Fatty acid esterification and chylomicron formation during fat absorption in rat: III. Positional relations in triglycerides and lecithin

MALCOLM WHYTE,* DEWITT S. GOODMAN,‡ and ARTHUR KARMEN§

Laboratory of Metabolism and Laboratory of Technical Development, National Heart Institute, Bethesda, Maryland

SUMMARY Chylomicron triglyceride and lecithin obtained after feeding mixtures of three or four free fatty acids to rats were hydrolyzed using pancreatic lipase and phospholipase A respectively. The distribution of fatty acid mass and radioactivity in the substrate materials and in the cleavage products was determined by gas–liquid chromatography.

The incorporation of exogenous (labeled) fatty acids into different positions was nearly random in the triglycerides but markedly nonrandom in lecithin where saturated acids, especially stearic acid, were predominantly esterified at the α'-position and polyunsaturated fatty acids at the β-position.

Specific radioactivity measurements were interpreted as showing greater than random amounts of endogenous (unlabeled) palmitic acid on the α'-position of lecithin and, to a small extent, of endogenous linoleic acid on the β-position of triglyceride.

KEY WORDS chylomicron · fatty acids · exogenous (C^{14}) · endogenous · incorporation · triglyceride · lecithin · positional relations · rats

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* Visiting Scientist, National Heart Institute, 1961–62. Permanent address: Kanematsu Memorial Institute, Sydney Hospital, Sydney, Australia.

‡ Recipient of Career Scientist Award of the Health Research Council of the City of New York under Contract I-399. Present address: Department of Medicine, Columbia University College of Physicians and Surgeons, New York, New York.

§ Present address: Division of Nuclear Medicine, The Johns Hopkins Hospital, Baltimore, Maryland.

Reprints of this article should be requested from one of these addresses.

SINCE THE DISCOVERY THAT pancreatic lipase specifically attacks the α-position of triglycerides (1–3), it has been repeatedly demonstrated that the distribution of fatty acids on the α- and β-positions of most animal and vegetable triglycerides is not random (4–7). Saturated fatty acids are usually found esterified predominantly on the α-positions of triglycerides, and polyunsaturated fatty acids on the β-position. Notable exceptions include pig fat, which has the reverse relationship (4), and whale and seal blubber oil, which show a predominance of polyenoic acids on the α-position (8). Recent studies on fat absorption have shown that some asymmetry is usually present in lymph triglycerides formed during fat absorption and chylomicron formation (7, 9, 10). This is especially so when the fed fat includes triglyceride, because some is absorbed without being fully hydrolyzed; any original asymmetry is therefore partly retained and the opportunities for reesterification differ for the different sites. Even after free fatty acids have been fed slight asymmetry has been observed, with an excess of unsaturated fatty acids, especially linoleic, at the β-position, and saturated fatty acids, particularly stearic, at the α-positions (9). No consistent asymmetry has been reported for palmitic acid, and in fact Borgström (11) reported that dietary palmitic acid-C^{14} was randomly distributed in chylomicron triglyceride. Palmitic acid has sometimes been found in slight excess at the β-position (9, 10).

Lecithins from practically all sources, including human chylomicrons (12, 13), have been found to show a marked

TABLE 1 COMPOSITION OF THE DIETS AS DETERMINED BY GLC AND BY RADIOASSAY OF THE GLC EFFLUENTS

Diet	Total	% Distribution 16:0	18:0	18:1	18:2
	Mass				
	mg				
A	276	12.6	0.3	77.0	10.0
B	269	11.3	0.6	12.2	76.0
D	285	7.1	7.1	43.3	42.5
	Radioactivity				
	cpm × 10^{-6}				
A	17.31	40	0.3	32	28
B	17.61	40	0.4	30	30
D	28.60	26.6	29.2	24.9	19.4

concentration of saturated fatty acids on the α-position, and unsaturated, especially polyunsaturated, fatty acids on the β-position. Most of these studies have involved the use of phospholipase A, usually from snake venom, which is now known to hydrolyze the fatty acid from the β-position of lecithin (14–16).

