104. SHEEP BODY FATS

2. COMPONENT GLYCERIDES OF PERINEPHRIC AND EXTERNAL TISSUE FATS FROM THE SAME ANIMAL

BY THOMAS PERCY HILDITCH AND
YOUSSEF ABDEL HAMID ZAKY

From the Department of Industrial Chemistry, University of Liverpool

(Received 19 August 1941)

THE glycerides present in the perinephric and external tissue fats of a sheep have been studied by means of the procedure described in detail in the case of an ox depot fat [Hilditch & Paul, 1938], and later applied to the perinephric and outer back fats from a pig by Hilditch & Pedelty [1940]. For the present purpose the fats chosen for investigation were from the animal (ewe no. 4) referred to in the preceding communication [Hilditch & Pedelty, 1941], since this sheep, which had been reared from 70 to 150 lb. live weight on the 'super-maintenance' ration (cf. p. 932), furnished the comparatively large quantities of fat required for work of this kind.

EXPERIMENTAL

The essential data were obtained by methods which were described in full in connexion with ox depot fat glycerides [Hilditch & Paul, 1938] and also summarized in the subsequent communication on pig depot fats [Hilditch & Pedelty, 1940]. Description of the procedure may therefore here be confined to a brief recapitulation of the various stages.

(a) The sheep fats (perinephric 750 g., and external tissue fat 580 g.) were each systematically crystallized from acetone (at 0° or room temperature, at various concentrations and for varying periods), with the result that a number of fractions were obtained and ultimately grouped, when sufficiently similar in iodine value, into three main fractions in the case of each fat.

(b) The component acids in each fraction were determined by ester-fractionation.

(c) The amounts of fully saturated glycerides present in the fractions more sparingly soluble in acetone (i.v. below 40) were determined by oxidation of a portion of each fraction with KMnO_4 in acetone solution. When sufficient fully saturated glycerides (10 g. or more) were available, their component acids were determined by ester-fractionation; otherwise, the latter were calculated as mixtures of palmitic and stearic acids from the observed equivalent of the fully saturated glycerides.

(d) In the fractions most soluble in acetone (in which any tri-unsaturated glycerides and most of the di-unsaturated glycerides are concentrated), the amount of tri- C_{18} glycerides was determined, in the form of tristearin, by hydrogenating a portion as completely as possible and separating the hydrogenated product, by crystallization from anhydrous ether, into a series of fractions. The equivalents of the fractions obtained were determined, and the component acids of the least soluble fraction obtained by ester-fractionation and

calculated as a mixture of tristearin with palmitodistearin. Small proportions of incompletely hydrogenated material passed with the more soluble fully saturated glycerides into the fractions relatively soluble in ether, and were calculated as oleodistearin from the iodine values of the fractions concerned.

In view of recent findings [Atherton & Hilditch, 1941] that migration of acyl groups between triglyceride molecules occurs slowly, and to a small extent, during hydrogenation at 170–180°, the hydrogenations were conducted as far as possible at 95° by means of Raney nickel catalyst, and completed by a short treatment at 170° after addition of a small quantity of active nickel on kieselguhr.

The whole of the data obtained as described above permit the component glycerides in each fraction to be grouped, according to the proportions of palmitic, stearic and unsaturated C_{18} acids present, into (a) dipalmitomono- C_{18} glycerides, palmitodi- C_{18} glycerides, and tri- C_{18} glycerides, and (b) fully saturated, mono-unsaturated-disaturated, di-unsaturated-monosaturated and tri-unsaturated glycerides. From the proportions of these various classes, the individual components in each fraction of the original fat can be deduced within fairly close limits. (The minor component acids, for this purpose, have to be included with appropriate major components: lauric, myristic, tetradecenoic and hexadecenoic acids are included with palmitic, arachidic with stearic, and octadecadienoic and unsaturated C_{20-22} acids with oleic acid. The minor component acids of the sheep fats, other than octadecadienoic (2–3%), together formed 5–6% of the total fatty acids, so that probably about 15% of the glycerides contained one of the minor component acyl groups.)

