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Fatty acid esterification and chylomicron formation during fat absorption: 2. Phospholipids*

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SUMMARY

Studies were conducted on the over-all fatty acid specificity of the mechanisms involved in chylomicron lecithin formation during fat absorption in the rat. Synthetic mixtures of fatty acids containing trace quantities of C^{14}-labeled fatty acids were administered by gastric intubation to rats with cannulated thoracic ducts. The chylomicron lipids were first separated on silicic acid columns into cholesterol ester, glyceride, and phospholipid fractions. The results obtained with the sterol ester and glyceride fractions are described in the accompanying paper. The phospholipids were rechromatographed to obtain pure lecithin, and determinations were made of the total fatty acid radioactivity, total mass, and distribution of mass and radioactivity, among the several fatty acids of lecithin, by gas-liquid chromatography.

Different fatty acids fed to the rat were not incorporated to equal extents into chylomicron lecithin. The incorporation showed a marked relative specificity for stearic acid and a lesser specificity for linoleic acid. Oleic acid was incorporated least of all into lecithin. The incorporation of different dietary fatty acids varied with the nature of the diet, but the addition of fatty acids from endogenous sources was of such magnitude as to make this change less evident, so that the over-all fatty acid pattern of lecithin was relatively constant and independent of the composition of the diet. The endogenous contribution to lecithin fatty acids was greater than the endogenous contribution to the sterol ester or triglyceride fatty acids of the same chylomicron sample. This endogenous contribution to lecithin also varied from fatty acid to fatty acid, being greatest with palmitic acid and least with oleic acid.

The general features of chylomicron formation are discussed in detail. Lecithin carried a relatively large share of stearic acid, and relatively very little oleic acid, within the chylomicron.

During the intestinal absorption of long-chain fatty acids, a small fraction of the absorbed fatty acids appears in lymph as chylomicron phospholipid. The extent to which this occurs for each major fatty acid under different dietary conditions is not entirely clear. A number of studies in both rats and humans, given a single labeled fatty acid on any one occasion, has shown that palmitic, oleic, and linoleic acids are incorporated to much the same extent, with 2–6% of the lymph radioactivity being found in the phospholipid fraction (2–9). On the other hand, Hanahan and Blomstrand (10) reported that after feeding labeled palmitic acid to rats, 10% of the lymph radioactivity was in lecithin, whereas after labeled oleic acid, there was less than 0.5%. Clément and Mead (11) also found a low recovery of radioactivity (1–6% of total) in lymph phospholipids after feeding oleic acid-C^{14}. By contrast, stearic acid has been observed to be incorporated into lymph phospholipids to a much greater extent than the other three fatty acids, accounting for 8 to 20% of the total lymph radioactivity

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in rats and humans (5, 7, 12). In addition, it is known that the esterification of a given fatty acid may be almost exclusively in either the α- or β-position of lymph lecithin (4, 5).

In the accompanying paper (13), information was presented on cholesterol esterification during fat absorption. The present report is a continuation of these studies and deals with the phospholipid fraction of chylomicrons formed after the ingestion of mixtures of labeled and unlabeled palmitic, stearic, oleic, and linoleic acids. A general discussion of the incorporation of different fatty acids into the glycerides, cholesterol esters, and phospholipids of the chylomicron is also presented.

EXPERIMENTAL METHODS

The studies reported here were conducted with materials collected during Experiments II and III, described in the accompanying paper (13). Most of the methods used were the same as those used in the preceding study and are described there in detail. In brief, synthetic mixtures of fatty acids containing trace quantities of C^{14}-labeled fatty acids were administered by gastric tube to rats. Following this, chyle from the thoracic duct was collected for 24 hr via a polyethylene cannula, and the chylomicron fraction was separated by high-speed centrifugation. The lipids were extracted; separated by silicic acid chromatography into glyceride (G), cholesterol ester (E), and phospholipid (P) fractions; and assayed for radioactivity by liquid scintillation counting. Each lipid was then treated to form methyl esters of the fatty acids, which were analyzed by gas-liquid chromatography. Equipment was used that provided a direct read-out of radioactivity as well as mass in the GLC effluent (14). Through the use of an internal standard, the total fatty acids in each lipid were measured by GLC, as well as the fatty acid composition and the distribution of radioactivity.