We have recently reported studies showing that the processes involved in the formation of different chylomicron lipid esters in rats display different patterns of fatty acid specificity (17, 18). Thus, chylomicron triglyceride formation showed no specificity in the utilization of palmitic, oleic, or linoleic acids, and displayed a slight relative discrimination against stearic acid. In contrast, cholesterol ester formation showed a relative specificity for oleic acid, and lecithin formation a strong specificity for stearic and linoleic acids. In addition, a considerable portion of each chylomicron lipid was found to have originated from a source other than the diet, with the extent of this endogenous contribution differing from one lipid ester to another, and differing from one fatty acid to another within each lipid class.

The present paper reports a continuation of these studies, and deals with the positional relationships of fatty acids incorporated into chylomicron triglycerides and lecithin formed after the simultaneous ingestion of three or four labeled free fatty acids.

METHODS

Pure triglyceride and lecithin were obtained by further chromatography of the chylomicron lipid fractions of 4 rats used in studies described previously (17,18). The procedures which were employed to collect and isolate the several lipid fractions have been described in detail (17, 18). In brief, synthetic mixtures of free fatty acids containing trace quantities of C^{14}-labeled free 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 and separated on silicic acid columns into glyceride (G), cholesterol ester (E), and lecithin (L) fractions. Each lipid fraction was then analyzed for the distribution of fatty acid mass and radioactivity by gas–liquid chromatography.

The designations of the rats and diets used here are identical with those employed previously (17, 18), and refer to the same lymph collections. Table 1 describes the composition of the diets. Rats 3 and 4 had been previously maintained on Purina laboratory chow. Chyle was collected following test meals of mixtures of labeled palmitic, oleic, and linoleic acids with oleic acid as the major constituent in Experiment 3A (diet *A*) and linoleic acid in 4B (diet *B*). Each rat had had a different test meal 24 hr prior to the one under scrutiny here and there may have been some carry-over of fatty acid mass and radioactivity from the preceding diet. A small amount of labeled stearic acid was present as a contaminant in the test meals. Rats 5 and 6 had been maintained for 7 weeks prior to the experiment on a diet of Purina laboratory chow with additional 10% (by weight) of oil (corn:olive 3:1) and additional 1% of cholesterol. Their chylomicron collections were made following test feeding with a mixture of labeled palmitic, stearic, oleic, and linoleic acids in which the latter two fatty acids provided the main bulk (diet *D*). Neither rat had been given other test meals previously.

Preparation and Lipolysis of Triglycerides

The triglyceride (G) fractions used in some of the previous studies (17, 18) were rechromatographed on 5 g silicic acid columns in order to remove the small quantities of free fatty acids and partial glyceride present in the G fractions. Triglyceride (TG) fractions were obtained by elution with 75 ml of benzene–*n*-hexane 3:2 (v/v). Examination by thin-layer silicic acid chromatography showed that the material obtained was pure triglyceride. These TG samples contained 50–60% of the total radioactivity applied to the columns and present in the whole fractions. Comparison of the fatty acid patterns of the G and TG samples showed that the fatty acids of TG were, on the average, slightly more saturated than those of G; a somewhat more unsaturated fraction of glycerides must therefore have been retained on the column. It has previously been shown (17) that selective loss of microgram amounts of linoleic acid does not occur during silicic acid chromatography as performed here.

