SUMMARY

1. The thiol groups produced when wool is treated with $NaHSO_3$ can be methylated by methyl iodide or methyl bromide; the *S*-cysteinesulphonate groups are unaffected. A similar reaction occurs when wool is treated simultaneously with $NaHSO_3$ and dimethyl sulphate.

2. A technique for the isolation of *S*-methylcysteine from hydrolysates of *S*-methylated wools by partition chromatography of the *N*-acetylated amino-acids is described.

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Human-milk Fat

1. COMPONENT FATTY ACIDS

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It has long been known that human-milk fat has marked differences in analytical characteristics from cow-milk fat. Thus Halden & Grün (1929), surveying the data then available, quoted ranges of observed values as follows: Reichert-Meissl 1·4–3·4, Polenske 1·5–2·2, iodine value 36–54, and saponification equivalent 268–273; they suggested that the chief fatty acids are oleic (52–54%) and palmitic (25–35%), with myristic, lauric and probably decanoic acids, and possibly small quantities of octanoic, hexanoic and butyric acids. Elsdon (1928) has recorded the following characteristics for a mixed sample of human-milk fats at very early stages of lactation: Reichert-Meissl 3·4, Polenske 1·9, Kirschner 2·0, i.v. 35·9, and has also observed the following average figures for carbohydrate, protein and fat in human milk, based upon over 500 specimens:

Stage of lactation	Lactose (g./100 ml.)	Protein (g./100 ml.)	Fat (g./100 ml.)
1–7 days	6·8	2·1	2·86
8–28 days	6·8	1·6	3·58
1–9 months	6·9	1·3	3·44

According to these figures the proportions of lactose, protein and fat in human milk do not differ greatly from those of cow's milk.

Bosworth (1934) separated the acids from about 3 lb. of human-milk fat, by fractional distillation of the methyl esters, into 30 fractions. The original fat had sap. equiv. 273·4, i.v. 56·2, Reichert-Meissl value 2·5, Polenske value 0·1, and contained 1·1%

of unsaponifiable matter. Bosworth stated that the amount of butyric and hexanoic acids present must have been very small, and that at least 0·02% of decenoic and 0·58% of tetradecenoic acid were present, but did not deduce the proportions of any other acids from the ester-fractionation data. He made, however, the important observation that, whereas the octadecadienoic acids of cow-milk fat contain little, if any, ordinary or 'seed fat' linoleic acid (*cis-cis*-$\Delta^{9,12}$-octadecadienoic acid), the octadecadienoic acids of human-milk fat include considerable proportions of the latter, as shown by isolation of the characteristic tetrabromostearic acid, m.p. 113° and insoluble in light petroleum, on addition of bromine.

Mr P. N. Williams (Central Research Laboratories, Messrs Lever Brothers and Unilever, Ltd.) informs us that he has employed the ester-fractionation data given by Bosworth to calculate the approximate composition of the human-milk fat mixed fatty acids, with the following result: butyric *nil*, hexanoic *trace*, octanoic 0·2, decanoic 1·9, lauric 5·8, myristic 13·5, palmitic 23·7, stearic 2·6, arachidic 0·2, behenic 1·1, decenoic *trace*, tetradecenoic 0·5, hexadecenoic 4·1, oleic 37·1 and octadecadienoic 9·2% (wt.).

Dr S. K. Kon, of the National Institute for Research in Dairying, Shinfield, has recently been engaged in work on human milk (collected mainly from two localities), in the course of which there became available small quantities of human-milk fat and larger quantities of human-milk fatty acids from which the unsaponifiable matter had been separated; these specimens were very kindly placed by him at our disposal, and we have thus been enabled to effect the component acid determinations recorded in this paper. If, in the near future, larger specimens of human-milk fat become available, we hope also to study the component glycerides present and compare the latter, as well as the component fatty acids as a whole, with those in cow-milk fat.

EXPERIMENTAL

The material available was in two forms, the fat itself and a solution of the K salts of the mixed fatty acids. Since the latter were the larger samples, they were more suitable for detailed examination by ester fractionation, and we attach more quantitative significance to the resulting data than may be perhaps attributed to the analyses of the smaller specimens of the fats. The results obtained from the two specimens of mixed fatty acids will therefore be considered first.

Human-milk fat (as mixed fatty acids)

In these instances, the fats had been saponified, at the National Institute for Research in Dairying, by short boiling with ethanolic KOH, after which the mixture had been diluted with water and exhaustively extracted with ether in order to remove the unsaponifiable or non-fatty components. We received the accumulated soap solutions from milk of early lactation (first 3 weeks of lactation; collected 4. iii. 43–8. v. 43), and from full lactation milk (collected 3–21. iii. 43 and 22. iv. 43–5. vi. 43).

