Fatty Acids of Bovine Milk Glycolipids and Phospholipids and Their Specific Distribution in the Diacylglycerophospholipids[1]

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Abstract

Milk lipids were fractionated by silicic acid column chromatography and preparative thin-layer chromatography (TLC). Ceramide monohexoside (CMH), ceramide dihexoside (CDH), phosphatidyl ethanolamine (PE), phosphatidyl choline (PC), phosphatidyl serine (PS), and sphingomyelin (Sph) were isolated, and the purity of each was checked by infrared spectroscopy and TLC. The diacylphospholipids were hydrolyzed with phospholipase A and the products separated by TLC. Fatty acid methyl esters were prepared from the various fractions and analyzed by gas chromatography.

The glycolipids, CMH and CDH, and Sph contained large amounts of long-chain saturated fatty acids, especially $C_{22:0}$, $C_{23:0}$, and $C_{24:0}$. PE, PS, and PC contained C_{10}-C_{22} normal and branched-chain saturated fatty acids, and C_{15}-C_{20} unsaturated fatty acids (mainly monoenes). The distributions of saturated acids between the α'- and β-positions were respectively: PE, 46 and 11%; PS, 65 and 19%; and PC, 72 and 53%. PC was exceptional in that there was 10.8% myristic acid in the β-position and only 5.6% in the α'-position. PE and PS were similar in composition except that in PE oleic acid was evenly distributed, and in PS was largely in the β-position. In general, PC was much more saturated than PE or PS, and there was no overall pattern governing the specific distribution of the fatty acids in the three diacylphospholipids. Comparison with PC from other bovine tissues and from egg lecithin showed that fatty acids are located much less specifically in milk phospholipids than in PC from other sources.

Introduction

BOVINE MILK CONTAINS 0.03–0.04% phospholipids, which are important components of the milk fat globule membrane. They are involved in formation of the fat globules and affect the physical properties and flavor of milk and milk products during processing and storage (3,17,22,37,38). Extraction and purification of the individual classes of milk phospholipids present numerous difficulties, and various methods have been used to investigate them in recent years (3,8,13,27,29,34–37). From these studies, the approximate amounts of the major components of the phospholipid fraction of milk lipids are estimated to be 3% ceramide monohexoside (CMH), 3% ceramide dihexoside (CDH), 30% phosphatidyl ethanolamine (PE), 1% phosphatidal ethanolamine, 8% phosphatidyl serine (PS), 5% phosphatidyl inositol (PI), 28% phosphatidyl choline (PC), 3% phosphatidal choline, and 19% sphingomyelin (Sph). Recent evidence (8), however, indicates that these lipids are to a large extent the breakdown products of labile glycophospholipid and proteolipid complexes.

Smith and Lowry (36) determined the fatty acid composition of the cerebroside, PE, PS, PC, and Sph components of total milk phospholipids separated by silicic acid column chromatography. Results of PE, PS, PC, and Sph analyses by other investigators (3,13,22,37) agree with those of Smith and Lowry.

The data obtained in those studies showed marked differences in fatty acid patterns of the various phospholipid classes. Hawke (13) showed differences in specific distributions of fatty acids in the diacylglycerophospholipids, but he did not examine PE and PS separately.

The materials used in those studies may not have been completely pure or representative of the major phospholipids or glycolipids. A reason would be the inherent difficulty of separating PE from PS and PC from Sph by normal silicic acid column chromatography methods. Further, lipids are subject to partial separation, varying with their degree of unsaturation, and this may sometimes have contributed to nonrepresentative fractions being used.

The present work was undertaken to isolate pure glycolipids and phospholipids more representative of those originally in milk than were separated in previous studies, and to determine the fatty acid compositions of these lipids. In addition, the specific distribution of fatty acids in the diacylglycerophospholipids was studied by phospholipase A hydrolysis. Preparative thin-layer chromatography (TLC) was employed to separate milligram amounts of the various lipids for analysis by TLC, infrared, and GLC techniques.