An emulsion of triglycerides was prepared, submitted to attack by pancreatic lipase (steapsin, Nutritional Biochemicals Corp, Cleveland, Ohio.) for 30 min at 37°, and the final products [free fatty acids (FFA), and remaining lipid (R)] were extracted and separated by

the methods described by Korn (7). It is known that this method does not give perfect separation of the outer from the inner fatty acid chains of triglycerides. Providing about 67% of the total fatty acids are hydrolyzed, however, the R fraction is composed predominantly of β-monoglyceride while α- and α′- fatty acids make up most of the FFA fraction. Thus, for example, Table 2 presents the results obtained with cocoa butter. The FFA released in this instance accounted for 75% of the total mass of fatty acids. The fatty acid composition of the original triglyceride and of the R fraction resembled the results obtained by Savary and Desnuelle (6), who examined only the isolated β-monoglyceride, and those of Mattson and Volpenhein (5), who isolated total monoglycerides. Comparison of these results suggests that our R fraction contained some fatty acids from the α-position, and the FFA fraction some from the β-position, but that the degree of cross-contamination (perhaps due to incomplete lipolysis of outer ester bonds, or to spontaneous isomerization of β- to α-esters, or both) was small and should not impair a qualitative and semiquantitative assessment of the results. It is not possible to give a precise estimate of the magnitude of error introduced by considering the FFA and R fractions as representative of the α- and β-fatty acids, respectively, but it is presumed to be small. Any error due to this cause would lead to underestimation of any nonrandom positional distribution of fatty acids.

Preparation and Lipolysis of Lecithin

The preparation and analysis of the lecithin (L) samples have been fully described (18). The L fractions were pure and contained more than 90% of the total lecithin content of the chylomicron lipid.

The enzymatic cleavage of lecithin was carried out by a modification of the method used by Long and Penny (19). Aliquots of the L fractions, containing about 1.0 mg of lecithin, were reduced to dryness in a centrifuge tube under a stream of nitrogen, dissolved in 0.2 ml of chloroform, and mixed with 1.8 ml of diethyl ether. A small amount (0.06 ml) of freshly prepared enzyme solution (phospholipase A from *Crotalus adamanteus*, obtained from Ross Allen Reptile Institute, Silver Springs, Fla.), of concentration 5 mg/ml in 0.01 M $CaCl_2$, was added. The tube was tightly stoppered, thoroughly mixed by shaking, and then allowed to stand in a water bath at 37° for about 1.5 hr, till no more precipitate formed. The lysolecithin (LyL) was removed by centrifugation and washed twice with diethyl ether. The lysolecithin and the free fatty acids (FFA) from the supernatant solution (plus washings of LyL ppt) were dried under nitrogen and dissolved in chloroform. Thin-layer silicic acid chromatography as in (18) showed that complete cleavage occurred and that the resulting products were pure. The recovery of mass and radioactivity was quantatitive.

Fatty Acid Analyses

Fatty acid methyl esters were prepared from aliquots of the original unhydrolyzed materials and the products of cleavage and were analyzed for total mass and radioactivity and for the distribution of mass and radioactivity among the several fatty acids, by the gas–liquid chromatographic procedures described previously (17, 18). Heptadecanoic acid was used as an internal mass standard. Radioactivity was always recovered quantitatively through methylation.

RESULTS

The distributions of mass and radioactivity among the fatty acids of the unhydrolyzed samples of triglyceride and lecithin and of their cleavage products are listed in Table 3. Ideally, 67% of the triglyceride fatty acid mass would be liberated by pancreatic lipase if hydrolysis of the α-positions were complete and the β-position were left intact. Calculations of mass in the products of Experiment 3A, achieved by triangulation of the GLC peaks and reference to the internal standard, showed that 69% of the total mass recovered in the two (FFA + R) fractions obtained after cleavage appeared as free fatty acids. The same FFA fraction contained 68% of the radioactivity. Corresponding figures for the FFA released from the other samples were: 4B, 67% of mass and 66%

TABLE 2 Analysis of Cocoa Butter and of the Two Fractions Obtained after Hydrolysis with Pancreatic Lipase

Results obtained by two other groups of workers are also shown, together with the composition of the α-fatty acids calculated from the observed composition of the original triglyceride and the monoglyceride fractions. The composition of the α-fatty acids in the present study has also been calculated by assuming that the R fraction was solely β-monoglyceride.

