LXI. THE GLYCERIDE STRUCTURE OF BUTTER-FATS.

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THE glyceride structures of five butter-fats from cows fed under definite conditions have been investigated by isolation of sufficient of their fully-saturated glycerides to permit in each case of a detailed analysis of the fatty acids present in the latter by the ester-fractionation process. The component fatty acids of the whole butter-fats had already been determined [Hilditch and Sleightholme, 1930]. Combination of the analytical results for the whole fats with those for the fully-saturated glyceride portions furnishes much information as to the distribution of saturated and unsaturated acids among the glycerol molecules, and also throws some light on certain aspects of the distribution of the individual saturated acids. The objects of this communication are (i) to summarise the experimental data on the examination of the fully-saturated glycerides present in these five butter-fats and (ii) to discuss the general features of the glyceride structure of butter-fats revealed by these figures in conjunction with those formerly obtained for two New Zealand butter-fats [Hilditch and Jones, 1929] and, more recently, for an Indian cow ghee [Bhattacharya and Hilditch, 1931].

The various specimens will be referred to throughout this paper as follows.

- I. Shinfield, Berkshire; October 1928, cows partly on pasture, partly on winter diet.
- II. Shinfield, Berkshire; March 1929, cows on winter diet with added coconut cake ration.
- III. Shinfield, Berkshire; March 1929, cows on winter diet with added soya bean cake ration.
- IV. Shinfield, Berkshire; May 1929, cows on early summer pasture.
- V. Palmerston North, New Zealand; December 1928, cows on early summer pasture.
- A and B. New Zealand butter, market samples [Hilditch and Jones, 1929].
- CG. Indian cow ghee from pasture-fed cows [Bhattacharya and Hilditch, 1931].

The butters I-IV were collected from members of the same herd of ten Shorthorn cows at the National Institute for Research in Dairying and V was

supplied by Dr F. H. McDowall from the Dairy Research Institute at Palmerston North; details of the feeding of the cows in question will be found in our former paper [1930, pp. 1099, 1100], and the general analytical characteristics of these five butter-fats and the data for the component fatty acids present in the whole fats have also been recorded [1930, pp. 1100, 1105, 1106].

Fully-saturated glyceride content of butter-fats I-V.

Determinations of fully-saturated glyceride content were carried out by the method which has been fully described in several recent papers [*cf.* Hilditch and Jones, 1929], the fat, in quantities of 100 g., being dissolved in acetone (1 litre) and oxidised by gradual addition of powdered potassium permanganate (400 g.) to the gently boiling solution. The only modification which has been made in the procedure as formerly described is that it has been found useful to have suspended in the solution clean dry silver sand (about 300 g.), which lessens the accumulation of a hard cake of mixed fat, oxidation products and manganese oxides, and facilitates its removal for subsequent reduction of the oxides of manganese by means of sodium bisulphite and dilute sulphuric acid. After separating as much of the acidic products of oxidation as possible by washing the ethereal solution with dilute aqueous ammonia, the crude fully-saturated glycerides were recovered from the washed ethereal solution and in all cases possessed a negligible iodine absorption (less than 0.2 %). Usually 500–600 g. of each fat was oxidised, 100 g. at a time, in order to provide an adequate quantity of purified fully-saturated glycerides for the detailed analysis of the component fatty acids present.

The crude fully-saturated glycerides thus obtained were further purified from any remaining acidic products of oxidation by adding a boiling saturated solution of potassium carbonate to the melted crude glycerides in an evaporating basin, with vigorous stirring, until a stiff paste was formed; this was immediately boiled with a large quantity of water until clarification was complete, allowed to settle, and the aqueous solution siphoned away from the upper layers of liquid fat and emulsion. This washing process was repeated until no emulsified paste was left, when a further washing with boiling aqueous potassium carbonate was given and the residual fat finally washed free from soap and alkaline carbonate by the above method. The alkaline and aqueous washings were collected together and thoroughly shaken with ether, and in this way two weighed fractions of fully-saturated glycerides were obtained, "A," the greater part of the whole, consisting of almost completely neutral fat, and "B," the product recovered from the ether extraction of the alkaline and aqueous washings, smaller in quantity than "A" and still somewhat contaminated with acidic material. The ether-extracted washings were treated with mineral acid and the acidic oxidation products were recovered: the acid value of the latter was observed and employed to estimate acidic products still present in fraction "B," on the assumption that the acid value of acidic

products in "B" was approximately the same as that of those removed during the purification.

These data suffice to determine the fully-saturated glyceride content of the original fat; from the saponification equivalents of the purified fully-saturated glycerides "A" and of the original butter-fat the molar percentages of fully-saturated glycerides follow, whilst, knowing the proportion of unsaturated acids present in the original fat from the earlier detailed analyses [1930, p. 1005], it is also possible to estimate the molar proportions of saturated acid present in the mixed saturated-unsaturated (and completely unsaturated) glycerides—the "association-ratio."

The results thus obtained for butter-fats I–V are collected in Table I.

