FORMATION OF HIGHER GLYCERIDES FROM MONOPALMITIN AND PALMITYL-COA BY MICROSOMES OF RAT INTESTINAL MUCOSA[1]

John R. Senior[2] and Kurt J. Isselbacher

Department of Medicine, Harvard Medical School, and the
Medical Services, Massachusetts General Hospital, Boston, Massachusetts

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As a result of the intestinal digestion of fats, considerable quantities of free fatty acids and monoglycerides are made available for absorption into the epithelial cells of the intestinal mucosa. After passage across the cells the fatty acids appear in the lymph chylomicrons largely as triglycerides. It has been shown that the initial step in this reesterification involved conversion of the fatty acids to water-soluble and chemically reactive coenzyme A (CoA) derivatives within the mucosal cells, utilizing energy supplied by adenosine triphosphate (ATP) and catalyzed by a long chain fatty acid thiokinase (Senior and Isselbacher, 1960). The resynthesis of the triglycerides which appear in the lymph has been thought to occur exclusively by the pathway of esterification of L-α-glycerophosphate by the activated fatty acids. Recently, however, it has been proposed by Clark and Hübscher (1961) that direct acylation of the monoglycerides to higher glycerides might also occur. These investigators found increased incorporation of C^{14}-labeled fatty acid into glycerides when monoglycerides, especially monoolein, were added to incubation mixtures of rabbit gut mucosal mitochondria, ATP, and CoA; furthermore, they found that polyoxyethylene sorbitan monolaurate (Tween 20), which

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[2] Special Research Fellow, National Institute of Arthritis and Metabolic Diseases.

inhibited glyceride formation by the glycerophosphate esterification pathway, did not reduce production of higher glycerides using monoolein to stimulate incorporation. Monoglyceride absorption into the intestinal mucosal cell has been shown to be significant, as indicated by studies in which both the glycerol and fatty acid portions of the monoglycerides were isotopically-tagged (Reiser and Williams, 1953; Skipski, Morehouse and Deuel, 1959).

In order to demonstrate the direct condensation of palymityl-CoA and monopalmitin to diglyceride and thence to triglyceride, it was concluded that a simpler system not involving several sequential enzymatic steps, addition of cofactors such as ATP, or detergents such as Tween would provide more definite evidence for this alternate pathway of glyceride synthesis. To show that monoglycerides were indeed esterified to higher glycerides and to exclude the possibility of transesterification, experiments were carried out with the C^{14}-label located in the glycerol moiety of the monopalmitin acceptor and with unlabeled palmityl-CoA as an acyl group donor.

In the synthesis of the labeled monopalmitin, glycerol-1-C^{14} was converted to DL-isopropylideneglycerol-1,(3)-C^{14} by exhaustive reflux with anhydrous acetone and petroleum ether, with total reflux of the solvent vapors into a Barrett receiver as a water trap. The isopropylideneglycerol was esterified with freshly redistilled palmityl chloride in quinoline, the acetone removed by acid hydrolysis, the product twice recrystallized from diethyl ether and finally purified on a silicic acid column. The DL-glyceryl-1(3)-C^{14}-1-palmitate[3] moved as a single monoglyceride spot in several solvent systems on thin layer plates of silicic acid, melted at 71-72° and had a specific activity of 101,000 cpm per mg. Palmityl-CoA was prepared from the same palmityl chloride and CoA in 50% tetrahydrofurane (freshly redistilled over $LiAlH_4$ and used at once to avoid peroxides) under N_2 at pH 7.5-8.0, and after purification (Seubert, 1960) made up as 0.0024 M solution in 0.01 M phosphate buffer, pH 5. Microsomes were prepared by sedimentation at 105,000 x g for 30

[3] This compound will be referred to as monopalmitin-C^{14}.

minutes from neutral isotonic mannitol homogenates of mucosal cells from fasted rat jejunum, after removal of cell debris, nuclei and mitochondria by prior centrifugation. The pellets of microsomal particles were washed and resuspended in isotonic KCl, buffered to pH 7.0 with 0.01 M potassium phosphate; this particulate fraction showed negligible cytochrome oxidase activity or DNA content, and had a protein concentration of 3-4 mg. per ml. by the biuret method.

