# Composition of Bovine Milk Lipids<sup>1,2</sup>

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## ABSTRACT

Recent literature on the composition of milk lipids is reviewed and discussed. Many additional exotic fatty acids, mainly branched chain, keto, hydroxy and isomers containing double bonds, have been identified, often with the aid of mass spectrometry. It is estimated that ca. 500 fatty acids have been found in milk lipids. Components of lipid classes have been isolated and their structure determined, e.g., glucosyl and lactosyl ceramide, sphingomyelin, ether lipids, etc. The fatty acid composition of "protected" milk is discussed. This milk, obtained from cows fed polyunsaturated oils encapsulated with formaldehyde-treated casein, contains about five times more linoleic acid than regular milk. The future of research on milk lipids is promising, as there are still many intriguing problems of separation and identification.

## INTRODUCTION

An era of unprecedented activity in the separation and identification of milk lipid classes and their components began in 1956 when James and Martin (1) published the first gas liquid chromatographic (GLC) analysis of milk fat. At about the same time column chromatography was put into use, followed shortly thereafter by application of thin layer chromatography (TLC). Thus for the first time investigators of milk lipids had available relatively simple and reliable methods for the separation and tentative identification of milk lipids and fatty acids. These and other procedures were rapidly and intensively applied, resulting in thorough and nearly complete elucidation of the kinds of lipids present in milk and of the components in these classes. In 1967, we collated the information then available upon the fatty acid composition of milk lipids (2). At that time ca. 150 fatty acids had been positively and tentatively identified. In 1970, Morrison (3) published a comprehensive review of the composition of milk lipids, listing and describing the many lipids found in milk fat during the period of intensive research starting in 1956. Since about 1967, milk lipids have not received as much attention as previously, but work continues. This review describes some of the information that has appeared since the publication of Morrison's paper. The effects of various environmental, genetic and nutritional factors upon the

<sup>2</sup>Contribution No. 528, Storrs Agricultural Experiment Station, University of Connecticut, Storrs.

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Composition of Lipids in Whole Bovine Milka

Lipid	Wt %		
Hydrocarbons	Trace		
Sterol esters	Trace		
Triacylglycerols	97 - 98		
Diacylglycerols	0.28 - 0.59 <sup>t</sup>		
Monoacylglycerols	0.016 - 0.038		
Free fatty acids	0.10 - 0.44		
Free sterols	0.22 - 0.41		
Phospholipids	0.2 - 1.0		

<sup>a</sup>Adapted from Morrison (3).

bJensen et al. (9).

fatty acid composition of milk lipids are not included. The triglyceride structure of milk fat is reviewed in the accompanying paper by Kuksis (4). Discussion of lipid flavor components is omitted as these have been recently described by Forss (5), and milk fat globule membrane lipids are included in a review by Brunner (6). In general, only bovine milk lipids are discussed.

## LIPID CLASSES

#### Triacylglycerols and Related Compounds

The composition of bovine whole milk lipids (Table I) presents no surprises. The partial acylglycerols and free fatty acids are in part leftovers from biosynthesis, since they are always present in milk that has been extracted immediately after being drawn, before much lipolysis could occur and without any intervening separation treatment other than TLC. If the diacylglycerols are involved in biosynthesis, it is possible that they are enantiomeric and probably the sn-1,2-isomer. The configuration could be readily determined by Brockerhoff's stereospecific analysis (7), but it would be difficult to accumulate enough material for the analysis. It seems likely that diacylglycerol contents of 4.4-6.6% reported by Boudreau and DeMan (8) are too high and probably resulted from the isolation technique employed. Unless freshly drawn milk is heated or extracted immediately, lipolysis commences and is accelerated by pipeline milking. Processed milk always contains partial acylglycerols and free fatty acids resulting from lipolysis, but the quantities are small (9).