Table I. *Determination of fully-saturated glyceride (F.S.G.) content.*

(i) <i>Analytical data.</i>										
Purification of crude fully-saturated glycerides										
No.	Weight taken g.	Crude F.S.G. g.	Weight taken g.	"A"		"B"		Acidic products		F.S.G. % (weight)
				g.	Acid No.	g.	Acid No.	g.	Acid No.	
I	601.9	182.4	61.8	42.2	0.5	9.5	8.8	9.9	153.9	25.4
II	606.5	248.0	244.2	204.6	0.3	26.7	6.5	12.9	170.0	38.5
III	501.2	183.3	181.3	150.8	0.4	24.6	9.9	5.9	149.5	35.05
IV	202.1	57.8	57.2	32.6	0.6	17.5	14.3	7.2	190.6	24.35
V	303.5	120.9	120.9	102.3	1.2	11.8	14.4	6.8	258.3	37.4

(ii) <i>Summary of observed values.</i>						
No.	Iodine value	Acids in whole fat		Fully-saturated glycerides		"Association ratio" in non-fully-saturated part Mols. saturated acid per mol. unsaturated acid
		Saturated % (mol.)	Unsaturated % (mol.)	Weight %	Mols. %	
I	41.3	63.0	37.0	25.4	29.1	0.92
II	31.6	72.4	27.6	38.5	41.3	1.11
III	34.8	70.0	30.0	35.05	38.2	1.07
IV	41.6	61.9	38.1	24.35	27.2	0.94
V	34.5	70.2	29.8	37.4	39.6	1.07

Fatty acid compositions of fully-saturated glycerides of butter-fats I–V.

The purified fully-saturated glycerides "A" were converted into their mixed fatty acids and analysed as described in our former communication [1930], the operations being somewhat simplified by the absence of unsaturated acids and consequent omission of the lead salt separation of the non-volatile acids. An illustration of the results of this method of analysis in detail as applied to the whole acids of butter-fat IV was given in the paper referred to, and in the present cases only the final amounts of each acid as determined in the analyses are recorded.

Direct analysis of the saturated fatty acids combined in the acidic products of oxidation of mixed saturated-unsaturated glycerides cannot be effected owing to the presence of nonoic and azelaic acids as products of oxidation,

Table II. *Summarised analytical data for fatty acids from fully-saturated glycerides of butter-fats I-V.*

Acid	Volatile acids g.	Acids non-volatile in steam g.	Total g.	% (weight) % (mol.) (excluding unsaponifiable matter)	
Fully-saturated glycerides of butter-fat I.					
	8.39	92.7	101.09		
Butyric	4.70	—	4.70	4.7	11.7
Caproic	2.80	—	2.80	2.8	5.3
Caprylic	0.67	0.76	1.43	1.4	2.2
Capric	—	3.28	3.28	3.3	4.2
Lauric	—	4.79	4.79	4.7	5.2
Myristic	—	13.69	13.69	13.6	13.2
Palmitic	—	50.40	50.40	50.0	43.1
Stearic	—	19.60	19.60	19.5	15.1
Unsap.	0.22	0.18	0.40	—	—
Fully-saturated glycerides of butter-fat II.					
	15.45	175.2	190.65		
Butyric	7.21	—	7.21	3.8	9.2
Caproic	5.94	—	5.94	3.1	5.8
Caprylic	1.75	1.82	3.57	1.9	2.8
Capric	0.55	10.08	10.63	5.6	6.9
Lauric	—	19.73	19.73	10.4	11.1
Myristic	—	40.72	40.72	21.4	20.1
Palmitic	—	80.54	80.54	42.3	35.4
Stearic	—	21.87	21.87	11.5	8.7
Unsap.	—	0.44	0.44	—	—
Fully-saturated glycerides of butter-fat III.					
	10.72	114.6	125.32		
Butyric	4.67	—	4.67	3.7	9.2
Caproic	4.29	—	4.29	3.4	6.4
Caprylic	1.04	1.53	2.57	2.1	3.1
Capric	0.72	5.54	6.26	5.0	6.3
Lauric	—	7.40	7.40	5.9	6.4
Myristic	—	25.67	25.67	20.5	19.6
Palmitic	—	53.20	53.20	42.5	36.1
Stearic	—	20.63	20.63	16.5	12.6
Arachidic	—	0.50	0.50	0.4	0.3
Unsap.	—	0.13	0.13	—	—
Fully-saturated glycerides of butter-fat IV.					
	9.12	95.45	104.57		
Butyric	4.795	—	4.795	4.6	11.4
Caproic	2.815	—	2.815	2.7	5.1
Caprylic	0.88	0.94	1.82	1.7	2.7
Capric	0.63	3.71	4.34	4.2	5.3
Lauric	—	5.75	5.75	5.5	6.0
Myristic	—	16.41	16.41	15.7	15.1
Palmitic	—	48.17	48.17	46.2	39.5
Stearic	—	20.20	20.20	19.4	14.9
Unsap.	—	0.27	0.27	—	—
Fully-saturated glycerides of butter-fat V.					
	13.51	157.3	170.81		
Butyric	7.63	—	7.63	4.5	11.2
Caproic	4.09	—	4.09	2.4	4.6
Caprylic	1.51	2.22	3.73	2.2	3.4
Capric	—	6.84	6.84	4.0	5.1
Lauric	—	8.21	8.21	4.8	5.3
Myristic	—	26.27	26.27	15.4	14.9
Palmitic	—	79.11	79.11	46.5	39.9
Stearic	—	34.34	34.34	20.2	15.6
Unsap.	0.28	0.31	0.59	—	—

but the approximate composition of the unsaturated glycerides can be estimated by the difference between the respective acids present in the fully-saturated portion and in the whole fat as in Table III.

