Structure of Bovine Milk Fat Triglycerides: II. Long Chain Lengths[1]

W. C. BRECKENRIDGE and **A. KUKSIS**, Banting and Best Department of Medical Research and the Department of Biochemistry, University of Toronto, Toronto, Canada

ABSTRACT

The long chain triglycerides of bovine milk fat were isolated by thin layer chromatography, and their chemical structure determined by combined thin layer and gas liquid chromatography, and a stereospecific analysis of a molecular distillate of butteroil of comparable composition. The milk fat fraction (39% of total) contained C_8-C_{20} fatty acids which were distributed among the glycerides of 40-56 acyl carbon atoms in a manner not unlike that found for the same acids in the short chain triglycerides. Although individual triglycerides were not identified, the specific distribution of the fatty acids could best be accounted for by assuming a common pool of long chain 1,2-diglyceride precursors from which the bulk of both short and long chain triglycerides are synthesized by a stereospecific introduction of C_4-C_{18} fatty acids in position 3 of sn-glycerol. This hypothesis is compatible with the results of stereospecific analyses of the short and long chain fractions and of the total butteroil. It is supported by the nonrandom distributions demonstrated for the molecular weights of the milk fat triglycerides of different degrees of saturation.

INTRODUCTION

Both plasma lipids and de novo synthesis in the udder are known to contribute fatty acids to the synthesis of milk fat (1). Previous studies (2) would appear to exclude the possibility that the plasma triglycerides are incorporated intact into the milk fat. Precursor triglycerides must therefore be broken down at least partially to allow for a reesterification with fatty acids of both short and long chain lengths (3). Since the identification of the partial glyceride precursors might yield information about the mechanism of milk fat assembly, there has been much speculation regarding the potential intermediates (4-6). The experimental findings have been limited to a demonstration of specific association (7,8) and positional placement (9,10) of fatty acids in the short chain triglycerides, and a general non-randomness in the molecular weight distribution of the triglycerides in milk fat (11).

In the present study a detailed investigation has been made of the molecular association and positional and overall distribution of fatty acids in the long chain triglycerides. The data have been compared to the results of similar earlier analyses of the short chain triglyceride fraction and appropriate inferences have been made.

MATERIALS AND METHODS

The chemical reagents, solvents, chromatographic materials and analytical standards were as described (8,10). The long chain triglyceride fractions of milk fat were as obtained (8) during the isolation of the short and medium chain length triglycerides. Another long chain triglyceride fraction of milk fat was obtained as a residue by molecular distillation of butteroil (12). This material (D-3; 50% of total butteroil) was used for the stereospecific analyses.

Thin Layer Chromatography

The long chain triglycerides were isolated as outlined for the short and medium chain lengths (8). Plates of Adsorbosil-3 (20×20 cm, 250μ thick) were prepared by standard methods, and 5-10 mg of milk fat applied per plate as a band. The bands were developed in heptane-isopropyl ether-glacial acetic acid (60:40:4 v/v/v) and the lipid bands located by spraying with dichlorofluorescein. The triglycerides were recovered by elution with 5% methanol in diethyl ether. The long chain triglycerides were resolved on the basis of degree of unsaturation using similar plates made up of silica gel G containing 10% silver nitrate (8). About 5-10 mg of triglyceride was applied per plate and the plate developed with 0.65% methanol-chloroform (v/v). The bands were located and the lipids recovered as above.

Gas Liquid Chromatography

Triglycerides were analyzed on Aerograph 204-1B Dual Channel Gas Chromatograph (Varian-Aerograph, Walnut Creek, California). The instrument was equipped with dual col-

[1]Presented in part at the AOCS meeting, Philadelphia, October, 1966.

umns (stainless steel tubes, 2 ft x ⅛ in. OD, packed with 2% JXR on Gas Chrom. Q, 100-120 mesh). The columns were conditioned and operated as previously described (11) The chromatographic system was calibrated with mixtures of standard triglycerides, tributyrin through tristearin.