Experimental

Isolation of Glycolipids and Phospholipids

Most of the materials and methods have recently been described in detail (26). Beef heart PC and beef brain Sph were commercial samples (26), plasmalogens were removed from the PC by mild acid hydrolysis (40), and pure PC was recovered by TLC as described below. Spray-dried buttermilk powder was used as a convenient source for the preparation of replicate samples of milk glycolipids and phospholipids (3,7,35,37). Figure 1 shows the procedure used to extract and isolate the lipids studied. In Step 1, the total lipids were extracted from 20 g of powder with the monophasic $CHCl_3$-MeOH-H_2O solvent system of Bligh and Dyer (4), and were purified by partitioning between $CHCl_3$ and MeOH-H_2O phases. The solvent was removed with a rotary vacuum evaporator, and the lipids were taken up in a small volume of $CHCl_3$. In step 2, the lipids were applied

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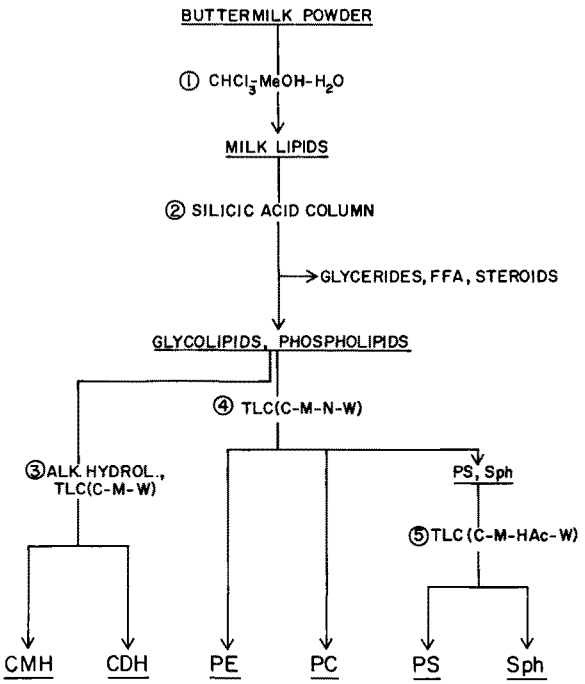

FIG. 1. Scheme for the isolation of bovine milk glycolipids and phospholipids from buttermilk powder. Details are given in the text.

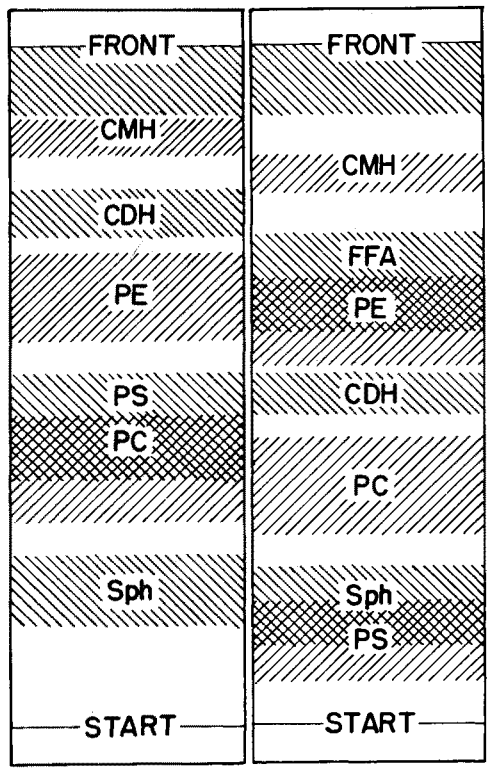

FIG. 2. TLC of a mixture of milk glycolipids and phospholipids on silica gel G plates developed with (left) $CHCl_3$-MeOH-CH_3COOH-H_2O (65:25:8:4, v/v) or (right) $CHCl_3$-MeOH-NH_4OH-H_2O (65:35:1:4, v/v). The diagrams show preparative-scale separations of the lipids.

to a silicic acid column (20 g silicic acid, 10 g Celite), and the glycerides, free fatty acids (FFA), and steroids were eluted with 500 ml diethyl ether. The glycolipids and phospholipids were then eluted with 300 ml $CHCl_3$-MeOH (1:1)[3] and 200 ml MeOH. The combined eluates were taken to dryness and redissolved in a few ml $CHCl_3$-MeOH (1:1) for preparative TLC on silica gel G.

To illustrate the principles of the preparative and analytical TLC procedures used in the subsequent steps, Figure 2 shows the relative positions of the major glycolipid and phospholipid components of milk on plates developed in two TLC solvent systems. When lipid loads were small and detection methods were sensitive, most of these lipids were completely resolved, but on the preparative scale there was some overlapping of adjacent components. Best TLC results were obtained by using freshly activated plates (110 C, 1 hr) which were cooled under glass plates, and then exposed only along the bottom edge during application of lipids. Since lipid R_f values and separations were rarely perfectly reproducible, the TLC behavior of the lipids is given as a diagram. A PS-Sph band obtained by TLC in the alkaline solvent system (Fig. 2, right) can be rerun in the acidic system, and the difference in R_f will permit a clean separation of the PS and Sph (Fig 2, left).