Table III. *General composition (weight) of butter-fats I-V.*

	Whole fat	Fully- saturated	Non-fully- saturated
Butter-fat I.			
	100 g.	25.4 g.	74.6 g.
Glyceryl residue $C_3H_5\equiv$	5.05	1.4	3.65
Butyric acid	2.95	1.1	1.85
Caproic "	1.65	0.65	1.0
Caprylic "	1.5	0.35	1.15
Capric "	2.0	0.8	1.2
Lauric "	3.25	1.15	2.1
Myristic "	6.55	3.25	3.3
Palmitic "	27.5	12.0	15.5
Stearic "	7.2	4.7	2.5
Arachidic "	0.85	—	0.85
Oleic "	38.0	—	38.0
Linoleic "	3.5	—	3.5
Butter-fat II.			
	100 g.	38.5 g.	61.5 g.
Glyceryl residue $C_3H_5\equiv$	5.2	2.15	3.05
Butyric acid	3.2	1.4	1.8
Caproic "	1.9	1.15	0.75
Caprylic "	1.05	0.7	0.35
Capric "	3.05	2.0	1.05
Lauric "	6.9	3.75	3.15
Myristic "	16.2	7.75	8.45
Palmitic "	25.6	15.4	10.2
Stearic "	4.55	4.2	0.35
Oleic "	30.05	—	30.05
Linoleic "	2.3	—	2.3
Butter-fat III.			
	100 g.	35.05 g.	64.95 g.
Glyceryl residue $C_3H_5\equiv$	5.2	1.95	3.25
Butyric acid	3.4	1.2	2.2
Caproic "	1.9	1.15	0.75
Caprylic "	1.6	0.7	0.9
Capric "	3.5	1.65	1.85
Lauric "	6.2	1.95	4.25
Myristic "	10.0	6.8	3.2
Palmitic "	24.8	14.1	10.7
Stearic "	7.9	5.45	2.45
Arachidic "	1.1	0.1	1.0
Oleic "	31.0	—	31.0
Linoleic "	3.4	—	3.4
Butter-fat IV.			
	100 g.	24.35 g.	75.65 g.
Glyceryl residue $C_3H_5\equiv$	5.05	1.35	3.7
Butyric acid	3.1	1.1	2.0
Caproic "	1.2	0.6	0.6
Caprylic "	1.15	0.4	0.75
Capric "	2.1	1.0	1.1
Lauric "	3.8	1.3	2.5
Myristic "	9.9	3.6	6.3
Palmitic "	24.8	10.6	14.2
Stearic "	6.2	4.4	1.8
Oleic "	38.8	—	38.8
Linoleic "	3.9	—	3.9

Table III (*contd.*).

	Whole fat	Fully- saturated	Non-fully- saturated
Butter-fat V.			
	100 g.	37.4 g.	62.6 g.
Glyceryl residue $C_3H_5\equiv$	5.15	2.05	3.1
Butyric acid	3.3	1.6	1.7
Caproic "	1.6	0.85	0.75
Caprylic "	1.25	0.8	0.45
Capric "	2.9	1.4	1.5
Lauric "	3.85	1.7	2.15
Myristic "	10.55	5.45	5.1
Palmitic "	25.9	16.45	9.45
Stearic "	10.9	7.1	3.8
Arachidic "	0.6	—	0.6
Oleic "	29.75	—	29.75
Linoleic "	4.25	—	4.25

DISCUSSION.

As already mentioned, we desire to review here the data for all the cow butter-fats which have now been submitted to examination by these methods, *i.e.* the present series of five specimens together with two former (market) samples of New Zealand butter and the recently-investigated specimen of Indian cow ghee. We thus have at disposal two Berkshire pasture-fed (late autumn and early summer), one Indian pasture-fed and three New Zealand (probably all pasture-fed) butters, with two other Berkshire butters from cows on winter diet supplemented by specific oil-cakes.

We find that our results lead to the consideration of two separate aspects of the glyceride structure of the butter-fats, namely, (i) the general distribution of unsaturated acids throughout the glycerides, which appears to be independent of the particular saturated acid components of the fat; and (ii) certain regularities in the occurrence of the saturated acid components in all the fats except those two in which added oil-cake formed a prominent part of the diet.

General distribution of unsaturated acids in the butter glycerides.

