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Review

The composition and biosynthesis of milk lipids

G. A. Garton

Rowett Research Institute, Bucksburn, Aberdeen, Great Britain

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Apart from the obvious importance of milk as the natural food of the infant mammal and, in the case of cows' milk, as food for man, its ready availability has invited chemical and biochemical study. In respect of milk fat in particular, its special place in the history of lipid chemistry is noteworthy; it was included by Chevreul in his classical studies of 150 years ago (1), from which he deduced that fats are ester-like substances composed of glycerol and fatty acids, of which he isolated butyric, caproic, and capric acids from butter. Since that time, many investigations on the chemistry of milk lipids have been reported and much has been written about their possible biochemical origin.

Information available on the formation of milk fat has been extensively reviewed, most recently by Folley and McNaught (2), Folley (3), Glascock (4), Hele (5), Hele and Popják (6), Luick (7), and Shaw and Lakshmanan (8). Though the work on lipogenesis has lately attracted considerable attention in view of the widespread general interest in this field, many purely chemical studies have been conducted on milk lipids with results that have not been summarized and discussed collectively since 1956–57, when this was done by Jack and Smith (9), by Shorland and Hansen (10), and by Hilditch in his well-known book (11).

It is therefore intended in this review to survey present knowledge of the chemical composition of milk lipids, then to consider current views on the origin of milk fatty acids and their incorporation into glycerides in the mammary gland, and finally to discuss milk lipids in relation to diet and digestion, dealing only briefly with material that has previously been discussed in detail.

I. THE LIPIDS OF MILK

The lipid content of normal milk from healthy animals in full lactation widely varies from species to species, and, to a lesser extent, within species, as is well known in the case of milk from cows of different breeds. Colostrum may contain more lipid than does the normal milk subsequently secreted (*vide* proximate analyses quoted by Garton [12]), and, in the cow and in man, the lipid content of normal milk is known to progressively increase during its withdrawal from the mammary gland (13). Proximate analyses of milk (12) include lipid values ranging from 1.6% (horse) to 36.6% (whale); for the milks most extensively studied (cows' milk and human milk), the amount of lipid present is usually about 4%.

The lipids consist essentially of triglycerides, together with very small proportions of other lipids including phospholipids, cholesterol, squalene, lanosterol, free fatty acids (9), traces of monoglycerides resulting from lipolysis (14), and the recently-discovered bound aldehydes, glycerol ethers, and keto-compounds, which are described later. Much detailed work has been done on the structure of the fat globules (see King [15]) showing that the phospholipids present (about 0.3% of the total lipid) are integral constituents of the globule membrane, with which are associated small amounts of "high-melting" triglycerides and possibly cholesterol.

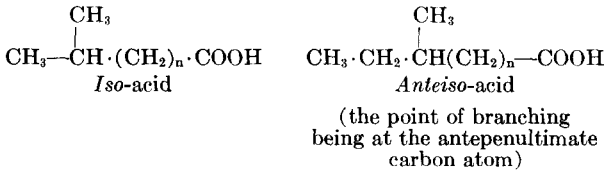
COMPONENT FATTY ACIDS

The over-all pattern of the principal component fatty acids of milk fats was established several years ago, many of the analyses being due to Hilditch and his

co-workers who employed the techniques of ester distillation and alkali isomerization in this regard. Results thus obtained, representative of the composition of the milk fats of several species of mammals, are shown in Table 1.

One of the most striking features to emerge from these investigations was that milk fats of herbivores, especially those of ruminant animals, differed from the corresponding depot fats and from the milk fats of other species in that they contained significant amounts of steam-volatile components, mainly butyric, caproic, caprylic, and capric acids. Because of the resultant wide variation in the molecular weights of the acids present in these milk fats, the values in Table 1 are given as molecular percentages rather than percentages by weight in order to show the relative number of molecules of each constituent fatty acid. The difference between herbivores and other mammals in respect to the steam-volatile fatty acid of their milk fats and between the composition of the milk fats of ruminant and non-ruminant herbivores (e.g., the relatively high content of octadecatrienoic acid in horse milk fat *vis-à-vis* that in bovine milk fat) will be discussed later in relation to the specialized digestive processes of herbivores.

Saturated Fatty Acids. The results of ester-fractionation analysis depended on the fundamental assumption that the acids present represented those of homologous series of saturated and unsaturated *n*-straight-chain components, each possessing an even number of carbon atoms; the composition of each ester fraction was determined from its saponification equivalent, its iodine value, and (where appropriate) its behavior on alkali isomerization. For a long time, many saturated acids, which it was deduced were present, were not formally identified, though for most components a melting point, saponification equivalent, iodine value, and combustion analysis were recorded. However, about ten years ago, as a result of detailed investigations of fractions of methyl esters of fatty acids derived from cow milk fat, Shorland and



his co-workers discovered that saturated branched-chain fatty acids (*iso*-acids, *anteiso*-acids, and a multi-branched acid), and saturated *n*-fatty acids with an odd number of carbon atoms were present, though only as a very small proportion of the total fatty acids. In Table 2 are listed these minor constituents of bovine milk fatty acids that have been isolated to date.

Other work in Shorland's laboratory (39, 40) failed to show the presence of odd-numbered, volatile fatty acids in freshly-prepared milk fat of cows and sheep; this was unexpected since these had been reported in goat milk fat (47) and (in extreme traces) in ruminant depot lipids (41, 42). It was shown, however, that autoxidized butter fat contained some volatile, odd-numbered fatty acids (39).

In their review of the component acids of bovine milk fat, Shorland and Hansen (10) point out that the unequivocal identification of the saturated acids of milk fats requires stringent analytical criteria (e.g., mixed melting points with the authentic acids or their

TABLE 2. BRANCHED-CHAIN SATURATED FATTY ACIDS AND ODD-NUMBERED SATURATED FATTY ACIDS ISOLATED FROM BOVINE MILK FAT

Nature of Fatty Acid	Estimated % (w/w) of Total Acids	Reference
Branched-chain acids		
Iso-acids		
11-Methyldodecanoic acid	0.05	(25)
12-Methyltridecanoic acid*	0.05	(26)
13-Methyltetradecanoic acid	0.37	(27)
14-Methylpentadecanoic acid	Tr.	(28)
15-Methylhexadecanoic acid*	Tr.	(29)
10(?)-Methylheptadecanoic acid†	Tr.	(30)
Anteiso-acids		
(+)-10-Methyldodecanoic acid	0.01	(25)
(+)-12-Methyltetradecanoic acid	0.43	(27)
(+)-14-Methylhexadecanoic acid	0.41	(31)
Multi-branched acid		
3,7,11,15-Tetramethylhexadecanoic acid	0.05	(32); see also (33, 34)
Odd-numbered n-acids		
Undecanoic acid	Tr.	(35)
Tridecanoic acid	0.03	(25)
Pentadecanoic acid	0.82	(27)
Heptadecanoic acid	Tr.	(36)
Nonadecanoic acid	Tr.	(37)
Heneicosanoic acid	0.05	(37)
Tricosanoic acid	0.06	(37)

* From hydrogenated glycerides.

† This acid is isomeric with stearic acid, though it is not an *iso*-acid as defined by Weitkamp (38) and Shorland and Hansen (10); i.e., an acid with a methyl side-chain on the carbon atom adjacent to the terminal methyl group (see generic formula in text).