In Step 3 (Fig. 1), CMH and CDH were isolated as described recently (27). $CHCl_3$-MeOH-H_2O (65:25:4) was used as the developing solvent. In Step 4, the lipids were separated by preparative TLC using $CHCl_3$-MeOH-NH_4OH-H_2O (65:35:1:4), and the three main bands were detected with 2′,7′-dichlorofluorescein or Rhodamine 6G. The bands were scraped off, and the lipids were recovered by a scaled-down Step 1 procedure. In Step 5, the PS and Sph in the lowest band (Fig. 2, right) were separated by TLC using $CHCl_3$-MeOH-HAc-H_2O (65:25:8:4), and were recovered as in Step 1. After TLC with solvents containing HAc, the plates were exposed to NH_4OH vapor so that the lipids could be detected readily with 2′,7′-dichlorofluorescein or Rhodamine 6G. Sph was treated with mild alkali (4 vol. $CHCl_3$-MeOH, 1:1, and 1 vol. 0.2 *N* KOH for 30 min at 30C), acidified with dilute HCl, and Sph was recovered in the $CHCl_3$ phase of a Folch wash (26). The PE, PC, PS, and Sph were precipitated from acetone and stored under acetone or in $CHCl_3$ at 0C. The acetone precipitation removed any FFA which were present, and care was taken to recover phospholipids not precipitated initially from the acetone. PI was never satisfactorily isolated and was not included in this study.

[3] All solvent mixtures are given as parts by volume.

Qualitative Examination of Lipids

Infrared spectra (Fig. 3) were obtained from thin films of each lipid on NaCl plates. In addition, each lipid was examined by TLC before and after mild alkaline hydrolysis (see Sph above) using both alkaline and acidic TLC solvents (Fig. 1, Steps 4 and 5). The spots were compared with spots for reference compounds described elsewhere (26).

CMH and CDH were the samples recently identified (27). The absence of absorption bands around 5.8 μ in their infrared spectra show that no FFA or esterified fatty acids were present.

The spectrum of PE compares closely with published spectra of PE from natural sources (31,34). Milk PE does not contain much plasmalogen (8,29); this is confirmed by a lack of absorption at 6.0 μ, a wavelength characteristic of the vinyl ether group (10). Absorption near 6.1 and 6.5 μ attributable to NH_2 (31) was enhanced by the presence