Fraction and Study	% Distribution			
	16:0	18:0	18:1	18:2
Triglyceride				
Present study	28	32	36	4
Savary and Desnuelle (6)	27	35	34	3
Mattson and Volpenhein (5)	27	33	35	2
"β"-fatty acids				
Present study (R fraction)	4	6	83	6
Savary (β-monoglyceride)	2	1	88	9
Mattson (total monoglyceride)	2	2	85	11
"α"-fatty acids				
Present study (FFA fraction)	35	44	19	2
Present study (calculated as TG minus R)	40	45	12	2
Savary (calculated as TG minus β)	40	52	7	1
Mattson (calculated as TG minus "β")	40	48	10	0

of radioactivity; 5D, 72% and 74%; 6D, 83% and 85%. The amounts of TG substrate used for hydrolysis were not measured precisely, because of uneven dispersion of the TG emulsions. Therefore, Table 3 lists only the distribution of mass and radioactivity for the unhydrolyzed substrates, and the total recovery of material could not be quantified.

Small discrepancies exist between the measured composition of R and the expected composition of the β-chains as calculated from the measurements made on TG and FFA (assumed to represent the α-chains). The measured values for palmitic and stearic acids were greater and for linoleic acid smaller than the calculated values. These discrepancies could have arisen, at least in part, from imperfect separation of outer and inner chains and from spontaneous isomerization. In addition, however, the stearic acid content of R plus FFA consistently exceeded what would have been expected from the original TG. The cause of this was not discovered.

The lecithin cleavage and the recovery of the products of cleavage appeared to be complete. Hydrolysis of sample 3A resulted in 51% of the fatty acid mass and 59% of the radioactivity appearing in the FFA fraction, which was presumed to have been derived from the β-position. Corresponding figures for the FFA liberated from the other samples were: 4B, 49% of mass and 40% of radioactivity; 5D, 48% and 26%; 6D, 47% and 28%. The composition of the FFA as measured closely resembled but was not identical with what would be expected from calculations based on the original lecithin and the LyL fraction. The discrepancies were most evident, though of doubtful significance, for palmitic acid, for which the measured values were consistently smaller than the calculated values: 6 vs. 8, 6 vs. 13, 4 vs. 8, and 5 vs. 6 respectively for the four experiments. For other acids the discrepancies were smaller and inconsistent. The reason for this finding is not clear.

Inspection of Table 3 reveals that the fatty acid patterns of the two fractions derived from TG closely resembled each other and that of their parent triglycerides, indicating a nearly random distribution of fatty acids between the α- and β-positions. This applied to each sample, even though three different dietary, and hence triglyceride, patterns were concerned. There was, however, some indication of positional differences in the arrangement of fatty acids, with stearic acid

TABLE 3 DISTRIBUTION OF MASS AND RADIOACTIVITY IN THE FATTY ACIDS OF CHYLOMICRON TRIGLYCERIDE AND LECITHIN, AND IN THE PRODUCTS OF THEIR ENZYMATIC CLEAVAGE