TABLE 1. FATTY ACID COMPOSITION OF MILK FATS DETERMINED BY ESTER FRACTIONATION ANALYSIS*

	Molecular Percentages of Fatty Acids									
Fatty Acid	Cow	Cow (colostrum)	Sheep	Goat	Pig	Horse	Man	Man (colostrum)	Seal	Whale
Saturated										
Butyric	10.5	2.6	7.5	7.5		1.1	—	0.8	—	—
Caproic	4.6	1.6	5.3	4.7		1.9	—	0.2	—	—
Caprylic	1.3	0.5	3.5	4.3	2.4	4.4	—	0.1	—	—
Capric	2.7	1.6	6.4	12.8		7.9	2.5	1.4	—	—
Lauric	2.6	3.2	4.5	6.6		6.8	8.3	3.4	—	—
Myristic	9.6	9.5	9.9	11.8	1.8	7.4	8.7	5.7	3.4	12.1
Palmitic	23.4	31.7	21.6	24.1	28.3	15.4	22.8	28.9	17.8	19.0
Stearic	9.7	11.8	10.3	4.7	6.1	2.4	8.3	7.2	2.8	3.3
Chain-length > C_{18}	0.6	0.6	0.8	0.4	N.d.†	0.2	0.8	2.3	—	0.3
Unsaturated										
Decenoic	0.3	0.1	0.2	0.3	N.d.	1.3	—	0.1	—	—
Dodecenoic	0.2	0.2	0.2	0.3	N.d.	1.2	0.2	0.1	—	—
Tetradecenoic	1.0	0.7	0.6	0.8	N.d.	1.9	0.6	0.2	2.0	1.7
Hexadecenoic	2.1	2.7	2.0	2.2	8.8	7.2	3.8	3.0	13.9	8.9
Octadecenoic	28.6	28.5	21.6	16.5	35.0	16.3	33.9	35.1		
Octadecadienoic	1.8	2.5	4.3	2.8	14.0	6.6	7.7	5.9	36.3	26.1
Octadecatrienoic	—‡	0.4	—	—‡	N.d.	14.0	—	0.2		
Chain-length > C_{18}	1.0	1.8	1.3	0.2	3.6	4.0	2.4	5.4	23.8	28.6
Reference	(16)	(17)	(18)	(18)	(19)	(18)	(20)	(21)	(22)	(23)

* See text for details of unsaturated acids and of fatty acids now known to occur in trace amounts.
† N.d. = not determined.
‡ Trace amounts subsequently revealed by alkali isomerization of distilled ester fractions (24).

derivatives, X-ray diffraction measurements) additional to those formerly deemed sufficiently adequate; from the overwhelming abundance of *n*-straight-chain acids with an even number of carbon atoms, however, it is clear that the identity of the saturated components already claimed (Table 1) was basically well established. It is, however, surprising that it was not until 1953 that lauric acid, long since presumed present in bovine milk fat, was definitely identified (43). In addition to the series of even-numbered, saturated, *n*-fatty acids listed in Table 1 as present in bovine milk fat, Hansen, Shorland, and Cooke (37) have recently reported the isolation of trace amounts of several high molecular weight saturated *n*-acids, namely, eicosanoic (arachidic) acid, docosanoic (behenic) acid, tetracosanoic (lignoceric) acid, and hexacosanoic (cerotic) acid, representing 0.21, 0.07, 0.05, and 0.06%, respectively, of the total fatty acids.

Ester-distillation analysis of milk fats has now been largely superseded by chromatographic methods, which require much less starting material and much less time to perform. With the advent of the application of reversed-phase partition chromatography to the separation of nonvolatile, saturated fatty acids (44), this was applied in 1951 to the even-number *n*-acids of goat milk fat by Popják et al. (45), and, with modification to embrace odd-numbered acids, to the saturated components of bovine milk fat by Garton and Lough (46). In 1956, James and Martin (47) published their brilliant paper on the separation of higher fatty acids by gas-liquid chromatography (GLC); included in this work was an analysis of goat milk fatty acids, which indicated the presence of many minor components. This was followed by similar studies on bovine milk fat by Hawke (39) and on human milk fatty acids by Insull and Ahrens (48). Subsequently, various groups of investigators, notably Gerson et al. (122), Patton et al. (49), Gander and co-workers (50, 51), and Herb and co-workers (53, 52), have applied GLC to the detailed analysis of bovine milk fatty acids. These studies have amply confirmed the presence and proportions of the main component saturated acids and have added to the list of minor components. In one of the most recent studies, Herb et al. (53) found a total of no less than 64 acids, 27 of which were present at a concentration of <0.1%, accounting for only 1.0% of the total fatty acids. Identity of the many minor components was based solely on their relative retention times before and after hydrogenation and on the assumption that the branched-chain acids were saturated and possessed only one methyl branch of the *iso* or *anteiso* type. Evidence of this kind must be taken

cum grano salis, particularly conclusions drawn from relative retention times regarding the number of carbon atoms in an ester, even if it be saturated. The need for positive chemical identification of a component is borne out by the study of Sonneveld et al. (32), who isolated 3,7,11,15-tetramethyhexadecanoic acid (not reported by Herb et al. [53]) from bovine milk fat; GLC data alone would not permit a structure to be assigned, even provisionally, to this acid. The so-called "high-melting" triglycerides of milk (which, as mentioned earlier, form part of the structure of the milk-fat globule membrane) were first isolated in 1933 by Palmer and Wiese (54). With the advent of GLC, it became possible to identify the constituent fatty acids; Patton and Keeney (55) and Thompson, Brunner, and Stine (56) reported that palmitic acid accounted for more than half the total fatty acids, the remainder consisting of stearic and myristic acids together with traces of other components.

Unsaturated Fatty Acids. In Table 1, the unsaturated acids are listed "generically" without reference to chemical structure. The elucidation of chemical structure has presented many problems over the years (10, 11), particularly in bovine milk fat to which the following account refers unless otherwise indicated.

No unsaturated acid having less than ten carbon atoms has been found in milk fat, though as early as 1912 Smedley (57) deduced the presence of unsaturated acids of chain length less than 18 carbon atoms. Evidence for the presence of decenoic, dodecenoic, tetradecenoic (myristoleic), and hexadecenoic (palmitoleic) acids was subsequently forthcoming from various groups of workers (for references see [10], [11], and [58]); Hilditch and Longenecker (59) established that the double bond in each acid was predominantly, if not exclusively, in the 9,10 position relative to the carboxyl group. More recent investigations have confirmed the absence of positional isomers of hexadec-9-enoic acid (60), and have shown that *trans* components are present (61) to the extent of as much as 20% of the hexadecenoic acid (60), contrary to the former assumption that this group of monoethenoid acids was entirely of the *cis* configuration. An odd-numbered unsaturated acid (heptadec-*cis*-9-enoic acid) has been identified (62) as 0.06% of the total fatty acids of butter fat, and GLC evidence (53) suggests that others are present in trace amounts.

It is to the C_{18} group of unsaturated fatty acids that most attention has been paid, since they comprise a considerable proportion of the total fatty acids of milk fats. For a long time, it was considered that the monoethenoid component consisted entirely of oleic acid (octadec-*cis*-9-enoic acid), though about 35 years ago doubt was cast on this assumption by Hilditch and Jones (63), who found that the dihydroxy acid that resulted from the alkaline $KMnO_4$ oxidation of the C_{18} unsaturated acids of butter fat had a somewhat indefinite melting point lower than that of dihydroxystearic acid prepared from oleic acid. At about the same time, Bertram (64) isolated from butter fat traces of a solid, unsaturated C_{18} acid (octadec-11-enoic acid) to which he ascribed a *trans* structure and gave the trivial name vaccenic acid; this structure was confirmed by Rao and Daubert (65) and by Gupta et al. (66). In addition to vaccenic acid, small amounts of other oleic acid isomers have been reported in cow milk fat (66–69), buffalo milk fat (67), and sheep milk fat (66). In their detailed study of cow milk fat, Backderf and Brown (60) deduced that the C_{18} unsaturated components included octadec-*trans*-16-enoic acid and possibly the *cis* isomer of vaccenic acid; no evidence was found for the presence of elaidic acid (the *trans* isomer of oleic acid). The unequivocal (chemical) identification of octadec-*trans*-16-enoic acid (as 0.2% of the total fatty acids of butter fat) was achieved very recently by Hansen and Cooke (70). In human milk fat, Brown and Orians (71) obtained evidence for the occurrence of isomers of oleic acid and, from GLC data, Insull and Ahrens (48) estimated that about 7% of the total fatty acids were of this form (cf 29% as "normal" oleic acid) and isolated an octadec-11-enoic acid as the principal component.

