154. THE COMPONENT ACIDS AND GLYCERIDES OF SOME INDIAN OX DEPOT FATS

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AN investigation of four specimens of depot fats from oxen from three widely separated districts in India has disclosed considerable differences between their composition and that of corresponding fats from English, American or Australian cattle. The Indian ox depot fats are relatively much less unsaturated (I.V. 26–31 as compared with 40–50) whilst the content of palmitic acid, in three out of the four instances examined, approaches 40 % (mol.) of the total fatty acids (instead of the more usual 30 ± 3 %). In addition to component acid determinations on each of the fats, the component glycerides of the two fats with the highest and lowest contents of palmitic acid have therefore been studied by the procedure applied to an English ox depot fat by Hilditch & Paul [1938].

The Indian fats about to be described included the depot fats of a bullock and of a cow from Bombay, of a cow from Calicut (Malabar), and those of animals of both sexes from Calcutta. These were respectively obtained for us by the Director of Industries, Bombay, the Kerala Soap Institute, Calicut, and the Calcutta works of Messrs Lever Brothers and Unilever, Ltd., to all of whom we express our cordial thanks. These fats were prepared in India under qualified supervision, and we were informed that the diets of the animals concerned were of the following general nature: in the Bombay area oxen usually feed on grass, rice, jowar straw, wheat bran and cotton-seed, whilst in the Calicut (and probably also the Calcutta) area the main diet is dry paddy straw and fresh grass. The fats were taken from one or more depots (probably mainly the perinephric tissues) of one or more animals of the sex and locality mentioned. The Bombay fats contained over 1 % of free fatty acid (as oleic) and were neutralized before their detailed examination was commenced; the free acidity of the Calicut fat was negligible.

The general analytical characteristics of the fats are given in Table 1.

Table 1. *Analytical characteristics of Indian ox depot fats*

	Sap. equiv.	I.V.	Free fatty acid % (as oleic)	Unsap. %	Fat M.P. (open tube)	Mixed acids setting-point
Bombay (cow), original	280·1	26·6	1·4			
Bombay (cow), neutralized	282·2	26·5	0·1	0·35	50·5°	47·6°
Bombay (bullock), original	282·0	26·1	2·4			
Bombay (bullock), neutralized	283·2	25·8	0·1	0·3	50·0°	48·5°
Calcutta, original	283·2	31·0	1·3	0·2	49·0°	48·4°
Calicut (cow), original	283·5	31·1	0·2	0·4	50·5°	48·7°

Component acids of the Indian ox depot fats

Each fat (*ca.* 150 g.) was converted into the mixed fatty acids, and the latter submitted to the usual lead salt separation from alcohol. The methyl esters of the "solid" acids were distilled from a Willstätter bulb, and those of the "liquid" acids through an electrically heated and packed column, following our customary procedure. From the analytical characteristics of the ester-fractions thus obtained the composition of the mixed fatty acids was calculated, with the final results given in Table 2.

Table 2. *Component acids of Indian ox depot fats*

	Bombay (cow) (neutralized)		Bombay (bullock) (neutralized)		Calcutta (original)		Calicut (cow) (original)	
Component acids:	%(wt.)	%(mol.)	%(wt.)	%(mol.)	%(wt.)	%mol.)	%(wt.)	%(mol.)
Saturated:								
Lauric	0·1	0·2	0·2	0·3	0·2	0·3	0·3	0·5
Myristic	4·5	5·2	3·7	4·4	2·4	2·8	3·1	3·7
Palmitic	41·4	43·3	37·1	39·1	36·8	38·8	32·9	34·7
Stearic	24·3	22·9	29·4	27·9	26·8	25·5	29·3	27·9
Arachidic	0·5	0·4	1·2	1·0	0·4	0·4	—	—
Unsaturated:								
Tetradecenoic	0·4	0·5	0·4	0·5	0·3	0·4	0·4	0·4
Hexadecenoic	1·3	1·4	1·0	1·0	2·1	2·2	1·5	1·6
Oleic	26·4	25·1	25·9	24·8	29·2	28·0	30·7	29·5
Octadecadienoic	1·0	0·9	0·9	0·9	0·9	0·9	1·3	1·3
C_{20-22} unsaturated	0·1	0·1	0·2	0·1	0·9	0·7	0·5	0·4