The soap solutions were concentrated until all the ethanol had been removed, then made just acid with H_2SO_4 and distilled in steam for some hours as described for cow-milk fats (Hilditch & Sleightholme, 1930; Hilditch & Thompson, 1936; Hilditch & Longenecker, 1938).

The aqueous condensates from the steam distillations were exhaustively extracted with ether, and the dried ethereal solutions were distilled (Table 1).

Table 1. *Steam-volatile acids from human-milk fatty acids*

	Stage of lactation	
	Early	Full
Total fatty acids steam-distilled (g.)	68·2	130·1
Total steam-volatile acids obtained (g.)	0·45	1·73
Ether-extracted aqueous condensate (ml.)	2000	2840
N/10 KOH required for neutralization (ml.)	11·40	34·0
Acidity as butyric acid (g.)	0·10	0·30
Ether recovered by distillation (ml.)	2000	1960
N/10 KOH required for neutralization (ml.)	1·65	3·45
Acidity as butyric acid (g.)	0·01	0·03
Distilled up to 140°/760 mm. (g.)	Nil	0·42
N/10 KOH required for neutralization (ml.)	—	31·8
Acidity as butyric acid (g.)	—	0·28
Residue from distillation (g.)	0·34	1·12
Equivalent	200·8	193·3
i.v.	15·1	10·6

The data in Table 1 show that, if all the acidity due to acids of low molecular weight is credited as butyric acid, the percentage of the latter in the total human-milk fatty acids was only 0·15 (early lactation) and 0·45 (late lactation); moreover, no hexanoic or octanoic acids were detected. The characteristic odour of butyric acid was, however, not present in either instance, and in fact there is no evidence that this acid was present. The ethanol used in saponifying these specimens of milk fat was not pre-treated by refluxing with NaOH and subsequent distillation, and the observed lower acids were probably mainly acetic acid from traces of esters present therein (cf. Hilditch & Sleightholme, 1930, p. 1101). This reasoning is supported by the still lower acidities (as butyric acid) recorded for the corresponding steam-volatile acids from the specimens of human-milk fat hydrolyzed by us (*vide*

infra) when ethanol pre-treated with NaOH was employed. We therefore consider that the present evidence justifies the conclusion that butyric acid is not present, unless in minute traces, in human-milk fat, and the acidities observed as in Table 1 (other than the residual acids of mean equivalent *c.* 200) are not included in the final computation of the human-milk component fatty acids.

The acids non-volatile in steam were separated into two groups in the usual way by the differing solubility of their lead salts in ethanol, and the methyl esters of each group were fractionated in a vacuum through an electrically heated and packed column. From appropriate ester fractions, lauric, myristic, palmitic and stearic acids were formally identified with authentic specimens by m.p. and mixed m.p. The acids from an ester fraction (sap. equiv. 295·1, i.v. 102·5) which contained only unsaturated acids of the C_{18} series were oxidized in dilute ice-cold alkaline solution with $KMnO_4$, when they yielded 9:10-dihydroxystearic acid, m.p. 131–131·5° (unchanged when mixed with a specimen of the acid prepared from oleic acid): traces of tetrahydroxystearic acid were also isolated.

The acids (5·0 g.) from a similar ester fraction (sap. equiv. 294·8, i.v. 102·4) were dissolved in light petroleum and bromine was added until a slight excess was present, when a crystalline precipitate (0·62 g., m.p. 108–110°) separated after standing at 0°; on recrystallization from light petroleum this melted at 110–112° (mixed with crystalline tetrabromostearic acid of m.p. 113·5°, prepared from cotton-seed oil, m.p. 112·5–113°). On the other hand, addition of bromine to a solution of similar acids (5·0 g., i.v. 102·0) in ether led to the separation of less than 0·01 g. of indefinite crystalline material insoluble in ether at 0°. The amount of tetrabromostearic acid obtained from the unsaturated C_{18} acids indicates that at least 60 % of the octadecadienoic acids present therein was the form of linoleic acid present in seed fats.