The situation in respect of the C_{18} diethenoid fatty acids of milk fat is somewhat more confused, despite many attempts to characterize the positional and geometrical isomers present (for references see Hilditch [11], Jack et al. [61, 72], Shorland and Hansen [10], and Scott et al. [58]). Since the iodine value of the C_{18} unsaturated acids of bovine milk fat indicated that acids other than monoethenoid components were present, numerous attempts were made (many of them by Hilditch and his collaborators) to isolate linoleic acid (octadeca-*cis*-9,*cis*-12-dienoic acid) as the petroleum ether-insoluble tetrabromide, a compound well-characterized from studies on the fatty acids of many seeds. From these experiments (which did not yield tetrabromolinoleic acid) and from oxidation studies, it was concluded that, at most, only traces of linoleic acid were present and that *cis-trans* isomers constituted the bulk of the C_{18} diethenoic acids; a similar conclusion was reached (18) regarding the octadecadienoic acid in the milk fat of two other ruminants, the goat and the sheep. In 1949, however, White and Brown (73) claimed to have isolated tetrabromolinoleic acid from butter fat in amount corresponding to about 70% of the

octadecadienoic acid. In a very recent paper from the same laboratory (74), a similarly high proportion of linoleic acid was reported, the remainder consisting of positional isomers having widely-separated double bonds probably of *cis-trans* configuration (cf Scott et al. [58]).

Another consideration regarding the octadecadienoic acids is whether the double bonds are separated by three carbon atoms (i.e., —CH=CH—CH_2—CH=CH—, as in linoleic acid) or whether they are "conjugated" (i.e., have the structure —CH=CH—CH=CH—). Complementary to many of the studies just discussed, ultraviolet spectroscopy has been employed to examine this fraction of butter fat acids before and after treatment with hot alkali, which effects a partial rearrangement of nonconjugated structures to the conjugated form showing specific absorption bands. It has thus been demonstrated on numerous occasions (10) that conjugatable and preconjugated forms exist, and that the latter comprise *cis-trans* or *trans-trans* forms (58).

The complexity of the octadecadienoic acids of bovine milk fat and the apparently conflicting evidence regarding the occurrence of linoleic acid is discussed later in this review in relation to the effects of rumen bacteria on the fatty acids of the animals' feed.

In contrast to the mixture of isomeric forms of octadecadienoic acid found in ruminant milk fat, that present in horse milk fat is probably entirely linoleic acid (18) and that in human milk fat contains a high proportion of linoleic acid (20, 48, 71).

The octadecatrienoic acid present in small amount in bovine milk fat and forming a significant proportion of horse milk fat (Table 1) has, in each case, been identified (18, 75) as consisting almost entirely of linolenic acid (octadeca-*cis*-9, *cis*-12, *cis*-15-trienoic acid), though traces of a conjugated isomer have been detected in butter fat (see Shorland and Hansen [10]).

Unsaturated acids of longer chain length containing 4, 5, and possibly 6 double bonds have been shown, by alkali isomerization, to be present in the milk fats of several species (10, 71, 76); the tetra- and penta-unsaturated acids of bovine butter fat have the all-*cis* configuration (58). The recent GLC analyses by Herb et al. (53) indicated the presence of C_{20} and C_{22} di-, tri-, tetra- and pentaunsaturated acids. Arachidonic acid (all *cis*-eicosa-5,8,11,14-tetraenoic acid) was isolated, as its octabromide, from butter fat by Bosworth and Sisson (77) some 30 years ago.

GLYCERIDE STRUCTURE

From the very large number of fatty acids identified in milk fats, it is evident that the possible number of different mixed triglycerides that may exist is legion. However, it is possible to group them into fairly well-defined major types as a result of fatty acid analyses of fractions derived from milk fat. As described by Hilditch (11), early studies included fractional crystallization and oxidative removal of unsaturated acids using $KMnO_4$ in acetone; these were followed by the more refined technique of low-temperature crystallization from acetone or ether to separate groups of glycerides of different degrees of unsaturation. By this means, the probable glyceride structures of the quantitatively more important components of cow milk fat and Indian buffalo milk fat were determined by Achaya and Hilditch (78) as shown in Table 3.

TABLE 3. FATTY ACID STRUCTURE OF THE PRINCIPAL GLYCERIDES OF BOVINE MILK FATS

Component Acid Residues* in Triglycerides	Cow Milk	Indian Buffalo Milk
	moles %	*moles %*
16:0, <16, <16	14	14
16:0, 18:0, <16	13	17
16:0, 18:1, <16	12	16
16:0, 18:0, 18:1	10	10
16:0, 18:1, 18:1	4	4
18:0, 18:1, <16	5	5
18:1, 18:1, <16	6	6

* 18:1 includes oleic acid and its isomers; <16 includes all acids containing fewer than 16 carbon atoms. Data of Achaya and Hilditch (78).

It is evident that these glycerides are very similar in the general pattern of their component acids and that this is particularly well marked in respect of the distribution of palmitic acid. Fully saturated glycerides were found to comprise 35 and 40%, respectively, of the cow and buffalo milk fats, values that accord well with those calculated (34 and 37%) on the basis of random distribution of these acids among the available glycerol molecules. No simple triglycerides or glycerides containing three unsaturated fatty acids were found in either of the milk fats, though presumptive evidence for the presence of traces of tripalmitin in a sample of American butter fat was obtained by Haab, Smith and Jack (79).

Using the low-temperature crystallization technique, Hilditch and Meara (80) were able to obtain fairly detailed information on the principal glyceride types of human milk fat (Table 4). This analysis showed that about half of the milk fat comprised glycerides containing at least two unsaturated fatty acid residues. The total fatty acids of the whole fat contained 33% of octadecenoic acid and 17% of other unsaturated acids

TABLE 4. PROBABLE GLYCERIDE COMPOSITION OF HUMAN MILK FAT

Glyceride Type	Moles % in Total Glycerides	
Fully saturated		
Di-C_{10-14}-monopalmitin	1.7	9.1
Mono-C_{10-14}-dipalmitin	1.7	
Mono-C_{10-14}-palmitostearin	5.7	
Monounsaturated,* disaturated		
Monounsaturated-C_{10-14}-palmitin	20.3	39.6
Monounsaturated-C_{10-14}-stearin	1.9	
Monounsaturated-dipalmitin	3.5	
Monounsaturated-palmitostearin	13.9	
Diunsaturated,† monosaturated		
Mono-C_{10-14}-diunsaturated	23.6	42.7
Palmito-diunsaturated	19.1	
Triunsaturated†	8.6	

* Almost entirely mono-oleo-glycerides.

† Containing one radical of linoleic acid or other unsaturated acid, the remaining one (or two) radicals being oleic acid. Data of Hilditch and Meara (80).

TABLE 5. COMPARISON OF GLYCERIDE COMPOSITION OF BOVINE AND HUMAN MILK FATS*

Structural Characteristic of Triglyceride	Moles % of Total Glycerides		
	Cow	Indian Buffalo	Human
Containing			
(*a*) One radical of myristic acid or a saturated acid of shorter chain-length	52	52	53
(*b*) Two radicals of myristic acid or saturated acids of shorter chain-length	21	22	2
(*c*) One palmitic acid radical	64	70	60
(*d*) Two palmitic acid radicals	8	8	6

* After Hilditch (11).

(with octadecadienoic acids predominating), thus providing sufficient of these for it to be concluded that each of the di- and triunsaturated glycerides contained, respectively, one and two octadecenoic acid radicals, the remaining acyl residue being one of the other unsaturated acids. The rest of the milk fat was largely composed of triglycerides containing palmitic acid, octadecenoic (oleic) acid, and either stearic acid or one of the C_{10}–C_{14} saturated acids.

Table 5 shows that there is a marked general resemblance in the pattern of the glyceride structure of human and bovine milk fats; monopalmito-glycerides accounted for about 90% of the total palmitic acid of both milk fats. The saturated fatty acids of shorter chain length are present mainly in glycerides that contain two different acyl groups, such as palmitic acid and octadecenoic (oleic) acid, the smaller proportion of these shorter-chain acids in human milk fat being reflected in a correspondingly smaller proportion of this type of glyceride.

