CCXXXI. THE COMPONENT GLYCERIDES OF AN OX DEPOT FAT

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(*Received 1 September 1938*)

The general composition of the depot fats of the ox and the pig was indicated by Banks & Hilditch [1931; 1932] by consideration of the proportion of fully saturated glycerides present in these fats together with the percentage composition of the fatty acids in the whole fats and in their fully saturated glycerides. Later, in the case of a pig depot fat, Hilditch & Stainsby [1935] obtained somewhat more detailed information by its study after it had been progressively hydrogenated to varying extents, coupled with the determination of tristearin in the completely hydrogenated fat. More recently it has been found possible to obtain an approximate estimate of at all events the main component glycerides present, in solid fats of comparatively simple fatty acid composition, by preliminary systematic crystallization of the fat from acetone. This procedure, although usually incapable of yielding definite individual mixed glycerides, causes a division of the fat into sparingly soluble portions in which mono-unsaturated-disaturated (and fully saturated) glycerides predominate, and more soluble portions in which the di-unsaturated glycerides (and tri-unsaturated glycerides when present) are concentrated. The fat is thus divided into two or three fractions, each of which is investigated as follows:

(*a*) the component acids are determined by ester-fractionation;

(*b*) a portion is hydrogenated and the tristearin content of the product determined;

(*c*) where necessary, the fully saturated glycerides present are isolated and determined; the component acids present in the fully saturated glycerides, and the tristearin content of the latter, are determined.

In addition to the data for fully saturated glycerides, it is then possible, in each fraction of the fat, (i) to estimate the proportions of mono- and of di-unsaturated glycerides (or of di- and tri-unsaturated glycerides) and (ii), knowing the tri-C_{18} glyceride content (determined as tristearin in (*b*)), to estimate the proportions of mono-C_{18}- and di-C_{18}-mixed glycerides in which another homologous acid (palmitic) is present. With these data, and taking into account the known general order of solubility in acetone of, for example, oleodistearin, dioleostearin, oleopalmitostearin, oleodipalmitin and palmitodiolein, it is usually possible to give a detailed, approximately quantitative statement of the component glycerides in each portion of the fat, and therefrom to deduce that of the whole fat.

This method has been used in the investigation of a number of solid seed fats, including cacao butter, mowrah fat and shea fat, by Hilditch *et al.* [1936; 1938, 1, 2, 3]. The present communication describes its application to a typical ox depot fat. Here the procedure is somewhat more complicated owing to the comparatively large proportions of fully saturated glycerides present, and to the circumstance that animal depot fats contain a somewhat greater number of minor component acids than any of the seed fats to which the method has yet been applied.

Experimental

The ox depot fat employed was obtained by extracting with acetone the perinephric tissue from a Shorthorn heifer. It had I.V. (Wijs) 38·7, sap. equiv. 284·0, and contained 0·12 % of unsaponifiable matter. The fatty acids (185·9 g.) obtained on hydrolysis of 200 g. of the fat were submitted to lead salt separation by the modified Twitchell method [Banks *et al.* 1933], and the resulting acid fractions were converted into methyl esters and fractionally distilled. The esters of the "solid" acids were distilled from a Willstätter bulb; those of the "liquid" acids were distilled through the electrically heated and packed column described by Longenecker [1937]. The final results of the analysis are summarized in Table I.

Table I. *Component acids of ox depot fat*

Component acids	"Solid" (56·6 %)	"Liquid" (43·4 %)	Total	% (wt.)	% (mol.)
Saturated:					
Lauric	—	0·50	0·50	0·5	0·7
Myristic	0·68	2·04	2·72	2·7	3·2
Palmitic	27·17	3·23	30·40	30·4	32·2
Stearic	23·63	—	23·63	23·7	22·6
Unsaturated:					
Tetradecenoic	—	0·41	0·41	0·4	0·5
Hexadecenoic	—	1·65	1·65	1·7	1·8
Oleic	5·08	33·49	38·57	38·6	37·1
Octadecadienoic	—	2·00	2·00	2·0	1·9
Unsaponifiable:	0·04	0·08	0·12	—	—

Crystallization of the ox depot fat from acetone

About 1 kg. of the fat was subjected to systematic crystallization from acetone at 0° or room temperature as follows.