Timmen and Dimick (10) characterized the major hydroxy compounds in milk lipids by first isolating the compounds as their pyruvic ester-2,6-dinitrophenylhydrazones. Concentrations as weight per cent of the compounds from bovine herd milk lipids were: 1,2-diacylglycerols, 1.43; hydroxyacylglycerols, 0.61; and sterols, 0.35. Lipolysis tripled the diacylglycerol content. The usual milk fatty acids were observed, except that the diacylglycerols lacked 4:0 and 6:0, again indicating that these lipids were probably intermediates in milk lipid biosynthesis.

Diol lipids are not listed but might be present. These compounds, discussed in a recent review by Bergelson (11), have been detected in several mammalian tissues and in seeds. If present in milk lipids, diol lipids would be masked by triacylglycerols.

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Phospholipid Composition of Bovine Milka

Phospholipid	Mol %
Phosphatidylcholine	34.5
Phosphatidylethanolamine	31.8
Phosphatidylserine	3.1
Phosphatidylinositol	4.7
Sphingomyelin	25.2
Lysophosphatidylcholine	Trace
Lysophosphatidylethanolamine	Trace
Total choline phospholipids	59.7
Plasmalogens	3b
Diphosphatidyl glycerol	Trace
Ceramides	Trace
Cerebrosides	Trace

<sup>a</sup>Adapted from Reference 3. bReference 6, p. 561. <sup>c</sup>Reference 12. <sup>d</sup>References 14, 15.

<sup>&</sup>lt;sup>1</sup>One of eight papers presented in the symposium "Milk Lipids," AOCS Meeting, Ottawa, September 1972.

We know now that milk triacylglycerols are an exceedingly complex mixture of enantiomeric and racemic compounds probably numbering many thousands of molecular species. As mentioned, milk triacylglycerol structure is reviewed in the accompanying paper by Kuksis (4).

In addition to triacylglycerol, milk lipids contain the so-called neutral lipids, alkyl and alk-1-enyl ether diacyl-glycerols (3).

## **Phospholipids**

The phospholipids in milk are presented in Table II. There have been no new additions to the list since mid-1968, but several of the components have been more thoroughly analyzed, as will be disucssed later.

Diphosphatidyl glycerol (cardiolipin) is found in lactating mammary tissue at levels 20-30 times the amount found in milk (12). The authors attributed this difference to selectivity during milk secretion.

Lysophosphatides are presumably always present, but whether or not these are artifacts of isolation or fragments of biosynthesis is unknown. A determination of the structure of these compounds, e.g., sn-1- or sn-2-monoacyl, should provide useful information.

Phosphatidylserine has received attention from Boatman et al. (13) who found contents of 2.4-3.4%, as percentage of total lipid P. In the same paper, 37.6-40.9% quantities of phosphatidylethanolamine were noted as well as ceramide mono- and dihexosides (cerebrosides).

Cerebrosides were earlier observed by, among others, Nutter and Privett (14) and have been recently analyzed more completely by Kayser and Patton (15), who observed partitioning of the compounds between milk fat globules (73%) and the serum (27%) portion of milk. They also identified glucosyl and lactosyl ceramides. Morrison and Hay (16) have published comprehensive data on the composition of both these compounds.

Lipids containing phosphonate groups have not been detected in milk lipids but may be present, since several have been identified in rumen protozoa (17).

Milk sphingolipids have been investigated by Fujino and Fujishima (18) and Morrison and Hay (16), all of whom identified the fatty acids and sphingosine bases present. These data will be presented later.

#### Sterols

These compounds are found in the unsaponifiable fraction of milk lipids and are mostly cholesterol with some lanosterol. Recently Brewington et al. (19) confirmed the presence of the latter sterol and identified two new constituents, dihydrolanosterol and  $\beta$ -sitosterol. Some of the many sterol precursors of cholesterol may also be present in trace amounts but have not been isolated and identified.

Keenan and Patton (20) have reported on the cholesterol esters of milk lipids. These represent ca. 0.1 of the sterol content of the milk.

#### Hydrocarbons

Milk lipids contain small quantities of various hydrocarbons: carotenoids, squalene, etc. (3,21). These compounds have not been investigated much in recent years. Waxes, which are present in plants, have not been found in milk lipids. These esters are undoubtedly hydrolyzed in the rumen; however the long chain alcohols should be detectable.