In a recent analysis of cow butter fat, in which fractional crystallization was employed to segregate glyceride types, Bhalerao, Johnson, and Kummarow (81) deduced that the saturated and unsaturated fatty acids were distributed approximately at random among the glycerol molecules; Boatman, Decoteau, and Hammond (82), in a study of the trisaturated glycerides of butter fat, found that the amounts agreed well with those calculated for random distribution and that there was no preferential inclusion or exclusion of any of the principal saturated fatty acids present in the fat as a whole. A similar conclusion regarding the random distribution of the saturated and unsaturated acids was reached by Ast and Vander Wal (83) from studies in which pancreatic lipase was used to split off the fatty acids from the 1 and 3 (α, α') positions of the triglyceride molecules. Table 6 shows the values calculated by Ast and Vander Wal from their experimental results and from data derived from a similar investigation by Patton, Evans, and McCarthy (84).

In relation to the *intra*molecular structure of the glycerides, however, there is evidence from the studies involving selective hydrolysis by pancreatic lipase (83–85) of a definite tendency toward esterification of fatty acids in specific positions in the triglyceride molecule, as had previously been indicated from the analyses of fractions of butter fat obtained by countercurrent distribution (79). Butyric acid is apparently esterified exclusively in the 1-position of bovine milk glycerides (86); it was found (85) that the proportions of capric, lauric, myristic, myristoleic, and palmitoleic acids were greater in the 2-position than in the 1-position of the glycerides and that the converse applied to stearic acid and the unsaturated C_{18} acids. Palmitic acid showed only a very slight tendency to preferential esterification in the 2-position, though in milk fat from cows deprived of feed for several days, this acid was present in enhanced concentration in the 2-position of the glyceride molecules. This observation may be of significance in relation to the synthesis of milk fat in the udder (*vide infra*).

PHOSPHOLIPIDS

Analyses of the fatty acids of the mixed phospholipids of cow butter fat of British and Swiss origin were made

TABLE 6. TYPES OF TRIGLYCERIDE IN COW MILK FAT

Glyceride Type*	Sample Number 1†	2†	3‡
	moles %	*moles %*	*moles %*
S S S	24.5 (24.5)§	30.9 (31.2)	28.1 (28.2)
S U S	13.4 (14.7)	9.9 (14.8)	13.7 (14.8)
S S U	30.6 (29.4)	35.0 (29.6)	31.0 (29.6)
U S U	9.5 (8.8)	9.9 (7.0)	8.7 (7.8)
U U S	16.8 (17.6)	11.2 (14.1)	14.6 (15.6)
U U U	5.2 (5.2)	3.1 (3.3)	4.0 (4.1)

* S = saturated fatty acid, U = unsaturated fatty acid.

† Experimental results of Ast and Vander Wal (83).

‡ Calculated by Ast and Vander Wal (83) from data of Patton et al. (84).

§ Values in parentheses are those expected on the basis of a random distribution of fatty acids.

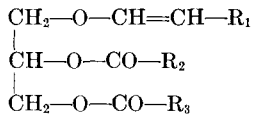
by Hilditch and Maddison (87). In confirmation of earlier studies, no steam-volatile fatty acids were detected; the major components were (1) palmitic acid, (2) C_{18} unsaturated acids (mainly monounsaturated), and (3) acids of longer chain length (both saturated and unsaturated), together with small amounts of myristic, hexadecenoic, and stearic acids. A previous report (88) that the fatty acids of milk phospholipids contained 70% of "oleic" acid was not confirmed, the proportions of monounsaturated C_{18} acid being 23.5 and 32.5%, respectively, in the fatty acids of the British and Swiss samples. Smith and Jack (89) found that saturated acids and monounsaturated acids each comprised about 40% of the total phospholipid acids, the remainder being nonconjugated di-, tri- tetra-, and pentaunsaturated acids, together with a small amount of conjugated diunsaturated acid.

In 1955, Baliga and Basu (90) fractionated the phospholipids of milk of cows, sheep, goats, and Indian buffaloes and found that they comprised lecithins ca. 30%, sphingomyelins ca. 25%, and cephalins (by difference) ca. 45%; values of a similar order were subsequently reported by other workers (91–94). In addition to the phospholipid classes mentioned above, Smith and Freeman (94) found 6% of cerebrosides; Rhodes and Lea (93) showed that the cephalins contained phosphatidyl ethanolamine and phosphatidyl serine in the proportions of about three to one. In addition, these latter investigators and Smith and Lowry (95) reported that unsaturated fatty acids were present in both the 1- and 2-positions of the glycerophospholipids, an observation that contrasts with the finding (96) that the glycerophospholipids of ox liver contain unsaturated acids in the 1-position and saturated acids in the 2-position. Smith and Lowry (95) made a detailed GLC examination of the component fatty acids of the phospholipids of bovine milk. The phosphatidyl ethanolamines were the most unsaturated, containing more than 50% of octadecenoic acid; of the remaining acids, stearic, palmitic, and octadecadienoic predominated. The major fatty acids of the phosphatidylcholines were octadecenoic acid (34.3%), palmitic acid (26.1%), and stearic acid (8.2%); those of the sphingomyelins were mostly saturated, with long-chain acids (C_{22}, C_{23}, and C_{24}) contributing about 30% to the total. The cerebrosides were characterized by their high content of myristic, palmitic, and tricosanoic acids. Results very similar to those of Smith and Lowry were reported by Badings (97).

RECENTLY-DISCOVERED MINOR COMPONENT LIPIDS

Investigations concerning "off-flavors" that develop in milk and milk products have included studies of the "carbonyl" compounds present before and after autoxidation; in the course of this work, several novel trace components of milk lipids have been discovered.

Plasmalogens and Glyceryl Ethers. In 1955, Van Duin (98) reported the presence of plasmalogens in cow milk lipids and showed that a series of long-chain aldehydes arose from them under the influence of the acidity of butter serum; the identity of hexadecanal (palmitaldehyde) and octadecanal (stearaldehyde) was established and several other saturated and unsaturated long-chain aldehydes, possibly including branched-chain components, were detected. Subsequently, Schogt, Begemann, and Koster (99) found aldehydogenic lipids in milk fat from which the phosphorus-containing lipids had been removed; calculated as tetradecanal, they accounted for 50 mg/kg milk fat. The aldehydes were shown to be bound to glycerol as enol-ethers, mostly in the 1-position of the molecule, the remaining hydroxyl groups being esterified to fatty acids giving the structure:



The aldehydes were later shown (100) to consist predominantly of the branched-chain 12-methyltridecanal and 12- and 13-methyltetradecanal, together with smaller amounts of a branched C_{13} aldehyde and *n*-aldehydes containing 13, 14, 15, and 16 carbon atoms; the aldehydogenic lipids of butter also yielded branched-chain components (notably a C_{15} aldehyde), though the *n*-aldehydes hexadecanal and octadecanal were present in greater proportions. As with the

branched-chain fatty acids of milk fat (Table 2), the aldehydes of the milk lipids were of the *iso* or *anteiso* type. Free aldehydes were also found in the milk lipids and nonaldehydogenic glycerol ethers were detected in the nonsaponifiable matter (99).

Concurrently, Parks, Keeney, and Schwartz (101) used GLC to analyze and identify the aldehydes derived from the plasmalogens of butter serum and from phosphorus-free butter oil. Both groups yielded a variety of straight-chain and branched-chain saturated aldehydes, together with several mono- and diunsaturated components. Like Schogt, Begemann, and Recourt (100), these workers found that the major aldehydes from the plasmalogens were hexadecanal and octadecanal, while those from the phosphorus-free butter oil were mainly hexadecanal and branched-chain saturated aldehydes containing 14 and 15 carbon atoms.

Nonaldehydogenic glycerol ethers were independently found and investigated in detail by Hallgren and Larsson (102). These ethers were shown to comprise 0.01% of the lipids of cows' milk and 0.1% of those of human milk and to be of the alkoxydiglyceride type:

$$\begin{array}{l} CH_2—O—CH_2—R_1 \\ | \\ CH—O—CO—R_2 \\ | \\ CH_2—O—CO—R_3 \end{array}$$

Following their chromatographic separation from the nonsaponifiable matter, the dihydric ethers were converted to their dimethoxy derivatives, which were analyzed by GLC before and after hydrogenation. About 80% of the alcohols (R_1) in ether linkage consisted of hexadecanol (palmityl alcohol), octadecanol (stearyl alcohol), and octadecenol (oleyl alcohol); the remainder comprised several saturated, unsaturated, and branched-chain components containing from 16 to 24 carbon atoms.