(i) The fat (in three portions for convenience) was dissolved in acetone (5 ml. per g. of fat) and kept at room temperature overnight; after filtering the separated fat the solution was then cooled at 0° overnight and a further fraction of fat separated:

Fat taken	Separated at room temperature			Separated at 0°			Left in solution at 0°		
g.	No.	g.	I.V.	No.	g.	I.V.	No.	g.	I.V.
350	A_1	102	22·8	B_1	130	35·7	C_1	118	56·3
350	A_2	95	16·9	B_2	161	42·2	C_2	94	57·6
325	A_3	96	19·3	B_3	142	41·6	C_3	87	57·6

(ii) Corresponding fractions were then united and further crystallized as shown in the scheme below:

Fractions crystallized		Conditions of crystallization			Separated fat			Fat left in solution		
Nos.	g.	Acetone (ml. per g. fat)	Temp.	Time (hr.)	No.	g.	I.V.	No.	g.	I.V.
$A_1+A_2+A_3$	293	3 : 1	Atmospheric	16	**A_4**	**244**	**15·9**	A_5	49	41·1
$B_1+B_2+B_3$	433	5 : 1	0°	6	**B_4**	**347**	**37·1**	(further 16 hr. at 0°)		
			0°	16	B_5	27	40·4	**B_6**	**59**	**55·3**
A_5+B_5	76	5 : 1	0°	6	**D_1**	**66**	**40·6**	**D_2**	**10**	**53·8**

The eight fractions (A_4, B_4, B_6, D_1, D_2, C_1, C_2, C_3) finally obtained were then assembled into three groups of similar I.V. as follows:

No.	g.	I.V.	No.	g.	I.V.
A	244	15·9	A_4	244	15·9
B	413	37·5	B_4	347	37·1
			D_1	66	40·6
C	368	57·4	C_1	118	56·3
			C_2	94	57·6
			C_3	87	57·6
			B_6	59	55·3
			D_2	10	52·8

(The weights actually obtained were slightly diminished owing to withdrawal of samples for I.V. determinations, etc., and have been "corrected" in the above description to allow for this; the total weight so withdrawn amounted to about 5 g., or 0·5 % of the original fat employed.)

The component fatty acids in each of the fractions A, B, C were determined by lead salt separation and ester-fractionation as in the case of the whole fat (Table I). The final percentage compositions (wt. and mol.) found for the acids from each fraction are shown in Table II.

Table II. *Component acids of the separated fractions (A, B, C) of ox depot fat*

	A		B		C	
Saturated:	% (wt.)	% (mol.)	% (wt.)	% (mol.)	% (wt.)	% (mol.)
Lauric	—	—	0·2	0·2	0·3	0·4
Myristic	1·3	1·5	2·1	2·6	2·5	3·0
Palmitic	44·0	46·5	29·8	31·7	25·0	26·5
Stearic	31·8	30·3	25·8	24·7	12·2	11·7
As arachidic	5·5	4·8	0·4	0·3	0·1	0·1
Unsaturated:						
Tetradecenoic	0·3	0·4	0·3	0·4	0·7	0·9
Hexadecenoic	1·1	1·1	1·5	1·6	2·6	2·7
Oleic	16·0	15·4	38·8	37·4	47·4	45·8
Octadecadienoic	—	—	1·1	1·1	9·0	8·8
As C_{20-22} unsaturated	—	—	—	—	0·2	0·1

It will be observed that, of the minor component acids, arachidic acid is the only one concentrated in fraction A, in which fully saturated and mono-unsaturated-disaturated glycerides predominate. Myristic, tetra- and hexa-decenoic, and especially octadecadienoic and the unsaturated C_{20-22} acids, are found in the (mainly di-unsaturated) glycerides of fraction C, which are liquid at room temperature and most soluble in acetone.

Examination of fully saturated glycerides present in fractions A and B of the ox depot fat

The fully saturated glycerides were isolated, and their amounts determined, in each case by oxidation of the fats in acetone solution with powdered $KMnO_4$ by our usual procedure.

Fraction A: 80·2 g. gave 49·9 g. fully saturated glycerides (sap. equiv. 283·4, acid value 4·8, I.V. 0·2). *Fully saturated glycerides*: 59·0 % (wt.) or 60·0 % (mol.).

Fraction B: 200 g. gave 14·6 g. fully saturated glycerides (sap. equiv. 272·1, acid value 1·8, I.V. 0·4). *Fully saturated glycerides*: 7·1 % (wt.) or 7·4 % (mol.).