The acids (3·0 g.) from the residual esters of the unsaturated group (i.v. *c.* 165), remaining after the ester fractionation, were similarly brominated in

Table 2. *Component acids of human-milk fatty acids*

	Steam-volatile acids	Non-steam-volatile 'Solid' acids	Non-steam-volatile 'Liquid' acids	Total	Fatty acids % (wt.)	Fatty acids % (mol.)
	Fatty acids from early lactation					
	(0·6 %)	(38·3 %)	(61·1 %)			
As Butyric	(0·15)	—	—	(0·15)	—	—
Decanoic	0·15	—	2·59	2·74	2·7	4·2
Lauric	0·22	0·63	4·25	5·10	5·1	6·7
Myristic	—	5·30	2·74	8·04	8·1	9·2
Palmitic	—	20·25	2·16	22·41	22·5	22·9
Stearic	—	8·31	—	8·31	8·3	7·7
As Arachidic	—	0·99	—	0·99	1·0	0·8
As Decenoic	—	—	0·03	0·03	Trace	Trace
As Dodecenoic	—	—	0·09	0·09	0·1	0·1
As Tetradecenoic	—	0·01	1·29	1·30	1·3	1·5
As Hexadecenoic	—	0·40	2·64	3·04	3·1	3·1
Oleic	0·08	2·41	33·77	36·26	36·4	33·6
Octadecadienoic	—	—	7·84	7·84	7·9	7·3
Unsaturated C_{20-22}	—	—	3·53	3·53	3·5	2·9
Unsaponifiable	—	—	0·17	0·17	—	—
	Fatty acids from full lactation					
	(1·3 %)	(36·7 %)	(62·0 %)			
As Butyric	(0·46)	—	—	(0·46)	—	—
Decanoic	0·34	—	1·31	1·65	1·7	2·5
Lauric	0·40	0·49	5·47	6·36	6·4	8·3
Myristic	—	4·32	3·26	7·58	7·6	8·7
Palmitic	—	19·65	2·58	22·23	22·4	22·8
Stearic	—	8·97	—	8·97	9·0	8·3
As Arachidic	—	0·90	—	0·90	0·9	0·8
As Decenoic	—	—	0·02	0·02	Trace	Trace
As Dodecenoic	—	—	0·12	0·12	0·1	0·2
As Tetradecenoic	—	—	0·54	0·54	0·5	0·6
As Hexadecenoic	—	0·13	3·50	3·63	3·7	3·8
Oleic	0·10	1·78	34·59	36·47	36·6	33·9
Octadecadienoic	—	—	8·16	8·16	8·2	7·7
Unsaturated C_{20-22}	—	0·46	2·45	2·91	2·9	2·4
Unsaponifiable	—	—	—	—	—	—

ether at 0° and gave 0·42 g. of insoluble bromo-additive compounds which decomposed without complete melting at about 197°.

The general results of the two component fatty acid analyses are given in Table 2. From the observed fatty acid compositions, the saponification equivalents and i.v.'s of the original human-milk fats were calculated to be:

	Sap. equiv.	i.v.
Early lactation	274·0	56·0
Full lactation	274·9	54·7

Human-milk fat

In addition to the milk fatty acids described above, we had access to smaller specimens of human-milk fats, one from early lactation (50 g.) and one mainly from late stages of lactation (35 g.). The characteristics of these were as follows:

	Early lactation	Late lactation
Sap. equiv.	277·2	274·4
i.v.	52·1	48·2
Reichert-Meissl value	1·2	0·9
Polenske value	1·2	1·2
Kirschner value	0·6	0·5

The late lactation fat was almost white, the early lactation fat was full yellow in colour.

As much as possible of each fat (44·6 g. early, and 30·4 g. late lactation) was hydrolyzed and the acidified soaps were distilled in steam as usual. The quantities being so small, the aqueous condensate was filtered and titrated directly with N/10 KOH (the filtered traces of insoluble acids, together with slight amounts of solid acids separated in the condenser tube, were dissolved in ether and added to the non-steam-volatile acids). The acidities of the aqueous condensates were as follows:

	N/10 KOH required (ml.)	Calc. as butyric acid (g.)	Calc. as butyric acid (% of total milk fat acids)
Early lactation fat	2·30	0·020	0·05
Late lactation fat	1·85	0·016	0·06

The non-steam-volatile acids were separated as follows by crystallization of their lead salts from ethanol:

	Total fatty acids (g.)	'Solid' acids (g.)	'Solid' acids (%)	'Liquid' acids (g.)	'Liquid' acids (%)
Early lactation fat	41·3	17·6	42·6	23·7	57·4
Late lactation fat	28·0	12·6	45·0	15·4	55·0

The results of the corresponding ester-fractionations are summarized in Table 3.

Some of the data in Table 3, in which the quantities of esters available for fractionation were undesirably restricted, may be relatively less accurate than the corresponding figures in Table 2. This applies more especially to the saturated acids, in which resolution into binary mixtures of esters may in some cases be less complete than usual. On the other hand, the values for the unsaturated acids (with the possible exception of the unsaturated C_{20-22} group) are of the usual order of accuracy, and in particular the amounts of oleic and octadecadienoic acids (based upon the iodine values of the fractions consisting wholly of unsaturated C_{18} esters) should fall within the usual limits of accuracy of the fractionation method.