The most notable feature in the molar percentage data in Table 2 is the small content of unsaturated acids (27–33 %) compared with that obtaining in all English, North and South American and Australian ox depot fats hitherto examined; in the latter the unsaturated acids have never fallen below 40 %, and approach 50 % in some instances. On the other hand, the proportion of stearic acid in the Indian fats, although substantial, does not exceed that observed in the more saturated (perinephric) ox depot fats from the other localities mentioned. In marked contrast to what has been uniformly observed in English, American and Australian depot fats, not only of oxen, but also of sheep and pigs [Hilditch, 1940], the deficiency in oleic acid in these Indian ox depot fats is made up not by a corresponding proportion of stearic acid but by an increased proportion of palmitic acid.

The contents of palmitic acid in three of these fats are, indeed, much larger than have hitherto been observed in any animal depot fat. Reviewing all the available detailed data for ox depot fat component acids, Hilditch & Longenecker [1937] were able to conclude that "the palmitic acid content of nearly all tallows which have been analysed lies within the relatively constant limits of 30 (±3) % (mol.)". This statement also applies to the numerous pig depot fats, both English and North American, which have been investigated in detail; whilst, over a much wider range of land animals (including birds) palmitic acid is roughly constant at 25–30 % of the total component acids of their depot fats [cf. Hilditch, 1940]. An explanation of the higher proportions of this acid in the Indian ox depot fats cannot be suggested with certainty; it might be connected with species, diet or climate. There is nothing in the diet of these Indian oxen, as far as we are aware, to account for the unusually large percentages of palmitic acid; cottonseed, it is true, was mentioned as a constituent of the diet of the Bombay animals but, although cottonseed oil contains 20–25 % of palmitic acid, inclusion of this oil in the diet of pigs causes a diminution in the palmitic

acid content of their depot fats [Ellis *et al.*, 1931; Bhattacharya & Hilditch, 1931]. It is equally unlikely that any species difference between the Asiatic and European oxen is concerned in this respect, since the depot fats of animals of many widely different families share the common feature of a content of 25–30 % of palmitic acid in their component acids. In this connexion it may be mentioned that Dhingra *et al.* [1938; 1939] found the component acids of North Indian goat depot fats to contain 27–28 % of palmitic acid.

On the other hand, the low content of unsaturated acids, i.e. the marked tendency towards saturation in the fats as a whole, may well be connected with the tropical temperatures in which these Indian cattle are reared. Further, it is possible that the simple oleic-stearic acid relationship observed in the depot fats of oxen, pigs etc. breaks down when the content of stearic acid reaches a certain point. At least it is suggestive that the molar proportion of stearic acid in the present series, and in other animal depot fats, reaches but has not been observed to exceed 27–28 % of the total fatty acids. It may be that, when the content of oleic acid falls, as in these cases, much below 40 %, the proportion of palmitic acid is supplemented so that stearic acid does not rise above a possibly limiting amount of 27–28 %. However this may be, the augmentation of the palmitic acid content and the limitation of that of stearic acid has an important practical result as regards the consistency of the depot fat itself. The "melting-points" of the fats and the setting-points of their mixed fatty acids (Table 1) are little, if at all, higher than those of the more usual ox depot fats with acids which include 40 % or more of oleic acid and the customary 30 % of palmitic acid; on the other hand, a fat with 30 % or less of oleic acid, 30 % palmitic acid and the balance in the form of stearic acid would possess a considerably higher melting-point. The increase in the proportion of palmitic, instead of stearic, acid is undoubtedly the reason why these Indian ox depot fats melt at temperatures little different from those of European, American or Australian tallows which contain considerably more combined oleic acid. At the body temperature of the oxen, therefore, fats of either type will possess much the same consistency or degree of fluidity.

Component glycerides of the Bombay and Calicut cow fats

The fats of highest and lowest palmitic acid content were selected for study of their glyceride structure. The fats were each resolved by systematic crystallization from acetone into three fractions of differing consistency and I.V., and each fraction was then studied by the methods of Hilditch & Paul [1938]; in the present paper only the numerical data at each stage of the analysis of both fats need be recorded.

Bombay cow depot fat

The fat (901 g.) was resolved into three fractions (A, B, C) by crystallization from acetone at 0° and 20°; the analytical characteristics and component acid percentages for each fraction are collected in Table 3.

Fully saturated glycerides in fractions A and B. These were isolated quantitatively after oxidation of each fraction in acetone solution with powdered $KMnO_4$ in the usual way.