The component acids of the two fats, determined on the total fatty acids of each fat (preceding paper, p. 933), were found to be as below:

Acid	Perinephric fat		External tissue fat	
	% (wt.)	% (mol.)	% (wt.)	% (mol.)
Lauric	—	—	0.9	1.2
Myristic	2.3	2.8	3.1	3.7
Palmitic	26.2	28.0	28.3	29.9
Stearic	27.1	26.1	13.5	12.9
Tetradecenoic	0.3	0.3	0.2	0.2
Hexadecenoic	1.0	1.1	0.6	0.7
Oleic	38.7	37.5	50.8	48.9
Octadecadienoic	3.3	3.2	1.9	1.9
Unsaturated C_{20-22}	1.1	1.0	0.7	0.6

Glyceride structure of the perinephric fat

Systematic crystallization from acetone finally gave four fractions which were grouped for analysis into three fractions A, B, C as follows:

	Fractions isolated	
	g.	I.V.
A	255.2	19.3
B	188.8	39.5
C	{ 53.1	{ 61.5
	{ 250.7	{ 65.0

The proportions, characteristics and component acids of the three fractions are given in Table 1.

Table 1. *Fractions (A, B, C) separated by crystallization of the perinephric fat (747.8 g.) from acetone*

	A		B		C	
Wt. of fraction (g.)	255.2		188.8		303.8	
I.v.	19.3		39.5		64.4	
Sap. equiv.	286.6		288.6		285.9	
Glycerides % (wt.)	34.1		25.3		40.6	
Glycerides % (mol.)	34.2		25.1		40.7	
	% (wt.)	% (mol.)	% (wt.)	% (mol.)	% (wt.)	% (mol.)
Component acids:						
Lauric	—	—	—	—	0.5	0.6
Myristic	1.8	2.1	1.0	1.1	3.4	4.1
Palmitic	29.1	31.2	23.5	25.4	23.1	24.5
Stearic	44.4	42.8	30.4	29.5	9.3	8.9
Arachidic	2.6	2.3	2.6	2.3	0.3	0.3
Tetradecenoic	0.2	0.2	0.2	0.3	0.5	0.5
Hexadecenoic	1.2	1.3	1.1	1.2	2.1	2.3
Oleic	20.7	20.1	39.4	38.5	52.3	50.5
Octadecadienoic	—	—	0.8	0.8	7.9	7.7
Unsaturated C ₂₀₋₂₂	—	—	1.0	0.9	0.6	0.6

The fully saturated glycerides in the less soluble fractions A and B were determined, together with their component acids, with the results given in Table 2. (As a precaution, the most soluble fraction C was similarly examined; but 50 g. yielded, after two oxidations, only 0.3 g. of neutral material which still possessed i.v. 10.3. Fully saturated glycerides were thus completely absent from fraction C.)

Table 2. *Fully saturated glycerides present in fractions A and B of the perinephric fat*

	A*				B	
Wt. of fraction oxidized (g.)	100.5				100.0	
	(i)		(ii)			
Wt. of crude F.S.G. (g.)	27.2		15.5		4.0	
I.v. of crude F.S.G.	0.3		0.8		0.7	
Sap. equiv. of crude F.S.G.	288.0		281.4		274.9	
Acid value of crude F.S.G.	1.7		1.8		1.7	
% (wt.)	25.0		14.0		3.8	
% (mol.)	24.9		14.4		4.0	
	% (wt.)	% (mol.)	% (wt.)	% (mol.)	% (wt.)	% (mol.)
Component acids:						
Myristic	—	—	13.8	16.1	—	—
Palmitic	30.1	32.3	36.0	37.4	77.1	78.9
Stearic	69.0	66.9	46.7	43.6	22.9	21.1
Arachidic	0.9	0.8	3.5	2.9	—	—

* The crude fully saturated glycerides from fraction A were separated into two groups by crystallization from ether before detailed analysis.

In the most soluble fraction C the tri-C₁₈ glycerides were estimated by the hydrogenation and crystallization procedure described above. The hydrogenated product (ca. 80 g.) yielded the following fractions when crystallized from anhydrous ether:

	Least soluble				Most soluble
Wt. (g.)	32.9	19.2	3.1	3.1	18.8
I.v.	0.0	0.0	0.9	2.1	2.2
Sap. equiv.	290.6	287.5	282.1	282.0	284.0
% (wt.)	42.7	24.8	4.1	4.0	24.4
% (mol.)	42.2	24.8	4.2	4.1	24.7

The component acids in the least soluble portion were found (by ester-fractionation) to be palmitic 22.4, stearic 76.6, and C_{20-22} 1.0% (mol.), from which it was estimated to contain 32.8% (mol.) of tri- C_{18} glycerides (including the C_{20-22} group), corresponding to 13.9% (mol.) of tri- C_{18} glycerides in fraction C. The small i.v. of the more soluble fractions, reckoned as oleodistearin, amounted to 2.2% (mol.) of the fraction C. The total tri- C_{18} glycerides in fraction C thus amounted to 16.1% (mol.), corresponding to 6.5% (mol.) in the whole perinephric fat.