The fatty acids were determined by GLC of their butyl esters (1) using an F & M Model 402 Gas Chromatograph (F & M Scientific Corporation, Avondale, Pennsylvania) equipped with dual glass columns (4 ft x ¼ in. OD) containing 15% diethylene glycol succinate (DEGS) on 60-80 mesh Gas Chrom. P. Samples containing C_4-C_{18} fatty acids were determined by temperature programming from 70-220 C at 4 C/min. Long chain fatty acids were determined isothermally at 200 C. The instrument was calibrated with mixtures of standard butyl esters prepared by transbutylation of known amounts of high purity triglycerides (tributyrin through tristearin).

Values obtained from duplicate analyses of both standard triglycerides and fatty acid butyl esters showed a relative error of less than 3% for any peak comprising more than 10% of the sample, and less than 6% for any peak comprising less than 10% of the sample.

Stereospecific Analyses

The stereospecific examination of the long chain triglycerides of butteroil was performed essentially as described by Breckenridge (10), except that proportionally smaller amounts of material were used. The mixed 1,2- and 2,3-diglycerides were prepared from 1 g of fat. The lipolysis was stopped when approximately 50% of the total triglyceride had been hydrolyzed (4 min). The diglycerides were recovered by extraction with diethyl ether following dilution of the reaction mixture with methanol. They were purified by TLC on Adsorbosil-3 using the above described solvent system. About 40 mg of the phosphatidyl phenol was used for hydrolysis with phospholipase A.

RESULTS AND DISCUSSION

Preliminary Resolution

The resolution of milk fat triglycerides into short (SCT), medium (MCT) and long (LCT) chain triglycerides by TLC on plain silica gel was described in an earlier communication from our laboratory (8), as were the further analyses of the SCT and MCT fractions. The preparations of LCT obtained by this method contained triglycerides with 40-56 acyl carbons per molecule, and made up 38.7% and 39.8% of the total milk fat triglyceride. It should be noted that the LCT fraction contains several triglyceride groups with the same carbon numbers as the SCT and MCT fractions, but that these triglycerides do not possess identical fatty acid compositions. Thus in the SCT the C_{40} glycerides are largely comprised of 18,18,4, whereas in the LCT these must have been made up of combinations such as 16,14,10 or 16,18,8 since no butyric or caproic acids are present. The MCT contains both types of C_{40} triglycerides in appreciable amounts. This resolution of triglycerides within a molecular weight is due to differences in the polarity of these compounds and must have been operative also during the TLC of the C_{42}-C_{46} triglycerides which are represented in significant amounts in both MCT and LCT.

The preliminary segregation of butteroil triglycerides by molecular distillation is characterized by a separation based on differences in molecular weight only (12). Hence the triglycerides of corresponding carbon number in the various molecular distillates would be expected to contain identical fatty acid compositions. Therefore, while the overall triglyceride distributions were comparable, the distillation residue contained significant amounts of butyric and caproic acids, in contrast to the two preparations of LCT made by TLC. A removal of the more polar short chain triglycerides from the distillation residue prior to the stereospecific analysis was not necessary.

Combined TLC-GLC Analysis of LCT

Resolution of the long chain triglycerides on thin layers of silicic acid impregnated with silver nitrate gave a pattern more complex than anticipated for the relatively simple triglyceride mixture. Two bands were obtained for the monoenes and the dienes with two monoenoic and one saturated fatty acid per molecule. This was due to the presence of small amounts of elaidic acid in these primarily oleic acid-containing glycerides. Since oleic and elaidic acids are not readily resolved by the GLC of their butyl esters the *trans*-monoenes and the *trans*-dienes were pooled with the corresponding *cis*-isomers for the presentation of data. No differences were noted between oleic and elaidic acids in their association with other fatty acids in the glyceride molecules.