The data for all eight fats show consistently that the content of fully-saturated glycerides is, within close limits, simply a function of the relative proportions of saturated and unsaturated acids in the whole fat, irrespective of the nature of the saturated acids (the unsaturated acids are throughout almost constant in composition, *viz.* about 90 % oleic and about 10 % linoleic). In this respect the butter-fats resemble such other animal fats (beef and mutton tallows, lards, rabbit-fat) as have been examined, and also vegetable "pericarp" fats such as palm oil or stillingia tallow; all these are also not far removed in fully-saturated glyceride content (i) from glycerides of the same degree of unsaturation prepared synthetically by heating glycerol with a slight excess of the mixed fatty acids [*cf.* Bhattacharya and Hilditch, 1930], or

(ii) from that calculated on the assumption that the content of fully-saturated glycerides in mixed glycerides of saturated and unsaturated acids is proportional to the cube of the concentration of the total saturated acids. It will be remembered that vegetable seed-fats appear to be elaborated on entirely different and more "evenly distributed" lines [Collin and Hilditch, 1929].

The present series covers a relatively wide range of variation in the milk-fats from a single animal species and affords a more stringent comparison than has hitherto been possible of the relation of fully-saturated glyceride content to total saturated acid content in different specimens of a single type of fat. In Table IV will be found the iodine values and molar percentages¹ of saturated acids in the original butter-fats, together with the observed molar percentages of fully-saturated glycerides and (i) the latter calculated on the basis of proportionality to the cube of the total saturated acid present and (ii) the molar percentages of fully-saturated glycerides in synthetic mixed glycerides taken from the curve given by Bhattacharya and Hilditch [1930]; the "association ratio," or mols. of saturated acid present per mol. of unsaturated acid, in the non-fully-saturated glycerides is added in the final column.

Table IV. *Relationship between fully-saturated glyceride content and total saturated acid content of butter-fats.*

Butter-fat (cf. p. 507)	Iodine value	Total saturated acids % (mols.)	Fully-saturated glyceride content			"Association ratio" (observed)
			Observed % (mols.)	Calculated ($y \propto x^3$) % (mols.)	From synthetic glyceride curve (B and H) % (mols.)	
II	31.6	72.4	41.3	38.0	34.0	1.11
V	34.5	70.2	39.6	34.5	30.5	1.07
III	34.8	70.0	38.2	34.2	30.2	1.07
CG	36.0	67.9	33.7	31.5	27.5	1.07
A	38.0	67.3	33.8	30.5	26.5	1.04
B	39.4	66.1	31.5	28.7	25.0	1.03
I	41.3	63.0	29.1	24.5	21.5	0.92
IV	41.6	61.9	27.2	23.3	20.5	0.94

These relations are shown, probably more clearly, in Fig. 1, in which the appropriate portions of Bhattacharya and Hilditch's "synthetic" glyceride curve (unbroken line) and of the "calculated" curve (dotted) are plotted on an enlarged scale together with the observed values for the eight butter-fats. All the butter-fats contain rather more fully-saturated glycerides than would be the case if the fully-saturated glyceride content varied as the cube of the total saturated acids present, whilst, at this range of total unsaturation, the

¹ Throughout this discussion we are concerned with the relative number of molecules (or, perhaps, equivalents) of the various glycerides and fatty acids—the weight percentages usually employed are of little use in interpreting the structure of a complex mixture of glycerides of acids which differ widely (88 to 284) in molecular weight.

synthetic glycerides contain somewhat less fully-saturated components than the amount corresponding with this relationship. The relative regularity in the alteration of fully-saturated glyceride content with total unsaturation in the case of the butter-fats is perhaps more striking than the parallelism with either of the curves in question. The corresponding alteration in the proportions of saturated and unsaturated acids in the non-fully-saturated glycerides (Table IV, final column) should also be noted¹.

Fig. 1.

These data are considered to show that, as between saturated and unsaturated acids, the constitution of the mixed glycerides in butter-fat is determined by the relative concentration of these classes of acid; this proportion being given in any particular case, the mode of assemblage into triglyceride molecules appears to follow according to some fixed rule. Although the experimental methods only permit of differentiation between saturated and unsaturated acids, there is no reason to suppose that, normally, the case is different as between one saturated acid and another.

As regards the general composition of the non-fully-saturated glycerides of the eight fats, the molar "association ratios" in the final column of

¹ By way of contrast, the corresponding available data for the two seed-fats so far examined [Collin and Hilditch, 1929] in this region of relative unsaturation may be recalled:

Fat	Iodine value	Total saturated acids % (mols.)	F.S.G. content % (mols.)	"Association ratio"
Cacao butter	37.1	59.8	2.5	1.4
Illipé tallow	32.3	62.8	4.5	1.55

In seed-fats, so long as there is sufficient saturated acid present, there is a tendency towards a constant association of about 1.4 mols. saturated with 1 mol. of unsaturated acid in the "mixed" glycerides and, at this particular state of relative unsaturation, the content of fully-saturated glycerides is exceedingly small.

Table IV permit limiting values to be calculated for the possible proportions of either mono-unsaturated di-saturated and di-unsaturated mono-saturated glycerides, or mono-unsaturated di-saturated and tri-unsaturated glycerides which may be present; in the absence of any analytical method which will allow the amount of any one of these classes (*e.g.* triolein) to be determined, this is as far as we can go at present. On the other hand, there is little or no evidence that triolein occurs in any great amount in butter-fat, and considerable indirect evidence against this possibility [*cf.* Amberger, 1918]; and very probably the minimum limits for mono-unsaturated derivatives and the maxima for the di-unsaturated class given in Table V are not far removed from the actual proportions present.