The component acids in each fraction of fully saturated glycerides were determined by ester-fractionation with the results given in Table III.

Table III. *Component acids of the fully saturated glycerides in fractions A and B*

	A		B	
	% (wt.)	% (mol.)	% (wt.)	% (mol.)
Myristic	5·4	6·3	7·5	8·6
Palmitic	49·6	51·5	63·0	64·2
Stearic	45·0	42·2	29·5	27·2

A portion of the fully saturated glycerides from the least soluble fraction A of the ox fat was systematically crystallized from anhydrous ether into five fractions. From the sap. equiv. of these fractions it was estimated that the material contained 3·0% (mol.) of tristearin and 19·6% (mol.) of tripalmitin (the latter figure is probably somewhat higher than the truth, because traces of acidic products of oxidation left in the fully saturated glycerides may have lowered the equiv. of the portion most soluble in ether).

Lack of material prevented similar crystallization of the fully saturated glycerides from fraction B, which however only amounted to 3% of the whole fat, and were taken as a mixture of tripalmitin and dipalmitostearin in the proportions demanded by the fatty acid composition.

Determination of tri-C_{18} glycerides in each fraction (A, B, C) of the ox depot fat

A portion (50 g.) of each fraction was completely hydrogenated and then submitted to systematic crystallization from anhydrous ether. From the sap. equiv. of the six or seven crystal fractions thus obtained the proportion of tristearin was determined [cf. Hilditch & Stainsby, 1935]. The molar percentage, of course, corresponds with the molar percentage of glycerides composed exclusively of C_{18} acids in the original fats before hydrogenation. The amounts of tristearin determined by this procedure in the hydrogenated portions A, B and C are shown in Table IV.

Table IV. *Tristearin content of hydrogenated fractions A, B and C of the ox depot fat*

Fraction	% (wt.)	% (mol.)
A	18·4	17·7
B	15·6	15·1
C	9·7	9·3

Discussion

The evaluation of the data recorded in the experimental part requires some explanation both as regards general interpretation and as regards the manner in which the various minor component acids in the fat are considered. The discussion is based throughout, it should first of all be noted, on the molar (not weight) proportions of the various components; this has the primary advantage that in any given case the same numerical figure denotes the proportion of a component either in the form of glyceride or of the fatty acid in question. The data for the molar proportions of glycerides in fractions A, B and C of the ox depot fat, and for the increments of component acids present in each fraction (from the percentage figures given in Table II), are shown in Table V.

Comparison of the last column in Table V with Table I shows satisfactory accordance in the percentages of the component acids as determined (i) in the whole fat and (ii) from summation of the component acids recorded for each of the three fractions A, B and C. As would be expected, the components present in very small proportions are more apparent in the data for the subdivided parts,

Table V. *Proportions (mol. %) of glycerides and of their component acids in fractions A, B and C*

	A	B	C	
Weight of fraction:	244	413	368	
I.V.	15·9	37·5	57·4	
Sap. equiv.	283·3	284·9	285·3	
Unsaponifiable %	—	0·06	0·2	
Glycerides % (wt.)	23·8	40·3	35·9	
Glycerides % (mol.)	23·9	40·3	35·8	
Component acids (increments % mol):				Whole fat
Lauric	—	0·1	0·15	0·25
Myristic	0·35	1·0	1·05	2·4
Palmitic	11·1	12·8	9·5	33·4
Stearic	7·2	10·0	4·2	21·4
As arachidic	1·15	0·1	0·05	1·3
Tetradecenoic	0·1	0·2	0·3	0·6
Hexadecenoic	0·3	0·6	1·0	1·9
Oleic	3·7	15·1	16·4	35·2
Octadecadienoic	—	0·4	3·1	3·5
C_{20-22} unsaturated	—	—	0·05	0·05

in one or other of which they have become concentrated. Indeed, arachidic and unsaturated acids of the C_{20} and C_{22} series (which are found only in the residual fractions of the distilled esters) only became detectable in the analyses of the fat after separation by crystallization.