Fraction A. The fat (100·7 g.) yielded 73·7 % (wt.) or 74·3 % (mol.)[1] of fully saturated glycerides which were further separated into three sub-fractions (D,

[1] The component acid figures for fraction A (Table 1) indicate that the minimum proportion of fully saturated glycerides present (i.e. with all unsaturated acids as mono-unsaturated-disaturated glycerides) must be 74·5 %; this figure has therefore been used.

Table 3. *Fractions from acetone (Bombay cow depot fat)*

	A		B		C	
Weight (g.)	391·8		254·4		254·8	
I.V.	7·9		30·1		51·1	
Sap. equiv.	280·3		282·1		284·6	
Glycerides % (wt.)	43·5		28·2		28·3	
Glycerides % (mol.)	43·8		28·2		28·0	
Component acids:	% (wt.)	% (mol.)	% (wt.)	% (mol.)	% (wt.)	% (mol.)
Lauric	—	—	—	—	0·3	0·4
Myristic	6·1	7·1	3·8	4·4	4·5	5·4
Palmitic	42·8	44·7	39·9	41·9	32·0	33·6
Stearic	40·6	38·2	22·9	21·7	10·1	9·6
Arachidic	1·8	1·5	—	—	—	—
Tetradecenoic	Trace	0·1	0·2	0·3	0·9	1·0
Hexadecenoic	1·7	1·8	1·9	2·0	3·4	3·6
Oleic	7·0	6·6	30·0	28·6	44·6	42·5
Octadecadienoic	—	—	0·9	0·8	3·4	3·3
C_{20-22} unsaturated	—	—	0·4	0·3	0·8	0·6

E, F) by crystallization from ether. After component acid determinations on each sub-fraction, the glycerides present in each were calculated as binary mixtures of tripalmitin and dipalmitostearin, or of the latter and palmitodistearin,[1] with the results given in Table 4.

Table 4. *Components of fully saturated glycerides in fraction A (Bombay cow depot fat)*

Sub-fraction:	D	E	F	Total
Weight (g.)	35·9	25·5	12·8	74·2
Sap. equiv.	284·5	272·4	271·8	
Glycerides % (wt.)	48·5	34·4	17·1	
Glycerides % (mol.)	47·3	35·0	17·7	
Component acids:	% (mol.)	% (mol.)	% (mol.)	% (mol.)
Lauric	—	1·6	0·6	0·7
Myristic	1·0	13·5	22·2	9·1
Palmitic	43·5	55·7	45·5	48·1
Stearic	54·5	29·2	31·7	41·6
Arachidic	1·0	—	—	0·5
Component acid groups (increments % mol.):				
C_{16} ($+C_{14}+C_{12}$)	21·0	24·8	12·1	57·9
C_{18} ($+C_{20}$)	26·3	10·2	5·6	42·1
Probable component glycerides (increments % mol.):				
*Tri-"palmitin"	—	4·4	0·9	5·3
*Di-"palmito"-stearin	15·8	30·6	16·8	63·2
Palmitodistearin	31·5	—	—	31·5

* These will include, respectively, minor amounts of myristodipalmitin and myristopalmitostearins.

Fraction B. The fat (100·4 g.) yielded 11·0% (wt.) or 11·6% (mol.) of fully saturated glycerides, the component acids of which were lauric 1·1, myristic 32·3, palmitic 46·1 and stearic 20·5% (mol.). Its probable component glycerides were therefore tri-"palmitin" 39, di-"palmito"-stearin 61% (mol.).

[1] As usual, minor amounts of C_{14} and C_{12} acids are grouped, for component glyceride estimation, with palmitic acid, whilst stearic acid here includes minor amounts of arachidic acid and "oleic" acid those of the other minor component unsaturated acids.

Whilst the component acid data for the remaining mixed saturated-unsaturated glycerides of fractions A and B indicated that little or no tri-C_{18} glycerides were present therein, it was desirable to determine the proportion of tri-C_{18} glycerides, if any, in fraction C.

