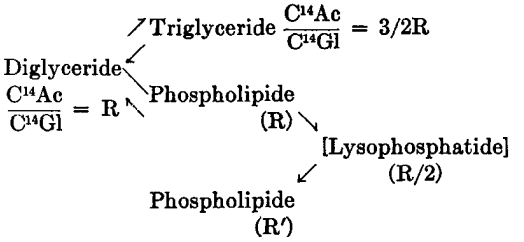
| Experiment No. | Tracer used | Triglyceride | | | Lecithin | |
|---|---|---|---|---|---|---|
| | | Total c.p.m. | 3/2R | R | Total c.p.m. | R |
| B | Acetate-$C^{14}$ | 12,200 | 1.13 | 0.75 | 35,200 | 1.95 |
| A | Glycerol-$C^{14}$ | 10,600 | | | 18,000 | |
| 2A | Acetate | 20,600 | 7.3 | 4.8 | 63,200 | 9.4 |
| | Glycerol | 2,800 | | | 6,720 | |
| 2B | Acetate | 16,100 | 5.3 | 3.5 | 38,300 | 8.2 |
| | Glycerol | 3,570 | | | 4,700 | |
| 6A | Acetate | 15,900 | 4.9 | 3.2 | 38,100 | 10.0 |
| | Glycerol | 3,230 | | | 3,800 | |
| 6B | Acetate | 12,800 | 3.5 | 2.3 | 27,600 | 9.9 |
| | Glycerol | 3,700 | | | 2,790 | |

stand for the counts per minute arising from labeled acetate and glycerol, respectively.



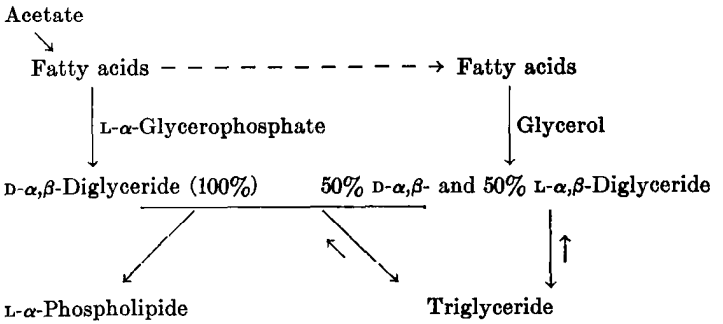

The data in Table I show that the ratio R is two to four times higher in the phospholipides than in the triglycerides. Although the specific activity of the glycerol portion is essentially the same in the two types of lipide, the fatty acid portion has a much higher specific activity in the phospholipides. This unexpected finding requires some modification of the above scheme.

One modification is given below.




This scheme is one in which the diglyceride serves as the common intermediate, but the phospholipide formed from it is capable of independent incorporation of fatty acids without utilizing a diglyceride intermediate. At the present time, phospholipide degradation is often considered to go to completion, once a fatty acid is removed (8), and a lysophosphatide acylating enzyme has not yet been demonstrated. The results of the experiments presented above, however, suggest the existence of an enzyme system which catalyzes exchange or synthesis of the fatty acid ester in lecithins.

Another hypothesis may be considered. Suppose the simple system above is modified so that the diglyceride pool is non-homogeneous, being formed by two independent pathways. One pathway involves the direct esterification of glycerol to give both D- and L-diglycerides. Here we shall consider that equal amounts of the isomers may be formed, taking into account the low specificity of acylating enzymes. The second pathway of diglyceride synthesis is one in which L-$\alpha$-glycerophosphate is acylated and the resultant phosphatidic acid is hydrolyzed to give only the D-$\alpha,\beta$-diglyceride (1).




According to this suggestion, only part of the molecules arising from the direct esterification of glycerol plus those from the glycerophosphate pathway can form L-$\alpha$-glycerophosphatides, whereas all the molecules may be available for triglyceride synthesis. This situation would lead to different

labeling patterns when the glycerophosphate is acylated from a different acyl pool from glycerol. From the data that have been obtained, we could postulate that L-α-glycerophosphate is the preferred acceptor of newly synthesized fatty acids, while free glycerol is acylated by fatty acids from a more dilute pool. Further experiments are in progress to examine the biosynthesis of glycerolipides with respect to these suggested schemes.

SUMMARY

In lung tissue slices, glycerol-$C^{14}$ produces triglycerides and phospholipides with similar specific activities, whereas acetate-$C^{14}$ leads to much higher activity in the phospholipides. These results suggest that the diglyceride unit of the phospholipides is metabolically different in some respect from that of the triglycerides. Possible schemes are presented to account for these observations.

BIBLIOGRAPHY

1. Weiss, S. B., Smith, S., and Kennedy, E. P., *Nature*, **178**, 594 (1956).
2. Weiss, S. B., and Kennedy, E. P., *J. Am. Chem. Soc.*, **78**, 3550 (1956).
3. Folch, J., Ascoli, I., Lees, M., Meath, J. A., and LeBaron, F. N., *J. Biol. Chem.*, **191**, 833 (1951).
4. Goddu, R. F., LeBlanc, N. F., and Wright, C. M., *Anal. Chem.*, **27**, 1251 (1955).
5. Lea, C. H., and Rhodes, D. N., *Biochem. J.*, **56**, 613 (1954).
6. Pritchard, E. T., *Federation Proc.*, **15**, 330 (1956).
7. Borgström, B., *Biochim. et biophys. acta*, **13**, 491 (1954).
8. Fairbairn, D., *J. Biol. Chem.*, **157**, 645 (1945).