The method of estimation of component glycerides employed in this work depends essentially on comparison of non-C_{18} and C_{18} acid components on the one hand, and of the total saturated and unsaturated acid components on the other. Complete detailed results can therefore only be obtained when the acids of the fat consist of one saturated non-C_{18} acid (e.g. palmitic), one saturated C_{18} acid (stearic) and unsaturated C_{18} acids (which, whether oleic or linoleic, etc., must be considered for the present purpose as one group; it is clearly impossible, by the use of methods involving hydrogenation, to distinguish between what were originally oleic or linoleic glycerides. To emphasize this, as in previous papers, the prefixes "oleic" or "oleo-" are employed when it is desired to indicate that all the C_{18} unsaturated components, and not merely oleic derivatives alone, are included).

In the seed fats investigated by this procedure, the acids present have been confined so far to palmitic, stearic, oleic and linoleic, with the exception of extremely small proportions of myristic or arachidic acids. In the case of ox depot fat (and of other animal fats which we are at present examining) the number of these minor components is increased and unfortunately includes, amongst others, about 2% of hexadecenoic acid (and traces of tetradecenoic acid), belonging simultaneously to the unsaturated group and to the group of non-C_{18} acids. In relation to the three major components (palmitic 33, stearic 21, oleic 35 ("oleic" 39), amounting together to 93% of the component acids) the quantity of any one of the minor components is almost insignificant; but it must be remembered that each mol. of a minor component will be associated almost invariably with 2 mol. of one or other of the three major components in a mixed triglyceride molecule, so that the total percentage of triglycerides involved is not of the order of 7%, but about 20%, of the whole fat. Whilst it is desirable to draw attention to this feature, it should equally be pointed out that the data presented in this paper are entirely valid from the point of view of the distribution of saturated non-C_{18} and C_{18} acids in the mixed glycerides of the depot fat,

and that the great predominance of palmitic acid in the former class ensures that the conclusions drawn later cannot be, in point of fact, very far from the actual state of affairs. It is perhaps not unreasonable to remark upon the fact that the presence of a number of minor component acids is the bugbear of detailed study of component glycerides in natural fats; in the rare absence of these, it is now possible to give an almost exact statement of the component glycerides present in a fat containing only three fatty acids.

For the purpose of the present discussion, myristic and lauric are included with palmitic glycerides, and arachidic with stearic glycerides, whilst the traces of unsaturated C_{20} and C_{22} acids are included in the "oleic" group. Hexadecenoic (and tetradecenoic) glycerides, which fall in the unsaturated non-C_{18} acid category, have been included also with palmitic glycerides, since on hydrogenation (for the tri-C_{18} glyceride determinations) they yield palmito- (or myristo-) glycerides. Accordingly, in terms of non-C_{18} ("palmitic"), stearic and "oleic" derivatives, the data in Table V, together with those for the fully saturated glycerides and contents of tri-C_{18} glycerides, may be transformed as shown in Table VI. In Table VI are also given the deduced proportions of palmitodi-C_{18}- and dipalmitomono-C_{18}-glycerides, and of mono- and di-unsaturated glycerides. Since the determination of the former and of tristearin, by crystallization of the hydrogenated fats from ether, is of a lower order of accuracy than the component acid analyses in Table II, the palmitodi-C_{18}- and dipalmitomono-C_{18}-glycerides have been calculated from the latter, after deduction of the proportion of tri-C_{18} acids present as tri-C_{18} glycerides. In fractions A and B, the fully saturated glycerides present are of course first dealt with, and the remaining acids accounted for in the various categories of mixed saturated-unsaturated glycerides.

Table VI. *Glyceride categories (% mol.) present in fractions A, B and C of ox depot fat*

	A		B		C	Whole fat
Glycerides	23·9		40·3		35·8	100·0
Component acids (increments):						
Palmitic	11·85		14·7		12·0	38·55
Stearic	8·35		10·1		4·25	22·7
"Oleic"	3·7		15·5		19·55	38·75
Component glycerides (increments):	Fully saturated	Mixed	Fully saturated	Mixed		
Tri-C_{18}	0·4	3·8	—	6·1	3·3	13·6
Palmitodi-C_{18}	5·8	0·8	—	24·9	29·0	60·5
Dipalmitomono-C_{18}	5·4	4·9	2·4	6·3	3·5	22·5
Tripalmitin	2·8	—	0·6	—	—	3·4
Mono-"oleo"-disaturated	—	8·0	—	28·1	12·9	49·0
Di-"oleo"-monosaturated	—	1·5	—	9·2	22·9	33·6

Consideration of the data in Table VI leads us to the statement of component glycerides in the fractions of the original fat, and consequently in the whole of the original fat, given in Table VII. These considerations are as follows.