DISCUSSION

The percentages (wt. and mol.) of the component acids observed in the four specimens of human-milk fat which have been examined are arranged for comparison in Table 4.

Subject to some variation in mean unsaturation (i.e., mainly, in oleic acid content), it would appear that human-milk fat component acids consist of saturated and unsaturated fatty acids in not far from equal proportions. In the unsaturated group oleic acid (the chief component acid of the whole fat) amounts to 30–37 % (wt.), the minor components hexa- and tetradecenoic acids occur in about the same proportions as in cow-milk fat and in many animal body fats, but decenoic and dodecenoic acids are probably present in even lower proportions than in cow-milk fat. The most arresting features of the unsaturated acids are the proportions of diethenoid C_{18} acids, unusually high for an animal fat and consisting to a large extent of the linoleic acid characteristic of vegetable seed fats, and the amounts, also relatively high for a land animal fat, of unsaturated acids of the C_{20} and C_{22} series.

The saturated acids are also quite specific, and present notable differences from those of cow-milk fats. Although palmitic acid (the main saturated acid at about 22–24 %), stearic and myristic acids are present in proportions similar to those in which they occur in cow-milk fats, the lower members of the series are quite different, and include only lauric and small proportions of decanoic acid. Whereas, in cow-milk fat, out of every 100 mol. of fatty acids, about 10 mol. consist of butyric acid, 4–5 mol. of hexanoic-octanoic acids, and 4–5 mol. of decanoic-lauric acids, in human-milk fat the three lower acids are not present and the combined amount of lauric and decanoic acids reaches 10–11 % (mol.), with lauric acid predominating.

The general picture suggested by these data is that, whatever is the mechanism of formation of the lower fatty acids in milk fats, this process is carried on much more extensively, and to a lower

Table 3. *Component acids of human-milk fat*

	'Solid' acids	'Liquid' acids	Total	Fatty acids % (wt.)	Fatty acids % (mol.)
		Early lactation fat			
	(42·6%)	(57·4%)			
Decanoic	—	0·76	0·76	0·8	1·2
Lauric	1·00	5·07	6·07	6·1	8·0
Myristic	7·56	3·11	10·67	10·8	12·4
Palmitic	21·73	2·62	24·35	24·6	25·2
Stearic	7·26	—	7·26	7·3	6·7
As Arachidic	1·72	—	1·72	1·8	1·5
As Decenoic	—	Trace	Trace	Trace	Trace
As Dodecenoic	—	0·10	0·10	0·1	0·1
As Tetradecenoic	0·03	0·38	0·41	0·4	0·5
As Hexadecenoic	0·57	2·72	3·29	3·3	3·4
Oleic	1·92	30·49	32·41	32·8	30·4
Octadecadienoic	—	6·23	6·23	6·3	5·9
Unsaturated C_{20-22}	0·81	4·83	5·64	5·7	4·7
Unsaponifiable	—	1·09	1·09	—	—
		Late lactation fat			
	(45·0%)	(55·0%)			
Decanoic	—	0·51	0·51	0·5	0·8
Lauric	2·26	4·63	6·89	7·0	9·0
Myristic	8·97	4·82	13·79	13·9	15·8
Palmitic	21·77	2·11	23·88	24·1	24·4
Stearic	9·50	—	9·50	9·6	8·8
As Decenoic	—	Trace	Trace	Trace	Trace
As Dodecenoic	0·03	0·07	0·10	0·1	0·1
As Tetradecenoic	0·03	0·88	0·91	0·9	1·1
As Hexadecenoic	0·56	2·22	2·78	2·8	2·9
Oleic	1·88	27·93	29·81	30·2	27·6
Octadecadienoic	—	5·43	5·43	5·5	5·1
Unsaturated C_{20-22}	—	5·34	5·34	5·4	4·4
Unsaponifiable	—	1·06	1·06	—	—