#### Lipoproteins

Lipoproteins in milk occur mainly as components of the milk fat globule membrane (6). The membrane is found in milk at a concentration of ca. 0.1% and is derived from plasma membrane which envelops the globule at secretion. Another lipoprotein is apparently present, a component obtained by ultracentrifugation (density, 1.063-1.21 g/ml)

TABLE III

Composition of Lipids from Milk Fat Globule Membranea,b

Lipid component	Per cent of membran lipids		
Carotenoids	0.45		
Squalene	0.61		
Cholesterol esters	0.79		
Triacylglycerols	53.4		
Free fatty acids	6.3 <sup>c</sup>		
Cholesterol	5.2		
Diacylglycerols	8,1		
Monoacylglycerols	4.7		
Phospholipids	20.4		

<sup>a</sup>Adapted from Reference 6.

bReference 24.

<sup>c</sup>Contained triacylglycerols.

from skim milk (22). The lipoprotein is not derived from the milk fat globule membrane and may be similar to the low density material isolated from disrupted milk microsomes by Berlin et al. (23), which also floated in salt solution of density 1.063. This lipoprotein contained 87%lipid, of which 52% was phospholipid and 35% neutral lipid. The cholesterol content was not determined.

The results of detailed analyses of the lipid composition of fat globule membranes from bovine milk have been published by Bracco et al. (24), Peereboom (25) and Prentice (26). These investigators found the high melting triacylglycerols and other lipids observed by others (6). Approximately 62% triacylglycerols were present—much less than in the parent milk. Among the hydrocarbons isolated, squalene was positively identified with indications by GLC of odd and even alkanes, and alkenes and polyunsaturated compounds between  $C_{13}$  and  $C_{38}$ . In addition to cholesterol, 7-dehydrocholesterol was detected; other hydrocarbons tentatively identified were carotenoids and tocopherols. Phospholipid classes were noted in relative quantities not greatly different from those in Table II.

Summarized data on milk fat globule membrane lipids can be seen in Table III. The free fatty acid and partial acylglycerol contents are probably too high. The former fraction contained some triacylglycerols (6). Bracco et al. (24) found lower quantities of all these lipid classes. These investigators determined the positional distribution of the component fatty acids within the membrane triacylglycerols, finding relatively larger quantities of 12:0, 14:0, 16:0 and 18:0 in position 2 than in unfractionated milk triacylglycerols.

## COMPOSITION OF LIPID CLASSES

There is little to add to our discussion (2) and that of Morrison (3) on the determination of milk fatty acids by GLC, other than the following. A superior method for the conversion of milk fat to methyl esters has been developed by Christopherson and Glass (27). A petroleum ether solution of milk fat is treated with either methanolic potassium hydroxide or sodium methoxide, resulting in rapid conversion of the acylglycerol fatty acids to methyl esters. The solution can be injected into the GLC instrument immediately. We have used this method for milk as well as many other fats and find it superior to all other methods of transesterification. Little or no loss of the short chain acid esters occurs, the loss being a problem that has plagued most other esterification methods for milk fat.

Many more fatty acids have been added to the list of those present in milk lipids that we compiled earlier (2). To summarize, the following fatty acids were believed to be present: even and odd saturated acids from 2:0 to 28:0; even and odd monoenes from 10:1 to 26:1 except for 11:1, positional and geometric isomers included; even acids from

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Positional and Geometric Isomers of Bovine Milk Lipid Monoenoic Fatty Acids<sup>a</sup> (wt%)

		cis	trans Isomers			
Position of double bond	14:1	16:1	17:1	18:1	16:1	18:1
5	1.0	tr			2.2	
6	0.8	1.3	3.4		7.8	1.0
7	0.9	5.6	2.1		6.7	0.8
8	0.6	Trace	20.1	1.7	5.0	3.2
9	96.6	88.7	71.3	95.8	32.8	10.2
10		Trace	Trace	Trace	1.7	10.5
11		2.6	2,9	2.5	10.6	35.7
12		Trace	Trace		12.9	4.1
13					10.6	10.5
14						9.0
15						6.8
16						7.5

<sup>a</sup>Adapted from Reference 28.