Tri-C_{18} glycerides in fraction C. The fat (120 g.) was hydrogenated as completely as possible (to I.V. 1·2) in presence of nickel at 170°, and the product crystallized exhaustively from dry ether at room temperature. The least soluble of the four sub-fractions obtained (42·7% (wt.) or 42·4% (mol.) of fraction C) contained as component acids myristic 1·5, palmitic 29·6, stearic 68·3, arachidic 0·5 and oleic 0·1% (mol.); this corresponds with a minimum content of 6·8% tri-C_{18} glycerides in this sub-fraction, or 2·9% in the whole fraction C. (The three more soluble fractions included respectively 44·7, 42·6 and 45·8% (mol.) of stearic acid in their component acids, suggesting the absence of tristearin.)

Table 5. *Probable component glycerides of the Bombay cow depot fat*

Fractions from acetone:	A		B		C	Total
Glycerides % (mol.)	43·8		28·2		28·0	100·0
Component acids (increments % mol.):						
Lauric	—		—		0·1	0·1
Myristic	3·1		1·2		1·5	5·8
Palmitic	19·6		11·8		9·4	40·8
Stearic	16·7		6·1		2·7	25·5
Arachidic	0·7		—		—	0·7
Tetradecenoic	Trace		0·1		0·3	0·4
Hexadecenoic	0·8		0·6		1·0	2·4
Oleic	2·9		8·1		11·9	22·9
Octadecadienoic	—		0·2		0·9	1·1
C_{20-22} unsaturated	—		0·1		0·2	0·3
	F.S.G. (32·6%)	Mixed (11·2%)	F.S.G. (3·3%)	Mixed (24·9%)	Mixed (28·0%)	
Component acid groups (increments % mol.):						
C_{16} (+C_{14}, C_{12})	18·9	4·6	2·6	11·1	12·3	49·5
Stearic	13·7	3·7	0·7	5·4	2·7	26·2
Oleic (+C_{20-22})	—	2·9	—	8·4	13·0	24·3
Component glyceride groups (increments % mol.):						
(*a*) Tripalmitin	1·7	—	1·3	—	—	3·0
Dipalmitomono-C_{18}	20·6	2·6	2·0	8·3	9·7	43·2
Palmitodi-C_{18}	10·3	8·6	—	16·6	17·5	53·0
Tri-C_{18}	—	—	—	—	0·8	0·8
(*b*) Fully saturated	32·6	—	3·3	—	—	35·9
Hexadeceno-disaturated	—	2·4	—	—	—	2·4
Mono-"oleo"-disaturated	—	8·8	—	24·6	17·8–17·0	51·2–50·4
Di-"oleo"-monosaturated	—	—	—	0·3	9·4–11·0	9·7–11·3
Tri-"olein"	—	—	—	—	0·8–Nil	0·8–Nil
Probable component glycerides (increments % mol.):						
Fully-saturated (35·9%):						
Tripalmitin	1·7	—	1·3	—	—	3·0
Dipalmitostearin	20·6	—	2·0	—	—	22·6
Palmitodistearin	10·3	—	—	—	—	10·3
Mono-"oleo"-disaturated (53·6–52·8%):						
Hexadecenopalmitostearin	—	2·4	—	—	—	2·4
"Oleo"-dipalmitin	—	0·2	—	8·3	9·7	18·2
"Oleo"-palmitostearin	—	8·6	—	16·3	8·1–7·3	33·0–32·2
Di-"oleo"-monosaturated (9·7–11·3%):						
Palmitodi-"olein"	—	—	—	0·3	9·4–10·2	9·7–10·5
Stearodi-"olein"	—	—	—	—	Nil–0·8	Nil–0·8
Tri-"olein" (0·8–Nil %)	—	—	—	—	0·8–Nil	0·8–Nil

From the foregoing data the probable component glycerides of the whole Bombay cow depot fat are deduced to be those shown in the final column (bottom) of Table 5.

Calicut cow depot fat

The fat (904 g.) was resolved into three fractions (A, B, C) by crystallization from acetone at 0° and 20°; the analytical characteristics and component acid percentages for each fraction are collected in Table 6.