The several diets used have been described in the accompanying paper (13). In Experiment II, the two diets, A and B, were similar in respect to the distribution of radioactivity among palmitic, oleic, and linoleic acids, but differed in that unlabeled oleic acid was the major component of A and linoleic acid of B. In Experiment III, the diet (D) contained stearic as well as the other three acids. Diets AC, BC, and DC were identical with A, B, and D, respectively, except for the addition of about 50 mg cholesterol per 0.3 ml of fatty acid mixture.

The additional methodology used in the study of the phospholipids was as follows:

Analysis of the P Fractions. As mentioned above, the total lipid extracts from chylomicrons were separated into G, E, and P fractions by chromatography on silicic acid columns. Each P fraction in Experiment II was analyzed by chromatography on cellulose paper impregnated with silicic acid (15). Lecithin was the major component in every case. Small amounts of other phospholipids, particularly phosphatidyl ethanolamine and phosphatidyl inositol, and of free fatty acids were also detected; these accounted for an average of 28% of the radioactivity of the P samples. In Experiment III, thin-layer chromatography was used to check the composition of the P fractions. Chromatography on layers of silica gel G in $CHCl_3$–MeOH–H_2O 70:30:4 showed that lecithin accounted for about 60% of the radioactivity of the P samples, and that free fatty acids accounted for much of the remainder.

Isolation of Lecithin. Pure lecithin was isolated from the P samples by silicic acid chromatography. Small columns (10 mm i.d.) were loaded with a slurry of 4 g silicic acid ("Unisil," 200-325 mesh, Clarkson Chemical Co.) in $CHCl_3$ and packed by gravity. The samples were applied in $CHCl_3$ solution, followed by elution of the cephalin fraction with 60 ml 20% methanol in $CHCl_3$ (v/v). Pure lecithin was eluted with 130 ml 40% methanol in $CHCl_3$, and the remaining lipids eluted with pure methanol. The total recovery of radioactivity from the columns was always close to 100%. Chromatography of the separate fractions on paper impregnated with silicic acid (Experiment II) or on thin layers of silica gel (Experiment III) showed that the lecithin had been almost quantitatively isolated, without contamination.

Preparation of Methyl Esters of the Fatty Acids of Phospholipids. Our first attempts to prepare methyl esters in the usual way (by refluxing with 2 to 9% H_2SO_4 in methanol at 65° for 16 hr or more) resulted in a poor and variable recovery of radioactivity and of mass as assessed by GLC. Preliminary saponification did not resolve the trouble, which was found to be due to difficulty in extracting the methyl esters with light petroleum ether. Meltzer has observed poor extractability of methyl stearate when dispersed in 50% methanol, and has postulated the formation of water-methanol barriers around the ester particles (16). He found that extraction of methyl stearate was improved by avoiding an excess of water, by the addition of NaCl or $CaCl_2$, and by centrifuging It is probable that similar barriers to extraction existed in the present experiments and were perhaps much more pronounced than usual because of the emulsifying action of the hydrolysis lecithin products.

TABLE 1. RECOVERY OF INTERNAL STANDARD MASS AND OF PHOSPHOLIPID FATTY ACID RADIOACTIVITY AFTER METHYLATION

P Fraction From Collection (Rat and Diet)	Recovery of Margaric Acid Mass	Recovery of Fatty Acid-C^{14}
	%	%
3A	76	70
4A	64	67
3B	62	60
4B	54	54
4AC	44	43
4BC	34	34

Evidence that the poor recoveries were due to poor extractability of the methyl esters of fatty acids was provided by the demonstration that all the fatty acids, including added free fatty acid, were equally affected. In these experiments, margaric acid (*n*-heptadecanoic acid) was always included in the methylating mixtures as an internal standard. Since this acid was added as a free fatty acid, it was reasonable to assume that it was effectively methylated. Nevertheless, the recovery of margaric acid, determined by comparing the area of its peak in the GLC record with that expected from areas of equal amounts of margaric acid methylated alone, varied widely and in parallel with the recovery of radioactivity from the accompanying phospholipid fatty acids, as shown in Table 1.