14:2 to 26:2 with some conjugated geometric isomers; polyenoic acids, 18:3-22:6, all even with some conjugated *trans* isomers; monobranched acids, 9:0-26:0 with the exception of 10:0, some of both iso and anteiso; multibranched acids, 16:0-28:0, both odd and even with three to five methyl branches and finally several keto, hydroxy and one cyclic acid. As mentioned in our paper and reiterated by Morrison (3), not all of these acids have been positively identified.

Hay and Morrison (28) studied the monoenoic positional and geometric isomers in bovine milk fat, finding a total of 44 cis 14:1, 16:1, 17:1, 18:1 and trans 16:1 and 18:1 isomers. The  $\Delta 9$  isomers and several of the positional isomers of 18:1 had been detected earlier (Table IV).

Strocchi and Holman (29), with the aid of argentation-TLC, GLC and mass spectrometry, identified more monoenoic acids as follows: *trans* 17:1 and 19:1, 20:1, 21:1, 22:1, 23:1 and 24:1. Notably missing was 11:1, either *cis* or *trans*. These acids were also not detected by Hay and Morrison (28).

Still to be completely identified are the large number of isomers that could result from positional and geometrical isomerization of *cis,cis-9*, 12-18:2. The same situation applies to 18:3. A few conjugated isomers of both acids have been found (2). Van der Wel and de Jong (30) have identified several *cis,trans* or *trans,cis* and *trans,trans* 18:2 positional isomers other than the 9,12 (Table V).

Strocchi and Holman (29) noted, but did not further characterize, two or more positional isomers of 18:2, 18:3, 20:2, 20:3, 20:4, 22:2, 22:3, 22:4, 22:5 and 24:3. Also detected were conjugated isomers of 18:2 and 18:3.

Hansen (31) isolated and identified 4,8,12-trimethyltridecanoic acid from milk fat. The acid was the DD diastereoisomer, and phytol was believed to be the precursor. Earlier Ackman and Hansen (32) examined three butterfat samples for phytanic and pristanic acids by an improved GLC method, finding that two contained about twice as much DDD as LDD isomer while the third had approximately equal quantities of both. Egge et al. (33) found at least 50 branched chain fatty acids in human milk fat by identification with GLC-mass spectrometry following hydrogenation and enrichment of the acids by urea fractionation. They postulated that many of these were of bacterial origin produced in or absorbed from the intestinal tract. Strocchi and Holman (29) confirmed the presence of 2,6,10,14-tetramethylpentadecanoate, 3,7,11,15-tetramethylhexadecanoate and DDD and DDL phytanates in bovine milk lipids. Also identified were others, including 13:0 anteiso, 19:0 iso and anteiso and 21:0 iso and anteiso, which were not listed in our compilation (2). Monomethyl branched isomers of 15:0 and 17:0 other than iso and anteiso were absent, although several of both were observed in human milk lipids by Egge et al. (33).

One of the questions that has arisen during the preparation of this paper is: In view of the large number of exotic fatty acids in plant lipids (34,35) and in rumen lipids (17), why have not more fatty acids been detected in milk lipids? Where, for example, are the cyclopropane acids? One answer is that they could be hydrogenated to methyl branched and straight chain acids during passage through the rumen (36). Also, some of the acid-catalyzed esterification and transesterification procedures may completely destroy or alter cyclopropane acids. Anhydrous methanolic HCl apparently may be used without alteration of the cyclopropane acids, as can methanolic boron trichloride (37).

