ESTERIFICATION OF AN ETHER ANALOGUE OF 2-MONOSTEARIN IN ISOLATED SEGMENTS OF INTESTINAL MUCOSA[1]

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Various estimates of the lipids in the intestinal lumen during fat absorption have placed the amount of 2-monoglycerides at from 50 to 80% of the total monoglycerides recovered (Desnuelle and Constantin, 1955; Harris et al., 1955; Ahrens and Borgstrom, 1956). It has not been demonstrated that the 2-isomer is absorbed and utilized as a substrate for triglyceride synthesis as has been shown for 1-monoglycerides (Skipski et al., 1959; Tidwell and Johnston, 1960). A major problem in the study of 2-monoglyceride absorption has been the ease of acyl migration to the 1-monoglyceride which is favored at equilibrium by 9 to 1 (Mattson and Volpenhein, 1962). In experiments on subcellular mucosal fractions (Senior and Isselbach, 1962; Clark and Hubscher, 1963), both 1 and 2-monoglycerides apparently could serve as substrates for glyceride synthesis but it was not indicated whether the 2-monoglycerides had to be first converted to the 1-isomer in order to be utilized.

Acyl migration of monoglycerides apparently involves a nucleophilic attack on the carbonyl carbon by the vicinal hydroxyl groups of the glycerol moiety. It was thought that substitution of an ether linkage for the ester linkage might eliminate this problem and that the ether analogue might still retain the essential characteristics of a 2-monoglyceride. For this reason, glycerol-2-octadecyl-1-C^{14} ether (iso-batyl

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alcohol) was used as an analogue of 2-monostearin. This compound was found to be taken up intact and to act as a substrate for glyceride synthesis in everted sacs of rat small intestine.

Materials and Methods: Male Wistar rats, fasted 48 hours, were sacrificed and their intestines removed. Everted intestinal sacs were prepared as described by Wilson and Wiseman (1954). After incubation, the sacs were homogenized and extracted three times with hot ethanol-ether (1:1). The pooled extracts were then evaporated to dryness under nitrogen and re-extracted with n-hexane. The lipids obtained were separated by thin-layer silicic acid chromatography in a n-hexane-acetone-acetic acid (89:11:3) solvent system which was allowed to ascend 15 cm from the origin. The plates were air dried and divided into ten 1.5 cm sections which were scraped off into test tubes. The lipids were eluted from the scrapings with 10% acetone in ethyl ether except for the origin which was eluted with methanol. The eluates were then centrifuged at low speed to bring down the silicic acid and aliquots were removed and evaporated to dryness for radioactivity determinations in a liquid scintillation system.

The glycerol-2-octadecyl-1-C^{14} ether was prepared by condensing 1,3-benzylideneglycerol (Hill et al., 1928) with octadecyl-1-C^{14} iodide as described by Davies et al. (1934). The product was purified by silicic acid column chromatography and melted at 63-64°C. The isocyanate derivative melted at 83-84°C.

Results and Discussion: Table 1 shows the thin-layer chromatography profiles of glycerol-2-octadecyl-1-C^{14} ether and its fatty acid derivatives which were obtained in this solvent system. Chromatogram A shows the migration of the pure ether analogue while chromatogram B shows the migration obtained after a sample of the ether was esterified with excess oleyl chloride for 25 hours at room temperature in anhydrous chloroform. This treatment converted it to the 1,3-diolein derivative which corresponds to a triglyceride. After partial hydrolysis of this derivative in 0.1N

alcoholic KOH at 70°C for 1 minute, an intermediate compound was obtained (Chromatogram C) which was the 1-monoolein derivative and corresponds to a 1,2-diglyceride. Since the synthetic compounds displayed the same migration pattern in this system as mono-, di-, and triglycerides, this was construed to be additional evidence for their identity. The chromatogram (D) of the intestinal sac lipids had three radioactive bands which corresponded to those obtained with the synthetic compounds. After hydrolysis in 0.1N alcoholic KOH for one hour at 70°C, the synthetic as well as the biosynthetic derivatives yielded the original ether (Chromatograms E and F).

Table 1

THIN-LAYER CHROMATOGRAPHY OF GLYCEROL-2-OCTADECYL-1-C^{14} ETHER AND ITS OLEIC ACID DERIVATIVES

		Per Cent of Total Radioactivity					
		CHROMATOGRAM*					
	Section	A	B	C	D	E	F
Solvent Front	10	-	-	-	-	-	-
	9	-	87	28	11	-	-
	8	-	7	8	-	-	-
	7	-	-	-	-	-	-
	6	-	-	-	-	-	-
	5	-	3	13	16	-	-
	4	-	-	-	-	-	-
	3	-	-	-	-	-	-
	2	97	-	47	65	93	94
Origin	1	-	-	-	-	-	-

* Explanation of Chromatograms:

A Pure glycerol-2-octadecyl-1-C^{14} ether.