When recovery was poor, the methyl esters of all component fatty acids, both saturated and unsaturated, were affected to an equal extent. This is shown in Table 2 by the near identity of the mass distribution of the 4BC phospholipid fraction, determined after two separate methylations with widely differing recoveries.

The effects of the presence of lecithin and of different concentrations of mineral acid were demonstrated by tests made with an artificial mixture of palmitic acid-1-C^{14} and the methyl esters of fatty acids of soap. These were refluxed in solutions of methanol containing varying amounts of H_2SO_4, with or without the addition of small amounts of lecithin. An equal volume of water was added, and the methyl esters were extracted three times with light petroleum ether; sufficient time was allowed, in each extraction, for any signs of emulsion to clear. The recovery of radioactivity in the three extractions is given in Table 3. It is clear that the presence of lecithin reduced the recovery of radioactivity and that higher concentrations of acid improved recovery. In those solutions where recovery was incomplete, more radioactivity was recovered by leaving some petroleum ether on the surface of the methanol phase and carrying out further extractions 2 hr later.

TABLE 2. COMPARISON OF THE FATTY ACID COMPOSITION OF TWO PORTIONS OF THE SAME P SAMPLE AFTER METHYLATIONS WITH VERY DIFFERENT RECOVERIES

Recovery		Distribution of Mass						
Margaric Acid Mass	Radioactivity	16:0	16:1	18:0	18:1	18:2	20:1	20:4
%	%				%			
34	34	30.2	2.9	13.2	7.3	34.9	1.4	8.9
96	90	29.7	2.7	15.6	8.8	32.9	1.7	8.6

TABLE 3. EFFECTS OF ADDING LECITHIN AND OF INCREASING AMOUNTS OF ACID IN METHANOL ON THE EXTRACTABILITY OF METHYL PALMITATE-1-C^{14}

Concentration of H_2SO_4 in CH_3OH	Recovery of C^{14}	
	Lecithin Absent	Lecithin Present
%	%	%
2	87	70
5	93	73
10	100	84

As a routine procedure, the samples were refluxed, as described, in 5% (v/v) H_2SO_4 in methanol, followed by the addition of an equal volume of water and four or five extractions with excess petroleum ether. Sufficient time was allowed to elapse between extractions to ensure good recoveries. The mixtures were centrifuged when any persistent interfacial emulsion was encountered. The recovery of radioactivity and of the added margaric acid mass was always checked, and ranged from 89–103% in the results presented.

RESULTS

The total mass and radioactivity and their distribution among the fatty acids of each of the phospholipid (P) fractions and the purified lecithins are shown in Tables 4 and 5. The average values for the distribution of mass and radioactivity in the chylomicron lecithins after each diet are presented in Table 6. The values for the diets and for the triglyceride fractions from the same lymph samples are shown for comparison.

In Experiment II (Table 4), an average of 78% of the mass and 72% of the radioactivity of the P fractions was found in lecithin. The lecithin samples showed only minor changes in composition following administration of the two very different diets (A and B), in contrast to the large changes observed in the tri-

TABLE 4. RECOVERY AND DISTRIBUTION OF MASS AND RADIOACTIVITY IN THE FATTY ACIDS OF THE CHYLOMICRON P FRACTIONS AND LECITHINS IN EXPERIMENT II

Rat and Diet	Mass								Radioactivity				
	Total	Distribution							Total	Distribution			
		16:0	16:1	18:0	18:1	18:2	20:1	20:4		16:0	18:0	18:1	18:2
	mg				*%*				*cpm* × 10^{-6}		*%*		
						P Fractions							
3A	20.68	22.2	3.0	10.6	24.0	32.2	2.8	4.2	0.794	34	5	20	41
4A	3.95	25.9	1.2	16.8	20.3	28.1	tr	7.6	0.0659	30	3	19	47
4AC	36.09	26.3	1.6	12.6	17.1	35.4	0.9	6.1	1.169	40	8	19	32
3B	7.01	26.8	4.1	14.3	12.0	27.6	2.6	12.4	0.0931	39	6	22	33
4B	21.53	26.7	1.3	11.7	11.2	39.6	2.3	5.3	0.483	38	5	23	34
4BC	11.01	29.7	2.7	15.6	8.8	32.9	1.7	8.6	0.1997	36	9	23	33
						Lecithins							
3A	15.04	25.1	2.7	9.8	21.2	34.1	2.6	3.8	0.540	32	5	20	42
4A	3.88	26.3	1.6	15.0	16.2	30.1	0.8	8.3	0.056	20	5	19	56
4AC	26.46	26.3	2.7	11.3	14.5	37.2	0.7	7.4	0.8766	32	7	20	40
3B	6.19	28.0	3.9	13.9	10.9	26.5	2.8	13.0	0.0614	32	4	17	47
4B	16.44	31.9	1.4	10.9	9.5	39.8	2.1	4.3	0.3429	33	8	19	39
4BC	10.56	31.9	2.8	12.8	7.9	34.3	1.0	9.3	0.1458	31	10	24	34