Table V. *Limiting values for the molar percentages of the four types of mixed glycerides in butter-fats.*

Butter	Iodine value	"Association ratio"	Glycerides (mols. %)			
			Fully saturated	Mono-unsaturated-di-saturated	Di-unsaturated-mono-saturated	Tri-unsaturated
II	31.6	1.11	41.3	33.9-46.3	24.8-0	0-12.4
V	34.5	1.07	39.6	33.3-46.8	27.1-0	0-13.6
III	34.8	1.07	38.2	34.0-47.9	27.8-0	0-13.9
CG	36.0	1.07	33.7	36.5-51.4	29.8-0	0-14.9
A	38.0	1.04	33.8	35.0-50.6	31.2-0	0-15.6
B	39.4	1.03	31.5	35.8-52.1	32.7-0	0-16.4
I	41.3	0.92	29.1	31.0-51.0	39.9-0	0-19.9
IV	41.6	0.94	27.2	33.0-52.9	39.8-0	0-19.9

Whilst these general characteristics of butter-fat glyceride structure may prove to be of some importance with regard to the biochemical elaboration of milk-fats, they also have a practical bearing on the variation in the "melting point" or softness of butters. It is evident that increase in oleic acid content of butter, according to the above data, will be reflected in a disproportionate increase in softness, since not only is the ratio of unsaturated to saturated acids increased in the saturated-unsaturated mixed glycerides, but the quantity of the latter increases considerably with augmentation of the oleic acid. For example, in 100 mols. of fat, butter-fat IV contains about 73 mols. of mixed glycerides in which the unsaturated acids are in excess of the saturated, whereas butter-fat II contains only about 59 mols. of mixed glycerides in which there are 11 mols. of saturated acid for every 10 of unsaturated. Whilst, therefore, the fully-saturated glycerides (which are of course the highest-melting components of butter) have been reduced by 14 mols. in every 100 of total fat, the mixed glycerides contain relatively more combined oleic acid, again leading to the production of components of lower melting point. These data also illustrate the obvious reasons for the varying differences which obtain between the melting or softening point of most natural fats and the corresponding (and usually much higher and sharper) melting points of their respective mixed fatty acids; the melting points of individual simple triglycerides being, on the other hand, of the same order

and usually somewhat higher than those of the fatty acid from which they are derived [Joglekar and Watson, 1930].

Distribution of the individual fatty acids in the butter-glycerides.

In order to discuss several features of this aspect of the glyceride structure it will be necessary to refer to the molar percentages of each acid combined in the whole fat, the fully-saturated glycerides and the remaining glycerides; these are collected in Tables VI, VII and VIII. The butter-fats are arranged in increasing order of total unsaturation except that, since the fats II and III (from cows whose food contained large amounts of specific oil-cakes) show certain differences from the remaining (pasture-fed) fats, the data for samples II and III are placed at the end of each table.

Table VI. *Molar distribution of fatty acids in the whole fat.*

Fat	...	V	CG	A	B	I	IV	II	III
Butyric acid		9.2	6.9	9.2	8.4	8.4	8.9	9.0	9.6
Caproic	"	3.4	4.0	3.7	3.9	3.5	2.7	3.9	3.0
Caprylic	"	2.2	2.2	1.4	1.3	2.7	2.0	1.7	2.8
Capric	"	4.2	4.9	2.7	2.8	2.9	3.0	4.3	5.1
Lauric	"	4.7	6.7	3.7	4.6	4.1	4.7	8.3	7.5
Myristic	"	11.5	10.9	10.2	11.0	7.2	10.9	17.2	10.7
Palmitic	"	25.0	26.8	25.7	26.2	27.1	24.3	24.1	23.7
Stearic	"	9.5	5.5	10.2	7.1	6.4	5.4	3.9	6.7
Arachidic	"	0.5	—	0.5	0.8	0.7	—	—	0.9
Oleic	"	26.1	28.4	28.9	30.8	33.9	34.6	25.7	27.0
Linoleic	"	3.7	3.7	3.8	3.1	3.1	3.5	1.9	3.0

Table VII. *Molar distribution of fatty acids in the fully-saturated glycerides.*

Fat	...	...	V	CG	A	B	I	IV	II	III
Molar % F.S.G.	...		39.6	33.7	33.8	31.5	29.1	27.2	41.3	38.2
Butyric acid			11.2	11.2	11.0	10.5	11.7	11.4	9.2	9.2
Caproic	"		4.6	5.1	6.5	4.9	5.3	5.1	5.8	6.4
Caprylic	"		3.4	0.5	1.8	5.0	2.2	2.7	2.8	3.1
Capric	"		5.1	4.4	3.3	3.1	4.2	5.3	6.9	6.3
Lauric	"		5.3	6.1	4.1	4.7	5.2	6.0	11.1	6.4
Myristic	"		14.9	15.5	17.9	17.0	13.2	15.1	20.1	19.6
Palmitic	"		39.9	43.0	39.6	39.3	43.1	39.5	35.4	36.1
Stearic	"		15.6	14.2	15.8	15.2	15.1	14.9	8.7	12.6
Arachidic	"		—	—	—	0.3	—	—	—	0.3