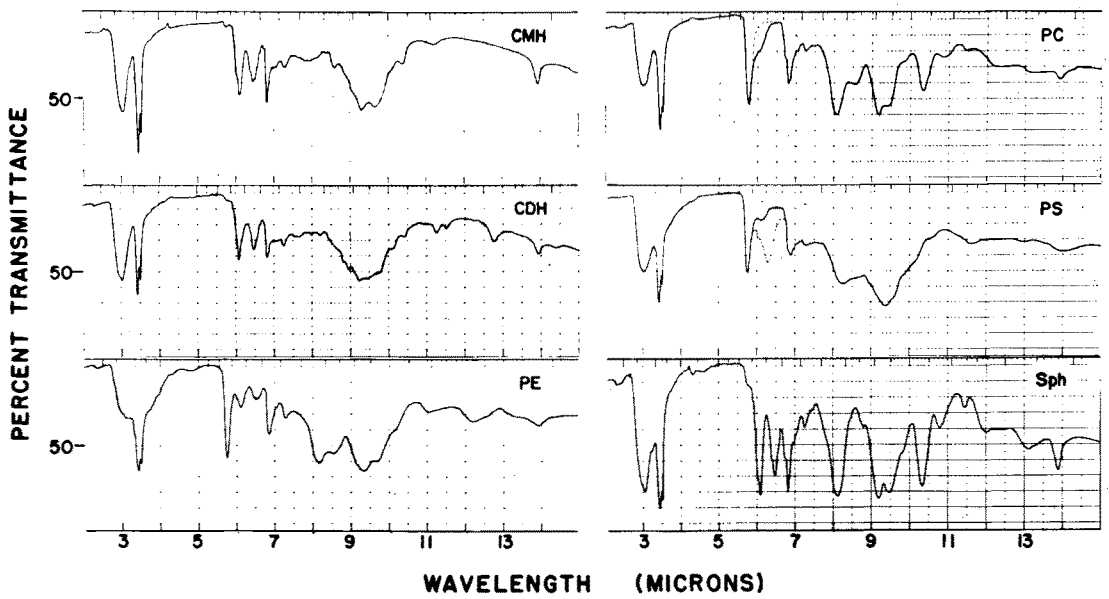

FIG. 3. Infrared spectra of bovine milk glycolipids and phospholipids prepared from thin films of lipids on NaCl plates: Ceramide monohexoside (CMH) hexose = glucose; Ceramide dihexoside (CDH), hexose + glucose = galactose; phosphatidyl ethanolamine (PE); phosphatidyl choline (PC), dotted line indicates normal diacyl PC spectrum 5.75—6.3 μ; phosphatidyl serine (PS) in acid form, dotted line shows change due to Na^+ salt form; sphingomyelin (Sph).

of a trace of amide, identified by TLC as CDH. No other impurities were detected.

The infrared spectrum of PC compares closely with published spectra of natural PC (10,31,34) except in the region of 5.75—6.1 μ. The difference may be attributed, at least in part, to absorption at 6.0 μ from the plasmalogen form present (29). However, there is no apparent explanation for the failure to resolve the ester C =O absorption at 5.75 μ from the vinyl ether absorption at 6.0 μ. The PC sample was shown by TLC to be free of impurities.

The infrared spectra of PS in acid and sodium salt forms agree with published spectra (16,31). TLC revealed no impurities in the sample.

The infrared spectrum of Sph corresponds closely to published spectra of natural Sph (31,34). The sample showed no ester absorption at 5.75 μ and was homogeneous when examined by TLC.

TABLE I

Fatty Acid Compositions (mole %) of Bovine Milk Glycolipids and Sphingomyelin

Fatty acid[a]	CMH	CDH	Sph
12:0	0.8	0.4	0.3
13:0	0.1	0.1	Tr[b]
*i*14:0	0.1	0.2	0.1
14:0	6.3	2.7	2.5
*i*15:0	0.7	0.3	Tr
*ai*15:0	0.7	0.4	Tr
15:0	1.6	0.7	0.4
15:1	0.2		
*i*16:0	0.1	0.2	0.1
16:0	29.8	25.0	22.1
16:1	2.6	1.2	0.8
*i*17:0	0.4	0.4	Tr
*ai*17:0	0.5	0.9	0.2
17:0	1.1	0.5	0.6
17:1	0.4	0.2	Tr
*i*18:0	0.1	0.2	0.1
18:0	11.1	16.2	4.5
18:1	16.7	13.9	5.0
18:2	2.7	2.3	0.9
18:3?	0.5		
19:0	0.7	0.8	0.2
*i*20:0		Tr	0.1
20:0	0.6	1.6	0.6
20:1	0.2	1.1	0.1
21:0	0.4	0.5	0.8
21:1			0.1
22:0	5.4	9.3	14.7
22:1	0.6		0.2
23:0	8.6	12.5	27.0
23:1	0.7		1.0
24:0	5.1	7.1	14.8
24:1	1.1	1.3	1.9
25:0			0.6
26:0			0.2
Saturated	74	80	90
Unsaturated	26	20	10

[a] *i* = *iso*, *ai* = *anteiso*
[b] Tr = trace = amounts in the range 0.01–0.04%. Other values are given to nearest 0.1%.

Phospholipase A Hydrolysis of PE, PC, and PS

PE, PC, and PS were hydrolyzed overnight at room temperature with snake (*Ancistrodon piscivorus piscivorus*) venom phospholipase A in the ethereal system of Saito and Hanahan (32). The apparent ph of the PE and PS solutions was adjusted to ~7.4 with ethereal NH_4OH (20).

Hydrolysis proceeded rapidly with PC, somewhat less rapidly with PE and comparatively slowly with PS. The degree of hydrolysis was measured by TLC of an aliquot of each hydrolysate, using the acidic solvent (Fig. 1, Step 5), and by determining the P content (25) of the original and lysophospholipid bands. Hydrolysis of PC, PE, and PS to the lyso forms was respectively >90%, >85%, and 75–80% complete.