The GLC patterns of the triglycerides of the

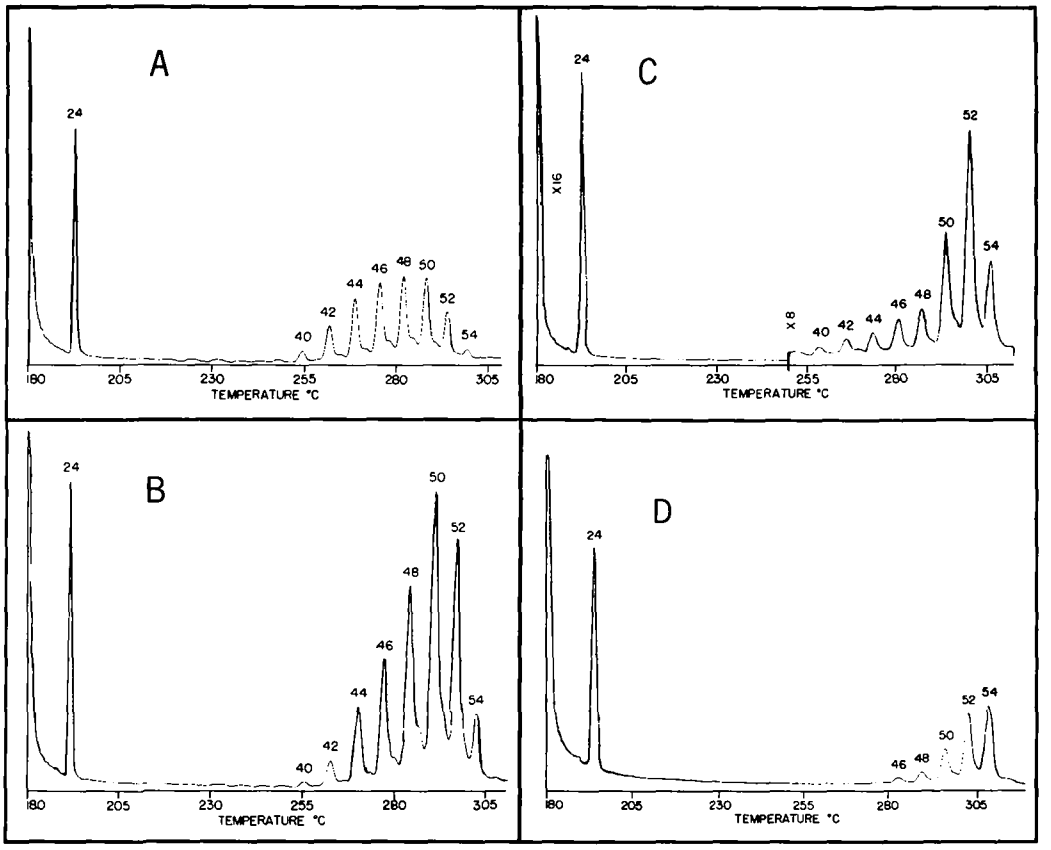

FIG. 1. GLC of long chain triglycerides of various degrees of unsaturation; A, saturates; B, monoenes containing one *cis*-monounsaturated acid; C, dienes containing two *cis*-monounsaturated acids; D, trienes containing three monounsaturated acids and dienes containing one linoleic acid residue. Temperature program as shown. Other operating conditions as stated in Ref. 8. Peak 24, trioctanoin used as internal standard.

saturates, monoenes, dienes and trienes are shown in Figure 1. As noted previously with the short and medium chain fractions (8), segregation of the saturated and unsaturated triglycerides by argentation TLC greatly improves the GLC resolution of adjacent even and odd carbon number peaks. It allowed a better estimate of the triglycerides of odd carbon number than was possible by GLC of the total milk fat triglycerides.

The composition of the triglycerides and the fatty acids of the various classes of saturation of the long chain fractions are shown in Tables I and II. In the saturates, which comprise 16.5% of the LCT, the major fatty acids are myristic (17.7%), palmitic (39.4%) and stearic (25.4%), while the major triglyceride types are C_{44} (13.9%), C_{46} (17.2%), C_{48} (16.4%) and C_{50} (15.6%). This suggests a broad range of triglycerides involving all the combinations of the saturated fatty acids in the sample. A small amount (1.4%) of tristearin is also present. In the monoenes (36.7% of the fraction) the major acids are palmitic (30.6%), stearic (17.3%) and oleic (30.6%), while the major triglycerides are C_{48} (17.6%), C_{50} (25.3%) and C_{52} (18.4%). The diene band (27.7%) made up of two monoenoic and one saturated fatty acid contained palmitic (16.0%) and oleic (59.3%) as major acids and C_{52} (37.1%) as major triglyceride. This suggests the occurrence of the 16,18:1, 18:1 triglyceride. The dienes made up of two saturated acids and linoleic acid migrated with the trienes comprised of three monoenoic acids and together accounted for 12.9% of the long chain fraction. The major acids were palmitic (18.5%), oleic 45.8%) and linoleic (13.2%), while the major glycerides were C_{52} (31.5%) and C_{54}