Table 3. *Probable component glycerides (% mol.) present in the constituent fractions and in the whole ewe perinephric fat*

	A		B		C	Whole fat
Glycerides	34.2		25.1		40.7	100.0
Component acids (increments):						
Lauric	—		—		0.3	0.3
Myristic	0.7		0.3		1.7	2.7
Palmitic	10.7		6.4		10.0	27.1
Stearic	14.6		7.4		3.6	25.6
Arachidic	0.8		0.6		0.1	1.5
Tetradecenoic	0.1		0.1		0.2	0.4
Hexadecenoic	0.4		0.3		0.9	1.6
Oleic	6.9		9.6		20.6	37.1
Octadecadienoic	—		0.2		3.1	3.3
Unsaturated C ₂₀₋₂₂	—		0.2		0.2	0.4
	F.S.G. (13.4%)		Mixed (20.8%)		F.S.G. (1.0%)	Mixed (24.1%)
Groups of component acids (increments):						
C ₁₆ (+ C ₁₄ , C ₁₂)	5.4	6.5	0.8	6.3	13.1	32.1
Stearic (+ C ₂₀)	8.0	7.4	0.2	7.8	3.7	27.1
'Oleic' (+ C ₂₀₋₂₂)	—	6.9	—	10.0	23.9	40.8
Groups of component glycerides (increments):						
Tripalmitin	—	—	0.4	—	—	0.4
Dipalmitomono-C ₁₈	2.9	1.5	0.6	—	5.1	10.1
Palmitodi-C ₁₈	10.2	16.5	—	18.9	29.1	74.7
Tri-C ₁₈	0.3	2.8	—	5.2	6.5	14.8
Fully saturated	13.4	—	1.0	—	—	14.4
Mono-'oleo'-disaturated	—	19.4	—	18.1	9.7-16.3	47.2-53.8
Di-'oleo'-monosaturated	—	1.4	—	6.0	31.0-17.9	38.4-25.3
Tri-'olein'	—	—	—	—	Nil-6.5	Nil-6.5
Probable component glycerides						
Fully saturated (14.4%):						
Tripalmitin	—	—	0.4	—	—	0.4
Dipalmitostearin	2.9	—	0.6	—	—	3.5
Palmitodistearin	10.2	—	—	—	—	10.2
Tristearin	0.3	—	—	—	—	0.3
Mono-'oleo'-disaturated (47.2-53.8%):						
Hexadecenopalmitostearin	—	1.5	—	—	—	1.5
'Oleo'-dipalmitin	—	—	—	—	5.1	5.1
'Oleo'-palmitostearin	—	16.5	—	18.1-12.9	4.6-11.2	39.2-40.6
'Oleo'-distearin	—	1.4	—	Nil-5.2	—	1.4-6.6
Di-'oleo'-monosaturated (38.4-25.3%):						
Palmitodi-'olein'	—	—	—	0.8-6.0	24.5-17.9	25.3-23.9
Stearodi-'olein'	—	1.4	—	5.2-Nil	6.5-Nil	13.1-1.4
Tri-'olein' (Nil-6.5%)	—	—	—	—	Nil-6.5	Nil-6.5

Applying the methods of interpretation described in the earlier communications cited to the foregoing data, we arrive at the general statement of probable component glycerides in the ewe perinephric fat given in Table 3. Where alternative possibilities arise, the results are given, as usual, in the form of limiting values for 'oleo'-palmitostearin, palmitodi-'olein', steardo-'olein' and tri-'olein'.¹

Glyceride structure of the external tissue fat

Systematic crystallization from acetone yielded eight portions which were finally grouped for analysis into three sets, A, B, C, as follows:

Fractions isolated		Fractions studied		
g.	I.V.	No.	g.	I.V.
102.8	26.7	A	179.7	29.7
25.2	33.8			
51.7	35.6			
20.9	45.0	B	29.2	45.4
8.3	46.5			
18.2	54.0	C	370.8	59.7
108.0	58.7			
244.6	61.9			

The proportions, characteristics and component acids of the three composite portions A, B, C, are given in Table 4.