Fraction A. The fully saturated components are derived from the estimated amounts of tripalmitin and tristearin, those of dipalmitostearin and palmitodistearin following from the component acid percentages in Table III. In the mixed saturated-unsaturated glycerides, the 4·9% of dipalmitomono-C_{18} glycerides must be "oleo"-dipalmitin. Of di-"oleo"-monosaturated glycerides, palmitodi-"oleins" are relatively soluble in acetone and may safely be considered absent from the sparingly soluble fraction A; the 1·5% of di-"oleo"-

glycerides is therefore credited as stearodi-"olein" and the remaining 2·3 % of tri-C_{18} glycerides as "oleo"-distearin, whilst the 0·8 % of palmitodi-C_{18} glycerides is taken as "oleo"-palmitostearin.

Fraction B. After reckoning the small amount of fully saturated glycerides (from the fatty acid analysis, Table III) as tripalmitin (0·6) and dipalmitostearin (2·4), and the remaining 6·3 % of dipalmitomono-C_{18} glycerides as "oleo"-dipalmitin, the 6·1 % of tri-C_{18} glycerides is assumed to be stearodi-"olein". (Some of the latter might be, perhaps, "oleo"-distearin, but since the amount of this relatively insoluble glyceride in the least soluble fraction A is only 2·3 % of the whole fat, it is certain that the proportion in fraction B will be definitely less than this.) Of the 9·2 % of di-"oleo"-glycerides, the remaining 3·1 % is then palmitodi-"olein", and the rest of the 28·1 % of mono-"oleo"-glycerides appears as "oleo"-palmitostearin.

Fraction C. From the proportions of saturated and unsaturated acids in this portion, its components lie between the following limits:

	% (of whole fat)
Mono-"oleo"-glycerides	12·9–24·4
Di-"oleo"-glycerides	22·9– 0
Tri-"oleins"	0–11·4

Since, however, the total amount of tri-C_{18} glycerides in C is only 3·3 % of the whole fat, there cannot at most be more than 3·3 % of tri-"olein" present (the corresponding figures in this case being 16·3 % of palmitodi-"olein", with 12·7 % of "oleo"-palmitostearin). But the presence of probably nearly 6 % of stearodi-"olein" in fraction B implies almost certainly that appreciable amounts of this glyceride will be present in the most soluble fraction C, and therefore, whilst the experimental data do not permit a final subdivision into tri-"olein" and stearodi-"olein", the probability is very great that nearly all the 3·3 % of tri-C_{18} glycerides in fraction C is, in fact, stearodi-"olein". The experimental figures, in fact, show almost conclusively that tri-"oleins", if not wholly absent, can only be present in quite insignificant amounts in the specimen of ox depot fat investigated.

The proportions of the remaining components of fraction C, once the nature of the tri-C_{18} unsaturated glycerides has been settled, follow as in the preceding cases of fractions A and B.

Table VII. *Probable component glycerides (% mol.) of the ox depot fat*

	A		B		C	Whole fat	
	Fully saturated	Mixed	Fully saturated	Mixed		Exact figure	(in round nos.)
Fully saturated glycerides (17·4 %):							
Tripalmitin	2·8	—	0·6	—	—	3·4	3
Dipalmitostearin	5·4	—	2·4	—	—	7·8	8
Palmitodistearin	5·8	—	—	—	—	5·8	6
Tristearin	0·4	—	—	—	—	0·4	†
Mono-"oleo"-disaturated glycerides (49·0 %):							
"Oleo"-dipalmitin	—	4·9	—	6·3	3·5	14·7	15
"Oleo"-palmitostearin	—	0·8	—	21·8	9·4	32·0	32
"Oleo"-distearin	—	2·3	—	—	—	2·3	2
Di-"oleo"-monosaturated glycerides (33·6 %):							
Palmitodi-"oleins"	—	—	—	3·1	19·6	22·7	23
Stearodi-"oleins"	—	1·5	—	6·1	3·3	10·9	11
Tri-"oleins"	—	—	—	—	*	*	*

* Traces of triolein, probably not exceeding 1 % of the fat, may be present.
† Tristearin is present to the extent of less than 1 % of the fat.

The ox depot fat investigated thus consists of about one-third of "oleo"-palmitostearins, and one-quarter of palmitodi-"oleins", with (in progressively diminishing proportions) "oleo"-dipalmitin, stearodi-"olein", dipalmitostearin and palmitodistearin. Very small amounts of tripalmitin, tristearin and (possibly) tri-"olein" are also present.