TABLE I
Composition of Long Chain Triglycerides of Bovine Milk
(moles %)

	Saturates[b]		Monoenes[b]		Dienes[b]		Trienes[b]		Polyenes[b]		Recovery[c]	Original[d]
TG[a]	Band	Total	Band	Total	Band	Total	Band	Total	Band	Total	Total	Total
38	0.3	—	—	—	—	—	—	—	—	—	—	—
40	3.0	0.5	0.4	0.2	0.7	0.2	—	—	—	—	0.9	1.0
41	0.4	0.1	—	—	—	—	—	—	—	—	0.1	—
42	8.3	1.4	2.3	0.8	1.5	0.4	—	—	—	—	2.6	3.0
43	1.8	0.3	0.6	0.2	—	—	—	—	—	—	0.5	0.5
44	13.9	2.3	6.8	2.5	2.6	0.7	0.7	0.1	—	—	5.6	5.9
45	3.5	0.6	1.1	0.4	—	—	—	—	—	—	1.0	0.7
46	17.2	2.8	11.1	4.1	5.6	1.5	3.5	0.5	2.4	0.2	9.1	9.3
47	4.0	0.7	2.0	0.7	0.4	0.1	—	—	—	—	1.5	1.2
48	16.4	2.7	17.6	6.4	7.8	2.2	8.1	1.0	5.6	0.4	12.7	13.5
49	3.4	0.6	3.5	1.3	1.7	0.5	—	—	—	—	2.4	2.0
50	15.6	2.6	25.3	9.3	19.9	5.5	17.7	2.3	15.2	0.9	20.6	21.5
51	2.6	0.4	4.0	1.5	4.4	1.2	—	—	—	—	3.1	2.2
52	8.0	1.3	18.4	6.8	37.1	10.3	31.5	4.0	26.7	1.6	24.0	24.5
53	0.2	—	1.8	0.7	2.2	0.7	—	—	—	—	1.4	1.0
54	1.4	0.2	5.0	1.8	15.6	4.3	36.2	4.7	41.6	2.6	13.6	13.0
56	—	—	—	—	0.6	0.1	2.1	0.3	7.1	0.4	0.8	0.7
58	—	—	—	—	—	—	—	—	—	0.1	0.1	—
	100.0	16.5	100.0	36.7	100.0	27.7	100.0	12.9	100.0	6.2	100.0	100.0

[a] Triglycerides identified by total number of acyl carbon atoms.

[b] Saturates, monoenes, dienes, trienes and polyenes are triglyceride types containing 0, 1, 2, 3, >3 double bonds per triglyceride molecule.

[c] Values obtained by proportional summation of the triglyceride types differing in degree of saturation.

[d] Values obtained for the sample before fractionation.

(36.2%). The polyenes (6.3%) contained oleic, linoleic and linolenic acids in various combinations with each other and with one saturated fatty acid to give largely C_{52} (26.7%) and C_{54} (41.6%) triglycerides. Tables I and II also compare the values reconstituted for

TABLE II
Fatty Acid Composition of Long Chain Triglycerides of Bovine Milk
(moles %)