After hydrolysis, each sample was evaporated to dryness, redissolved in a small volume of $CHCl_3$-MeOH (1:1), and applied to TLC strips. The strips were developed with diethyl ether-hexane-HAc (70:30:1), and FFA were detected by spraying only the top third of each strip with 2′,7′-dichlorofluorescein (MeOH in this reagent would otherwise deactivate the remaining silica gel so that the next TLC separation would not be possible). The FFA band was removed and the acids recovered by extracting the silica gel with $CHCl_3$-MeOH (3:1). The TLC strip was then redeveloped with the acidic solvent (Fig. 1, Step 5), and lysophospholipids ($R_f \cong 0.5 \times R_f$ of diacyl compound) were detected

TABLE II

Specific Distribution of Fatty Acids (mole %) of Bovine Milk Diacylglycerophospholipids

Fatty acid[a]	PE			PS			PC		
	α′	β	total	α′	β	total	α′	β	total
10:0						0.2	0.2	0.7	
11:0	0.1	0.2	0.1	0.5	0.1	0.1	Tr	0.1	0.2
12:0	0.3	0.6	0.3	1.1	0.5	0.8	0.3	0.8	0.7
13:0	Tr	0.1	Tr	0.4	0.1	0.1	0.1	Tr	0.1
*i*14:0	0.2	0.1	Tr	0.1	0.1	0.1	0.2	0.1	0.1
14:0	1.9	1.3	1.5	4.0	2.2	2.4	5.6	10.8	8.4
14:1	0.1	0.1	0.1				0.1		0.1
*i*15:0	0.1	0.1	0.1	0.3	0.2	0.2	0.3	0.4	0.4
*ai*15:0	0.3	0.3	0.3	0.5	0.5	0.4	0.3	0.6	0.5
15:0	0.6	0.4	0.5	0.6	0.5	0.6	1.6	2.4	2.1
15:1	0.1	0.1	0.2	0.4	0.3	0.3	0.2	0.3	0.2
*i*16:0	Tr	0.1	0.1		0.2	0.2	0.4	1.0	0.6
16:0	19.7	4.7	11.7	24.5	7.6	13.8	41.9	30.6	36.4
16:1	1.2	2.2	2.1	1.7	2.3	1.5	0.6	1.2	0.6
*i*17:0	0.4	0.2	0.2	0.3	0.2	0.3	0.4	0.5	0.5
*ai*17:0	0.4	0.3	0.3	1.3	0.5	0.6	1.0	1.0	1.0
17:0	1.3	0.9	0.9	1.3	0.7	0.9	1.3	0.6	0.9
17:1	0.3	0.6	0.5	0.7	0.6	0.6	0.2	0.3	0.2
*i*18:0	0.1	Tr	0.1	0.1	0.1	0.1	0.1	0.1	0.1
18:0	19.0	1.3	10.5	28.0	3.9	15.1	17.5	2.4	11.1
18:1	45.8	47.8	46.7	25.8	46.2	39.6	20.3	27.8	25.7
18:2	2.9	21.4	12.4	1.6	18.3	10.1	2.7	9.2	5.3
conj. 18:2[tt]	0.5	0.2	0.3		0.4	0.6	0.3	0.5	0.4
18:3	1.1	4.5	3.4	1.2	3.0	2.4	0.8	1.8	1.1
conj. 18:3[ttt]	0.4	1.8	1.0		1.0	0.6	1.1	1.0	0.6
19:0	0.4	0.4	0.4	0.7	0.4	0.7	0.6	0.2	0.3
*i*20:0	0.1	0.1	0.1		0.2	0.2	0.1	0.1	0.1
20:0	0.7	0.1	0.3	1.0	0.2	0.4	0.3	0.2	0.3
20:1?	0.1	0.2	0.2		0.1		0.1	0.2	0.1
20:3	0.2	2.2	1.4	1.2	2.0	1.7		1.6	1.0
20:4	0.2	3.0	1.9	1.1	2.7	1.5	0.2	1.2	0.7
21:0	0.1	0.1			0.4	1.1			
21:1		0.3	0.1	1.5	0.6	0.8	0.2	0.3	0.3
22:0	0.1	0.1	0.2		0.4	0.4			0.1
Unknowns	1.1	3.7	2.3		4.0	1.8	1.1	2.0	0.3
Saturated	46	11	28	65	19	39	72	53	64
Unsaturated	54	89	72	35	81	61	28	47	36

[a] *i* = iso; *ai* = anteiso; conj . . . [tt] = conjugated . . . *trans,trans*.

with 2′,7′-dichlorofluorescein and recovered from the silica gel by extracting with $CHCl_3$-MeOH (1:4).