It is interesting to compare the detailed results obtainable by the procedure which has now been employed with those obtained by earlier methods. By simple but intensive crystallization of a sheep depot fat, Bömer *et al.* [1907; 1909] detected about 3% of tristearin with 4–5% of dipalmitostearin and some palmitodistearin. Banks & Hilditch [1931; 1932] were able to give limiting values for the mixed saturated-unsaturated glycerides by determining the total molar proportions of the fully saturated glycerides present. In the ox depot fat which has now been studied this procedure would have enabled us only to state the approximate components as fully-saturated 17·4%, mono-"oleo"-disaturated from 49 to 66%, di-"oleo"-monosaturated from 34 to 0% and tri-"oleins" from 0 to 17%. Determination of the tri-C_{18} glyceride content of the whole fat, coupled with consideration of the major component acids and the fully saturated glycerides, as applied by Hilditch & Stainsby [1935] to a pig depot fat, would have permitted a somewhat narrower range of the chief possible components: for example, 0–13·6% tri-"olein", 13·6–0% stearodi-"olein", 20–7% palmitodi-"olein", 34–47% "oleo"-palmitostearin, with 15% "oleo"-dipalmitin and 17·4% of fully saturated glycerides.

The procedure which has now been described, however, has demonstrated that tri-"oleins" are unlikely to be present in more than traces, and cannot in any case form more than 3% of the fat; whilst it has also indicated the presence of very small proportions (about 2%) of "oleo"-distearin. It therefore follows that the proportions of each of the component glycerides are defined by the approximate figures given in the final column of Table VII. (Even if the 3% of tri-C_{18} glycerides in fraction C were calculated alternatively as stearodi-"oleins" and as tri-"oleins", the possible limits of the component glycerides concerned would be narrowed to 0–3% tri-"olein", 11–8% stearodi-"olein", 23–20% palmitodi-"olein" and 32–35% "oleo"-palmitostearin.)

The possession of approximate figures for the chief component glyceride percentages permits some further discussion of the characteristic glyceride structure of ox depot fat in contrast to the "evenly distributed" type of glyceride structure encountered in the majority of natural fats. As mentioned earlier, the glycerides of several solid seed fats which conform closely to the "evenly-distributed" type have been investigated by the present method, and it has been found [Hilditch, 1938] that the observed proportions therein of the di-"oleo"-glycerides, and to a less extent the mono-"oleo"-glycerides, can be fairly closely reproduced by a simple calculation based upon the proportions of the component acids in the whole fat. If the chief component acids of the ox depot fat were assembled into a mixture of component glycerides on the lines of a typical seed fat (in which case there would be negligible proportions of fully saturated glycerides present), the application of the calculation referred to would suggest the probable presence of about 10% palmitodi-"olein", 6% stearodi-"olein", up to 62% of "oleo"-palmitostearin and 22% or more of "oleo"-dipalmitin—proportions which, naturally, differ widely from those observed in the foregoing analysis. If, on the other hand, allowance is made for the 17·4% of fully saturated glycerides known to be present, application of the numerical calculation to the component acids (28·0% palmitic, 15·8% stearic and 38·8% "oleic") of the mixed saturated-unsaturated glycerides would suggest the following composition

for the latter: 22% palmitodi-"olein", 12% stearodi-"olein", up to 35% of "oleo"-palmitostearin and 14% or more of "oleo"-dipalmitin. These figures are remarkably close to those actually observed (Table VII), but the implication of the coincidence, if it be more than a fortuitous one, is not at present altogether clear. Until the results of some contemplated parallel investigations of other animal depot and milk fats become available, it is therefore deemed desirable merely to draw attention to this feature without further comment, other than that an "even distribution" of the component acids of the mixed saturated-unsaturated glycerides seems not inconsistent with the hypothesis [Hilditch & Sleightholme, 1931; Banks & Hilditch, 1932; Hilditch, 1937, 1, 2] that the stearo-glycerides in the stearic-rich animal depot fats have resulted from saturation of preformed oleo-glycerides.