	Saturates[b]		Monoenes[b]		Dienes[b]		Trienes[b]		Polyenes[b]		Recovery[c]	Original[d]
FA[a]	Band	Total	Band	Total	Band	Total	Band	Total	Band	Total	Total	Total
8:0	1.3	0.2	—	—	—	—	—	—	—	—	0.2	0.2
10:0	4.8	0.8	2.0	0.7	1.4	0.4	—	—	—	—	1.9	2.3
12:0	5.9	1.0	3.0	1.1	2.1	0.6	0.7	0.1	2.4	0.2	3.0	2.7
14:0	17.7	2.9	10.0	3.7	6.5	1.8	4.6	0.6	9.3	0.6	9.6	9.8
14:1	—	—	—	—	2.1	0.6	0.8	0.1	1.1	0.1	0.8	0.8
15:0[e]	3.1	0.5	1.3	0.5	0.5	0.2	—	—	—	—	1.2	1.0
16:0	39.4	6.5	30.6	11.2	16.0	4.4	18.5	2.4	19.7	1.2	25.7	24.4
16:1	—	—	2.8	1.0	5.2	1.4	5.4	0.7	3.6	0.2	3.3	3.2
17:0[e]	2.4	0.4	2.4	0.9	1.5	0.4	1.6	0.2	0.7	—	1.9	1.8
18:0	25.4	4.2	17.3	6.4	5.4	1.5	7.1	0.9	8.0	0.5	13.5	15.7
18:1	—	—	30.6	11.2	59.3	16.4	45.8	5.9	32.0	2.0	35.5	35.4
18:2	—	—	—	—	—	—	13.2	1.7	—	—	1.7	1.5
18:3	—	—	—	—	—	—	—	—	11.5	0.7	0.7	0.6
20:2	—	—	—	—	Trace	Trace	2.3	0.3	9.4	0.6	0.9	0.6
20:3	—	—	—	—	—	—	—	—	1.5	0.1	0.1	Trace
20:4	—	—	—	—	—	—	—	—	0.8	—	—	—
	100.0	16.5	100.0	36.7	100.0	27.7	100.0	12.9	100.0	6.2	100.00	100.00

[a] Fatty acids identified by the number of carbon atoms in the fatty acid residue and number of double bonds.

[b] Saturates, monoenes, dienes, trienes and polyenes are triglyceride types with 0, 1, 2, 3, >3 double bonds per glyceride molecule.

[c] Values obtained by proportional summation of the fatty acid composition of the triglyceride types differing in degree of saturation.

[d] Values obtained for the fatty acid composition of the sample before fractionation.

[e] Consists of normal and iso-branched acids.

TABLE III

Bovine Milk Fat Composition (moles %)

Triglycerides			Fatty Acids		
Carbon No.[a]	Recovery[b]	Original[c]	Carbon No.[d]	Recovery[b]	Original[c]
26	0.1	0.3	4:0	9.4	9.8
28	0.6	1.2	6:0	4.6	4.9
30	1.2	1.5	8:0	1.8	2.2
31	0.1	Trace	10:0	3.2	4.0
32	2.6	2.6	12:0	3.6	3.4
33	0.2	0.1	14:0	10.1	10.2
34	5.3	5.6	14:1	0.4	Trace
35	0.8	0.4	15:0[f]	1.4	1.4
36	10.4	11.3	16:0	23.7	23.4
37	1.3	0.6	16:1	2.7	1.7
38	14.4	15.5	UNK[g]	0.1	—
39	1.0	0.5	17:0[f]	1.3	1.5
40	12.1	11.8	18:0	10.0	12.5
41	0.6	N.D.[e]	18:1	24.5	22.4
42	6.6	6.7	18:2	2.2	1.6
43	0.4	N.D.[e]	18:3	0.6	1.0
44	4.9	4.8	20:2	0.4	Trace
45	0.5	N.D.[e]			
46	4.7	5.0			
47	0.6	N.D.[e]			
48	5.5	6.4			
49	0.9	N.D.[e]			
50	8.3	9.7			
51	1.2	N.D.[e]			
52	9.5	10.2			
53	0.5	N.D.[e]			
54	5.4	5.8			
56	0.3	Trace			

[a] Triglycerides identified by total number of acyl carbon atoms.

[b] Values obtained by proportional summation of the components after fractionation of the sample on thin layers of silicic acid impregnated with silver nitrate.

[c] Values obtained by an analysis of the sample before fractionation.

[d] Fatty acids identified by total number of carbon atoms and number of double bonds.

[e] Not determined.

[f] Consists of normal and iso-branched acids.

[g] Identity not determined.

the triglycerides and fatty acids from the subfractions with the results of the analyses of the original LCT fraction. The good agreement between these data indicates that the chromatographic fractionations were not accompanied by extensive losses of any specific molecular species.