Ketones and Keto-Acids. An investigation of the volatile carbonyl compounds present in evaporated milk showed that acetone, pentanone-2, and heptanone-2 were present (103); other work (55) on the nonsaponifiable matter of the "high-melting" triglyceride fraction of milk fat revealed the presence of tridecanone-2 and pentadecanone-2. These observations prompted a detailed study of the methyl ketones of milk fat by Patton and Tharp (104), who isolated, as their 2,4-dinitrophenylhydrazones, a complete homologous series of methylketones with an odd number of carbon atoms (acetone to pentadecanone) from the steam-distillate and nonsaponifiable matter of cows' fresh milk. However, with the exception of acetone, none of the ketones were present before steam-distillation or saponification of the lipids. It seems likely, as suggested by Patton and co-workers (103, 104), that β-keto acids initially present in the milk fat may be the precursors of these methyl ketones, since such acids are known to undergo decarboxylation quite readily on heating.

Very recently, Keeney, Katz, and Schwartz (105) have reported that trace amounts of keto acids are present in milk in glyceride combination. Saturated keto-acids, containing 10 to 18 carbon atoms, and unsaturated C_{18} keto acids were detected by GLC. Keto-stearic acid predominated, and a detailed chemical examination of this component showed that it was a mixture of isomers having keto groups in the 8, 9, 10, 11, 12, and 13 positions, the molecular proportions being about 1, 39, 28, 10, 5, and 17% respectively. No isomer with a 3-keto group (i.e., a β-keto acid), such as might give rise to a methyl ketone, was detected, though it should perhaps be noted that heptadecanone-2 was not among the ketones isolated by Patton and Tharp (104). Boldingh and Taylor (106) also reported the isolation of traces of β-keto acids from butter fat as well as traces of δ-keto acids, δ-lactones, δ-hydroxy-lactones, and unsaturated γ-lactones.

This is a field in which further studies may be quite revealing, not only in connection with the origin of methyl ketones but possibly in relation to the biosynthesis of unsaturated fatty acids (see below).

II. THE ORIGIN OF MILK LIPIDS

ORIGIN OF FATTY ACIDS

For some years now, it has been appreciated that the fatty acids of milk lipids (glycerides) arise from two distinct sources—from blood plasma lipids and by direct synthesis in the mammary gland. It is not intended here to trace, in detail, the development of the work that led to this conclusion. Suffice it to refer the reader to the reviews cited in the introduction and to that of Garton (107), and to concentrate on recent findings in this field of research in relation to the relative contribution of the fatty acids from each source, the fractions of plasma lipids that may be involved, and the mechanism of intramammary synthesis of fatty acids.

Plasma Constituents as a Source of Milk Fatty Acids. Between 1935 and 1940, it was demonstrated that fatty acids of blood lipids can be incorporated into milk lipids; fatty acids not normally found in milk lipids (e.g., iodinated fatty acids, long-chain acids of cod liver oil) were fed to cows, and, subsequently, these acids were detected in the milk fat. With iodinated fatty acids, it was shown that their concentration in milk fat was directly related to that in the plasma neutral fat.

At about the same time, arteriovenous studies established that neutral lipids (glycerides and possibly cholesterol esters) were removed from the blood plasma flowing through the udder of the lactating cow.

In 1956, Glascock, Duncombe, and Reinius (108) fed tritium-labeled stearic acid, either as free acid or as triglyceride, to lactating goats and to a cow and tentatively concluded from analyses of the milk lipids subsequently secreted that the contribution of dietary fatty acids to milk fatty acids was probably not more than about 25%. In a later similar experiment in which a glyceride containing tritium-labeled stearic acid was fed to a lactating cow, evidence was obtained (109) that plasma lipids contained a quantitatively minor (unidentified) component of very high specific activity; the possibility that this plasma lipid fraction may be involved in milk fat synthesis remains to be investigated. Riis, Luick, and Kleiber (110) infused plasma containing P^{32}- and C^{14}-labeled lipids into a lactating cow, the plasma having been obtained from a donor (nonlactating) cow to which P^{32}-labeled phosphate and acetate-1-C^{14} had been administered. From a consideration of the resultant specific radioactivities of the lipids of the plasma and milk of the animal, it was calculated that about 50% of the fatty acids of the milk lipids were derived from plasma lipids and that, in addition to triglycerides, cholesterol esters and phospholipids are involved in the transport of fatty acids to the mammary tissue. That plasma phospholipids can be metabolized in the mammary gland and can also be synthesized in the tissue was earlier shown in experiments with lactating rabbits by Garton and Popják (111) and Garton (112). Lossow and Chaikoff (113) injected Na octanoate-1-C^{14} and tripalmitin containing palmitic acid-1-C^{14} intravenously into lactating rats and found that, within 24 hr, 45% of the octanoate and 60% of the palmitic acid appeared in the lipids of the milk and mammary gland, though what proportion of the milk fatty acids originated from plasma lipids was not determined.

By the examination of plasma lipids before and after perfusion of whole blood through the excised udders of lactating cows, Lough et al. (114) endeavored to obtain further information on the possible role of the different lipid fractions in mammary gland metabolism. A loss of triglycerides from the plasma was observed (confirming the findings of earlier arteriovenous difference studies) and, in some experiments, of cholesterol esters also; the cholesterol esters were apparently removed intact by the gland, since a concomitant increase in plasma free cholesterol was not found. Plasma phospholipids sometimes showed an increase and sometimes a decrease after perfusion, an observation possibly related to the previously mentioned finding (111, 112) that phospholipids can be both synthesized and broken down in lactating mammary tissue. In another perfusion experiment, Laurys-sens et al. (115, 116) found that plasma free fatty acids (FFA), which represent a quantitatively minor proportion of plasma lipids, can be rapidly taken up and incorporated into udder glycerides. Following the addition, as the albumin complex, of stearate-1-C^{14} to the perfusing blood, the gland glycerides contained significant amounts of labeled oleic acid in addition to stearic acid. Direct desaturation of stearic acid to give oleic acid apparently occurred; the significance of this process as a source of unsaturated acids for milk fat and the possible quantitative importance of FFA as precursors of milk lipids would seem to merit further investigation in the living animal.

Stemming from the suggestion in 1945 by Folley (117) that acetate might be a precursor of milk fatty acids, experimental evidence (from his laboratory and that of Popják) demonstrating this in vitro and in vivo was not long in forthcoming (118, 119). Later studies from these laboratories, and from those of Kleiber and of Shaw in the USA and of Peeters in Belgium, emphasized the important part played by plasma volatile fatty acids (especially acetate and, in the ruminant, β-hydroxybutyrate too) as carbon sources for production of long-chain fatty acids in the lactating mammary gland. Neither the propionate nor the butyrate of ruminant plasma is apparently incorporated to any great extent into milk fatty acids, though propionate can be the precursor of the odd-numbered fatty acids in milk fat and, following its degradation, to even-numbered fatty acids (120, 121); valerate has been shown (122) not to be a direct precursor of odd-numbered fatty acids of cow milk fat, though these can arise from propionate, which results (along with acetate) from the in vivo degradation of valerate. While β-hydroxybutyate can undoubtedly be metabolized to yield fatty acids of chain length up to 10 carbon atoms (8), the intermediary pathway is not clear though it seems unlikely, as pointed out by Glascock (4), to involve butyrate.

That plasma glucose can play a part in mammary lipogenesis was first demonstrated by Folley and French (118, 123). Working with mammary tissue slices, they observed that those from the sheep gland could utilize acetate alone, while those from the rat gland required glucose in addition to acetate. Subsequently, this difference between the ruminant and the nonruminant animal was confirmed and investigated in more detail by different groups of workers (2). For example, Popják, Hunter, and French

(124) showed that, in the rabbit mammary gland, glucose significantly contributed to the pool of C_2 units for fatty acid synthesis, while Kleiber and his colleagues (125, 126) found that in the living cow, only a very small proportion of C^{14}-glucose administered intravenously was incorporated into milk fatty acids though it was utilized for milk glyceride-glycerol synthesis. This aspect of glucose metabolism and also its possible involvement in the pentose phosphate cycle in relation to lipogenesis is referred to later in this review.