Rat and Diet*	Lipid Fraction†	Total	Mass % Distribution								Total	Radioactivity % Distribution			
			16:0	16:1	18:0	18:1	18:2	18:3	20:1	20:4		16:0	18:0	18:1	18:2
		mg									*10^{-3} × cmp*				
3A	TG	—‡	14	1	1	57	23	—	3	tr	—‡	42	2	32	24
	FFA	2.21	16	1	4	57	19	—	4	tr	93.30	42	2	35	20
	R	1.00	15	1	1	61	20	—	2	tr	43.90	43	1	33	23
	L	—	25	3	10	21	34	1	3	4	—	32	5	20	42
	LyL	0.352	42	3	18	23	8	tr	5	tr	11.95	45	6	30	18
	FFA	0.364	6	4	2	19	60	2	tr	8	17.08	15	—	8	76
4B	TG	—	19	1	3	28	44	—	6	tr	—	42	3	31	24
	FFA	5.27	19	1	5	28	41	—	6	—	205.03	42	2	33	23
	R	2.60	20	1	2	28	45	—	4	tr	105.62	46	3	27	23
	L	—	32	1	11	10	40	tr	2	4	—	31	5	17	46
	LyL	0.374	51	1	19	8	16	tr	2	2	12.38	33	8	19	39
	FFA	0.361	6	1	3	8	71	1	tr	10	8.06	27	—	8	65
5D	TG	—	16	1	9	40	29	—	3	2	—	27	30	30	13
	FFA	2.370	16	1	12	41	26	—	4	1	145.00	25	33	30	12
	R	0.925	19	1	7	37	32	—	2	2	51.00	32	26	27	15
	L	—	27	1	18	8	35	tr	1	10	—	14	53	12	21
	LyL	0.766	46	1	32	11	3	—	1	1	30.30	13	69	12	6
	FFA	0.712	4	tr	1	6	72	1	—	17	12.10	25	7	11	57
6D	TG	—	15	1	8	42	32	—	2	1	—	29	30	26	15
	FFA	1.876	15	1	10	43	29	—	2	tr	118.54	26	31	27	16
	R	0.372	18	1	5	36	35	2	—	2	21.30	40	17	28	14
	L	—	25	1	20	9	39	tr	tr	7	—	11	59	11	19
	LyL	0.682	44	1	35	11	8	—	1	—	31.20	9	73	14	6
	FFA	0.600	5	1	2	7	69	1	—	15	11.20	35	3	5	57

* See previous reports (17, 18) for composition of the diets and other details.

† TG = triglyceride; FFA = free fatty acids (mostly from α-position of TG or from β-position of L); R = remaining partial glycerides (β-monoglyceride predominantly); L = lecithin; LyL = lysolecithin.

‡ Only the distributions of mass and radioactivity are listed for the unhydrolyzed substrates.

TABLE 4 ESTIMATED PERCENTAGE OF THE MASS OF EACH FATTY ACID CARRIED IN THE β-POSITION

A random distribution of any given fatty acid would result in 33% on the β-position of TG and 50% on the β-position of L.

Experiment	% Fatty Acid					
	16:0	16:1	18:0	18:1	18:2	20:4
Triglycerides						
3A	32	37	13	35	34	—*
4B	34	30	19	33	35	—
5D	38	33	22	31	38	—
6D	38	37	20	30	38	—
Lecithin						
3A	13	53	10	44	88	100
4B	10	46	14	50	81	87
5D	8	27	2	35	90	94
6D	11	40	6	39	89	100

* Accurate values for the distribution of 20:4 in TG were not obtained because of the relatively small amounts of 20:4 present.

being more abundant in the FFA fraction (mostly α-chains) and linoleic acid slightly in excess in the R fractions (mostly β) in every instance. Arachidonic acid, when present, was concentrated on the β-position. Slightly more palmitic acid was present in the β-position in the samples produced after diet *D* (which contained stearic acid). These features are more clearly seen in Table 4, which gives estimates of the percentage of the mass of each fatty acid found in the β-position. As the calculations have been based on the assumption that the FFA released by pancreatic lipase have come solely from the α-positions and that the remaining glyceride fragment is β-monoglyceride (and that isomerization has not occurred), any nonrandom distribution will be underestimated, as explained previously. Any departure from a figure of 33% suggests a nonrandom distribution.

In contrast to the triglycerides, lecithin showed extreme nonrandomness in composition and a striking consistency of pattern in spite of marked differences in the diets. The distribution of each fatty acid between the two positions on the lecithin molecule is summarized in Table 4, assuming that LyL and FFA represent α- and β-chains respectively. Any significant departure from the figure of 50% (percentage of the fatty acid mass esterified at the β-position) indicates a nonrandom distribution. Saturated fatty acids and polyunsaturated fatty acids were almost exclusively situated on the α- and β-positions respectively, while the monoenes were more evenly distributed.