Comparable compositions of fatty acids for the long chain triglycerides of butteroil have been reported by Blank and Privett (13). They found approximately the same amount of fully saturated glycerides with nearly identical fatty acid composition. While the fatty acid complements of the monoenes and dienes were also quite similar, the proportions of the two unsaturation classes were different. According to Blank and Privett (13) the monoenes were much higher (57% vs. 36.7%) the dienes slightly lower and the trienes and polyenes

TABLE IV

Triglyceride Types of Bovine Milk Fat (moles %)

Types	Experimental	Random
Saturates	32.9	39.0
Monoenes	37.6	38.7
Dienes		
SMM[a]	16.4	12.7
SSD[b]	5.3	2.7
Trienes	5.4	5.0
Polyenes	2.4	1.9

[a] Triglycerides containing one saturated and two monoenoic fatty acids.

[b] Triglycerides containing two saturated and one dienoic fatty acid.

present only in trace amounts. Furthermore, the ratios of myristic, palmitic and stearic acids are very similar throughout all the classes of unsaturation in both studies, but the total amount of the saturated acids progressively decreases with increasing amounts of the unsaturated acids. These relationships suggest that the fatty acid combinations within each triglyceride group may remain relatively constant but that the overall amount of each unsaturation class may vary with the milk fat preparation.

Table III shows the reconstitutions of the triglycerides and fatty acids from the SCT, MCT and LCT fractions of bovine milk fat. These values compare very well with the results of the overall analyses of the original milk fat, and should therefore represent a valid description of the detailed distribution of the fatty acids in milk fat. The relative error of these estimates is ± 5% or less. Table IV compares the experimental and the random estimates of the various triglyceride types grouped on the basis of uniform degree of unsaturation. Although the dienes and trienes were not always cleanly resolved, reliable estimates of their proportions could be obtained from the fatty acid composition of the overlapping portions of the thin layer bands. While the amount of the saturates is slightly lower than the random value, and that of the dienes slightly higher, the estimates for the monoenes, trienes and polyenes approximate the values predicted by random distribution. Clearly, analyses at this low level of fractionation do not indicate any high degree of preference for the formation of any specific triglyceride types.

Table V compares the experimental and the random distributions of the triglycerides by molecular weight in the various saturation classes. For purposes of simplified presentation the values for the dienes, trienes and polyenes were combined. It can now be seen

TABLE V
Triglyceride Distribution of Bovine Milk Fat (moles %)

	Saturates		Monoenes		Combined dienes, trienes, and polyenes	
TG[a]	Experimental	Random[b]	Experimental	Random[b]	Experimental	Random[b]
<26	....	3.0	....	....	....	....
26	0.4	3.5	....	1.6	....	....
28	1.7	3.3	0.1	1.7	....	....
30	3.1	3.0	0.6	1.3	....	....
31	0.2	0.2	....	....	....	....
32	5.4	4.3	1.8	1.7	0.3	0.5
33	0.5	0.4	....	....	....	....
34	11.8	6.0	3.2	2.1	0.7	0.9
35	1.9	0.7	0.3	....	....	....
36	19.8	11.3	8.9	5.2	1.7	0.9
37	2.2	1.2	1.5	0.5	....	....
38	16.6	10.9	19.4	10.9	5.4	3.2
39	1.2	0.9	1.5	0.8	0.3	....
40	9.3	6.9	13.4	9.8	13.6	10.9
41	0.8	0.7	1.0	0.3	....	....
42	5.7	7.0	6.5	6.2	7.5	5.9
43	0.6	0.7	0.6	0.3	....	....
44	4.2	4.1	5.2	5.7	5.1	3.6
45	0.8	1.0	0.6	0.5	0.3	....
46	3.8	5.3	5.2	8.0	4.8	5.0
47	0.8	1.5	0.9	1.0	....	0.5
48	3.2	8.2	7.1	10.9	5.6	6.4
49	0.7	1.5	1.4	1.8	0.7	0.5
50	3.0	5.2	9.7	14.5	12.6	14.5
51	0.5	1.0	1.5	1.8	2.2	1.8
52	1.5	2.8	7.0	10.3	21.8	25.4
53	....	0.2	0.7	0.5	0.7	1.4
54	0.3	0.5	1.9	2.6	15.3	18.6
56	....	....	....	....	1.7	....