Table 4. *Fractions (A, B, C) separated by crystallization of the external tissue fat from acetone*

	A		B		C	
Wt. of fraction (g.)	179.7		29.2		370.8	
I.V.	29.7		45.4		59.7	
Sap. equiv.	285.4		285.9		285.5	
Glycerides % (wt.)	31.0		5.0		64.0	
Glycerides % (mol.)	31.0		5.0		64.0	
	% (wt.)		% (wt.)		% (wt.)	
Component acids:	% (mol.)		% (mol.)		% (mol.)	
Myristic	2.6	3.1	1.9	2.3	3.6	4.3
Palmitic	31.0	32.9	29.9	31.7	29.0	30.8
Stearic	30.7	29.4	17.5	16.7	7.5	7.2
Arachidic	0.5	0.5	—	—	0.4	0.4
Tetradecenoic	Trace	Trace	—	—	0.2	0.3
Hexadecenoic	0.8	0.9	3.3	3.5	0.8	0.8
Oleic	34.4	33.2	46.5	44.9	53.9	51.9
Octadecadienoic	—	—	0.9	0.9	3.2	3.1
Unsaturated C ₂₀₋₂₂	—	—	—	—	1.4	1.2

The small quantity of fraction B necessitated the computation of its component glycerides directly from the component acid proportions but, in view of its I.V., it is most unlikely that it contained either fully saturated or tri-unsaturated glycerides.

The fully saturated glycerides (and their component acids) were determined in the case of fraction A with the results shown in Table 5, the crude product being separated into two portions by crystallization from ether.

The terms 'oleo-', 'oleic', 'olein', denote the inclusion of octadecadienoic with oleic compounds.

Table 5. *Fully saturated glycerides present in fraction A of the external tissue fat*

Wt. of fraction oxidized (g.)	A			
	100.1			
	(i)		(ii)	
Wt. of crude F.S.G. (g.)	13.55		4.64	
I.v. of crude F.S.G.	0.3		0.8	
Sap. equiv. of crude F.S.G.	282.1		277.7	
Acid value of crude F.S.G.	1.7		1.8	
% (wt.)	13.1		4.4	
% (mol.)	13.2		4.5	
Component acids:	% (wt.)	% (mol.)	% (wt.)	% (mol.)
Myristic	5.9	7.0	—	—
Palmitic	36.4	38.3	69.9	72.0
Stearic	57.4	54.5	30.1	28.0
Arachidic	0.3	0.2	—	—

A portion (89 g.) of the most soluble fraction C, after complete hydrogenation, gave the following fractions when crystallized from anhydrous ether:

	Least soluble				Most soluble
Wt. (g.)	25.1	10.2	11.1	5.4	37.3
I.v.	0.0	0.0	0.5	2.1	2.3
Sap. equiv.	290.0	287.7	285.3	282.4	286.0
% (wt.)	28.2	11.5	12.4	6.0	41.9
% (mol.)	28.0	11.4	12.5	6.1	42.0

The component acids in the least soluble portion were found (by ester-fractionation) to be palmitic 25.1, stearic 73.8, and C_{20-22} 1.1% (mol.), corresponding with an estimated proportion of 24.8% (mol.) of tri- C_{18} glycerides (including the C_{20-22} group), or 6.9% (mol.) in the whole of fraction C. The slight unsaturation in the more soluble fractions, in terms of oleodistearin, corresponded to 3.9% (mol.) of fraction C. The total tri- C_{18} glycerides in fraction C were thus estimated to form 10.8% (mol.) of this fraction, or 6.9% (mol.) of the total external tissue fat.

Consideration of the above data led to a final estimate of the glycerides probably present in each fraction A, B and C, including limiting values in certain cases (Table 6).