Endogenous Synthesis of Fatty Acids in Mammary Tissue. Following the outstanding work some ten years ago of Popják and Folley and their collaborators, which demonstrated the ability of the mammary tissue of both ruminants and nonruminants to effect the synthesis of saturated *n*-fatty acids from acetate, it was considered for several years that the process involved was probably a reversal of β-oxidation. Studies in Popják's laboratory (127–129), in which cell-free preparations of mammary tissue were used, showed that coenzyme A (CoA), adenosine triphosphate (ATP), and a reduced pyridine nucleotide were required for fatty acid formation from acetate; in addition, an enhanced synthesis was observed when malonate was added and also when air was used as the gas phase instead of O_2. The reason for this stimulation was not apparent at the time, though it subsequently became evident from the studies of Wakil and his co-workers (130–132) and from investigations in Brady's laboratory (133, 134) that a source of CO_2 was of fundamental importance in fatty acid synthesis by particulate-free preparations of animal tissues (pigeon liver and chicken liver). This work and other related studies (reviewed in detail by Wakil [135–137]) showed that this biosynthetic pathway was different from a reversal of β-oxidation. Fatty acid chains of up to 16 carbon atoms (palmitic acid) are now known to be elaborated from acetyl CoA, which condenses with HCO_3^- to yield malonyl CoA in the presence of ATP, Mn^{++} ions, and acetyl CoA carboxylase (a biotin-containing enzyme). Malonyl CoA condenses, in turn, with acetyl CoA to yield, by reaction with reduced triphosphopyridine nucleotide (TPNH), CO_2, CoA, and a saturated fatty acid; thus successive condensations yield palmitic acid, 2 carbon atoms of which come directly from acetyl CoA and the remaining 14 carbon atoms from malonyl CoA. As discussed by Folley and Greenbaum (138) and Folley (3), the TPNH requirement could be met by the breakdown of glucose by the pentose phosphate pathway in the mammary gland.

Propionyl CoA can take the place of acetyl CoA (which probably explains the occurrence of *n*-odd-numbered fatty acids in milk and other tissue lipids), but other higher acyl CoA derivatives apparently cannot undergo chain-lengthening in this manner, nor can substituted acyl CoA compounds such as acetoacetyl CoA and β-hydroxybutyryl CoA. The nature of the chemical form of the intermediates in chain-lengthening is still not certain and may vary from one tissue to another (137, 139).

Whatever be the intermediates in fatty acid chain-elongation in the mammary gland, the importance of the malonyl CoA pathway is soundly established. In 1960, Ganguly (140) showed that microsomal and soluble-enzyme preparations of many ox tissues including brain, adipose tissue, and mammary gland rapidly synthesized saturated fatty acids from acetyl CoA and malonyl CoA, and that the carboxylation of acetyl CoA was the rate-limiting step; noteworthy is the observation that the relative proportions of butyric acid, caproic acid, and (collectively) the C_8–C_{16} acids synthesized by the mammary gland system were similar to those present in cows' milk fat (Table 1).

A reinvestigation of fatty acid synthesis by soluble preparations of lactating rat mammary gland by Dils and Popják (141) showed the malonyl CoA route to be of major importance; all the cofactors required in the systems described by Wakil and by Brady were necessary for optimal synthesis. It was shown that, though free malonate strongly stimulated the formation of fatty acids, it was not incorporated into the carbon chains. It was suggested (141) that free malonate might suppress the deacylation of acetyl CoA or inhibit a reversible decarboxylation of malonyl CoA; evidence favoring the latter hypothesis has very recently been obtained by Coniglio and Popják (142), who also isolated an alkali-labile derivative of C^{14}-malonic acid that resulted from the incubation of soluble preparations of rat mammary tissue with ATP, CoA, Mn^{++}, and either C^{14}-acetate plus HCO_3^- or unlabeled acetate plus $H^{14}CO_3^-$; acetate and CoA could be replaced by acetyl CoA. Dils and Popják (141) found that several intermediates of the citric acid cycle, formerly thought necessary to stimulate fatty acid synthesis, were no longer required. The principal products of synthesis were the saturated *n*-fatty acids C_8–C_{18}, together with a small proportion of oleic acid, though, compared with milk fatty acids, a somewhat greater proportion of lauric and myristic acids was produced. A number of unidentified components were present, the behavior of which on GLC suggested that they were of high molecular weight or "of high polarity due to the presence of oxygen functions beyond the ester group." Dils and Popják suggest that these substances may be intermediates in fatty acid oxidation or synthesis. This

suggestion may well be pertinent to the proposition that keto acids may be intermediates in chain-elongation of fatty acids (135) and to the previously-noted finding of Keeney et al. (105) that keto acids are present in milk glycerides and other tissue lipids. Keeney et al. direct attention to the possibility that an unsaturated acid may arise from a keto acid by cleavage, in the presence of reduced pyridine nucleotide, of the carbon-oxygen bond of a phosphate ester of the enol form of the keto acid. Thus the observation of Dils and Popják (141), that the rat mammary gland preparations to which mitochondria or microsomes were added showed a suppressed synthesis of C_{10}–C_{16} acids and an increased production of oleic acid and unidentified (?keto) acids, appears particularly cogent. The lower homologues of oleic acid present in milk fat (Table 1) were shown by Hilditch and Longenecker (59) to have their double bonds in the 9,10 position, indicating that they may all originate in a similar manner; Hilditch and Longenecker suggested that a combined oxidation and reduction of oleic acid might be involved, though, in the light of the finding that stearic acid can undergo desaturation in the udder to give oleic acid (115, 116), these shorter-chain unsaturated acids may be similarly derived from the corresponding saturated homologues.

Work similar to that of Dils and Popják was concurrently being conducted in Chaikoff's laboratory (143, 144), though the conditions found for optimal synthesis of fatty acids from acetate by lactating rat mammary gland were somewhat different from those reported by the British workers; an absolute requirement for citrate was noted and the stimulating effect of free malonate was observed only in the presence of citrate. The fatty acids synthesized were qualitatively similar to those obtained by Dils and Popják, though the proportion of C_8–C_{12} acids produced was much greater.

Whereas the work on ox mammary gland preparations showed that the malonyl CoA route to saturated fatty acids was effected by both microsomal and soluble-enzyme fractions (140), subcellular particles depressed this synthesis in the soluble system of the rat gland, indicating a species difference; this possibility is supported by the unpublished observations of Dils and Popják (cited in [141]) that preparations of rabbit mammary gland require both microsomes and soluble enzymes for maximal synthesis of fatty acids from acetate. Similar studies with rat liver tissue (145, 146) have demonstrated that fatty acid synthesis requires both microsomes and soluble enzymes in about the same proportions (on a protein basis) as are present in the original liver cells.

A second pathway of fatty acid synthesis (the so-called "mitochondrial system") is known to exist in several tissues, and its distribution and *modus operandi* have been discussed in detail by Wakil (135, 137). The system relies on the synthetic ability of some enzymes known to participate in β-oxidation plus TPNH-α, β-unsaturated fatty acyl CoA reductase, and possibly a condensing enzyme. Both TPNH and DPNH are required for synthetic ability, which consists of carbon-chain elongation by successive addition of two-carbon (acetyl CoA) units. It seems that stearate arises from palmitate in this manner, and arachidonate from linoleate. Liver (ox, rat, pigeon) is a good source of this system, though to what extent, if any, it participates in mammary synthesis of fatty acids for milk lipids is not known. Dils and Popják (141) and Abraham et al. (143), found that avidin (by combining with biotin) almost completely inhibited fatty acid synthesis in rat mammary gland preparations, though Dils and Popják point out that no attempt was made to investigate optimal conditions for the possible production of fatty acids from acetate by a mitochondrial system.