[a] Triglycerides identified by number of acyl carbon atoms per molecule.

[b] Amount calculated from probability equations.

that the experimental distributions deviate greatly from those predicted by calculation. In all three classes of unsaturation the amounts of short chain triglycerides greatly exceed the values predicted by random association of the fatty acids, while the long chain glycerides are present in lower proportions. In this respect these non-random patterns are similar to the non-random distributions of triglycerides noted for total butteroil (11).

Further resolution of these triglycerides could be obtained by preparative GLC, which, preceded by argentation TLC, would yield relatively simple mixtures of glycerides of uniform molecular weight and uniform degree of unsaturation. Since this method was unlikely to give the amounts of material required for a stereospecific analysis, the idea was abandoned for the time being in favor of a stereospecific analysis of a molecular distillate of butteroil with a fatty acid and triglyceride composition comparable to that of the LCT fraction.

Stereospecific Analysis

A stereospecific analysis of the long chain triglyceride fraction of butteroil (LCTB) was desirable for several other reasons. Thus, a comparison of the overall fatty acid composition of the butteroils analyzed by McCarthy et al. (12), Blank and Privett (13) and Pitas et al. (9) shows that these oils are remarkably similar as are the short (SCTB) and long (LCTB) chain triglyceride fractions prepared from them by TLC (13) and molecular distillation (12). This suggests that the preparations of butteroil possibly vary less in their composition than those of the milk globule fats. Furthermore, stereospecific analyses have already been reported for whole butteroil (9) and for the SCTB fraction obtained by molecular distillation (10). In addition, Blank and Privett (13) had reported the fatty acid composition of position 2 of the LCTB fraction and had made comparisons to the original oil.

Table VI gives the fatty acid composition of the three positions of the glycerol molecule (numbered relative to sn-glycerol-3-phosphate) for the LCTB fraction along with the composition previously reported for the SCTB (10) fraction and the whole butteroil (9). While there were differences in the fatty acid compositions of the original butteroils analyzed by Pitas et al. (9) and by the authors (10), the relative distributions of the major long chain fatty acids between the 1 and 2 positions are in good agreement in all three samples. Thus both capric and lauric acids are present in the highest concentrations in position 2, and about twice as much myristic is found in position 2 as in position 1, in all fractions. The amounts of palmitic acid (32.8-41.1%) are comparable in the two positions but much less of it occurs in position 3 (4.9-10.0%). Stearic acid is preferentially incorporated in position 1 in all cases, while oleic is only slightly higher in position 1 than in the other two positions. In contrast to the specific placement of the short chain acids in position 3 of the SCTB found by us (10), Pitas et al. (9) reported some short chain acids also in position 1 and 2. The latter acids might have been concentrated in the C_{26}-C_{30} triglycerides not present in the short chain distillate examined, as trace amounts of dibutyryl triglycerides have been reported to occur in milk fat by Nutter and Privett (7). The fatty acid composition of position 2 of the triglycerides of the whole butteroil and the LCTB fraction is also very similar to that

TABLE VI

Positional Distribution of Fatty Acids in Butteroil Triglycerides (moles %)