Cyclopropene acids, consumed by the cow in cottonseed oil cake, may be hydrogenated in the rumen (36). Cows fed cottonseed oil yielded high melting milk fat, which was the result of accumulation of saturated fatty acids and decrease in oleic acid content. The major cyclopropene acid present is sterculic, which is a potent inhibitor of fatty acid desaturase enzymes. It seems likely that the effect of cottonseed oil fed to cows discussed above might be caused

TABLE V

Location of	Double	Bonds in Unconjugated
18:2	Isomers	of Milk Lipids <sup>a</sup>

cis, cis	cis, trans or trans, cis	trans, trans	
11, 15	11, 16 and/or 11, 15	12.16	
10, 15	10, 16 and/or 10, 15	11, 16 and/or 11, 15	
9, 15	9, 15 and/or 9, 16	10, 16 and/or 10, 15	
8, 15 and/or 8, 12	8, 16 and/or 8, 15	9, 16 and/or 9, 15	
7, 15 and/or 7, 12	and/or 8, 12	and/or 9, 13	
6, 15 and/or 6, 12			

<sup>a</sup>Adapted from References 3 and 30.

by the inhibitory effect on desaturase enzymes by sterculic acid. It follows that the acid may not be hydrogenated in the rumen. This could be easily tested in vitro. Sterculic acid administered intravenously to cows causes an increase in the stearic acid content of the milk fat during treatment (38).

If an investigator uses appropriate methods of concentration, such as urea inclusion, argentation TLC, etc., most of the many fatty acids found in plant lipids probably could be detected in milk. One deterrent to such an endeavor is the very low concentration at which many of these acids may occur in milk fat.

## **Protected Milk**

Ruminant milk fat contains relatively low concentrations, ca. 2%, of polyunsaturated fatty acids as a result of biohydrogenation of dietary lipids in the rumen. Australian investigators found that a polyunsaturated oil encapsulated in sodium caseinate by spray drying, followed by treatment with formaldehyde to prevent proteolysis of the protein, was protected against ruminal hydrogenation (39). For example, the 18:2 content of milk fat from a cow fed protected particles of safflower oil was 35.2% as compared to 2.0% for the control animal. Protected oils are hydrolyzed in the abomasum and the fatty acids absorbed in the small intestine, thereby avoiding hydrogenation (40). Effects of feeding protected corn and peanut oils to cows on the fatty acid composition of milk fat are presented in Table VI. The 14:0, 16:0 and 18:0 contents are reduced while the amounts of 18:2 were increased about five-fold. Similar increases were observed in plasma and depot fats. Investigators at the USDA (41) confirmed the findings of the Australian workers, similarly noting that the 18:2 content of cows' milk fat could be increased from 3 to 35% by feeding protected safflower oil. Thus it is possible, if proved necessary, to markedly increase the polyunsaturated fatty acid content of milk fat. It seems that a more economical method would be to homogenize the oil into either skim or whole milk.

### **Phospholipids**

Morrison (3) has tabulated the fatty acid composition of milk phospholipids and some of his data can be seen in Table VII. Few data have appeared since Morrison's review, but Boatman et al. (13) have determined the composition of phosphatidylserine and phosphatidylethanolamine, and these data are also shown in Table VII. The differences in composition can be attributed largely to the variations resulting from nutritional, environmental and genetic ef-

TABLE VI

Effect of Feeding Protected Corn and Peanut Oils on Fatty Acid Composition of Bovine Milk<sup>a</sup>

	Fatty acid of	Fatty acid composition of milk lipids, wt%				
Fatty acids	Corn oil	Peanut oil	Control			
14:0	7.9	9.7	11.9			
16:0	20.5	22.1	31.1			
18:0	9.8	11.0	13.5			
18:1	28.8	25.3	29.5			
18:2	20.1	20.5	4.2			
18:3	1.8	2,9	2.7			
Others	11.1	8.5	7.1			

<sup>a</sup>Oils entrapped in formaldehyde treated casein. Adapted from Reference 40.

fects.