glycerides (see Table 6). The distribution of radioactivity among the fatty acids of lecithin greatly differed from that of the diets, but was essentially the same after each different diet. Relatively less oleic acid-C^{14} and more linoleic acid-C^{14}, and particularly stearic acid-C^{14} was found in lecithin than in the diet.

In Experiment III (in which the diet (D) contained four, rather than three, labeled fatty acids), the results were similar. Except for the presence of relatively more stearic acid, the fatty acid composition of the lecithin fractions was very similar to that observed after diets A and B. Relatively much more stearic acid-C^{14} and somewhat more linoleic acid-C^{14} was incorporated into lecithin, compared to palmitic or oleic acids.

Table 5 also includes the complete analysis of the P fraction of the "1.006 bottom" of the lymph from sample 5DC remaining after removal of the chylomicrons by centrifugation. The distribution of mass and radioactivity in this sample was similar to that observed in the corresponding fraction of the chylomicron lipid.

From the specific radioactivity of each of the lecithin fatty acids in Experiment III, divided by the corresponding specific activity in the fed fat, the fractional contribution of the diet to each fatty acid was cal-

TABLE 5. RECOVERY AND DISTRIBUTION OF MASS AND RADIOACTIVITY IN THE FATTY ACIDS OF THE CHYLOMICRON P FRACTIONS AND LECITHINS IN EXPERIMENT III

Rat and Diet	Mass								Radioactivity				
	Total	Distribution							Total	Distribution			
		16:0	16:1	18:0	18:1	18:2	20:1	20:4		16:0	18:0	18:1	18:2
	mg				*%*				*cpm* × 10^{-6}		*%*		
						P Fractions							
5D	27.59	23.8	0.8	18.6	12.3	33.4	1.2	9.8	1.217	15.0	49.9	15.0	20.0
6D	18.93	23.4	0.9	19.0	12.9	36.6	0.7	6.5	0.7455	15.7	46.9	16.7	21.7
5DC				Not analyzed					1.381		Not analyzed		
6DC				Not analyzed					0.964		Not analyzed		
						Lecithins							
5D	22.87	26.8	1.3	17.6	8.5	35.2	0.6	9.7	0.6231	14.5	53.4	11.5	20.9
6D	12.99	24.9	0.8	19.5	9.1	38.7	tr	6.9	0.4227	10.9	58.8	11.3	18.7
5DC	21.84	27.4	0.8	19.7	9.1	33.2	1.1	8.7	0.7179	13.0	64.0	8.8	13.9
6DC	15.85	24.3	1.1	18.7	8.8	38.2	tr	8.4	0.5282	15.6	55.1	12.4	16.8
						"1.006 Bottom": P Fraction							
5DC	4.50	25.7	0.6	19.2	9.4	32.4	1.4	11.3	0.2373	17.0	53.6	13.4	15.9

TABLE 6. THE AVERAGE VALUES FOR THE DISTRIBUTION OF MASS AND RADIOACTIVITY AMONG THE FATTY ACIDS OF THE DIETS AND OF THE CHYLOMICRON LECITHIN FRACTIONS OBTAINED AFTER EACH DIET*