Table VIII. *Molar distribution of fatty acids in the mixed saturated-unsaturated glycerides.*

Fat	...	...	V	CG	A	B	I	IV	II	III
Molar % M.S.-U	...		60.4	66.3	66.2	68.5	70.9	72.8	58.7	61.8
Butyric acid			7.9	4.8	8.3	7.3	7.2	7.8	8.6	9.6
Caproic	"		2.6	3.4	2.3	3.3	2.9	2.2	2.7	2.5
Caprylic	"		1.3	3.1	1.1	—	2.8	1.8	1.0	2.4
Capric	"		3.6	5.1	2.3	2.6	2.4	2.2	2.4	4.1
Lauric	"		4.4	7.0	3.4	4.7	3.7	4.3	6.4	8.2
Myristic	"		9.2	8.6	6.4	8.4	5.0	9.4	15.1	5.4
Palmitic	"		15.0	18.6	18.9	20.1	20.9	18.7	16.3	16.1
Stearic	"		5.6	1.1	7.5	3.3	3.1	2.2	0.5	3.3
Arachidic	"		1.0	—	0.7	1.1	1.0	—	—	1.2
Oleic	"		43.2	42.7	43.4	44.8	46.7	46.7	43.7	42.5
Linoleic	"		6.2	5.6	5.7	4.4	4.3	4.7	3.3	4.7

Further, we have found it convenient to compare the component acids in the following groups: (i) the lower acids from butyric to lauric inclusive, (ii) myristic and palmitic; and (iii) the total amount of acids containing 18 carbon atoms in the molecule (the very small quantities of C_{20} acids recorded in some of the analyses are included with this group). The molar percentages of these groups present in each fat and in its two subdivisions are shown in Table IX.

Table IX. *Molar distribution of butyric-lauric, myristic-palmitic, and C_{18} acids in the butter-fats.*

Section	Fat	...	...	V	CG	A	B	I	IV	II	III
	Acids										
Whole fat	{ Butyric-lauric			23.7	24.7	20.7	21.0	21.6	21.3	27.2	28.0
	{ Myristic-palmitic			36.5	37.7	35.9	37.2	34.3	35.2	41.3	34.4
	{ C_{18} acids			39.8	37.6	43.4	41.8	44.1	43.5	31.5	37.6
Fully saturated	{ Butyric-lauric			29.6	27.3	26.7	28.2	28.6	30.5	35.8	31.4
	{ Myristic-palmitic			54.8	58.5	57.5	56.3	56.3	54.6	55.5	55.7
	{ Stearic			15.6	14.2	15.8	15.5	15.1	14.9	8.7	12.9
Saturated-unsaturated	{ Butyric-lauric			19.8	23.4	17.4	17.9	19.0	18.3	21.1	26.8
	{ Myristic-palmitic			24.2	27.2	25.3	28.5	25.9	28.1	31.4	21.5
	{ C_{18} acids			56.0	49.4	57.3	53.6	55.1	53.6	47.5	51.7
	{ (Oleic-linoleic			49.4	48.3	49.1	49.2	51.0	51.4	47.0	47.2)

The six butter-fats from cattle on normal diets. The cows from various districts which were fed on pasture (or on pasture with roots, etc., but no added oil-cake) yielded butters which show much similarity in many features of their glyceride structure.

(i) In the total fatty acids of each whole butter-fat (Table VI), the amounts of (a) butyric-lauric, (b) myristic-palmitic and (c) stearic, oleic and linoleic acids tend severally towards more or less constant values. This approximate constancy extends to some of the individual acids, especially palmitic, myristic and perhaps butyric; but the amount of stearic acid is more variable. Yet the total amount of C_{18} acids is of much the same order throughout, variations due to the substantially different amounts of unsaturated acids in the butters being counterbalanced partly by variation in the amount of stearic acid, partly by the gross effect of minor variations in all the other saturated acids.

(ii) Although, as the total unsaturation in the fats varies, the amount of fully-saturated glycerides differs widely throughout the series, their composition (Table VII) is remarkably constant; especially noteworthy is the regular proportion of stearic acid (15 ± 0.8 mols. %) in contrast to its variability (5–10 mols. %) in the whole fats. All the saturated acids are present in both fully- and non-fully-saturated parts of the fats, but the tendency noticed by Hilditch and Jones [1929] for the lower acids to associate slightly more than the higher saturated acids with the unsaturated acids is confirmed by the present more extended results.

The inference can fairly be drawn, for these "normal diet" butters, that,

whilst the whole fats vary in unsaturation over a range of 34.5–41.3 in iodine value (61.9–70.2 mols. % total saturated acids), the composition of the fully-saturated glycerides (which comprise from 27 to 40 mols. % of the whole fat) is in every case much the same.

(iii) In the non-fully-saturated glycerides (Table VIII), some approximation to constancy in the molar content of the three groups of acids mentioned follows automatically from the general tendencies noted in (i) and (ii) above. The proportion of unsaturated acids (Table IX, last line) increases slowly, but on the whole steadily, with the total unsaturation of the butter-fats as a whole, but in less than direct proportion to the molar percentage of unsaturated acids present in the whole fat.

