preparation which had been added to the pool and which gave the normal vitamin B_{12} absorption spectrum, the complex exhibited some differences. The ratios of the absorbancies at 361/550 nm and 361/520 nm were 4.1 and 4.2, respectively, compared with 3.3 and 3.75 for the cyanocobalamin. In addition, there was the usual protein absorbance at lower wavelengths.

In the ultracentrifuge, complex I appeared to contain a trace of a slower-sedimenting impurity, also seen in disc electrophoresis, and amounting to 5 % at the most. Gel filtration of the complex through polyacrylamide (Biogel P-60) did not remove the impurity. The extrapolated sedimentation constant of complex I was 5.5 ± 0.1.

In our previous report[3] the isolated complexes S and I were shown to be the sole carriers of IF activity in gastric juice. The IF activity of complex S was again confirmed in this study.

The slightly lower sedimentation constant of complex I compared with that of complex S, and its absence in stomach mucosa[9] and in gastric juice neutralized in the stomach[6] appears to indicate that it is a breakdown product of the original intrinsic factor molecule.

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The Positional Distribution of Fatty Acids in Triglycerides and Lecithins of Human Chylomicrons

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Analyses of the positional relations of the fatty acids in human lymph lecithins[1-3] have demonstrated that the distribution of fatty acids on the α- and β-position is non-random. Saturated fatty acids are usually found esterified predominantly on the α-positions of the lecithins and polyunsaturated fatty acids on the β-position. A similar distribution of the fatty acids is also found in human bile and liver lecithins.[4] The existing data in the literature do not give any information about the structure of the various triglycerides in man. Such information is necessary in order to be able to elucidate the metabolic interrelations between phospholipids and triglycerides in man.

Experimental studies on the stereospecific mechanisms involved in fat absorption in animals have shown that some asymmetry is usually present in lymph triglycerides during chylomicron formation,[5-7] with a tendency to more unsaturated fatty acids at the β-position and saturated fatty acids at the α-position.

Studies on the specificity of fatty acid esterification during fat absorption in man have recently been reported from this laboratory.[8,9] In these studies, mixtures of several ^{14}C-labelled fatty acids were fed to patients with thoracic duct fistula, and the distribution of labelled and unlabelled fatty acids was measured for several different lymph lipids. Chylomicron triglyceride formation showed no specificity

for one fatty acid relative to another with slight discrimination against stearic acid. The formation of chylomicron lecithin showed a marked relative specificity for linoleic acid and a lesser specificity for stearic acid.

In the present work these studies have now been extended to the determination of the positional distribution of the fatty acids in the triglycerides and lecithins of human thoracic duct lymph.

Experimental. Human thoracic duct lymph lipids were prepared from lymph obtained from case 3 and 4 in the experiments of Blomstrand *et al.*[9,10] In connection with a scalene node biopsy the thoracic duct was cannulated with a polyethylene tubing. After fasting over night Case 3 was fed a liquid formula meal containing 15 μC palmitic acid-1-^{14}C + 2 g palmitic acid, 15 μC oleic acid-1-^{14}C + 2 g oleic acid, and 15 μC linoleic acid-1-^{14}C + 2 g linoleic acid together with 18 g egg white and 20 g of glucose mixed in 150 ml of water. Case 4 was fed a liquid formula meal containing 11.25 μC palmitic acid-1-^{14}C + 1.5 g palmitic acid, 11.25 μC stearic acid-1-^{14}C + 1.5 g stearic acid, 11.25 μC oleic acid-1-^{14}C + 1.5 g oleic acid and 11.25 μC linoleic acid-1-^{14}C + 1.5 g linoleic acid together with 10.2 g of egg white and 11.3 g of glucose mixed in 71 ml of water. (The labelled material was obtained from Radiochemical Centre, Amersham, England).

The lymph samples analysed were collected 5 h after feeding the formula meal. Extraction of lymph was carried out as described previously.[5] Silicic acid chromatography was employed to separate the total lipids into cholesterol esters, glycerides, and phospholipid fractions. The sterol ester fraction was eluted with benzene in hexane 15:85 (v/v) and the triglycerides with benzene. The purity of the triglycerides was tested with thin layer chromatography. The column was next eluted with chloroform followed by elution of the total phospholipids with methanol. The lymph phospholipids were rechromatographed on silicic acid and the lecithins purified with chloroform:methanol.[4] The purity of the lecithins was tested with thin layer chromatography.