DISCUSSION

If the molar percentage proportions of the component acids in the whole fats, determined by summation of the data from the three fractions into which the fats were separated by crystallization from acetone (Tables 3 and 6), are compared with those obtained from the ester-fractionation analyses of the total fatty acids from each fat (p. 941), it will be seen that very close accord is shown by the figures for the perinephric fat, and somewhat less close, but on the whole satisfactory, agreement in the case of the external tissue fat. In both fats, however, resolution into several fractions has, as frequently happens, led to the appearance of minor component acids (lauric, arachidic), which were not detected in the analyses of the total fatty acids, and to apparent increases in the proportions of other minor component acids (hexadecenoic, octadecadienoic). Inspection of Tables 1 and 4 shows, moreover, that the minor component octadecadienoic acid is concentrated in the most soluble (mainly diunsaturated) glyceride fractions, whilst arachidic acid is mainly found in the least soluble (largely disaturated) fractions. This is merely a consequence of the circumstance

Table 6. *Probable component glycerides (% mol.) present in the constituent fractions and in the whole ewe external tissue fat*

	A	B	C	Whole fat
Glycerides	31.0	5.0	64.0	100.0
Component acids (increments):				
Myristic	1.0	0.1	2.7	3.8
Palmitic	10.2	1.6	19.7	31.5
Stearic	9.1	0.8	4.6	14.5
Arachidic	0.1	—	0.3	0.4
Tetradecenoic	Trace	—	0.2	0.2
Hexadecenoic	0.3	0.2	0.5	1.0
Oleic	10.3	2.2	33.3	45.8
Octadecadienoic	—	0.1	2.0	2.1
Unsaturated C ₂₀₋₂₂	—	—	0.7	0.7
	<div style="display: flex; justify-content: space-around; width: 100%;">F.S.G. (5.5%)Mixed (25.5%)</div>			
Groups of component acids (increments):				
C ₁₆ (+C ₁₄)	2.9	8.6	1.9	23.2
Stearic (+C ₂₀)	2.6	6.6	0.8	4.8
'Oleic' (+C ₂₀₋₂₂)	—	10.3	2.3	36.0
Groups of component glycerides (increments):				
Tripalmitin	0.2	—	—	0.2
Dipalmitomono-C ₁₈	2.7	—	0.1	12.4
Palmitodi-C ₁₈	2.6	24.9	4.9	44.7
Tri-C ₁₈	—	0.6	—	6.9
Fully saturated	5.5	—	—	5.5
Mono-'oleo'-disaturated	—	19.3	2.5	20.1-27.0
Di-'oleo'-monosaturated	—	6.2	2.5	43.9-30.1
Tri-'olein'	—	—	—	Nil-6.9
	Probable component glycerides			
Fully saturated (5.5%):				
Tripalmitin	0.2	—	—	0.2
Dipalmitostearin	2.7	—	—	2.7
Palmitodistearin	2.6	—	—	2.6
Mono-'oleo'-disaturated (41.9-48.9%):				
Hexadecenopalmitostearin	—	0.9	0.5	—
'Oleo'-dipalmitin	—	—	0.1	12.4
'Oleo'-palmitostearin	—	17.8	1.9	7.7-14.6
'Oleo'-distearin	—	0.6	—	—
Di-'oleo'-monosaturated (52.6-38.7%):				
Palmitodi-'olein'	—	6.2	2.5	37.0-30.1
Stearodi-'olein'	—	—	—	6.9-Nil
Tri-'olein' (Nil-6.9%)	—	—	—	Nil-6.9

that, whilst nearly all the triglyceride molecules contain at least one radical of the most abundant acids oleic and palmitic, the minor component acids will only contribute one acyl group to any triglyceride molecule in which they are present. Thus the most likely combinations of the respective acids would be octadecadieno-oleo-saturated or arachido-palmito-unsaturated glycerides, of respectively large and small relative solubility in acetone.

The general glyceride structure of the two ewe fats conforms with that of other animal depot fats rich in stearic acid. This is indicated in the first place by the proportions of fully saturated glycerides which, when plotted against the respective proportions of saturated acids in the total fatty acids, fall on the

curve for this relationship characteristic for animal depot and milk fats [Hilditch, 1940, pp. 233, 237]. The more detailed examination of each fat shows that the chief component glycerides present are 'oleo'-palmitostearins and palmitodi-'oleins', which together form about 65% of the perinephric fat and nearly 75% of the external tissue fat. In the more saturated perinephric fat (the greater saturation being due to greater proportions of combined stearic acid) 'oleo'-palmitostearins occur to the extent of about 40%, whilst in the external tissue fat (with less stearic and more oleic acid in combination) these are reduced to about 30%, and over 40% of palmitodi-'oleins' are present. These facts, together with the presence of 10% of palmitodistearin in the perinephric fat, support the view that the stearo-glycerides of these and similar animal depot fats have been produced by hydrogenation of preformed oleo-glycerides.