Table 6. *Fractions from acetone (Calicut cow depot fat)*

	A		B		C	
Weight (g.)	372·9		271·3		259·8	
I.V.	11·6		34·9		54·0	
Sap. equiv.	282·6		283·6		285·3	
Glycerides % (wt.)	41·3		30·0		28·7	
Glycerides % (mol.)	41·4		30·0		28·6	
Component acids:	% (wt.)	% (mol.)	% (wt.)	% (mol.)	% (wt.)	% (mol.)
Lauric	—	—	Trace	Trace	1·3	1·7
Myristic	6·4	7·6	3·0	3·6	2·7	3·2
Palmitic	33·5	35·3	34·7	36·6	25·7	27·2
Stearic	45·1	42·8	23·3	22·2	12·7	12·2
Arachidic	1·5	1·3	—	—	—	—
Tetradecenoic	0·1	0·1	0·3	0·4	0·8	1·0
Hexadecenoic	0·8	0·9	1·3	1·4	2·4	2·5
Oleic	12·6	12·0	35·9	34·4	49·9	47·9
Octadecadienoic	—	—	1·5	1·4	4·1	4·0
C_{20-22} unsaturated	—	—	—	—	0·4	0·3

Fully saturated glycerides in fraction A. The fat (101 g.) yielded on oxidation in the usual manner 63·1 % (wt.) or 63·7 % (mol.) of fully saturated glycerides which were further separated into two sub-fractions (D, E) by crystallization from ether. The component acids present in each sub-fraction were determined; their molar percentages and the deduced binary mixtures of palmitostearins are given in Table 7.

Table 7. *Components of fully saturated glycerides in fraction A (Calicut cow depot fat)*

Sub-fraction:	D	E	Total
Weight (g.)	38·8	24·9	63·7
Sap. equiv.	283·1	274·6	
Glycerides % (wt.)	60·9	39·1	
Glycerides % (mol.)	60·2	39·8	
Component acids:	% (mol.)	% (mol.)	% (mol.)
Lauric	—	3·7	1·5
Myristic	4·1	15·5	8·6
Palmitic	41·3	42·9	42·0
Stearic	54·6	34·7	46·6
Arachidic	—	3·2	1·3
Component acid groups (increments % mol.):			
C_{16} ($+C_{14}+C_{12}$)	27·4	24·7	52·1
C_{18} ($+C_{20}$)	32·8	15·1	47·9
Probable component glycerides (increments % mol.):			
Di-"palmito"-stearin*	21·9	34·3	56·2
Palmitodistearin	38·3	5·5	43·8

* This will include minor amounts of myristo-(lauro-)palmitostearins.

Fully saturated glycerides in fraction B. The fat (119·6 g.) yielded 6·0 % (wt.) or 6·3 % (mol.) of fully saturated glycerides, of which the component acids were

myristic 35·9, palmitic 27·9 and stearic 36·2% (mol.). Its probable component glycerides were about 90% myristopalmitostearins (included in Table 8 with di-"palmito"-stearin) and 10% palmitodistearin.

Tri-C_{18} glycerides in fraction C. The fat (120 g.) was hydrogenated to I.V. 1·4 and separated into three fractions of increasing solubility in dry ether at room temperature. The least soluble of these fractions (41·0% (wt.) or 40·4% (mol.) of fraction C) contained as component acids palmitic 24·5, stearic 75·0, arachidic 0·4 and oleic 0·1% (mol.); this corresponds with a minimum content of 26·5% tri-C_{18} glycerides in this sub-fraction, or 10·7% in the whole fraction C. (The two more soluble fractions included respectively 56·5 and 51·2% (mol.) of stearic acid in their component acids, indicating substantial absence of tristearin.)

From the above data the probable component glycerides of the whole Calicut cow depot fat were deduced with the results shown in Table 8.