ORIGIN OF GLYCERIDES

Source of Glycerol. Although it was thought until fairly recently that the glycerol of milk glycerides originated entirely from plasma glycerides absorbed by the mammary tissue, evidence has now accumulated showing that plasma glucose is a precursor, in the lactating gland, of significant amounts of the glycerol required for milk lipid synthesis.

In studies in which C^{14}-labeled acetate was administered to a lactating goat, Popják, Glascock, and Folley (147) obtained evidence from specific radioactivity measurements of an apparent precursor-product relationship between lactose and the glyceride-glycerol of the milk fat. They concluded that glucose, from which lactose is rapidly formed, is the carbohydrate precursor of glycerol in the mammary gland. A similar conclusion was reached from experiments in which C^{14}-labeled glucose was administered to lactating rabbits (124) and cows (148). Following the intravenous injection of C^{14}-labeled glucose into lactating cows, Luick and Kleiber (149) were able to show that at least 70% of the glycerol-carbon of the milk fat was derived from plasma glucose, though this could have taken place by direct synthesis in the gland or by way of plasma glyceride synthesized in the liver and subsequently absorbed by the udder. A further experiment by Luick (150) indicated that intramammary synthesis is probably a pathway of quantitative significance. Various C^{14}-labeled substances were infused into the

udders of lactating cows, and a comparison was made between the C^{14}-content of the glycerol obtained from milk fat secreted by a quarter of the udder that had been infused and that obtained simultaneously from a noninfused quarter. It was thus found that glycerol was synthesized from glucose, but not from acetate, propionate, or butyrate; free glycerol was also incorporated directly into milk glycerides—confirming an earlier observation by Wood et al. (151), who injected C^{13}-labeled glycerol into the pudic artery of a lactating cow.

Though the foregoing experiment of Luick demonstrated that glucose can be converted to glycerol in the mammary tissue of the living cow, it should be mentioned that no significant conversion was observed by Balmain (152) in mammary tissue slices or in a study in which labeled glucose was added to blood perfusing the excised udder of a cow (153). The reason for these negative findings is not clear, though in the perfusion of the udder it is possible, as suggested by Wood et al. (148), that the experiment did not last a sufficient length of time. Hardwick and Linzell (154), however, in a series of perfusion experiments using udders from lactating goats, found that the addition of labeled glucose to the perfusing blood gave rise to milk triglyceride-glycerol of high specific activity; similar high specific activities were found in the milk lactose and the respired CO_2. The secretion of milk was shown in earlier perfusion experiments (155) to depend on glucose being available, the energy for secretion apparently coming from a metabolic route not available to acetate—such as the pentose phosphate pathway.

Assembly of Glycerides. Before the ability of mammary tissue to synthesize fatty acids was known, it was considered that milk glycerides represented plasma glycerides absorbed by the gland and secreted in a modified form. To account for the presence of short-chain acids in esterified form, Hilditch proposed (11, 78), with particular reference to the glyceride structure of ruminant milk fat, that these arose by ω-oxidation of preformed unsaturated acid residues, especially oleic acid groups. Later, this hypothesis was modified and Hilditch suggested (156) that some oleic and possibly other unsaturated fatty acid residues present in plasma triglycerides might undergo acyl exchange with short-chain saturated acids synthesized in the mammary gland. This, of course, poses the question of the fate of the displaced unsaturated acids; so far, no evidence is available to show that such an exchange of acyl groups takes place, though Kumar and his colleagues (86, 157) have observed an exchange in vitro between butyric acid and long-chain acids of cow milk glycerides under the influence of pancreatic lipase.

There is support for the suggestion (107, 150) that synthesis of triglycerides can take place in mammary tissue. As noted earlier in this review, Luick (150) found that glycerol (as such or derived from glucose) could be incorporated into milk glycerides, and Patton et al. (158) have recently shown that hydrolysis of glyceride-bound fatty acids can take place in the udder followed by resynthesis of the liberated acids into new triglycerides. In this study, either 1- or 2-monopentadecanoin was infused into the udder of a lactating goat, and it was found that milk triglycerides subsequently secreted contained pentadecanoic acid esterified in both the 1- and 2-positions of the same glycerol molecule. Further, Luick and Lucas (159) found that, when free C^{14}-labeled stearate was similarly infused into a cow's udder, it was incorporated into milk triglycerides, and, as previously mentioned, Lauryssens et al. (115, 116) showed that the perfused udder from a lactating cow assimilated plasma free stearate into triglyceride combination.

In their study of milk glyceride structure, McCarthy et al. (85) found that most of the component fatty acids were, to some extent, selectively esterified in either the 1- or 2-position of the glycerol molecule and that the site of selective esterification differed with some acids (e.g., capric, myristoleic, palmitic, and octadecenoic acids) from that observed in the triglycerides of plasma obtained from the same cow. In the milk glycerides, palmitic acid was apparently randomly distributed; in milk glycerides from a cow from which feed was withheld, palmitic acid was esterified to a greater extent in the 2- than in the 1-position. It was concluded that, in a normally-fed animal, plasma glycerides contributing to milk glycerides must undergo a rearrangement of their fatty acids or "there must be a supplementary synthesis of milk fat which tends to compensate by concentrating palmitate in the 2-position."

The sum of evidence currently available, admittedly somewhat fragmentary, suggests that these two postulated alternatives may well be but facets of a single process, namely *de novo* synthesis from a pool of fatty acids (as their CoA derivatives) and glycerol (in the form of L-α-glycerophosphate) in a manner similar to that known to take place in other tissues (160). Evidence that the main pathway of glyceride synthesis in the lactating rat mammary gland is via α-glycerophosphate has very recently been obtained by Dils and Clark (161).

III. MILK LIPIDS IN RELATION TO DIET AND DIGESTION

EFFECT OF CONDITIONS IN THE ALIMENTARY TRACT OF HERBIVORES

The somewhat bewildering complexity of unsaturated fatty acids present in the milk fat of ruminants is apparently associated with the presence of the forestomach or rumen in which the breakdown and modification of feed constituents takes place under the influence of rumen microorganisms (mainly bacteria). In addition to the bacterial breakdown of carbohydrates and proteins, feed lipids are affected in that, in the rumen, esterified fatty acids can be liberated by bacterial hydrolysis (162–164) and unsaturated fatty acids can undergo complete or partial hydrogenation (165, 166). Feed lipids, such as those of grass and other herbage, are rich sources of C_{18} *cis*-unsaturated fatty acids, particularly linolenic acid (167–169); because of the hydrogenating activity of rumen bacteria, these acids yield stearic acid together with a wide variety of geometrical (*trans*) and positional isomers of unsaturated acids (166). The isomers produced are remarkably similar to those formed by catalytic hydrogenation when double bonds have been shown to migrate along the carbon chain (170) and linoleate gives rise readily to a conjugated double bond system (171). Linolenate is hydrogenated particularly effectively by rumen microorganisms (164, 166), and there is chemical evidence (172) that the central double bond is preferentially attacked. Thus the unsaturated acids, wholly or partially hydrogenated, pass—largely in the form of free acids (164)—with the other digesta to the intestine; following their absorption and assimilation into plasma lipids, these structurally modified acids subsequently appear in the milk lipids. The presence, as such, of linoleic acid and linolenic acid in bovine milk fat indicates that they have probably escaped the effects of ruminal hydrogenation. The previously mentioned claims regarding the nature of the C_{18} fatty acids of bovine milk fats and seasonal variations in their amounts (see, for example, Hansen and Shorland [173], Mattsson [174], Stadhouders and Mulder [175], and Wood and Haab [176]) are probably attributable to differences in the rumen microflora of the animals and to differences between the amount and kind of feed lipids ingested.

In nonruminant herbivores, such as the horse, the milk is characterized by the presence of fairly high proportions of linoleic and linolenic acids (given in Table 1 as octadecadienoic and octadecatrienoic acids). This presumably reflects the absorption and utilization for milk glyceride synthesis of these acids derived unchanged from feed lipids; a confirmative corollary is the observation of Kaufmann, Volbert, and Mankel (177) that no *trans*-unsaturated fatty acids are present in the milk lipids of grazing mares. The presence in the milk lipids of omnivorous and carnivorous mammals of *trans*-unsaturated fatty acids and of positional isomers of the common unsaturated acids is almost certainly due to their having ingested lipid of ruminant origin, and, in the case of lactating women, of their having eaten margarine or other fat containing fatty acids that have been subjected to the influence of catalytic hydrogenation. Kaufmann et al. (177) found that *trans*-unsaturated fatty acids disappeared quite quickly from the milk lipids of lactating women when they were given a diet from which *trans* acids were absent.