	Mass Distribution							Radioactivity Distribution			
	16:0	16:1	18:0	18:1	18:2	20:1	20:4	16:0	18:0	18:1	18:2
	%							%			
Diet: A + AC	12.6	...	0.3	77.0	10.0	...	...	40	0.3	32	28
Triglycerides	15.3	1.1	1.8	50.0	29.2	2.0	0.4	38.0	1.0	31.4	29.6
Lecithin	25.9	2.6	11.1	16.9	35.6	1.3	6.3	31.5	6.2	20.0	41.3
Diet: B + BC	11.3	...	0.6	12.2	76.0	...	...	40	0.4	30	30
Triglycerides	18.2	2.3	3.1	21.4	49.4	3.5	1.5	38.2	1.7	29.9	29.5
Lecithin	31.2	2.3	12.0	9.2	35.6	1.8	7.5	32.4	8.1	20.1	38.6
Diet: D	7.1	...	7.1	43.3	42.5	...	...	26.6	29.2	24.9	19.4
Triglycerides	14.3	0.9	6.6	36.2	39.0	1.8	1.3	28.1	22.5	28.5	20.9
Lecithin	26.1	1.1	18.3	8.7	36.5	0.4	8.7	13.0	55.6	11.4	20.0
Diet: DC	7.1	...	7.1	43.3	42.5	...	...	26.6	29.2	24.9	19.4
Triglycerides	14.5	0.9	7.8	35.2	38.1	1.8	1.8	27.3	26.3	26.2	20.2
Lecithin	26.1	0.9	19.3	9.0	35.3	0.6	8.6	14.3	60.2	10.3	15.1

* The individual values that were averaged were "weighted" in proportion to their mass, in obtaining these tabulated values (see [13]). The corresponding values for the triglyceride fractions from the same chylomicron collections (13) are included for comparison.

culated. The results, shown in Table 7, show that remarkably large portions of each fatty acid in lecithin were derived from endogenous sources. The fractional contribution of endogenous fatty acids was greatest with palmitic acid (average of 94% endogenous) and least with oleic acid (average of 34% endogenous). Approximately 35% of the total lecithin fatty acids were derived from the diet, with 65% being of endogenous origin.

DISCUSSION

The differences between the P fractions and the lecithins were mainly due to the small amounts of free fatty acids present in the P fractions. The FFA fraction was not directly studied. Its composition, estimated by difference between the P and lecithin fractions reveals a distribution of radioactivity much more like that in the diet than that in the lecithin.

The lecithin samples were found to have fatty acid patterns that were hardly influenced by the composition of the fatty meal administered. The content of carbon-14, on the other hand, showed that the administered fat was being incorporated into the lecithin, so that its failure to reflect the composition of the fed fat could only have been caused by the relative specificities of the mechanisms involved in the formation of chylomicron phospholipid. The distribution of radioactivity in the lecithin fraction confirmed that this was the case.

In Experiment II, labeled palmitic, oleic, and linoleic acids were administered. Labeled stearic acid

TABLE 7. THE AVERAGE VALUES FOR THE SPECIFIC RADIOACTIVITY OF EACH FATTY ACID IN LECITHIN DIVIDED BY THE CORRESPONDING VALUE FOR THAT FATTY ACID IN THE DIET IN EXPERIMENT III*

	Relative Specific Radioactivity			
	16:0	18:0	18:1	18:2
Diet D	1.00	1.00	1.00	1.00
Triglycerides	0.30	0.47	0.77	0.66
Lecithin	0.04	0.21	0.66	0.35
Diet DC	1.00	1.00	1.00	1.00
Triglycerides	0.28	0.46	0.72	0.65
Lecithin	0.05	0.25	0.66	0.31

* As in Table 6, the individual values that were averaged were "weighted" in proportion to their mass. The corresponding values for the triglyceride fractions from the same chylomicron collections (13) are included for comparison.

was found in the phospholipid fraction isolated, although none had been intentionally fed. To determine if this acid was present as a contaminant in one of the other acids, these acids were analyzed and trace quantities of stearic acid-C^{14} were found. The amounts of stearic acid-C^{14} present in the diets were enough to account for the amounts recovered in the lymph phospholipids, provided that 35–50% of the dietary stearic acid-C^{14} had been incorporated into phospholipids. There was, of course, the additional possibility that some of the labeled stearic acid in lecithin resulted from conversion of one of the other labeled fatty acids or a fragment thereof into stearic acid. The data do not permit a clear choice between these possibilities. In either event, the finding of stearic