Table 8. *Probable component glycerides of the Calicut cow depot fat*

Fractions from acetone:	A		B		C	Total
Glycerides % (mol.)	41·4		30·0		28·6	100·0
Component acids (increments % mol.):						
Lauric	—		Trace		0·5	0·5
Myristic	3·2		1·1		0·9	5·2
Palmitic	14·6		11·0		7·8	33·4
Stearic	17·7		6·7		3·5	27·9
Arachidic	0·5		—		—	0·5
Tetradecenoic	Trace		0·1		0·3	0·4
Hexadecenoic	0·4		0·4		0·7	1·5
Oleic	5·0		10·3		13·7	29·0
Octadecadienoic	—		0·4		1·1	1·5
C_{20-22} unsaturated	—		—		0·1	0·1
	F.S.G. (26·4%)	Mixed (15·0%)	F.S.G. (1·9%)	Mixed (28·1%)	Mixed (28·6%)	
Component acid groups (increments % mol.):						
C_{16} (+C_{14}, C_{12})	13·8	4·4	1·2	11·4	10·2	41·0
Stearic	12·6	5·6	0·7	6·0	3·5	28·4
Oleic (+C_{20-22})	—	5·0	—	10·7	14·9	30·6
Component glyceride groups (increments % mol.):						
(*a*) Dipalmitomono-C_{18}	14·8	—	1·7	6·0	5·0	27·5
Palmitodi-C_{18}	11·6	13·2	0·2	22·1	20·5	67·6
Tri-C_{18}	—	1·8	—	—	3·1	4·9
(*b*) Fully saturated	26·4	—	1·9	—	—	28·3
Hexadeceno-disaturated	—	0·1	—	—	—	0·1
Mono-"oleo"-disaturated	—	14·9	—	23·9	15·5–12·4	54·3–51·2
Di-"oleo"-monosaturated	—	—	—	4·2	10·0–16·2	14·2–20·4
Tri-"olein"	—	—	—	—	3·1–Nil	3·1–Nil
Probable component glycerides (increments % mol.):						
Fully saturated (28·3%):						
Dipalmitostearin	14·8	—	1·7	—	—	16·5
Palmitodistearin	11·6	—	0·2	—	—	11·8
Mono-"oleo"-disaturated (54·5–51·3%):						
Hexadecenodistearin	—	0·1	—	—	—	0·1
"Oleo"-dipalmitin	—	—	—	6·0	5·0	11·0
"Oleo"-palmitostearin	—	13·1	—	17·9	10·5–7·4	41·5–38·4
"Oleo"-distearin	—	1·8	—	—	—	1·8
Di-"oleo"-monosaturated (14·2–20·4%):						
Palmitodi-"olein"	—	—	—	4·2	10·0–13·1	14·2–17·3
Stearodi-"olein"	—	—	—	—	Nil–3·1	Nil–3·1
Tri-"olein" (3·1–Nil %)	—	—	—	—	3·1–Nil	3·1–N

Comparison of the probable component glycerides of the depot fats from Bombay, Calicut and English oxen

It is interesting to compare the composition of the Bombay cow depot fat (unusually rich in combined palmitic acid) with that of the Calicut fat, which resembles the English ox depot fat [Hilditch & Paul, 1938] much more closely as regards its component fatty acids. The data for the component acids and the component glycerides (approximated to the nearest unit per cent) of these three ox depot fats are collected in Table 9. It is desirable again to point out that the methods involved in the present studies of component glycerides essentially restrict comparison to the three major component acids, palmitic, stearic and oleic, and that minor component acids have to be grouped with one or other of these three. Consequently the "oleo"-glycerides include the small amounts of octadecadienoic and C_{20-22} unsaturated acids, and stearo-glycerides also include traces of arachidic acid; whilst the "palmito"-glycerides take in, in addition to palmitic acid, the minor amounts of myristic (and lauric) and of hexa- (and tetra-) decenoic acids (apart from the least soluble portions of the Indian fats, where definite evidence of small amounts of hexadeceno-disaturated glycerides resulted). The circumstance that myristic acid amounts to 4–5% of the total component acids in ox depot fats involves the probable presence of about 12–15% of monomyristo-glycerides; these appear in Tables 6, 8 and 9 in the general category of di-"palmito"-mono-C_{18} glycerides, for the reason just mentioned.

Table 9. *Component acids (% mol.) and probable component glycerides (% mol.) of depot fats of Bombay, Calicut and English oxen*

	Bombay (cow) (present work)	Calicut (cow) (present work)	English (ox) (Hilditch & Paul [1938])
Component fatty acids:			
Lauric	0·1	0·5	0·2
Myristic	5·8	5·2	2·4
Palmitic	40·8	33·4	33·4
Stearic	25·5	27·9	21·4
Arachidic	0·7	0·5	1·3
Tetradecenoic	0·4	0·4	0·6
Hexadecenoic	2·4	1·5	1·9
Oleic	22·9	29·0	35·2
Octadecadienoic	1·1	1·5	3·5
C_{20-22} unsaturated	0·3	0·1	0·1
Probable component glycerides:			
Fully saturated:			
Tripalmitin	3	—	3
Dipalmitostearin	23	16	8
Palmitodistearin	10	12	6
Mono-"oleo"-disaturated:			
Hexadecenopalmitostearin	2	Trace	—
"Oleo"-dipalmitin	18	11	15
"Oleo"-palmitostearin	33–32	41–38	35–32
"Oleo"-distearin	—	2	2
Di-"oleo"-monosaturated:			
Palmitodi-"olein"	10–11	14–17	20–23
Stearodi-"olein"	0–1	0–3	8–11
Tri-"oleins"	1–0	3–0	3–0