The triglycerides were then subjected to the action of pancreatic lipase using the incubation conditions mainly according to Mattson and Volpenheim.[5] The monoglycerides and the free fatty acids after conversion to methyl esters were isolated using silicic acid chromatography in combination with thin layer chromatography. The component fatty acids of the monoglycerides were isolated; these fatty acids represent those present in the β-position of the original triglycerides.

The lecithin fractions isolated were pure as tested with thin layer chromatography. The enzymatic cleavage of lecithin was carried out mainly as described by Blomstrand,[4] using a freshly prepared enzyme solution (phospho-

Table 1. Distribution of mass and radioactivity among the fatty acids in human lymph triglycerides and lecithins and their hydrolytic products after hydrolysis by pancreatic lipase and phospholipase A, respectively. Patient 3 was fed a mixture of equal amounts of palmitic acid-1-^{14}C, oleic acid-1-^{14}C and linoleic acid-1-^{14}C. Patient 4 was fed equal amounts of palmitic acid-1-^{14}C, stearic acid-1-^{14}C, oleic acid-1-^{14}C and linoleic acid-1-^{14}C. Values are expressed as percentage of total fatty acid methyl esters and percentage of total radioactivity. The experimental conditions are given in previous reports.[5,9] TGFA = triglyceride fatty acids, MGFA = monoglyceride fatty acids, FFA = fatty acids released by the action of lipase resp. phospholipase A.

		Mass % Distribution				Radioactivity % Distribution			
		16:0	18:0	18:1	18:2	16:0	18:0	18:1	18:2
Patient 3	TGFA	25.6		36.7	35.9	26.3		39.9	33.7
	MGFA (β)	24.9		32.0	42.5	26.3		33.2	40.5
	FFA (α)								
	Lysolecithin FA (α)	5.2	1.6	15.4	77.2	9.8			90.2
	FFA (β)	51.3	34.1	8.8	1.1	57.6		42.4	
Patient 4	TGFA	26.4	15.0	29.9	29.0	26.3	17.7	30.2	25.8
	MGFA (β)	23.8	1.3	30.0	44.2	25.9	6.9	28.9	38.3
	FFA (α)	26.0	27.5	33.5	13.0	22.9	29.5	32.0	15.6

lipase A from *Crotaleus adamanteus*, Rosi Allen Reptile Institute, Silver Springs, Tla.). The lysolecithin and the free fatty acids from the reaction mixture were separated on silicic acid. Thin layer chromatography showed that the fractions obtained were pure.

Fatty acid methyl esters were prepared from aliquots of the original unhydrolyzed products and were analyzed for total mass and radioactivity among the different fatty acids by gas-radiochromatography as described by Blomstrand and Gürtler.[10]

Comments. The distribution of mass and radioactivity in the chylomicron triglycerides and lecithins and in their products after enzymatic cleavage are shown in Table 1. The results indicate that under the conditions of these experiments there are positional differences in the distribution of fatty acids in the human chylomicron triglycerides with stearic acid almost exclusively in the α-position of the triglycerides and linoleic acid mainly in the β-position.

Oleic acid and palmitic acid were represented in approximately equal amounts in the two positions. The lymph lecithins showed an extreme asymmetry in the distribution of the fatty acids with saturated fatty acids in the β-position and unsaturated fatty acids mainly in the α-position in accordance with earlier observation by Blomstrand, Dahlbäck and Linder.[2] The radioactivity in the fatty acids in the lymph lecithins was distributed even more non-randomly than the mass pattern. The radioactivity in the triglycerides was distributed in a similar fashion to the pattern of the mass. The results of this study demonstrate that there is a positional asymmetry in the distribution of fatty acids both in the triglycerides and lecithins of human chylomicrons. The distribution of the fatty acids on the triglyceride and lecithin molecules are, however, quite different suggesting that the major part of these molecules are produced via different biosynthetic pathways. A minor part of the chylomicron triglyceride and lecithin molecules have structural similarities in the distribution and incorporation of stearic acid and linoleic acid suggesting a common biosynthetic pathway, as described by Weiss, Kennedy and Kiyasu.[11]

In the formation of chylomicron triglycerides and lecithins, cleavage products from intraluminal hydrolysis of phospholipids and triglycerides also are involved.[13] The complex mechanisms involved in the formation of chylomicron triglycerides is also illustrated by experiments using α-glycerylethers [14] and β-glycerylethers [15] which indicate that both α- and β-monoglycerides can be utilized for the synthesis of triglycerides in the intestinal mucosa.

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