B After esterification of ether with oleyl chloride.

C After hydrolysis of triglyceride analogue in 0.1N alcoholic KOH for 1 minute at 70°C.

D Intestinal lipids after incubation with ether.

E After hydrolysis of triglyceride analogue in 0.1N alcoholic KOH for 1 hour at 70°C.

F After hydrolysis of intestinal lipids in 0.1N alcoholic KOH for 1 hour at 70°C.

The 1-monoolein derivative of the ether was the only radioactive band detected in the diglyceride area of the chromatograms. In other experiments (unpublished) where 1-monopalmitin-1-C^{14} was used, two distinct bands were found in this area and were identical to bands obtained when pure samples of 1,2- and 1,3-diglycerides were chromatogrammed[2]. The 1,3-diglyceride band had three times as much radioactivity as the 1,2-diglyceride and it appears that both diglyceride isomers may be intermediates in triglyceride synthesis depending on which monoglyceride isomer is presented as the substrate. Unless more than one monoglyceride acylase is present in rat intestinal mucosa, the enzyme has no positional specificity.

When 1-monopalmitin-1-C^{14} was used as a substrate, radioactivity was found in the phospholipid fraction which remains at the origin of the chromatogram in this system. Since 10-15% of the radioactivity in the intestinal lipids was present as free fatty acids due to hydrolysis of the monoglyceride, these free fatty acids are available for phosphatidic acid synthesis by the acylation of α-glycerol phosphate (Kennedy, 1957) as well as for monoglyceride acylation. Pieringer and Hokin (1962) reported a brain preparation which synthesized lysophosphatidic acid from monoglyceride and ATP so the possibility exists that monoglycerides are also phosphorylated in the mucosa. The failure to find any radioactivity in the phospholipid fraction when the ether analogue was used as a substrate suggests that monoglycerides are directly acylated in the mucosa to triglycerides via a diglyceride intermediate.

The addition of oleic acid to the incubation medium (Table 2) doubled the amount of each ether derivative synthesized along with a 14% increase in uptake. It has been suggested that monoglycerides form mixed micelles with fatty acids and bile salts (Hofmann and Borgstrom,

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Table 2

UPTAKE OF GLYCEROL-2-OCTADECYL-1-C^{14} ETHER BY INTESTINAL SACS AND ITS UTILIZATION AS A SUBSTRATE FOR GLYCERIDE SYNTHESIS WITH AND WITHOUT OLEIC ACID

	muMoles Glycerol-2-octadecyl-1-C^{14} ether			
Additions*	Free ether	1-Monoolein Deriv.	1,3-Diolein Deriv.	Total
None	383 ± 5#	58 ± 4	39 ± 2	480
4 umoles oleic acid	348 ± 6	121 ± 12	85 ± 4	554

* The incubation medium contained 5 ml 0.154 M sodium phosphate buffer pH 6.4, 20 umoles sodium taurocholate, 0.3% glucose, and 2 umoles glycerol-2-octadecyl-1-C^{14} ether (specific activity 31,000 cpm/umole). Sacs were incubated in this medium for 30 minutes at 37°C in a Dubnoff metabolic shaker under an atmosphere of 95% O_2 - 5% CO_2.

\# Standard Error.

1962) and it may be these small lipid aggregates which are absorbed. 1-glycerol ethers have been found to form micelles in bile salt solutions like 1-monoglycerides of the same chain length which in turn are solubilized equally as well as 2-monoglycerides (Hofmann, 1963). The polar ends of the fatty acids and monoglycerides are thought to be oriented in the same direction at the outer surface of the micelle while the non-polar ends combine to form a hydrocarbon center (Hofmann and Borgstrom, 1962). This would bring the free hydroxy groups of the monoglyceride into close proximity to the carboxyl group of the fatty acids and facilitate direct esterification into di- and triglycerides. It might also explain why free glycerol was not readily utilized as a substrate for glyceride synthesis in the mucosa as compared to monoglyceride glycerol (Tidwell and Johnston, 1962). Specificity may reside more in the amphipathic properties of the monoglycerides than in their chemical characteristics.

Summary: Evidence has been presented that glycerol-2-octadecyl-1-C^{14} ether is absorbed intact by the intestinal mucosa and is esterified by

fatty acids to produce compounds that correspond to di- and triglycerides. If this compound can be considered analogous to the actual 2-monostearin, the data suggest that 2-monoglycerides can act as a substrate for triglyceride synthesis during absorption.

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