(iv) The degree of concordance shown in the totals of the molar contents of butyric-lauric acids is of special interest in view of the importance of these acids as the characteristic acids of milk-fats. In the whole fats this figure appears to tend to a constant value of about 21–22, four of the six fats giving values between 20.7 and 21.6 (Table VI). The maximum variation in the combined butyric-lauric acid molar content of the six butter-fats is, indeed, much less than that in the corresponding Reichert-Meissl values; it is not unlikely that, with a reasonably rapid analytical method for the determination of combined butyric-lauric acid content, this figure would be a more certain index for milk-fats than the Reichert-Meissl value. We are endeavouring to ascertain whether a simplified procedure can be devised by means of which a measure of the total butyric-lauric acid content may be more readily obtained than by employment of the full "fractionation" analysis.

We might not have felt justified, at this stage, in stressing some of the foregoing likenesses in glyceride structure of these butters except for the fact that an examination of a number of beef tallows from different sources, now being made by Mr A. Banks in this laboratory, is yielding results of a similar and equally concordant nature. In the tallow investigation, an account of which it is hoped to publish at an early date, the number of individual component acids to be considered is much less than in butters and the regular features which we have been discussing stand out more sharply in consequence. At the same time, notwithstanding the complexity of the fatty acid mixture in butters, inspection of the foregoing tables on the lines suggested by this more recent work on the body-fats has shown that similar influences operate in the case of the milk-fats from pasture-fed sources.

It is interesting to find, further, that the combined molar contents of myristic and palmitic acids in the total butter-fats, and in their fully-saturated portions, are only a few mols. % lower than the corresponding average values for beef tallows. In other words, the molar contents of C_{18} acids in the tallows (*a*) as a whole or (*b*) in their fully-saturated components are respectively within a few units of the molar contents of all the acids, other than myristic and palmitic, (*a*) in butter-fat or (*b*) in its fully-saturated components. This naturally suggests that the lower fatty acids characteristic of butter are

elaborated at the expense of acids of the stearic series. We are not disposed to assert that the lower fatty acids present in butter necessarily result directly from stearic or oleic acid, but the data which we have put forward are definitely consistent with the view that a precursor of stearic or oleic acid in a tallow may, in the course of milk-fat metabolism, appear instead as a lower fatty acid, with the proviso that only one molecule of any of the lower acids is produced in milk-fat for each molecule of stearic (oleic) acid which otherwise would have resulted in a body-fat. We also consider that the present data confirm our former view that palmitic and (possibly) myristic acids stand apart from the remaining butter acids in many respects, and in particular we find it difficult at present to suppose that the sources of palmitic acid and of oleic, stearic, *etc.*, acids in the natural fats can be identical.

At first glance it may seem difficult to reconcile these regularities in glyceride structure with the variations in the component fatty acids of the same series of butter-fats which we discussed in our previous paper and which we showed to be somewhat large, and due more to conditions such as the change from outdoor to indoor life, general type of diet, or seasonal changes of temperature than to added fats given in the diet. It was pointed out, however [1930, p. 1110], that the chief variations due to such causes were in the content of unsaturated acids and of stearic acid, *i.e.* on the whole, in the balance between stearic, oleic and linoleic acids. Since the amount of fully-saturated glycerides depends on the relative amount of unsaturated acids present in the whole fat, this is a variable, indeed almost the only markedly variable, feature in the glyceride structure of the butters; for the rest, the assemblage of fatty acids into glycerides evidently operates in the same way whatever may be the individual acids concerned (the manner being very similar for such animal fats and vegetable "pericarp" fats as have been studied, but different in the case of vegetable seed-fats). Thus, when the factor due to variable unsaturation (the varying fully-saturated glyceride content) has been taken into account, close similarities are revealed in the respective fully-saturated and non-fully-saturated components of the butters—the constancy of the stearic acid content of the fully-saturated portions being, perhaps, the most illuminating feature.

The two butter-fats from cows whose diet included specific oil-cakes.

By way of contrast, the differences due to inclusion in the diet of coconut or soya bean cake appear more clearly from the component glyceride structures than from simple consideration of the total fatty acids of butters II and III [1930, p. 1111]; this will be seen by comparing the data in Tables VI–IX for Nos. II and III with those for the other six butters.

(i) *Coconut cake in diet (butter II)*. It was pointed out previously [1930] that the only distinctive features in the total acids of butter-fat II were increased amounts of myristic and lauric acids, with, however, less augmentation in the lauric, capric and caprylic acids than might have been anticipated

in view of the high lauric acid and appreciable capric and caprylic acid contents of coconut oil.

In the fully-saturated components of butter-fat II, however, the relative differences are more sharply defined: the lauric acid molar content (Table VII) is 11 (instead of about 5–6) and the myristic acid molar content is 20 (against 15–17). Since dilauromyristin is probably the major component of coconut oil [Collin and Hilditch, 1929], the figures suggest that to some extent this glyceride has passed through directly into the milk-fat, an inference which is supported by consideration of the butyric-lauric acid contents of the fully-saturated and non-fully-saturated parts of the fat.