The relative contributions of freshly absorbed dietary fatty acid and preexisting endogenous fatty acid were assessed for each of the four major fatty acids in each position of the triglyceride and lecithin samples by reference to the radioactivity data. The distribution of radioactivity in triglycerides (Table 3) was almost the same in both derived fractions, with only small differences which were similar to the mass differences noted earlier. Further detailed analysis of samples 3A and 4B was considered inadvisable because of possible confusion in interpretation due to carry-over of radioactive material from preceding test meals (see 17, 18). No such difficulties existed with samples 5D and 6D. The estimated amounts of exogenous and endogenous fatty acids contributing to the total fatty acid content of the different fractions, presumed to represent the different positions of the triglycerides, of 5D and 6D were very similar and have been averaged to give the results illustrated in Fig. 1. The general similarity of the α- and β-patterns is apparent. In addition, the relationships between exogenous and endogenous components apparently differed slightly in the two positions. This is indicated in Table 5, where the fatty acid distribution between the α- and β-positions is considered on the basis of a simple α,β-diglyceride. In triglycerides, stearic acid from both exogenous and endogenous sources seemed to be present to a lesser extent on the β- than on the α-position. Exogenous linoleic acid, and oleic acid from both sources, were present in approximately equal amounts in the two positions, but endogenous linoleic acid was more abundant on the β-position.

The radioactivity in lecithin was distributed nonrandomly in somewhat similar fashion to the mass pattern, except for palmitic acid (Table 3). Whereas only about 10% of the palmitic acid mass was present in the β-position, 25–80% of the palmitic acid-C^{14} was found in that position. The estimated exogenous and endogenous contributions to the over-all fatty acid

TABLE 5 PERCENTAGE OF EACH EXOGENOUS AND ENDOGENOUS FATTY ACID FOUND IN THE β-POSITION OF THE α,β-DIGLYCERIDE PORTION OF TRIGLYCERIDES AND LECITHINS AFTER DIET D

This calculation was performed for the triglycerides by assuming that the α- and α′-positions contained identical fatty acid distributions. The values listed are the average values for samples 5D and 6D. A random distribution of a fatty acid would result in 50% in the β-position.

Source of Fatty Acid	% of Diglyceride Fatty Acid in β-Position			
	16:0	18:0	18:1	18:2
Triglyceride				
Exogenous + endogenous (total mass)	55	35	46	55
Exogenous only (C^{14})	55	38	46	48
Endogenous (calculated)	55	32	47	65
Lecithin				
Exogenous + endogenous (total mass)	9	4	37	89
Exogenous only (C^{14})	56	3	20	80
Endogenous (calculated)	7	4	77	94

patterns of each position are illustrated in Fig. 1. The relative preference for saturated fatty acids in the α'-position, and the even greater preference for polyunsaturated fatty acid in the β-position are clearly seen. The β-position, but obviously not the α'-position, displayed the same fatty acid patterns for acids from both exogenous and endogenous sources. The contrast, emphasized in Table 5, was most striking with palmitic acid.

It should be noted that even though the various fractions produced by hydrolysis of TG and L might not have been composed exclusively of fatty acids from either the α- or β-positions, the assessment of exogenous–endogenous fatty acid relationship within each fraction should be valid.