It has already been explained that the experimental evidence, available from study of the group of mainly unsaturated glycerides most soluble in acetone, does not permit a decision as to whether the tri-C₁₈ glycerides therein are steardi-'oleins' or tri-'oleins', and therefore the results are given in terms of limiting values for these glycerides (Tables 3 and 6). From the general results obtained over a wide range of animal and vegetable fats in this laboratory, it appears increasingly doubtful whether, in instances such as the ewe fats now discussed, tri-unsaturated glycerides are present. At all events it is very unlikely that their proportions approach the upper limits given. For comparative purposes the presence of tri-'oleins' in the ewe fats may probably be safely ignored, and the approximate proportions of the component glycerides accepted as shown below (Table 7).

Table 7. *Component glycerides of ewe perinephric and external tissue fats (% mol.)*

	Perinephric	External tissue
% Stearic acid	25.6	14.5
Fully saturated:		
Tripalmitin	0.4	0.2
Dipalmitostearin	3.5	2.7
Palmitodistearin	10.2	2.6
Tristearin	0.3	—
Mono-'oleo'-disaturated:		
Hexadecenopalmitostearin	1.5	1.4
'Oleo'-dipalmitin	5.1	12.5
'Oleo'-palmitostearin	39.3	27.5
'Oleo'-distearin	1.4	0.6
Di-'oleo'-monosaturated:		
Palmitodi-'olein'	25.2	45.6
Stearodi-'olein'	13.1	6.9

If, after allowing for the composition of fully saturated glycerides present in the fats, the 'oleic' acid present is divided in proportion to the palmitic and stearic acids present in the remaining mixed ('oleo'-saturated) glycerides, and combined arithmetically with these two acids, a 'calculated' composition for the four mixed glycerides 'oleo'-dipalmitin, 'oleo'-palmitostearin, palmitodi-'olein' and steardi-'olein' is derived which represents the maximum 'even distribution' of these acids in the form of mixed glycerides. The accordance between these 'calculated' figures and the proportions actually observed was remarkable in the cases of ox depot fats, English [Hilditch & Paul, 1938] and Indian [Hilditch & Murti, 1940], but in two pig depot fats [Hilditch & Pedelty, 1940] there was little resemblance between the two sets of values. In the present

instances there is again considerable accordance, although the respective figures are not so similar as in the ox depot fats mentioned:

Ewe depot fats	Perinephric		External tissue	
	Found	'Calc.'	Found	'Calc.'
'Oleo'-dipalmitin	5	8	13	20
'Oleo'-palmitostearin	41	41	28	23
Palmitodi-'olein'	25	21	46	38
Stearodi-'olein'	13	16	7	14

The implication of the frequent resemblance between the observed and 'calculated' proportions of the four minor classes of animal depot fat glycerides is somewhat uncertain, but there now seems little doubt that the accordances hold fairly closely in the more saturated fats of the group (i.e. those in which the saturated acids form about 50 % or more of the total acids, and in which the stearic acid content approaches or exceeds about 20 %). Of the three ox, two sheep, and two pig fats which have now been examined, these coincidences occur in all but the pig fats (the least saturated of the series). The following explanation of these observations may, however, be put forward. It has been shown that the proportions of these four groups of glycerides can be similarly calculated from the proportions of palmitic, stearic and oleic acids in certain vegetable seed fats, in which these acids are all major components, and which follow the 'rule of even distribution' very closely [Hilditch & Stainsby, 1936; Hilditch & Ichaporia, 1938]; these fats, however, contain only minor quantities of fully saturated glycerides. If a similar 'evenly distributed' mixture of palmito-oleo glycerides and triolein (such as is believed to be the precursor of the stearic-rich animal depot fats) undergoes partial hydrogenation before deposition as reserve fat, the conversion of oleic acid into stearic groups may well follow a similar random course, with the result that, when a sufficient proportion of oleo-glycerides have been thus transformed, the composition of the mixture can be approximately simulated by an arithmetical calculation based on division of the oleic acid still present in proportion to the palmitic and stearic acid contents of the animal depot fats.