The glycerides in the Indian ox depot fats are of the mixed type characteristic of all other animal depot fats which have been examined, but it is instructive

to contrast the essential differences between the Bombay and Calicut specimens. The Calicut fat closely resembles the English fat in its palmitic acid content, the lower unsaturation of the Calicut fat being almost wholly accounted for by an increase of about 6% in stearic acid and a decrease of the same order in oleic acid as compared with the English fat. Concurrently, the glyceride structure is that hitherto regarded by us as typical for stearic-rich depot fats, and is fully explicable on the hypothesis of a biohydrogenation of preformed palmito-oleo-glycerides. Thus, the total proportions of monopalmitodi-C_{18} glycerides are 67% (Calicut) and 61% (English), but the more saturated Calicut fat contains about 6% more of both palmitodistearin and oleopalmitostearin than the English fat, whilst palmitodioleins are about 6% less in the Calicut than the English fat. Similarly the relative proportions of dipalmitostearin and oleodipalmitin are reversed in the two fats. The total amount of fully saturated components in the Calicut fat (28%) is consequently correspondingly larger than that in the English fat (17%).

The glyceride structure of the Bombay cow depot fat, however, marks a complete contrast to those of either of the other two fats. Here, as has already been indicated, stearic acid does not exceed the proportion in which it occurs in the Calicut fat, and the further fall in total unsaturation is compensated for by increase in the palmitic, and not the stearic, acid content. Whilst, therefore, the very low total unsaturation results in the presence of the highest proportion of fully saturated glycerides (36%) yet observed in an animal depot fat, these are for the most part dipalmito-glycerides. In the whole fat, the amount of monopalmitodi-C_{18} glycerides is only 53%, instead of approaching 70% as in other, more normal, ox depot fats hitherto examined [Banks & Hilditch, 1931; Hilditch & Paul, 1938]; dipalmitomono-C_{18} glycerides correspondingly exceed 40% compared with about 25% in the more usual types. (As already mentioned, the increase in dipalmitostearin and oleodipalmitin, instead of any further increase in palmitodistearin, has the effect of retaining the consistency or melting-point of this fat at about the same level as that of the Calicut or English ox depot fats which contain much more oleic acid.)

So far as the relative proportions of oleic and stearic groups in the various mixed glycerides are concerned, the Bombay cow fat exhibits the normal characteristics of a mixture of glycerides resulting from partial or complete hydrogenation of a mixture of preformed oleo-glycerides. Thus the proportion of fully saturated glycerides has the relationship to the proportion of saturated acids in the mixed acids of the fat which is in accordance with this hypothesis [Hilditch & Stainsby, 1935]; of the monopalmitodi-C_{18} glycerides, 21% are di-unsaturated, 60% mono-unsaturated and 19% palmitodistearin; whilst the dipalmitomono-C_{18} glycerides consist of 44% oleodipalmitin and 56% dipalmito-stearin. Comparison with the more unsaturated ox depot fat studied by Hilditch & Paul [1938, p. 1783] shows that the proportions of the more saturated groups of mono- and di-palmito-glycerides in the Bombay fat are increased, as would be expected on the hydrogenation hypothesis, and in spite of the unusually high proportions of palmitic acid (and, therefore, dipalmito-glycerides) present. It may be recalled that minor but progressive changes in the proportion of dipalmito-glycerides in depot fats have recently [Hilditch & Pedelty, 1940] been illustrated by comparison of pig and ox depot fats, in which a tendency for the more saturated (stearic-rich) fats also to contain slightly increased proportions of palmitic acid was observable. In the present instance, however, the increase in palmitic acid content of the Bombay cow depot fat compared with other animal depot fats is far more marked.