TABLE 8. THE RELATIVE SPECIFICITY OF INCORPORATION OF DIETARY FATTY ACIDS INTO CHYLOMICRON LECITHIN*

	Triglyceride				Lecithin			
	16:0	18:0	18:1	18:2	16:0	18:0	18:1	18:2
Diet: A + AC	1.00	..†	1.03	1.11	1.00	..†	0.79	1.87
Diet: B + BC	1.00	..†	1.04	1.03	1.00	..†	0.83	1.59
Diet: D + DC	1.00	0.82	1.05	1.02	1.00	3.87	0.85	1.78

* These are the average values after each diet, and the individual values being averaged were "weighted" in proportion to their mass. The corresponding values for the triglyceride fractions from the same chylomicron collections (13) are included for comparison.

† Since only traces of radioactivity were present in stearic acid in these diets, the relative preference for stearic acid could not be determined with accuracy in Experiment II.

acid-C^{14} emphasized that the processes involved in the formation of chylomicron lecithin showed a strong selectivity for stearic acid.

The subsequent studies (Experiment III), in which the diet contained labeled stearic acid in addition to the other three, confirmed this strong specificity for stearic acid. Although the diet contained 29.2% of its radioactivity in stearic acid, stearic acid-C^{14} comprised 53 to 64% of the total radioactivity of the lecithin samples.

The relative specificity shown for the several fatty acids can be estimated by examining the data as was done in the accompanying paper (13). The distribution of radioactivity between palmitic, oleic, and linoleic acids in diet A was 100:80:70. The corresponding average distribution in chylomicron lecithin was 100:63:131, thus showing selectivity for linoleic acid, and some discrimination against oleic acid relative to palmitic acid. Similar results were obtained with diet B, where the corresponding ratios were 100:75:75 for the diet and 100:62:119 for lecithin. In diet D, the distribution of radioactivity between palmitic, stearic, oleic, and linoleic acids was 100:114:94:73. The corresponding average distribution in chylomicron lecithin was 100:442:80:130. This indicates the striking specificity for stearic acid for lecithin synthesis. The lesser selectivity for linoleic acid and the relative discrimination against oleic acid are again evident.

These conclusions are also evident from the data presented in Table 8. This table compares the distribution of radioactivity among the lecithin fatty acids with the distribution in the diet, normalized to palmitic acid taken as 1.0. The almost 4-fold relative specificity for stearic acid, almost 2-fold specificity for linoleic acid, and slight discrimination against oleic acid relative to palmitic acid, is again apparent. The specificity for stearic acid is even more striking when considered in the light of the probability of positional specificity on the phosphoglyceride molecule. Positional specificity for stearic acid in lecithin synthesis has been suggested by other studies (5, 7, 12).

TABLE 9. THE AVERAGE VALUES FOR THE MASS DISTRIBUTION OF EACH FATTY ACID (TOTAL G + E + L) AMONG THE MAJOR LIPID CLASSES OF THE CHYLOMICRON*

Diet	Fraction	Distribution of Each Fatty Acid Among the Three Major Lipid Fractions					All Fatty Acids
		16:0	18:0	18:1	18:2	20:4	
		%					
A + AC	Glycerides	90.1	72.5	96.4	92.7	53.6	93.2
	Sterol Esters	1.5	2.1	1.8	1.1	0.4	1.6
	Lecithin	8.4	25.4	1.8	6.2	46	5.2
B + BC	Glycerides	86.6	75.2	94.6	93.5	72	91.2
	Sterol Esters	1.9	2.2	2.3	1.2	0.8	1.7
	Lecithin	11.5	22.6	3.1	5.3	27	7.1
D	Glycerides	86.1	80.8	96.9	92.4	65	93.5
	Sterol Esters	1.2	1.2	1.2	0.7	tr	1.0
	Lecithin	12.7	18.0	1.9	6.9	35	5.5
DC	Glycerides	87.0	83.6	95.0	92.2	75	91.2
	Sterol Esters	2.4	2.4	3.4	2.0	tr	2.6
	Lecithin	10.6	14.0	1.6	5.8	25	6.2

* The individual values being averaged were "weighted" in proportion to their mass.