Hay and Morrison (42) have analyzed the isomeric mono- and dienoic acids and the ethers in milk phosphatidylcholine and phosphatidylethanolamine. The composition of the major fatty acids in positions 1 and 2 (Table VIII), determined with the aid of phospholipase  $A_2$ , were in agreement with previous results (3). The types of fatty acids usually found in milk fat by argentation and gas liquid chromatography were observed. Phytanic acid was found only in the 1 position of the two phospholipids, while trans 17:1 and 18:1 acids were esterified mostly to this position. The cis 16:1, 17:1, 18:1 and trans 16:1 acids were more evenly distributed between the two positions. Trans monoenes were located about the same as the corresponding saturated acids. Neither cis nor trans positional isomers were selectively distributed. Cis, cis dienes, principally linoleic acid, were found mostly in the 2 position, but cis, trans and trans, trans isomers were more evenly distributed.

The composition of the alkyl and alkenyl esters present in very small quantities in milk phosphoglycerides (42) can be seen in Table IX; *trans* monoenes were not observed. The authors postulated that the branched chain compounds in the alkenyl ethers were derived from rumen microbial lipids.

Bracco et al. (24) have determined the fatty acid composition of milk fat globule membrane phospholipids. The compositions were not dissimilar from those in Table VII and the data from Bracco et al. for phosphatidylinositol are included.

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Morrison (3) presented earlier data on the fatty acid composition of these lipids. In a later paper Morrison and

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Fatty Acid Composition (mol%) of Various Bovine Milk Phospholipids<sup>a</sup>

Fatty acid			Phosphatidyl- choline	Sphingomyelin	Phosphatidyl- serine		Phosphatidyl- inositol	
12:0 Trace		b	0.7	0.3	3.6	1.6 <sup>b</sup>		
14:0	1.5	1.0	8.4	2.5	12.5	5.2	4.7	
15:0	0.5		2.1	0.4			1.3	
16:0	11.7	11.0	36.4	22.1	31.7	15.0	29.8	
16:1	2.1	1.1	0.6	0.8				
17:0	0.9		0.9	0,6				
18:0	10.5	13.0	11.1	4,5	13.0	30.0	31.8	
18:1	46.7	61.0	25.7	5.0	32.9	38.0	10.8	
18:2	12.4	12.0	5.3	0.9	4.9	7.3	6.9	
18:3	3.4	2.1	1.1			3.2	2.5	
20:3	1.4		1.0					
20:4	1.9		0.7			·		
22:0				14.7			3.9	
23:0	• <b>•</b> •			27.0				
24:0				14.8				

<sup>a</sup>Adapted from Reference 3. Minor acids omitted.

<sup>b</sup>Adapted from Reference 13, Minor acids omitted.

<sup>c</sup>From Reference 24, 3.8% 20:0 omitted.

#### TABLE VIII

Fatty Acid Composition (mol%) in 1 and 2 Positions of Bovine Milk
Phosphatidylethanolamine and Phosphatidylcholine <sup>a</sup>

	Phosphatidyle	ethanolamine	Phosphatidylcholine		
Fatty acid	1 position	2 position	1 position	2 position	
14:0	1.3	1.2	4.1	12.4	
16:0	10.7	4.6	34.4	27.7	
18:0	27.7	1.3	21.5	3.2	
18:1	52.0	27.7	25.5	36.7	
18:2	1.7	13.9	2,3	4.8	
18:3	0.8	4.2	0.4	1.0	

<sup>a</sup>Adapted from Reference 42. Most minor components omitted.

Hay (16) described the isolation and analysis of milk sphingomyelin, glucosylceramide and lactosylceramide. The long chain bases were similar in all compounds and consisted of normal, iso and anteiso saturated and unsaturated dihydroxy bases (Table X). The bases present in largest quantity were 18:1, 16:1, 17:1, 16:0, 18:0,iso 18:1and iso 17:1, with many branched chain bases occurring in smaller amounts. The major fatty acids, both normal and 2-hydroxy, were usually 22:0, 23:0 and 24:0 with some variations (Table XI). The *trans* acid content of total sphingomyelin fatty acids was 43-51%. Most of the *trans* isomers in sphingomyelin were shorter acids, e.g., 18:1 was 94.2% trans. Positional isomers of cis 23:1, 24:1 and 25:1 were mostly  $\Delta 9$  with small amounts of  $\Delta 7$ -14 present, whereas the *trans* isomers contained relatively large proportions of  $\Delta 10$  and 11.