DISCUSSION

The results of this study demonstrate the dramatic difference in the positional relationships of fatty acids in chylomicron triglycerides and lecithin. The arrangement in triglyceride was thought to be nearly random, with some preponderance of stearic acid in the α-position and of polyunsaturated fatty acids in the β-position. This applied even when different diets were used, though minor variations suggested that when stearic acid was freely available (diet *D*) it was preferred to palmitic acid for the outer chains, and that more palmitic acid was then found in the β-position. These findings agree with other reported observations (7, 9, 10). As already discussed, technical imperfections would probably lead to underestimation of the extent of positional asymmetry present. The observed positional relationships in triglycerides applied equally to newly absorbed, exogenous (labeled) fatty acids and to endogenously derived fatty acids, except for linoleic acid. Exogenous linoleic acid appeared to be distributed equally between the α- and β-positions but endogenous linoleic acid was present in greater than random amounts on the β-position.

Lecithin displayed extreme positional asymmetry, with saturated acids, especially stearic, predominantly esterified at the α'-position and polyunsaturated fatty acids at the β-position. These findings thus agree with the reported asymmetric incorporation of stearic and linoleic acids into human chylomicron lecithin (13). Both exogenous and endogenous fatty acids showed similar patterns in the β-position, suggesting that the β-fatty acids were largely derived from a single mixed pool. In contrast, the exogenous and endogenous fatty acids formed quite different patterns in the outer chains, as can be clearly seen in Fig. 1 and Table 5. A disproportionately large amount of endogenous palmitic acid was present in the α'-position. The exogenous pattern suggests that there was a less specific incorporation of available fatty acids into the α'- than into the β-position. This exogenous α'-pattern resembles that of the available dietary fatty acids (as seen in the triglycerides), but as distorted by specific selection of stearic acid, by deflection of polyunsaturated acids to the highly selective β-position, and possibly by the restricted number of available sites due to prior occupation by endogenous palmitic acid. For these reasons the incorporation of labeled (exogenous) palmitic acid into the two positions could vary with experimental circumstances and might explain why Hanahan and Blomstrand (20) found a random distribution in the lecithin of rat chylomicrons.

The fact that the triglyceride and lecithin patterns were so different raises the question as to whether or not their formation in the intestine involves a common biosynthetic pathway (21, 24). It is, of course, possible that entirely separate pathways exist, each with its own complexities, and with much more highly specific enzyme systems in the lecithin system than in the triglyceride system. Rhodes (23) adopted this interpretation of similar observations made in a study of the composi-

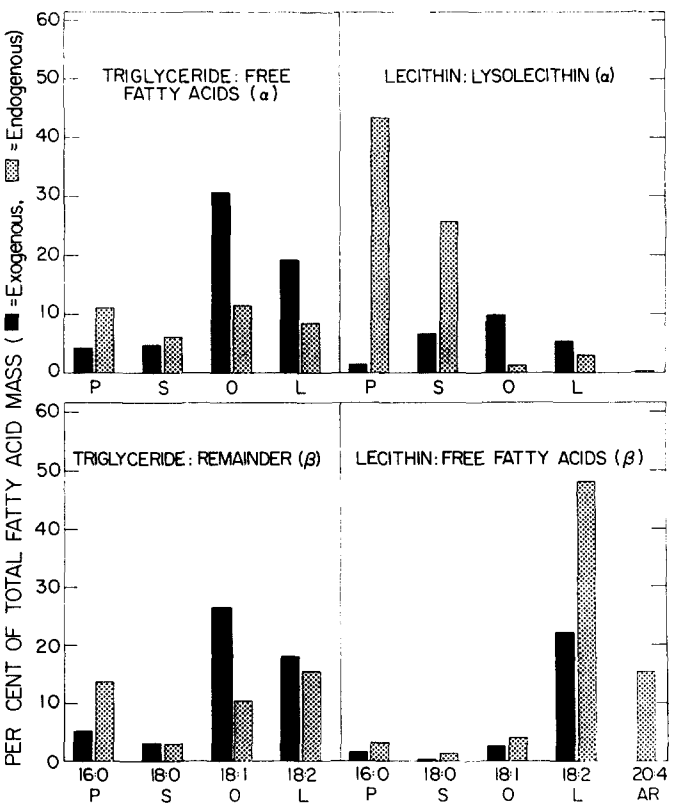

FIG. 1. Estimated distribution of exogenous and endogenous fatty acids on the α- and β-positions of chylomicron triglyceride and lecithin. The values shown are the average results from two collections with different rats after Diet *D*, and are expressed so that the sum of all fatty acids (exogenous + endogenous) in each position is 100%.