In herbivores, fermentation of carbohydrates by bacteria within the rumen or caecum (e.g., in the rabbit and the horse) is a source of volatile fatty acids consisting mainly of acetate, together with some propionate and butyrate. Following their absorption, these acids contribute to the endogenous synthesis of tissue lipids, including that of milk fat. The rumen bacteria also degrade proteins giving rise, *inter alia*, to branched-chain, volatile fatty acids; the aminoacid valine yields *iso*-butyric acid, and leucine and *iso*-leucine yield *iso*-valeric and 2-methyl butyric acids, respectively (178–180). It seems likely, as discussed by Shorland (181) and Hartman (182), that *iso*-butyric acid and 2-methyl butyric acid are incorporated into longer-chain branched acids, the former giving rise to the *iso*-series and the latter to the *anteiso*-series as found in bovine tissue (183) and milk lipids (Table 2). As pointed out by Keeney, Katz, and Allison (184), it was apparently assumed for some time that this synthesis took place within the animals' tissues, but no evidence has been forthcoming that shows this to be so. It is, however, evident from the studies of Keeney et al. (184) that the synthesis can be effected by rumen bacteria, which incorporate these branched-chain acids into long-chain components of their structural lipids; following their intestinal digestion, these microbial lipids pass into the tissues and milk lipids of the host animal. It was calculated from analyses of the rumen microbial lipids and those of the plasma and milk of a cow that more than half of the 5.5 g of C_{15} branched acid present in the milk lipids secreted during one day could have been derived from the lipids of the rumen microorganisms. Further, in vitro studies by Keeney and

his collaborators (185) showed that individual species of rumen bacteria required branched-chain volatile fatty acids as growth factors, and that, from *iso*-valerate, a strain of *Ruminococcus flavefaciens* produced leucine and branched-chain fatty acids containing 15 and 17 carbon atoms, together with a branched-chain C_{15} aldehyde that was present in the phospholipids presumably as plasmalogen. Similarly a strain of *R. albus*, which required *iso*-butyrate, largely converted this substrate into lipid components, principally C_{14} and C_{16} branched-chain acids and aldehydes.

The occurrence of 3,7,11,15-tetramethylhexadecanoic acid in bovine milk fat (32) and plasma lipids (186) suggests that its carbon skeleton may be derived intact from the phytol moiety of ingested chlorophyll; hydrolysis of chlorophyll, followed by reduction of the double bond of the phytol (possibly in the rumen) and oxidation of the alcohol group, would result in the formation of this multi-branched fatty acid.

EFFECT OF DIET

The influence of dietary lipids other than those of pasture on the milk fat output of cows and its fatty acid composition has been extensively studied. Experiments summarized by Hilditch (11) showed that specific fatty acids of dietary triglycerides were incorporated into milk glycerides; for example, the feeding of coconut oil or palm kernel oil led to increased proportions of lauric and myristic acids, and rape oil to the appearance of erucic acid as a glyceride component. The administration of cod liver oil led to a fall in milk fat output and to increased proportions of highly unsaturated fatty acids in the milk glycerides with a concomitant decrease in the proportion of steam-volatile fatty acids; similar results were obtained with other marine oils (see McDowall, Patchell, and Reid [187]). On the other hand, the feeding to cows of oils rich in C_{18} unsaturated fatty acids (e.g., linseed oil, groundnut oil, soyabean oil, cottonseed oil) may or may not increase the over-all unsaturation of the milk fat, depending on the amount of oil administered (11, 188, 189); this presumably reflects the capacity of the rumen bacteria for effecting hydrogenation of their component unsaturated acids. When the rumen was by-passed by the intravenous infusion of a cottonseed oil emulsion, this occasioned a marked increase in the linoleate content of cows' milk fat (189).

Of more practical importance is the effect on the proportion of fat in the milk of different "compounded" production rations fed to dairy cows. The optimal amount of lipid contained in the feed consumed has been the subject of many investigations and of controversial claims (190, 191), a discussion of which is outside the scope of this review. Suffice it to say that a certain minimum of feed lipid is apparently required and that this is probably of the order of 300–400 g/day (192). Of as much, if not more, importance is the carbohydrate (cellulose, starch) part of the ration which, by bacterial fermentation in the rumen, provides the volatile fatty acids (particularly acetate) required for the synthesis of higher fatty acids in mammary tissue. Rations that fail, for one reason or another, to yield sufficient acetate result in a diminished output of milk fat and a reduction in the proportion of its component short-chain fatty acids (see review by Rook [193]). It is relevant to note here that some years ago Smith and Dastur (194) found that fasting caused a reduction of 24.2% in the molecular proportion of C_4–C_{14} fatty acids in cow milk fat, while the C_{18} fatty acids (octadecenoic and stearic acids) increased by 24.7% to give glycerides resembling those of depot fat in their fatty acid composition. The question of why the milk fats of herbivores and, in particular, of ruminants should normally contain a greater proportion of short-chain acids than is present in the milk fats of other species has not yet been studied in any detail. It is evidently associated with the more abundant supply of acetate to the mammary tissue and the arresting of chain-lengthening, possibly as a result of rapid esterification of the shorter-chain acids as they are elaborated. Garton (195) found that the glycerides present in the secretory tissue of cows' udders, which were in the terminal stages of lactation, contained a much smaller proportion of short-chain acids and a markedly greater proportion of palmitic acid than did glycerides extracted from the secretory tissue of lactating cows. This suggests that, when rapid synthesis of milk fat was no longer taking place, chain-lengthening of short-chain acids had proceeded to a much greater extent.

The effects of alimentation on the milk lipids of other species have been much less thoroughly studied. Beare et al. (196) investigated the effect of various oils including maize oil and rapeseed oil on milk fat composition and on reproduction in rats. Those given rapeseed oil produced less young over four generations than did the animals given maize oil or a diet of low fat content. The erucic acid of the rapeseed oil was present in the milk glycerides. Linoleic acid was transferred to milk fat following the feeding of maize oil, while on the low-fat diet the milk fat produced was characterized by its high proportion of short-chain saturated fatty acids.

Many years ago, it was found (197–199) that the iodine value of human milk fat was increased following the ingestion of oils rich in unsaturated fatty acids;

more recently, Söderhjelm (76) showed that its polyunsaturated fatty acid content was enhanced after sesame oil, maize oil, or cod liver oil was added to the diet. Insull et al. (200, 201) studied the composition of the milk fat of a lactating woman who was given diets of different fat content and composition designed to favor (*a*) the transfer to milk fat of fatty acids from plasma lipids, and (*b*) the *de novo* intramammary synthesis of fatty acids. It was thus found that, without affecting the output of milk or milk fat, the component fatty acids of the milk fat resembled those of maize oil when this was administered as 40 or 70% of the total energy value of a diet that otherwise had an adequate (maintenance) or excess calorific value. However, when a fat-free diet deficient in energy value was given, the milk fat resembled human depot fat in the proportions of its component acids (cf the aforementioned observations [194] on a fasting cow). The feeding of a fat-free diet that provided excess calories resulted in the production of milk fat containing a very markedly increased content of lauric and myristic acids, which had been synthesized endogenously in the gland, no increased amounts of these acids being detected in the serum lipids. Albeit limited to one subject, this study points to the importance of the state of energy balance in determining the composition of milk fat.

Clearly, much remains to be done before the complex interrelationships of the various potential sources of fatty acids for milk glycerides are resolved and the problems of glyceride synthesis are better understood. Important as research on the cow and other ruminants may be, it is to be hoped that the future will see more investigations on other species (for example, a study of the utilization of plasma glucose *vis-à-vis* that of acetate for endogenous mammary synthesis of fatty acids) and establish the features of milk lipid formation that are common to all mammals.

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