It is evident from Table I that in the complete incubation system containing fresh microsomes and palmityl-CoA, over 40 per cent of the monopalmitin-C^{14} was converted to labeled di- and triglycerides. Although ATP has previously been shown to be necessary for the formation of palmityl-CoA by these particles under similar conditions (Senior and Isselbacher, 1960), it is apparent that the formation of higher glycerides from monopalmitin and palmityl-CoA proceeds without added ATP and is not stimulated by it. Furthermore, no labeled glycerides were isolated by silicic acid chromatography which contained any detectable phosphorus. It should also be noted in Table I that there was virtually a complete breakdown of the labeled monopalmitin to glycerol-C^{14} in the system in which palmityl-CoA was omitted. However in the incubations containing palmityl-CoA and in which higher glycerides were actively synthesized, only about half of the monopalmitin was hydrolyzed. Thus the microsomes appeared to contain both an active synthetic condensing enzyme and an active lipolytic enzyme, both of which were competing for the monopalmitin substrate. This lipase has been previously reported in the everted sac preparation of hamster intestine (Tidwell and Johnston, 1960), and suggested to be particularly active toward monoglycerides.

In additional studies, it has been found that in as short a time as five minutes over 40% of the monopalmitin may be converted to diglyceride, although in this brief interval less than 5% appeared as labeled triglyceride. Simultaneously, about 30% of the monopalmitin-C^{14} was hydrolyzed to free glycerol-C^{14} when palmityl-CoA was present, but over twice as much was split in the absence of palmityl-CoA. To date it has not been possible selectively

TABLE I

RAT GUT MICROSOMAL GLYCERIDE FORMATION FROM MONOPALMITIN-C^{14} AND PALMITYL-COA

Incubation System	Labeled Products Isolated After Incubation With 1 μmole of Monopalmitin-C^{14}				
	Glycerides			Glycerol (free)	Total (recovery)
	tri-	di-	mono-		
	μmoles				
Complete	0.200	0.215	0.007	0.555	0.98
Plus ATP, 10 μmoles	0.203	0.219	0.007	0.548	0.98
Minus palmityl-CoA	0.001	0.001	0.005	0.998	1.01
Minus microsomes	0.003	0.005	0.963	0.011	0.98
Boiled microsomes	0.002	0.006	0.964	0.017	0.99

The complete system contained 1 μmole synthetic monopalmitin-C^{14} (33,350 cpm), dispersed in 0.1 gm. bovine serum albumin, 200 μmoles potassium phosphate buffer pH 7.0, 80 μmoles KCl, 70 μmoles NaCl, 5 μmoles $MgCl_2$, 1.5 μmoles $CaCl_2$, 2.4 μmoles palmityl-CoA, and 1.9 mg. freshly prepared microsomal protein, in a total volume of 2.0 ml. Incubations at 38° were stopped after 30 minutes by the addition of 12 ml. ice cold methanol, chilling to 0°, and adding 24 ml. chloroform. Residual palmityl-CoA and free palmitate were extracted from the lipids at room temperature by partitioning twice against 0.05 M KCl-NH_4OH, pH 9. The upper phase aqueous layer was found to remove all of the free glycerol-C^{14} and none of the glycerides, to within 0.3%. Lipids from the dried lower phase were separated on silicic acid columns, checked on thin layer plates of silicic acid, and aliquots counted by liquid scintillation technique.

to inhibit the microsomal lipase, nor to separate it from the synthetic enzyme. Further characteristics of the microsomal lipase will be presented in a subsequent report.

These data demonstrate the ability of the microsomal fraction of rat jejunal epithelial cells to catalyze synthesis of di- and triglycerides by direct condensation of monoglyceride and activated fatty acids. While the quantitative significance of this step in fat absorption cannot be estimated, the existence of this pathway would appear relevant to the process of intestinal glyceride transport.

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