Preliminary Notes

PN 1295

Distribution of fatty acids in triglycerides synthesized from monoglycerides

The distribution of fatty acids between the 2- and 1,3-position of triglycerides has recently been examined with regard to the digestion and absorption of triglycerides in the rat[1,2]. By comparing the type of fatty acid in these positions in the fed triglycerides with those appearing in the thoracic duct lymph as well as the distribution obtained when only the fatty acids were administered, it was reported that approx. 80–85 % of the fatty acids did not show randomization between the 2- and 1,3-positions.

Two pathways for the biosynthesis of triglycerides have been reported in the intestinal mucosa. The first pathway[3–5] employs α-glycerophosphate as the backbone for the condensation of fatty acid-CoA units forming triglycerides via phosphatidic acids and diglyceride intermediates[6]. The second pathway was suggested by CLARK AND HÜBSCHER[4] and employs monoglyceride which is incorporated into triglycerides. This pathway has been confirmed employing glycerol-labeled monoglycerides[7] and doubly labeled monoglycerides[8]. Different intermediates for the synthesis of triglycerides from 1- and 2-monopalmitin have been suggested[9].

The distribution studies[1,2] could be interpreted in two ways. First of all, the intact absorption of the triglyceride molecule. This explanation, however, would be in conflict with the generally accepted views that between 50–60 % of the fatty acids of ingested triglycerides are hydrolyzed prior to absorption. The occurrence of the monoglyceride pathway in intestinal tissue provides a second interpretation of the distribution studies since the fatty acid present in the monoglyceride would positionally be fixed in resynthesis of triglycerides. To examine this latter possibility, the triglycerides synthesized from 1- and 2-monoglycerides by hamster mucosal homogenates were examined with regard to the positions of the fatty acids in the synthesized triglycerides.

In the reported investigation either 1- or 2-monopalmitin, labeled in the fatty acid portion of the molecule, was incubated with intestinal homogenates and palmityl-CoA and the lipids isolated under similar conditions to those previously reported[8]. ^{14}C-labeled 1-monopalmitin was synthesized according to the procedure of HARTMAN[10]. The 2-monopalmitin was labeled with ^{3}H and was synthesized by the method of DAUBERT[11]. Analysis by thin-layer chromatography for the 1- and 2-isomers according to the procedure of HOFMANN[12] gave the following results: [^{14}C]monopalmitin, 93 % 1-isomer and 7 % 2-isomer; [^{3}H]monopalmitin, 4 % 1-isomer and 96 % 2-isomer. The triglyceride fractions were obtained by the use of thin-layer chromatography[13]. The triglycerides were visualized by spraying with 10 % I_2 in methanol and outlined. Following the evaporization of the I_2, the area corresponding to the triglycerides

was scraped off and eluted with diethyl ether. The triglyceride fraction was confirmed by co-chromatography with a known sample of triglyceride.

The amount of enzymically synthesized triglycerides from either 1- or 2-monopalmitin was 0.400 μmole and 0.317 μmole, respectively. Each triglyceride was equally divided into two flasks. To one of the flasks was added pancreatic lipase and the remaining flask served as a control. The vessels were incubated for 10 min at 25° under the conditions given in Table I.

The lipids were then re-isolated by extraction with acidified chloroform–methanol (2:1, v/v) and the distribution of activity in the products of enzymic hydrolysis determined by the use of thin-layer chromatography followed by liquid scintillation counting[13]. Pancreatic lipase has been shown to be specific for the hydrolysis, primarily, of the ester bonds of the 1,3-positions of the triglyceride molecule[14,15].

TABLE I

THE DISTRIBUTION OF RADIOACTIVITY IN THE HYDROLYTIC PRODUCTS OBTAINED BY THE INCUBATION OF PANCREATIC LIPASE WITH THE TRIGLYCERIDES SYNTHESIZED FROM EITHER LABELED 1- OR 2-MONOPALMITIN

The vessels contained 1 mg pancreatic lipase (A. H. Robins Co., Inc., Richmond, Va. (U.S.A.)) suspended in 1 ml Tris–maleate buffer (pH 7), 0.43 ml 10% gum acasia and 28.6 mg sodium taurocholate.

Fraction	*% of recovered activity*	
	triglyceride synthesized from 1-monopalmitin*	*triglyceride* synthesized from 2-monopalmitin*
Monoglycerides	7.1	73.1
1,2-Diglycerides	0.0	5.9
Fatty acids	63.3	3.9
Triglycerides	29.5	17.0

* 200 mμmoles of the triglycerides synthesized from 1-monopalmitin and 158 mμmoles of the triglycerides from 2-monopalmitin were incubated with pancreatic lipase (EC 3.1.1.3).

As can be seen from Table I, the triglycerides enzymically synthesized from labeled 1-monopalmitin when subjected to hydrolysis by pancreatic lipase resulted in the appearance of activity primarily in the released fatty acids. The small amount of radioactivity present in the resulting monoglyceride can be explained on the basis of the small amount (7%) of ^{14}C-labeled 2-monopalmitin known to be present in the original substrate. Hydrolysis of the triglycerides synthesized from ^{3}H-labeled 2-monopalmitin produced almost exclusively labeled monoglycerides and only trace amounts of radioactive fatty acids. The small amount of labeled fatty acid can be explained on the basis of the presence of ^{3}H-labeled 1-monopalmitin (3%) in the original substrate and any isomerization that may have occurred. The failure to isolate an appreciable amount of labeled diglyceride is probably due to the high ratio of enzyme to substrate[16]. In the control vessels 99% of the added radioactivity was recovered in the unhydrolyzed triglyceride fraction. Similar results have been obtained at the early time intervals when the hydrolysis was followed with time.

It has recently been reported that the 2-monoglycerides are almost exclusively present in the lumen of the intestine[12]. HOFMANN AND BORGSTRÖM[17] suggested that the combination of monoglycerides, fatty acids, and bile salts in the form of micellar

solutions may be the form in which fats are absorbed and intestinal mucosa contains enzyme systems which can synthesize triglycerides from monoglycerides and fatty acids.

The reported results in combination with the above findings provide a possible explanation for what would appear to be conflicting results, namely the hydrolysis of 50–60% of the ingested triglyceride fatty acids and only a limited exchange between the 2- and 1,3-position of the triglyceride during the process of digestion and absorption. Via the resynthesis of triglycerides from the 2-monoglycerides and fatty acids as much as 66% of the total fatty acid ingested as triglycerides could be hydrolyzed. The hydrolyzed fatty acids would be derived primarily from the 1,3-positions of the triglyceride molecule because of the specificity of pancreatic lipase. The resulting 2-monoglycerides and fatty acids would then be resynthesized into triglycerides by enzymes of the intestinal mucosa. The reconstituted triglycerides would show a similar distribution between the 2- and 1,3-positions as the fed triglycerides. The reported findings would only be valid when the fatty acids utilized in the resynthesis of triglycerides were derived from the dietary sources. This has been shown to be the case experimentally[1,18].

The reported results provide additional evidence for the intact utilization of both the 1- and 2-monopalmitin for triglyceride synthesis without prior hydrolysis or isomerization of either isomer, since hydrolysis followed by the incorporation of the resulting fatty acids into triglycerides or the isomerization of the 1-isomer to the 2-isomer or *vice versa* would result in a change in the distribution between the 2- and 1,3-positions of the synthesized triglycerides.

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