The butyric-lauric acid content (Table IX) as a whole is much higher (35.8 mols. %) than in the fully-saturated components of the six "normal" butters (27–30 mols. %), whilst the palmitic and stearic acid contents are correspondingly low; the latter (9 mols. %) is in sharp contrast to the steady value of about 15 mols. % observed in the pasture-fed butters (fully-saturated glycerides). Thus the amounts of the individual components of the fully-saturated part of butter-fat II (except capric and caprylic acids) differ distinctly from those in the six butter-fats discussed above. On the other hand, the combined butyric-lauric acid content (21.1 mols. %) of the non-fully-saturated glycerides of butter-fat II is only slightly higher than the corresponding values for the normal fats—indeed the only notable divergence from the normal is an increase in the myristic acid and a minor increase in lauric acid. Consequently, practically all the differences in the butter-fat, caused by feeding coconut-fat (which contains about 85 % of fully-saturated components) to the cows, have been traced to variations from the normal composition in the fully-saturated part of the butter. Whilst, therefore, these more intimate analyses support our earlier conclusion that the greater part of the characteristic components of the coconut-fat have not appeared in the milk-fat, they clearly indicate the location of certain portions of the typical coconut fully-saturated glycerides which have, apparently, passed unchanged into the milk.

(ii) *Soya bean cake in diet (butter III)*. It has already been emphasised [1930] that the relatively large amount of linoleic acid in soya bean oil has little appreciable effect on the content of linoleic and oleic acids in the butter-fat, which is of the normal order. Examination of the component glycerides reveals (Table IX) that in butter III the butyric-lauric acid content of the non-fully-saturated components (26.8 mols. %) is much higher than the average value for the six "normal" butters (18–20 mols. %); myristic and stearic acids (Table VIII) are about normal and palmitic acid (16.1 mols. %) is definitely below the normal (18–20 mols. %). On the other hand, the glyceride structure of the fully-saturated part (except for a somewhat large content of myristic acid) is not far from the normal range of the six pasture-fed butters.

Thus whilst ingestion of the relatively highly saturated coconut-fat led

to variation from the normal in the character of the fully-saturated components of butter-fat II, the diet including soya bean oil (nearly all of which consists of unsaturated glycerides) has caused differences from the normal which are for the most part confined to the non-fully-saturated glycerides of butter-fat III. In the latter case, however, the observed differences do not involve any increase in the proportion of linoleic acid (the major component of soya bean oil) but are confined for the most part to augmentation of the butyric-lauric acid group; thus suggesting that the latter may, in part, be metabolic products from the soya bean oil acids which remain combined in the mixed saturated-unsaturated glycerides.

It will be recalled that the New Zealand December pasture-fed butter-fat V, which has almost the same iodine value as butter-fat III from the soya bean cake diet, resembles the latter in much of its total fatty acid composition [Hilditch and Sleightholme, 1930, p. 1110]; but the above study of the glyceride structure reveals the differences which in reality exist between the two fats. Indeed, unless investigation of glyceride structure be undertaken it appears that sufficient data cannot be provided for the adequate consideration of milk-fat metabolism.

SUMMARY.

Four English butter-fats from members of the same herd of cows fed on various diets and a New Zealand pasture-fed (December) butter-fat (the total fatty acid compositions of which have been previously discussed) have been oxidised in order quantitatively to determine their content of fully-saturated glycerides, and the fatty acids of the latter have been analysed in detail. The results obtained, with similar data for two other New Zealand butter-fats and an Indian cow ghee, are discussed with reference to the general glyceride structure of butter-fats.

The six butters from cows on "normal" (usually pasture-fed) diets show many features in common, whilst in two cases in which specific fatty oils had been fed to the cows the results stand apart in certain respects; but all eight fats conform to the usual rule (in animal fats) that the content of fully-saturated glycerides is a function of the proportion of the total saturated acids present in the mixed acids of the whole fat.

In the "normal" butters, moreover, the component acids of the fully-saturated parts are present in approximately the same quantitative amounts, the composition of the fully-saturated glycerides tending to be the same whatever the original unsaturation of the butter as a whole. Similar concordances are revealed in the combined butyric-lauric acid contents, and in the combined myristic-palmitic acid contents, of the whole fats and of their two divisions, fully- and non-fully-saturated glycerides, whilst the content of unsaturated acids in the non-fully-saturated glycerides increases steadily and slowly with increase of unsaturation in the fats as a whole.

When coconut-fat (a relatively saturated fat) formed part of the diet, the

fully-saturated part of the butter showed a marked increase in butyric-lauric acid content, but for the most part the composition of the non-fully-saturated glycerides was normal. With a soya bean (predominantly unsaturated) oil diet the structure of the butter glycerides was different: the fully-saturated components were not far removed from the normal in composition, but the lower acids of the non-fully-saturated portion were present in excess of the usual proportion.

Study of the glyceride structure of these eight butters, whilst confirming the general rules which have been observed to connect the amount of fully-saturated glycerides with the degree of unsaturation of animal fats, thus reveals influences due to diet more clearly than the consideration of the component fatty acids of the whole fats, and affords some additional evidence as to the mode of utilisation of ingested fat in milk-fat metabolism.

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