Morrison (43) reported that there were no trihydroxy saturated bases in bovine milk sphingomyelin, although these compounds had been detected in mammalian kidney. However, after a more thorough search, a ceramide from milk sphingomyelin (1% of the total), was isolated. This fraction contained several saturated trihydroxy bases, with C-18, C-16, C-17 and C-iso-18 being the most prominent.

Kayser and Patton (15) also identified the components of milk glucosyl and lactosyl ceramides (cerebrosides). Fatty acids of the cerebrosides bound to the fat globule membrane were mainly 20:0-25:0, 74.0 and 58.0% saturated for glucosyl and lactosyl ceramides, respectively. In skim milk, the proportions were lower with 28.0 and 17.0% saturates occurring in the two ceramides. Fujino and Fujishima (18) found 16 fatty acids in the free ceramides of bovine milk, with 90% consisting of 16:0, 22:0, 23:0 and 24:0. Seven accompanying sphingosine bases were observed with the C-16, 16-methyl-C-17 and C-18 compounds accounting for 83% of the total. According to the authors this was the first isolation of ceramide, in free form, from bovine milk.

The partitioning of fatty acids observed by Kayser and Patton (15) has important implications with regard to the milk component from which polar lipids are extracted. Patton and Keenan (44) had found that 42 and 58% of the lipid phosphorus in milk occurred in skim milk lipoprotein

Aliphatic chain		Alkyl ethers	Alkenyl ethers	Aliphatic chain		Alkyl ethers	Alkenyl ethers
br	13:0		0.2		16:1	0.7	4.6
	13:0	1.0	0.2	iso	17:0	1.2	1.6
br	14:0A	0.8	8.2	anteiso	17:0	1.5	3.8
br	14:0B	1.2			17:0	1.5	2.4
	14:0	3.6	3.4		17:1	0.6	
iso	15:0	1.4	6.7	iso	18:0	0.9	0.4
anteiso	15:0	2.4	20.1		18:0	25.5	4.8
	15:0	2.8	5.4		18:1	10.8	0.8
	15:1	0.3		iso	19:0	2.4	1.3
iso	16:0	1.0	3.2	anteiso	19:0		2.5
	16:0	30.4	30.4		19:0	0.8	

TABLE IX

<sup>a</sup>Adapted from Reference 42.

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Composition (wt%) of Dihydroxy Long Chain Bases in Bovine Milk Sphingolipids<sup>a</sup>

Base		Sphingomyelin	Glucosyl ceramide	Lactosyl ceramide	Bas	e	Sphingomyelin	Glucosyl ceramide	Lactosyl ceramide
	12:0	0.1		0.2		12:1	0.5	0.3	0.3
	14:0	0.1	0.5	0.4		13:1	0.4	0.5	0.7
	15:0	0.1	0,8	0.6	iso	14:1	0.2	0.3	0.2
iso	16:0		0.3	0.5	130	14:1	1.3	1.6	1.5
	16:0	8.2	2.1	3.6	iso	15:1	0.2	0.3	0.6
iso	17:0	0.2	~~~	0.6	anteiso	15:1	0.4	0.6	0.3
anteiso	17:0	0.2	0.5	0.9	uniciso	15:1	0.5	1.5	1.2
	17:0	1.1	1.0	1.6	iso	16:1		0.2	0.2
iso	18:0	0,4	0.8	0.3		16:1	21.0	10.4	11.6
	18:0	5.9	2.0	4.9	iso	17:1	1.2	0.8	1.3
iso	19:0	0,3			anteiso	17:1	0.8	1.1	1.4
anteiso	19:0	0.2				17:1	6.4	4.7	3.9
	19:0	0.2	1.3	1.5	iso	18:1	8.9	6.7	7.7
iso	20:0	0.2	1.6	1.4		18:1	33.5	48.1	37.6
	20:0	0.6	2.7	6.2	iso	19:1	1.8	2.2	2.0
					anteiso	19:1	3.5	3.2	2.4
						19:1	0.8	2.0	2.2
						20:1	0.8	1.9	1.5