Preparation of Fatty Acid Methyl Esters

Fatty acid methyl esters were prepared from CMH, CDH, and Sph by heating with BF_3-MeOH for 90 min at 100C (26). Methyl esters were prepared from PS by direct treatment with BF_3-MeOH. Because of th epresence of plasmalogen and cermide hexoside impurities, the total fatty acids of PE and PC were liberated by mild alkaline hydrolysis and, after acidification, were separated from the other hydrolysis products by TLC using diethyl ether-hexane-HAc (70:30:1). The FFA were recovered from the silica gel band by shaking with $CHCl_3$-MeOH (3:1) and were converted to methyl esters with BF_3-MeOH. After phospholipase A hydrolysis of phospholipids, methyl esters were prepared from the FFA and lysophospholipids by appropriate treatment with BF_3-MeOH.

Aliquots of the methyl ester samples were hydrogenated in methanol with platinum oxide catalyst (18) and extracted with pentane. Both the original and hydrogenated esters were purified by TLC on plates developed with benzene (26).

GLC of Methyl Esters

All methyl ester samples were chromatographed at 184C, before and after hydrogenation, in a Loenco 15A flame ionization gas chromatograph with a 6 ft × ¼-in. column packed with 12% stabilized diethylene glycol succinate polyester (DEGS) on 60–70 mesh Anakrom A. To facilitate identification of certain peaks in the chromatograms, representative samples were also chromatographed on a 4 ft × ¼-in. column packed with 12% Apiezon L on 60–70 mesh Anakrom AS operated at 220C.

Peaks on chromatograms were tentatively identified by their retention times relative to stearate or oleate, and by their response to hydrogenation. Reference retention data for methyl esters were obtained from known mixtures of methyl esters and from published data (1,2,14,19,21) for DEGS and EGS (ethylene glycol succinate polyester) columns.

Relative peak areas were measured as retention distance × peak height. GLC correction factors were calculated using several different standard methyl ester mixtures of compositions approximately the same as those of the lipids being analyzed. Analysis of National Heart Institute Fatty Acid Standard Mixture F, using these correction factors, agreed to within 3% of the stated composition for all components. Correction factors for minor components were obtained by interpolation.

Results

Identification of Methyl Esters by GLC

The DEGS column used for most of the GLC analyses gave excellent resolution and the relative retention times of unsaturated esters decreased very little with aging of the DEGS. All the fatty acids listed in Tables I and II have already been found in total milk lipids (9,21,23) but previous analyses of milk phospholipid fatty acids (3,13,22,36,37) have been less complete.

The saturated acids found ranged from C_{10} to C_{26}. The TLC and other techniques used were such that the more volatile acids and their methyl esters would not be recovered quantitatively, but other workers have shown that these short-chain acids are not present in milk phospholipids (3,9,13,22,36,37). Several branched-chain (*i* = iso, *ai* = anteiso) acids were identified tentatively: *i*14:0, *i*15:0, *ai*15:0, *i*16:0, *i*17:0, *ai*17:0, *i*18:0, and *i*20:0.

C_{14}—C_{24} monounsaturated acids were found, but there was no GLC evidence which could be used to

TABLE III

Specific Distribution of Major Fatty Acids (mole %) in Bovine Lecithins

Fatty acid	Milk[a]		Heart[b]		Blood plasma[c]		Liver[d]	
	α'	β	α'	β	α'	β	α'	β
14:0	6.3	12.5	1.8	1.8			0.9	0.4
16:0	46.8	35.3	57.2	16.7	43	1	20.8	1.4
16:1	0.7	1.4	9.3	7.2	4	30	3.2	2.0
18:0	19.5	2.8	17.7	8.4	51	1	45.7	Tr
18:1	22.7	32.1	9.3	38.2	2	36	19.0	18.3
18:2	3.0	10.6	0.7	24.8		20	3.1	17.4
18:3	0.9	2.1	3.0	2.2			0.5	2.0
20:3		1.9		0.7				
20:4	0.2	1.4	1.0			12	1.9	18.1
20:5							Tr	2.3
22:1							1.9	13.6
22:4							0.9	6.4
22:5							2.2	13.0
22:6							Tr	5.2
Saturated	72	51	77	27	94	2	67	2
Unsaturated	28	49	23	73	6	98	33	98

[a]Complete analysis given in Table II. Data recalculated to show only major acids, for comparison.
[b]Complete analysis available upon request. Data recalculated to show only major acids, for comparison.
[c]Data of Hanahan et al. (12).
[d]Calculated from data of Menzel and Olcott (23).