The hypothesis in question (with its extension to milk-fat glycerides) correlates the unusually large proportions of fully saturated glycerides present in many animal depot and milk fats with their possible formation by hydrogenation (depot fats) or other transformation (the lower saturated acids of milk-fats) of oleic groups already in combination as glycerides. The results of the present study may be briefly discussed in relation to this view. The ox depot fatty acids include, in molar proportions (Table VI), 38·5% palmitic (or, more strictly, non-C_{18} saturated acids), 22·7% stearic and 38·8% oleic (with octadecadienoic) acid, and of the glycerides 13·6% contain three C_{18}, 60·5% contain two C_{18}, and 22·5% contain only one C_{18} acyl group. The observed composition (Table VII) of these various groups was as follows:

	Tri-"oleo"	Di-"oleo"	Mono-"oleo"	Fully saturated
Tri-C_{18} glycerides	Nil or traces	10·9	2·3	0·4
Palmitodi-C_{18} glycerides	—	22·7	32·0	5·8
Dipalmitomono-C_{18} glycerides	—	—	14·7	7·8

If, in accordance with the above hypothesis, it be assumed that all these products have resulted from hydrogenation of preformed oleo-glycerides, their percentage proportions, at corresponding stages of the supposed hydrogenation, would be as below:

From	Unchanged %	1st stage %	2nd stage %	3rd stage %
Tri-"oleins"	Nil or traces	80	17	3
Palmitodi-"oleins"	37	53	10	—
Dipalmito-"oleins"	65	35	—	—

In catalytic hydrogenation of mixtures of mono-, di- and tri-oleo-glycerides in presence of nickel at 170–180° it has been shown [Hilditch & Jones, 1932; Bushell & Hilditch, 1937] that the process takes place in consecutive stages (i.e. only one oleo-group is saturated at a time) and that, whilst all three stages of hydrogenation proceed concurrently, the reduction of tri-oleo- to di-oleo-glycerides proceeds much more rapidly than that of di-oleo- to mono-oleo-glycerides, and the latter probably proceeds somewhat more rapidly than the production of saturated from mono-oleo-glycerides. Further, the relative concentrations of the different groups have an important influence on the amounts of the various semi-hydrogenated glycerides produced. The observed proportions of these products (illustrated in percentage form above) is that which would be expected if a biological hydrogenation process had followed the same course as addition of hydrogen by a nickel catalyst. Little or no tri-unsaturated glycerides are present, and the proportions of glycerides corresponding with the second and third stages of hydrogenation are much less than those of the initial

stages. Less of the original oleodipalmitin (as presupposed by the hypothesis) has been reduced than of the original palmitodiolein, but the amount of the latter present is nearly three times that of the former, and this is therefore also in keeping with the above-mentioned observations.

The observed presence of perhaps 2–3 % of tripalmitin in a fat which contains large proportions of stearic as well as palmitic acid is unusual. Tripalmitin has hitherto only been reported in cases in which the saturated acids of a natural fat consist almost wholly of palmitic acid (e.g. olive oil, palm oil, rabbit depot fat), and, when stearic and palmitic acids are both present in quantity (as in cacao butter and many other seed fats), any fully saturated components have been found to consist of mixed palmitostearins. The tripalmitin in ox depot fat is explicable, however, on the above "hydrogenation" hypothesis, since hexadecenoic acid is a minor component of ox depot fat, and saturation of any hexadeceno-palmitins present in the fat would, of course, yield tripalmitin.

Summary

A specimen of ox depot fat has been separated into fractions of varying degrees of solubility in acetone, and the component acids and glycerides present in each fraction have been investigated. The procedure leads to an approximate statement of the proportions of each of the major component mixed glycerides present in the fat. The approximate molar percentages of the more abundant glycerides are "oleo"-palmitostearin 32, palmitodi-"olein" 23, "oleo"-dipalmitin 15 and stearodi-"olein" 11 %; 17 % of fully-saturated glycerides are present, mainly dipalmitostearin and palmitodistearin, with very small amounts of tripalmitin and tristearin. Triolein is either absent, or only present in very small quantities. (Myristic, hexadecenoic, arachidic and other acids which are present to very small extents in the fat have to be included with palmitic acid in the analysis of the component glycerides.)

The proportions of the respective groups of tri-, di- and mono-"oleo"-glycerides, and of the fully-saturated glycerides, are fully consistent with the hypothesis that the stearo-glycerides in ox depot fat have resulted from saturation of preformed oleo-glycerides.

The assistance of the Department of Scientific and Industrial Research in a grant to one of us (S. P.) is gratefully acknowledged.

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