tion of hens' eggs. Lands (22) suggested the possibility of an inhomogeneous pool of diglycerides being formed (in lung tissue) by two independent paths, one involving the direct esterification of glycerol and the other proceeding first through α-glycerophosphate. It was then postulated that some of the products of the first path were not available for lecithin synthesis. Savary and Desnuelle (6) suggested that the asymmetry in lecithin appeared during acylation of α-glycerophosphate by the actions of two enzymes, one acting close to the phosphate radical and having a very strict specificity for unsaturated chains, and the other, probably less specific enzyme acting on the external chain. Rossiter and Strickland (25) put forward the same opinion.

In this regard it should be noted that several different pathways for diglyceride formation have been demonstrated in small intestinal mucosa. One pathway involves the sequence L-α-glycerophosphate → phosphatidic acid → diglyceride (26). A different pathway, involving the direct acylation of monoglyceride, has been described (27, 28). Significant incorporation of free glycerol into higher glycerides has also been reported (29).

The present findings are, however, not incompatible with the possibility that the triglyceride and lecithin in chylomicrons arise from a common D-α,β-diglyceride pool, as has been critically discussed by Weiss, Kennedy, and Kiyasu (24). Nevertheless, three qualifications would be necessary to bring this thesis into accord with the observations made in the present study. First, some enzymatic specificity must be postulated to operate in the synthesis of the diglyceride pool, so that there is some selective attachment of polyunsaturated fatty acids at the β-position and stearic acid at the α-position. These features were noticeable in both the triglycerides (to a small extent) and lecithins. Second, there would have to be a highly specific mechanism, possibly involving phosphorylcholine–glyceride transferase, for selecting for lecithin synthesis those diglyceride molecules which have the special configuration of β-polyunsaturated and α-saturated fatty acids. This has been suggested by others (21, 24), but experimental proof is lacking and technical difficulties apparently hinder definitive examination of the problem (24). Third, it would be necessary to postulate that not all of the triglyceride and lecithin present in chylomicrons had been synthesized de novo in the intestinal mucosa but that some previously formed or partially formed endogenous molecules had been added. This assumption is necessary to account for the observed discrepancies between exogenous and endogenous fatty acid patterns found in the different chains (Table 5 and Fig. 1). The preformed triglyceride precursors would differ from the newly synthesized triglycerides in having a slight excess of unlabeled, endogenous linoleic acid in the β-position (or whatever is the true source of the R fractions produced by the method of lipolysis used in this study). The characteristic feature of the preformed lecithin precursor would be endogenous palmitic acid in the α'-position. It can be calculated that the features observed in the triglycerides could be accounted for if about 5–10% of the molecules entering the TG-synthesizing pool already contained endogenous linoleic acid in the β-position. To account for the lecithin findings in this way about 40% of the lecithin precursor molecules would have had to enter the acylating pool already containing endogenous palmitic acid in the α'-position. Hypotheses as to the source of these partially formed endogenous precursors would be purely conjectural. Possibilities include cellular and plasma contributions, the direct absorption of endogenous lysolecithin (30, 31) containing palmitic acid in the α'-position, and a similar absorption of some endogenous β-monolinolein. These endogenous materials could arise from incomplete intraluminal hydrolysis of phospholipids and triglycerides derived from bile and from other alimentary secretions.

In any event, it is clear that not only are the positional relationships of chylomicron lecithin different from those of triglyceride, but that the positional relationships of the corresponding endogenous and exogenous fatty acids also differ significantly.

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