*Comparison of the chief component glycerides of pig, sheep
and ox depot fats*

The present work has completed a series of studies in which seven fats from the three species mentioned have been investigated by the acetone-crystallization procedure, leading to more detailed knowledge of the proportions of their various component glycerides than was hitherto available. It is therefore possible to review the series as a whole, with special reference to the manner in which the proportions of the minor groups of mixed glycerides are influenced by the proportions of oleic, stearic and palmitic acids present in the whole fats. For this purpose the data may be arranged in the form shown in Table 8.

In Table 8, the glyceride figures are based on the minimum limits of tri-'oleins' present (cf. p. 947); the small proportions of hexadecenopalmitostearins observed are included with the 'oleo'-palmitostearins; and the data are arranged in roughly decreasing order of general unsaturation. The palmitic acid content of the Bombay cow fat, and also the combined palmitic and myristic acid contents of the Calicut cow fat, are abnormally high, and this to some extent complicates comparison as regards the development of mixed stearoglycerides with increasing general saturation in the fats. Nevertheless, it is instructive to

Table 8. *Component glycerides of pig, sheep and ox depot fats (% mol.)*

Animal ... Fat ...	Ewe External	Pig Back	Pig Peri- nephric	Ewe Peri- nephric	Ox English	Cow Calicut	Cow Bombay
	(Present work)	[Hilditch & Pedelty, 1940]		(Present work)	[Hilditch & Paul, 1938]	[Hilditch & Murti, 1940]	
Component acids:							
Myristic (+ C ₁₂)	3.8	1.3	1.8	3.0	2.6	5.7	5.9
Palmitic	31.5	29.0	31.1	27.1	33.4	33.4	40.8
Stearic	14.5	13.8	17.6	25.6	21.4	27.9	25.5
Arachidic	0.4	—	—	1.5	1.3	0.5	0.7
Hexa- (+ tetra-)decanoic	1.2	2.7	2.4	2.0	2.5	1.9	2.8
Oleic	45.8	43.9	40.6	37.1	35.2	29.0	22.9
Octadecadienoic	2.1	7.2	5.3	3.3	3.5	1.5	1.1
Unsaturated C ₂₀₋₂₂	0.7	2.1	1.2	0.4	0.1	0.1	0.3
Component glycerides (approx.):							
Fully saturated:							
Tripalmitin	Trace	1	—	Trace	3	—	3
Dipalmitostearin	3	2	4	4	8	16	23
Palmitodistearin	2	2	5	10	6	12	10
Tristearin	—	—	—	Trace	—	—	—
Mono-‘oleo’-disaturated:							
‘Oleo’-dipalmitin	13	5	9	5	15	11	18
‘Oleo’-palmitostearin	28	27	34	41	32	38	34
‘Oleo’-distearin	1	—	—	2	2	2	—
Di-‘oleo’-monosaturated:							
Palmitodi-‘olein’	46	53	40	25	23	17	11
Stearodi-‘olein’	7	7	5	13	11	3	1
Tri-‘oleins’	0	3	3	0	0	0	0

compare the relative proportions of palmitodi-‘oleins’, ‘oleo’-palmitostearins and palmitodistearins as the proportion of stearic acid in the whole fat increases (Table 9).

Table 9. *Relative proportions of palmitodi-‘oleins’, ‘oleo’-palmitostearins and palmitodistearins*

Animal ... Fat ...	Pig Back	Ewe External	Pig Peri- nephric	Ox English	Ewe Peri- nephric	Cow Calicut	Cow Bombay
C ₁₈ fatty acids:							
Stearic	13.8	14.5	17.6	21.4	25.6	27.9	25.5
Oleic + octadecadienoic	51.1	47.9	45.9	38.7	40.4	30.5	24.0
Palmitodi-C ₁₈ glycerides (increments %):							
Palmitodi-‘oleins’	53	46	40	23	25	17	11
‘Oleo’-palmitostearins	27	28	34	32	41	38	34
Palmitodistearins	2	2	5	6	10	12	10
	82	76	79	61	76	67	55
Palmitodi-C ₁₈ glycerides (%):							
Palmitodi-‘oleins’	65	61	51	38	33	25	20
‘Oleo’-palmitostearins	33	37	43	52	54	57	62
Palmitodistearins	2	2	6	10	13	18	18

The progressive change in the relative proportions of the three types of palmitodi-C₁₈ glycerides, shown at the base of Table 9, is an extremely strong argument in favour of the hydrogenation hypothesis originally put forward by Banks & Hilditch [1931; 1932] to account for the varying proportions of fully saturated glycerides in these animal depot fats.