TABLE XI
Fatty Acids (wt%) of Bovine Milk Sphingolipids <sup>a</sup>

	Sphingomyelin		Glucosy	l ceramide	Lactosyl ceramide		
Fatty acid	Normal	Hydroxy	Normal	Hydroxy	Normal	Hydroxy	
12:0	0.1				0.1		
14:0	0.4	1.6	1.0	2.8	0.3	0.6	
15:0	0.1		0.3		0.1		
16:0	7.8	9.2	9.3	12.6	7.7	10.5	
16:1		0.8	1.4		0.3		
17:0	0.3	1,1	1.3	1.3	0.2	1.1	
18:0	1.6	6.2	13.7	8.4	3.3	3.7	
18:1	0.2	0.7	12.2		1.3		
18:2	0.2		2.0		0.2		
19:0	0.2	0.6	1.3		0.2		
20:0	0.6	0.6	0.9	4.5	1.1	1.2	
21:0	0.9	2.0	1.2	1.3	1.4	0.9	
21:1			0.1				
22:0	20.7	17.2	17.0	16.7	24.9	15.4	
22:1	0.7		0.9		0.6		
23:0	30.4	31.5	22.0	31.0	29.5	26.9	
23:1	5.0	2.5	3.4	1.7	6.6	6.3	
24:0	22.8	21.8	9.9	19.7	16.5	29.5	
24:1	4.0	1.5	2.1		3.7	4.1	
25:0	1.6	1.9			0.7		
25:1	1.6	1.0			1.4		
26:0	0,8						

<sup>a</sup>Adapted from Reference 16.

and in the milk fat globule membrane, respectively. The two sources of lipid phosphorus contained the same phospholipids, which in most cases had similar fatty acid compositions. Nevertheless the varying compositions of cerebrosides from the two sources, both from the same milk, indicate that the two sources are different in composition and are not, therefore, representative samples of milk polar lipids as in fresh whole milk.

#### **Sterol Esters**

Keenan and Patton (20) isolated and identified the cholesterol esters from cow, sow and goat milk and mammary tissue. The fatty acid compositions of the esters from the cow are tabulated in Table XII. The authors commented that the concentrations of monounsaturated, other than 18:1, and odd-numbered fatty acids in the cholesterol esters were greater than those found in milk triacylglycerols. For example, only traces of 13:1 are found in the latter.

## DISCUSSION

The major lipid classes and components of milk have apparently been identified and future research will probably involve the minor lipids, so-called because the quantities are small. Many additional fatty acids are undoubtedly present, dependent perhaps on what types of plants the cow consumes and on variations in the rumen fermentation. Some may be ephemeral. Thus many intriguing problems of isolation and identification remain.

Although a precise count has not been made, it is estimated from the papers reviewed herein that at least 300 fatty acids have been found in milk lipids, with some not as yet positively identified. Schwartz, in an accompanying paper (45) has added ca. 200 more to the list for a total of 500. An estimate of all of the individual lipid species would give a number in the very high thousands if the triacylglycerol species were included. Bovine milk, therefore, contains the most complex known lipid.

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## TABLE XII

#### Fatty Acid Composition of Cholesterol Esters from Bovine Milka

Fatty acid	Wt%	Fatty acid	Wt%
10:0	2.9	15:1	2.6
10:1	0.3	16:0	26.9
12:0	4.1	16:1	11.9
12:1	0.2	17:0	tr
13:0	tr	17:1	ND
13:1	11.0	18:0	6.7
14:0	6.9	18:1	13.7
14:1	0.5	18:2	10.1
15:0	2.1		

<sup>a</sup>Adapted from Reference 20.

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#### [Revised manuscript received February 26, 1973]