TABLE IV

Major Fatty Acids of Bovine Sphingomyelins (mole %)

Fatty acid	Milk[a]	Brain[b]	Blood plasma[c]	Blood erythrocytes[c]
14:0	2.5	0.9		
16:0	22.1	5.2	77	40
16:1	0.8		5	1
18:0	4.4	45.0	13	4
18:1	4.9	2.7	1	1
18:2	0.9	0.5	4	
20:0	0.6	1.2		
21:0	0.8	0.3		
22:0	14.7	5.3		11
23:0	27.0	4.5		2 (22:?)
23:1	1.0	0.3		
24:0	14.8	14.2		31
24:1	1.9	8.3		10 (24:?)
25:0	0.6	3.4		
25:1		2.5		
26:0	0.2	1.3		
26:1		3.3		
Saturated	90	82	90	86
Unsaturated	10	18	10	14

[a]Complete analysis given in Table I.
[b]Complete analysis available upon request.
[c]Data of Hanahan et al. (12).

designate *cis* or *trans* configurations, or the positions of the double bonds.

The diacylphospholipids contained several 18:2 and 18:3 acids. The main 18:2 GLC peak corresponded to the 9-*cis*,12-*cis* isomer, but nonconjugated *cis, trans* isomers (15) are included in the same peak. Conjugated *trans,trans*-18:2 was tentatively identified, but conjugated *cis,trans*-18:2, if present, was obscured by the linolenate peak. The main 18:3 component had the correct retention time for the all-*cis*-9,12,15 isomer, which is the nonconjugated 18:3 acid in milk lipids (9). A late peak was tentatively identified as a conjugated all-*trans*-18:3 acid.

The diacylphospholipids also contained several unsaturated C_{20} and C_{22} acids, as demonstrated by the 20:0 and 22:0 found after hydrogenation. Two of the C_{20} unsaturated acids were identified as 20:3 and 20:4. Milk fat contains conjugated and nonconjugated acids with 2 to 5 double bonds (9,17,33,35), determined by alkali isomerization. Higher proportions of these polyunsaturated acids are found in the phospholipids (17,35), particularly in the cephalin (PE + PS + PI) fraction (17). The present GLC results are in reasonable agreement with values found by alkali isomerization for nonconjugated dienes, trienes, and tetraenes, but are too low for conjugated dienes. The low result for conjugated dienes could be due to conjugated *cis,trans*-18:2 being included in the linolenate peak.

In any analysis of a complex mixture of methyl esters which is based entirely on GLC retention data there is always an element of uncertainty about peak identifications. Furthermore, although unsaturated fatty acid methyl esters have been reported to be stable during TLC (5), there is contrary evidence to show that during aerobic TLC there are losses of polyunsaturated esters which can be prevented by working under nitrogen (3) or with an antioxidant (42). Some phospholipids are very susceptible to autoxidation, and for this reason the overall time for analysis of the lipids was minimized, particularly those times when the TLC plates were not wetted with solvents. It is believed that serious oxidative changes were thereby avoided in the present study. However, it may be that some of the minor unidentified GLC peaks represented products of the autoxidation of unsaturated fatty acids.

Fatty Acid Composition of CMH, CDH, and Sph

Table I shows that the amide-linked fatty acids of CMH, CDH, and Sph are mainly saturated and include characteristically large amounts of 22:0, 23:0, and 24:0 acids. The amounts of the major fatty acids agree with previous analyses of milk CMH and Sph (3,22,36,37). CMH and CDH are structurally related, which is reflected in the similarity of their fatty acid compositions, and they are much less closely related to Sph than to each other, which is also seen in their fatty acid compositions.

The glycolipids and sphingomyelin are considered as a group because they contain amide-linked long-chain fatty acids but do not contain C_4—C_{10} or polyunsaturated acids. Most previous analyses of milk CMH (36) and Sph (3,22,36) included small amounts of atypical polyunsaturated acids, but, since no steps were taken to remove free or esterified acids from the sphingolipids before methyl ester preparation, these acids may be from impurities such as FFA, glycerides, or acylphospholipids. Table I shows that polyunsaturated acids were effectively absent, although CMH did contain a small amount of an acid which was either 18:3 or conjugated *cis,trans*-18:2.

