INCORPORATION OF PALMITIC ACID-H^3 INTO THE SPHINGOMYELINS OF THE INTESTINAL MUCOSA OF THE RAT DURING ABSORPTION

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In a previous study on the composition of the fatty acids of the phospholipids of the intestinal mucosa of the rat (1), we found that in the fasted animal the sphingomyelins contained about 20 moles per cent of palmitic acid. This value increased to 80 per cent two hours after ingestion of a diet containing 10 per cent lard (with 27 mole per cent of palmitic acid). It was of interest to see if these results could be explained by a preferential incorporation of palmitate into sphingomyelins during absorption by comparing the specific activities of these phospholipids after ingestion of labeled palmitic, stearic or oleic acids under identical experimental conditions. Since it has been shown in several studies (2,3,4) that palmitic acid serves as a precursor of sphingosine, we also measured the activity of the amino alcohol portion of the sphingomyelins.

The study was conducted with groups of 20 adult Wistar rats, each receiving, after a 24 hour fast, 4 g. of a synthetic diet containing 40 mg. of palmitic, stearic or oleic acids tritiated in the 9,10 positions (sp. act., 90,000 dis./min./mg.). The first two acids were incorporated into 360 mg. of olive oil and the last in 360 mg. of a mixture of 1/3 sunflower seed oil 2/3 mutton tallow so that the degree of unsaturation of the 3 fat mixtures would be approximately the same. The animals were sacrificed 2 hours after the feeding and the anterior 2/3 of the small intestines were removed and rinsed with 30 ml. of 9% NaCl and the mucosa were scraped off and collected. The lipids were extracted with cold

methylal-methanol and were separated into their various constituents by a combination of column and thin-layer silicic acid chromatography especially adapted to the separation of the phospholipids, particularly the sphingomyelins (1).

The identity and purity of the isolated sphingomyelins was assured by the absence of ester bonds, glycerol and sugar and by a $^{N}/_{P}$ ratio of 2. Choline was identified by Draggendorf's reagent and, after acid hydrolysis, the fatty acid and sphingosine fractions were obtained. The latter was determined by the spectrophotometric method of Lauter and Trams (5) using pure sphingosine as a reference. The radioactivity of the two fractions was measured by a Tracerlab LSC 10A scintillation counter.

The results are presented in Table I.

TABLE I

Specific Activities (dis/min./mg.) of the Sphingomyelins of the Intestinal Mucosa (Sphingosine and Fatty Acids) after Ingestion of Tritiated 16:0, 18:0 and 18:1

Fatty Acids Ingested	16:0		18:0		18:1	
Hours after ingestion	2	2	2	4	2	4
Sphingosine	1380	1037	60	24	28	12
Fatty acids	4940	3600	115	400	14	30

The results demonstrate conclusively that 2 hours after ingestion of labeled palmitic acid, the specific activities of both the sphingosine and the fatty acids are considerably higher than those obtained after administration of stearic or oleic acids.

To assure ourselves that in the case of stearic and oleic acids we were not simply dealing with a slower incorporation we made some measurements 4 hours after ingestion without finding any significant changes in the specific activities. The low values found in these cases could stem from a small contamination of these two acids with

palmitic or to their metabolic transformation into that acid. The stearic and oleic acids are well-absorbed, however, since it was found that 2 hours after their administration, the fatty acids of certain lipid fractions were up to 30 times more highly labeled than those in the sphingomyelins.

In the case of palmitic acid, on the other hand, the sphingomyelins had the highest specific activity of all the lipids. We conclude, therefore, that palmitic acid is preferentially incorporated into the sphingomyelins of the intestinal mucosa during absorption. The significance of this fact remains to be determined.

Since sphingosine itself has an appreciable specific activity (1/3 that of the sphingomyelin fatty acids) after ingestion of labeled palmitic acid, these in vivo experiments confirm, in the case of the intestinal mucosa the in vitro demonstration that in the brain, liver and spleen palmitic acid is the precursor of sphingosine (3,4).

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