General Features of Chylomicron Formation. The glyceride, cholesterol ester, and lecithin components of the chylomicron have so far been individually examined in this and the accompanying paper (13). The several fractions will now be considered together, to compare them in regard to the nature of their fatty acid composition and its response to diet, and to the relative part played by each in the transport of absorbed fat.

The distribution of the total chylomicron fatty acids among the three ester classes being studied, after each diet, is shown in Table 9. On the average, the glycerides contained 92% of all fatty acids, lecithin 6%, and cholesterol esters 1.8%. Lecithin contained much more than an average share of stearic and of arachidonic acids, and much less than an average share of oleic acid.

The total chylomicron fatty acids comprised, of course, newly absorbed dietary fat distinguishable by its radioactive label, and unlabeled fatty acid of endogenous origin.

The distribution of radioactivity of each fatty acid fed, among the three ester classes after each diet, is shown in Table 10. These data represent the distribution of the exogenous portion of each chylomicron fatty acid among the three lipid classes. On the average, the glycerides contained 95% of all the radioactivity, lecithin 3.7%, and cholesterol esters 1.3%. As discussed previously (13), addition of cholesterol to the test meal increased the proportion of total fatty acid mass and radioactivity found in the sterol ester fraction (compare results after diet DC with D in Tables 9 and 10). The addition of cholesterol to the test

TABLE 10. THE DISTRIBUTION OF RADIOACTIVITY OF EACH FATTY ACID (I.E., OF THE EXOGENOUS PORTION OF EACH FATTY ACID) AMONG THE MAJOR LIPID CLASSES OF THE CHYLOMICRON (TOTAL G + E + L)*

Diet	Fraction	Distribution of C^{14} in Each Fatty Acid Among the Three Major Lipid Fractions 16:0	18:0	18:1	18:2	All C^{14}
		%				
A + AC	Glycerides	95.7	...	95.8	93.8	95.0
	Sterol Esters	1.0	...	1.8	0.9	1.2
	Lecithin	3.3	...	2.4	5.3	3.8
B + BC	Glycerides	96.3	...	95.7	94.7	95.4
	Sterol Esters	1.0	...	2.2	1.2	1.4
	Lecithin	2.7	...	2.1	4.1	3.2
D	Glycerides	97.7	90.1	97.4	95.7	95.5
	Sterol Esters	0.4	0.6	0.9	0.5	0.6
	Lecithin	1.9	9.2	1.6	3.8	3.9
DC	Glycerides	96.7	90.0	95.7	95.4	94.3
	Sterol Esters	1.3	1.8	2.8	1.8	1.9
	Lecithin	2.0	8.2	1.5	2.8	3.8

* These are average values and the individual values being averaged were "weighted" in proportion to their mass.

meal did not significantly affect the proportion of total fatty acid mass and radioactivity found in the lecithin fraction.

From the specific radioactivity of each dietary fatty acid and the amount of radioactivity recovered in each fatty acid of each ester class, estimates can be made of the distribution of exogenous fatty acid mass among the different fatty acids within each lipid fraction. The results of these calculations are shown in Table 11. The distributions of the exogenous (labeled) fatty acids in the diets and in the glycerides are almost identical. The patterns of the other fractions illustrate, in different fashion, the fatty acid specificities that were apparent from the radioactivity data alone. This method of analysis also emphasizes the influence of diet on the nature of the exogenous fatty acids incorporated into each lipid class. Even lecithin contained very different patterns of exogenous fatty acids in response to diet. Since the lecithin samples had a fairly constant over-all fatty acid composition regardless of the diet, it is apparent that lecithin contained varying ratios of endogenous and exogenous fatty acids, depending on the dietary supply. The relative proportions of fatty acids of endogenous and exogenous origin also varied from one to another ester class. After each diet, the order of increasing endogenous contribution was glycerides, sterol esters, and lecithin. Thus, in Experiment III, fatty acids of endogenous origin accounted for an average of 43% of the total glyceride fatty acids, 54% of the sterol ester fatty acids, and 65% of the total lecithin fatty acids. Within each lipid class the extent of endogenous dilution varied from fatty acid to fatty acid (cf. Table 7).