Table 4. *Human-milk fat component acids*

Stage of lactation ...	Early (Table 2)	Early (Table 3)	Full (Table 2)	Late (Table 3)
i.v. of milk fat ...	56·0	52·1	54·7	48·2
Acid	Weight percentages			
Decanoic	2·7	0·8	1·7	0·5
Lauric	5·1	6·1	6·4	7·0
Myristic	8·1	10·8	7·6	13·9
Palmitic	22·5	24·6	22·4	24·1
Stearic	8·3	7·3	9·0	9·6
As Arachidic	1·0	1·8	0·9	—
As Decenoic	Trace	Trace	Trace	Trace
As Dodecenoic	0·1	0·1	0·1	0·1
As Tetradecenoic	1·3	0·4	0·5	0·9
As Hexadecenoic	3·1	3·3	3·7	2·8
Oleic	36·4	32·8	36·6	30·2
Octadecadienoic	7·9	6·3	8·2	5·5
Unsaturated C_{20-22}	3·5	5·7	2·9	5·4
	Molar percentages			
Decanoic	4·2	1·2	2·5	0·8
Lauric	6·7	8·0	8·3	9·0
Myristic	9·2	12·4	8·7	15·8
Palmitic	22·9	25·2	22·8	24·4
Stearic	7·7	6·7	8·3	8·8
As Arachidic	0·8	1·5	0·8	—
As Decenoic	Trace	Trace	Trace	Trace
As Dodecenoic	0·1	0·1	0·2	0·1
As Tetradecenoic	1·5	0·5	0·6	1·1
As Hexadecenoic	3·1	3·4	3·S	2·9
Oleic	33·6	30·4	33·9	27·6
Octadecadienoic	7·3	5·9	7·7	5·1
Unsaturated C_{20-22}	2·9	4·7	2·4	4·4

range of acids, in the production of cow-milk fat than in that of human-milk fat.

So far as may be concluded from the small specimen of late lactation fat of lower unsaturation than the rest, the amount of octadecadienoic acid, as well as that of oleic acid, has been reduced by about one-fifth; when increase of saturation is observed in cow-milk fats, diminution in unsaturated acids has been as a rule confined exclusively to oleic acid. Moreover, in cow-milk fats diminution in oleic acid is paralleled by increases in the contents of the lower saturated acids, whereas in the present instance the myristic acid content alone seems to be materially increased. No definite conclusion should be drawn here, however, until larger specimens covering a wide range of mean unsaturation have been studied.

Two points of some interest appear to be established by the analyses recorded in the course of this work. First, since cow's milk may be substituted for human milk in infant nutrition, it follows that the variation in the component acids of the two milk fats has little if any effect on the nutritive value of the milk. Secondly, any inference that butter from the cow is superior for human consumption to other forms of edible fat by reason of its characteristic milk fat composition is discounted, since the data presented demonstrate that human-milk fat, qualitatively and to some extent quantitatively, resembles modern types of margarine more closely than it does butter. The presence in human-milk fat of vegetable-seed fat linoleic acid, of definite proportions of lauric and myristic acids (characteristic of coconut and other 'nut oils') and of somewhat more unsaturated C_{20-22} acids than usual, coupled with the complete absence of butyric and other saturated acids of low molecular weight, cause it to be much more analogous to a margarine fat than to butter fat.

SUMMARY

1. The chief component acids of human-milk fat are oleic (30–37 %) and palmitic (22–24 %). Of unsaturated acids, diethenoid C_{18} acids (*c.* 7 %) include a considerable proportion of ordinary linoleic acid, unsaturated C_{20-22} acids amount to 3–4 %, and there are minor quantities of hexa- and tetradecenoic acids. The other saturated acids include stearic and myristic (*c.* 8–9 % each), lauric (5–7 %) and decanoic (2–3 %); no acids of lower molecular weight than decanoic are present.

2. The acids of human-milk fat differ from those of cow-milk fat in that they do not contain butyric or other acids below decanoic acid, and in their higher content of diethenoid C_{18} acids (including linoleic acid) and unsaturated C_{20-22} acids. Human-milk fat, in regard to its component acids, has more resemblance to a typical margarine fat-blend than to butter fat.

We offer cordial thanks to Dr S. K. Kon for placing the experimental material for this paper at our disposal, and to Mr H. Weatherall, F.I.C., of Messrs J. Bibby and Sons, Ltd., for assistance in the determination of some of the analytical characteristics of the milk fats.

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The 'Cyclization' of Vitamin A and Allied Compounds

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During the last two years a physico-chemical method for the estimation of vitamin A based on 'cyclization' and chromatographic separation of the 'cyclized' product has been devised in this laboratory. We hope to furnish a complete account of this method when further data have been accumulated with regard to comparison with biological assays. A recent paper by Shantz, Cawley & Embree (1943) anticipates to some extent our own conclusions with regard to the nature of 'cyclized' vitamin A and it appears desirable to place on record the results so far obtained. The best concentrate of 'cyclized' vitamin A which we obtained by chromatography on alumina had $E^{1\%}_{1\,cm.} = 2630$ at 370 mμ in *cyclo*-hexane, which agrees with the value given for material obtained in this way by