A somewhat similar comparison may be made in regard to the relative proportions of 'oleo'-dipalmitin and dipalmitostearin in the dipalmitomono- C_{18} group of glycerides. Here, however, the total proportion of the dipalmito-glycerides is partly dependent upon the palmitic acid content in the whole fats; the latter rises considerably above the average in the Indian cow depot fats with consequent increase in the amount of dipalmito-glycerides at the expense of the monopalmitodi- C_{18} group. Apart from increased proportions of dipalmito-glycerides from this cause, however, a tendency has previously been observed [Hilditch & Pedelty, 1940] for this group to increase in amount with increasing general saturation in the fats as a whole. The relevant data on this aspect for the seven fats for which information is now available are summarized in Table 10; in this case, since the minor quantities of lower saturated acids present are perforce included with palmitic acid in the evaluation of the main component glycerides, comparison is made with the combined amount of palmitic, myristic and lauric acids in the total acids of the fats.

Table 10. *Relative proportions of 'oleo'-dipalmitin and dipalmitostearin*

Animal Fat	...	Pig Back	Ewe External	Pig Peri- nephric	Ox English	Ewe Peri- nephric	Cow Calicut	Cow Bombay
Fatty acids:								
Palmitic (+ C_{14} , C_{12})		30.3	35.3	32.9	36.0	30.1	39.1	46.7
Stearic		13.8	14.5	17.6	21.4	25.6	27.9	25.5
Total unsaturated		55.9	49.8	49.5	41.3	42.8	32.5	27.1
Dipalmitomono- C_{18} glycerides (increments %):								
'Oleo'-dipalmitins		5	13	9	15	5	11	18
Dipalmitostearins		2	3	4	8	4	16	23
		7	16	13	23	9	27	41
Dipalmitomono- C_{18} glycerides (%):								
'Oleo'-dipalmitins		71	81	69	65	56	41	44
Dipalmitostearins		29	19	31	35	44	59	56

The total amount of dipalmito-glycerides in the depot fats bears a general, though somewhat irregular, relationship to the proportion of palmitic acid present in the whole fat; but the relative proportions of 'oleo'- and stearo-dipalmitins are, as in the monopalmito-di- C_{18} -glycerides, governed entirely by the proportion of stearic and unsaturated acids present and thus again support the view that production of the more saturated component glycerides involves hydrogenation of preformed 'oleo'-glycerides—in this case 'oleo'-dipalmitins.

SUMMARY

Specimens of perinephric and external tissue fats from the same ewe have been studied after resolution into fractions of varying degrees of solubility in acetone, and the approximate proportions of each of the major component mixed glycerides have been deduced from the resulting analyses (cf. Table 7). Sheep depot fats resemble ox and pig depot fats in that they are mainly composed of palmitodi-'oleins' and 'oleo'-palmitostearins, together with 'oleo'-dipalmitins, steardi-'oleins' and minor amounts of other mixed glycerides. Triolein is either absent, or present in very small proportions.

The detailed data now available for the component glycerides of three ox, two pig and two sheep depot fats (the pig and sheep fats comprising perinephric

and external tissue fats from the same animal) have been reviewed, with special reference to the respective proportions of palmitodi-'oleins', 'oleo'-palmito-stearins and palmito-distearins, and of 'oleo'-dipalmitins and dipalmito-stearins, in relation to (a) the general unsaturation and (b) the palmitic acid contents of each fat as a whole.

The present study is the conclusion of a programme of work undertaken on behalf of the Food Investigation Board, and is published by permission of the Department of Scientific and Industrial Research.

REFERENCES

- Atherton & Hilditch (1941). *J. chem. Soc.* p. 527.
Banks & Hilditch (1931). *Biochem. J.* **25**, 1168.
— (1932). *Biochem. J.* **26**, 298.
Hilditch (1940). The chemical constitution of natural fats, Chap. VII. London.
— & Ichaporia (1938). *J. Soc. chem. Ind., Lond.*, **57**, 44.
— & Murti (1940). *Biochem. J.* **34**, 1301.
— & Paul (1938). *Biochem. J.* **32**, 1775.
— & Pedelty (1940). *Biochem. J.* **34**, 971.
— (1941). *Biochem. J.* **35**, 932.
— & Stainsby (1936). *J. Soc. chem. Ind., Lond.*, **55**, 95 T.