The observations made in these studies reveal some of the great complexities of the processes involved in fat absorption and chylomicron formation. During the process of absorbing fat, there is added to the dietary material a considerable quantity of endogenous fatty acid, which, in the fasting state, is neither present in thoracic duct lymph nor incorporated into a particulate form such as the chylomicron. The composition and extent of the endogenous contribution varies with diet and among the ester classes, so that unjustifiable conclusions regarding the absorption and transport of fatty acids might be drawn if reliance were placed solely on the over-all fatty acid compositions of the chylomicron lipid fractions.

There are undoubtedly many factors, only one of which is the specificity of the esterifying enzymes, which influence the distribution of fatty acids among the different chylomicron lipid fractions. Our results, however, give estimates of the over-all specificities, which are presumably of physiological significance. Sterol ester synthesis shows a relative specificity for oleic acid. Lecithin synthesis strongly selects stearic acid, shows a lesser specificity for linoleic acid, and uses oleic acid least of all. In contrast, the incorporation of the dietary fatty acids into triglycerides occurs in proportion to their concentration in the diet.

The general metabolic significance of these findings, particularly in regard to cholesterol and phospholipid metabolism, is difficult to assess. Recent studies on the metabolism of chylomicron cholesterol ester in the rat

TABLE 11. THE AVERAGE PERCENTAGE DISTRIBUTION OF EXOGENOUS FATTY ACIDS AMONG THE DIFFERENT FATTY ACIDS WITHIN EACH LIPID FRACTION*

Fatty Acid	Diet: A + AC				Diet: B + BC				Diet: D + DC			
	Diet	G	E	L	Diet	G	E	L	Diet	G	E	L
16:0	12.6	12.1	7.7	13.1	11.3	10.8	8.3	7.5	7.1	7.0	4.0	4.9
18:0	<1	<1	<1	~5	<1	<1	<1	~5	7.1	5.7	5.1	18.9
18:1	77.0	76.2	85.3	63.0	12.2	12.1	20.1	6.7	43.3	44.8	56.9	25.2
18:2	10.0	10.7	6.2	19.2	76.0	74.6	70.1	80.2	42.5	42.5	34.0	51.0

* These values represent fatty acid mass of exogenous origin.

have shown that, after its entry into the vascular compartment, almost all the chylomicron cholesterol ester is taken up by the liver, followed by slow but extensive hydrolysis to free cholesterol and fatty acid in the liver (17). The details of the enzymatic hydrolysis of cholesterol esters in rat liver have also been partly defined (18). These latter studies suggested that the enzymatic capacity of rat liver to hydrolyze cholesterol esters is quite limited, since the demonstrable enzymatic activity was quite low. Because of this limited hydrolytic activity, it is possible that the observed fatty acid specificity for chylomicron cholesterol ester formation significantly affects the liver sterol ester composition, particularly in the presence of a high cholesterol diet. It is, of course, well known that, even in the absence of cholesterol feeding, a considerable enterohepatic circulation of cholesterol takes place. Studies by Klein and Martin (19) and by Morin et al. (20) have indicated that cholesteryl oleate predominates in the liver sterol ester accumulation that occurs during a high cholesterol diet. This predominance of cholesteryl oleate may, in part, reflect the specificity for oleic acid herein observed for chylomicron cholesterol ester formation. In addition, this predominant accumulation of cholesteryl oleate undoubtedly also reflects a specificity for oleic acid on the part of the liver enzymes involved in cholesterol ester synthesis (21).

It is generally accepted that triglycerides and lecithin are synthesized from a common diglyceride precursor (22), but the present study has revealed many points of difference between these two lipids as found in the chylomicron. Not only do their fatty acid patterns differ, but whereas the glyceride pattern changes with diet, the lecithin pattern is relatively inflexible. The proportion of endogenous fatty acids in lecithin is much greater than in glycerides, and the relative specificities toward incorporation of fatty acids are striking in lecithin and not apparent for triglycerides. None of these differences, however, precludes the possibility of a common precursor pool of diglycerides providing there is a highly specific mechanism for selecting diglycerides of a special type for lecithin synthesis. It is, for instance, possible that phosphoryl choline-glyceride transferase operates only on diglycerides whose fatty acid pattern satisfies the positional requirements of lecithin.

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