	Original			Position relative to sn-glycerol-3-phosphate								
				1			2			3		
FA[a]	SCT_B[b]	LCT_B[c]	Total[d]	SCT_B[b]	LCT_B[c]	Total[d]	SCT_B[b]	LCT_B[c]	Total[d]	SCT_B[b]	LCT_B[c]	Total[d]
4:0	18.3	1.5	11.3	....	....	5.0	....	....	2.9	53.9	4.5	43.3
6:0	7.5	2.2	4.8	....	....	3.0	....	....	4.8	24.3	6.6	10.8
8:0	2.0	1.6	2.3	....	Tr	0.9	0.9	Tr	2.3	5.1	4.8	2.2
10:0	3.5	2.9	4.2	0.9	1.2	2.5	4.3	2.8	6.1	5.3	4.7	3.6
12:0	3.1	3.5	3.9	3.1	1.7	3.1	6.5	3.9	6.0	–0.3	4.9	3.5
14:0	11.0	11.4	11.5	10.8	6.3	10.5	22.8	15.9	20.4	–0.6	11.8	7.1
14:1	1.0	Tr	....	0.5	Tr	....	2.4	Tr	....	–0.1	....	....
15:0[e]	1.8	3.8	....	2.3	1.9	....	4.4	3.7	....	–1.3	5.8	....
16:0	27.8	28.2	27.1	41.1	37.9	35.9	37.4	38.7	32.8	4.9	8.0	10.1
16:1	1.6	3.0	2.0	1.9	2.8	2.9	3.1	3.8	2.1	–0.2	2.4	0.9
17:0[e]	1.3	0.9	....	3.3	1.6	....	1.3	0.8	....	–0.7	–0.3	....
18:0	6.7	13.1	10.4	14.8	18.9	14.7	3.5	8.5	6.4	1.8	11.9	4.0
18:1	13.0	26.1	21.1	19.8	25.9	20.6	11.8	19.6	13.7	7.4	33.4	14.9
18:2	1.0	1.2	1.4	1.0	1.0	1.2	1.2	1.6	2.5	0.8	1.0	–0.5
20:2	0.4	0.5	....	0.5	0.8	....	0.4	0.6	....	0.3	0.1	....

[a] Fatty acids identified by number of acyl carbons and double bonds per molecule.

[b] C_{34}-C_{42} triglycerides isolated from butteroil by molecular distillation and analyzed earlier (10).

[c] C_{36}-C_{54} triglycerides isolated from butteroil by molecular distillation and described previously (12).

[d] Total milk fat triglycerides from butteroil as analyzed by Pitas et al. (9).

[e] Contains normal and iso branched chain fatty acids.

reported for these fractions by Blank and Privett (13) on the basis of pancreatic lipase hydrolyses.

Table VII gives the fatty acid composition of the 1,2-diglycerides of the SCTB, LCTB and the whole butteroil. These values have been derived by summing the fatty acids in positions 1 and 2 and dividing by two. Although the SCTB and LCTB fractions together account for only 80% of total butteroil, the 1,2-diglycerides appear to be very similar in their fatty acids. The greatest differences are in the content of capric and oleic acids in the diglycerides derived from the whole oil and the molecular distillates. This, however, could be attributed to the somewhat greater proportion of these acids in the lower molecular weight triglycerides in the more volatile distillates (R-1 and R-2, see Ref. 12) not subjected to the stereospecific analysis. Some of these and other minor discrepancies could have been due also to the slight differences in the original butteroils. In general, however, the 1,2-diglyceride compositions of the triglycerides of different molecular weights are similar. Since the SCT and LCT fractions derived from the milk globule fat by TLC possess overall compositions of fatty acids and triglycerides which are comparable to those of the SCTB and LCTB, it is likely that they also show similar positional associations of these acids.

The above results suggest the possibility that the 1,2-diglycerides could have been derived from a common pool during the biosynthesis of the milk fat. Such a hypothesis requires further testing by determining the molecular weight distribution of the 1,2-diglycerides, which is not possible when the phosphatidyl phenols are used as intermediates. Also, it would now appear experimentally feasible to pursue these studies using milk fats synthesized from appropriately labeled precursor lipids.

TABLE VII

Fatty Acid Composition of 1,2 Diglycerides (moles %)

FA	SCTB[b]	LCTB[b]	Total[b]
4:0	....	....	3.9
6:0	....	....	3.9
8:0	0.4	Trace	1.6
10:0	2.6	2.0	4.3
12:0	4.8	2.8	4.6
14:0	16.8	11.1	15.5
14:1	1.4	Trace	N.D.[c]
15:0	3.3	2.8	N.D.[c]
16:0	39.1	38.3	34.4
16:1	2.5	3.3	2.5
17:0	2.3	1.2	N.D.[c]
18:0	9.1	13.7	10.4
18:1	15.8	22.8	17.1
18:2	1.1	1.3	1.8
20:2	0.8	0.7	N.D.[c]

[a] Legends as in Table VI.

[b] Values determined by summation of the fatty acid compositions of positions 1 and 2 followed by normalization. Total values calculated from data of Pitas et al. (9).

[c] Not determined.

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