Fatty Acid Composition of PE, PS, and PC

Table II shows that PE was generally similar to PS in total fatty acid composition, although the latter contained a higher proportion of saturated acids. PC fatty acids were much more saturated than those of PE or PS. The distribution of 16:0 to 18:0 was approximately 1:1 in PE and PS and 3:1 in PC. The three diacylglycerophospholipids contained only small amounts of acids with more than 20 carbon atoms. The amounts of the major fatty acids found are in reasonable agreement with previously reported data (3,13,22,36,37) if allowance is made for variations in the feed of the cows.

Specific Distribution of Fatty Acids in PE, PS, and PC

Prior to the study of milk phospholipids, the method (starting from TLC preparation of PC) was evaluated by determining the specific distribution of the fatty acids in ovolecithin with phospholipase A. The data were in excellent agreement with published results (24,32,39) showing that the acids in the β-position are almost all unsaturated and those in the α-position include most saturated acids.

Although the milk phospholipids were always in-

completely converted to their lyso forms, the degree of hydrolysis was always sufficient to ensure completely representative α'- and β-fatty acid analyses (24). The only previous data available for milk phospholipids are those of Hawke (13), who examined PC and a mixture of PE and PS. Because of the similarity in the fatty acid composition of PE and PS (Table II), and because of the greater proportion of PE in milk phospholipids, his specific distribution results may be considered to be essentially those of PE alone. The general distribution of fatty acids in PE and PC found by Hawke and that found in the present study are remarkably similar. Excluding the minor components and those components of uncertain identity, the only significant difference between the PE results is the reversed specific distributions of the 14:0. In the present study, there was 1.9% α'- and 1.3% β-, whereas Hawke found 1.0%α'- and 1.7% β-position 14:0. There are no great discrepancies between the PC results of the present study and those of Hawke.

Comparing PE, PS, and PC (Table II), it is seen that the general tendency for saturated acids to be concentrated in the α'-position and unsaturated acids in the β-position is true for all three lipids. Exceptions are the 14:0 of PC, which is largely in the β-position, and the 18:1 of PE, which is almost evenly distributed. There must be a significant proportion of molecules containing two saturated acyl groups in PC, and saturated PE and PS molecules may also exist.

Discussion

Comparison of Phospholipids from Milk and Other Tissues

It is of interest to compare the distribution of fatty acids in the phospholipids of bovine milk and tissues. Such comparisons may assist in studies of the synthesis and structure of the milk fat globules, and differences in fatty acid composition may typify different functions of the phospholipids in biological membranes and other structures.

Hawke (13) concluded that the differences in fatty acid composition and specific distributions in milk PE + PS and PC are sufficient to exclude their direct biosynthesis from a common diglyceride or phosphatidyl serine precursor, and the present results (Table II) confirm this. Patton et al. (28) reviewed evidence suggesting that milk phospholipids originate from the cytoplasmic membrane which envelops the fat globule during its secretion from the mammary cell. They also showed that the mammary tissue phospholipids are very similar to milk phospholipids in phospholipid and total fatty acid compositions. Since milk lipids are partially derived from blood plasma lipids (9,28), the blood plasma phospholipids must themselves be considerably modified or supplemented in the mammary tissue to eventually form the milk phospholipids (Tables III and IV), or else the mammary and milk phospholipids may have been entirely synthesized *de novo* in the mammary tissue.

Milk PC is outstanding in that it contains 53% saturated acids in the β-position, and its β-position saturation is generally much greater than that in the PC of blood plasma or liver (Table III). The heart PC occupies an intermediate position, but the results here may be invalid because the β-fatty acids from the plasmalogen form (about 50% of the total PC) were excluded. Milk Sph is likewise quite different from Sph of other tissues (Table IV), its most characteristic feature being the large amount of 23:0 acid present. These phospholipids are from tissues in which they might be expected to fulfill very different roles, and it may be significant that their fatty acid compositions are so characteristically different. It is well recognized that phospholipids fulfill essential roles in tissues (11,30,41), and it is becoming apparent that the degree of fatty acid unsaturation is also important for biological functions (6). It seems reasonable to suppose that similar considerations may also apply to milk phospholipids in the structure and function of the milk fat globule membrane.

After the work described in this paper was completed, Galanos and Kapoulas published results (7,8) showing that milk phospholipids occur largely as labile glycophospholipid and proteolipid complexes. These complexes are readily degraded to the types of phospholipids studied in the present and previous work, and PC, for example, may be derived from several different glycophospholipids. Our knowledge of the nature and role of milk phospholipids will be greatly enhanced when the fatty acid structure of these lipid complexes is known.

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