The recent studies of Schoenheimer and his colleagues appear very suggestive in this connexion. Schoenheimer & Rittenberg [1937] observed that the body fat of mice, fed for 5 days with deuterostearic acid, contained deuteropalmitic acid; and, conversely, Stetten & Schoenheimer [1940], after feeding ethyl deuteropalmitate to rats for 8 days, found that the body fats contained deuterostearic acid, deutero-acids of shorter chain length than palmitic acid and deuterohexadecenoic acid. These workers conclude that interconversions of fatty acids which take place normally and continuously in the animal body include not only those due to hydrogenation or dehydrogenation, but also those in which the carbon chain may be increased or diminished by two carbon atoms at a time. Schoenheimer's conclusions, if applicable to the glycerides and not merely to the fatty acids comprised in animal fats, offer a possible explanation of the present instance in which the usual oleic-stearic balance in a depot fat appears to have been superseded, beyond a certain limit in stearic acid content, by an oleic-palmitic or stearic-palmitic interconversion. It is further possible that Stetten & Schoenheimer's observations on the degradation of palmitic (and hence of stearic or oleic) groups to acids of shorter chain length have a similar bearing on the characteristic structure of cow milk fat, which has been similarly co-related with a mixture of preformed palmito-oleo-glycerides as a result of studies of its component glycerides [Hilditch, 1937; Hilditch & Paul, 1940].

Somewhat similar inter-relationships between the amounts of different saturated acids have been observed occasionally in a few fats of marine animals. Lovern [1932] found that in three different depot fats of the sturgeon the total molar percentage of saturated acids was approximately constant (24 %), but that it was made up of variable amounts of myristic, palmitic and stearic acids, whilst palmitic and hexadecenoic acids, individually varying by several units per cent., amounted together to a constant proportion of 40–41 % of the total fatty acids. Again, in the fats from five depots of the tunny, Lovern [1936] observed progressive relationships between the total content of saturated acids, the proportion of stearic acid, and the average unsaturation of the unsaturated C_{18} acids.

In conclusion, a further reference may be made to the proportions of "oleo"-dipalmitin, "oleo"-palmitostearin, palmitodi-"olein" and stearodi-"olein" present in the Bombay and Calicut cow depot fats. If, after allowing for the composition of the fully saturated glycerides present in the fats, the "oleic" acid present is divided proportionately between the palmitic and stearic acids present as mixed "oleo"-saturated glycerides, and combined arithmetically with these two acids, a "calculated" composition for the four mixed glycerides referred to is derived which represents the maximum "even distribution" of these acids in the form of mixed glycerides. In the case of the English ox depot fat [Hilditch & Paul, 1938], the figures obtained by calculation agreed closely with those actually observed, but this was not so in two pig depot fats subsequently examined [Hilditch & Pedelty, 1940]. The corresponding data for the two Indian cow depot fats, however, again display considerable accordance between the observed and "calculated" proportions of the respective glycerides. This similarity, the implication of which is uncertain, is shown by the appended figures:

	Bombay cow		Calicut cow		English ox	
	Found	"Calc."	Found	"Calc."	Found	"Calc."
"Oleo"-dipalmitin	18	23	11	13	15	14
"Oleo"-palmitostearin	32	33	38	39	32	35
Palmitodi-"olein"	11	6	17	13	23	22
Stearodi-"olein"	1	3	3	7	11	12

SUMMARY

The component acids of four depot fats from oxen grown in various parts of India have been determined. All the fats were much more saturated than those of European or American animals, and contained only 27–33% of unsaturated acids (mainly oleic). The stearic acid content of the fats did not exceed 26–28%, however, and consequently the proportion of palmitic acid was in general considerably above the 30 ± 3% hitherto considered characteristic for ox depot fats; in one instance (Bombay cow fat) palmitic acid formed over 40% of the total fatty acids. The melting-points of the fats and the setting-points of their mixed fatty acids were of the same order as those of the more common and more unsaturated ox depot fats, owing to the presence of the increased proportions of palmitic acid.

The component glycerides of two of the cow depot fats have been studied in detail, and were found to conform with the general structure observed in other depot fats of oxen. In the Bombay cow fat the abnormal amount of palmitic acid was reflected in increased amounts of dipalmitostearin and oleodipalmitin but, apart from this, the general proportions of palmitodistearin, oleopalmitostearin, palmitodiolein etc. were in line with those in other ox depot fats, when allowance is made for the more highly saturated nature of the fat as a whole.

The apparent replacement of stearic acid, beyond a certain point, by palmitic acid is considered, with special reference to recent observations by Schoenheimer and to somewhat similar instances in certain marine animal fats.

REFERENCES

Banks & Hilditch (1931). *Biochem. J.* **25**, 1168.
Bhattacharya & Hilditch (1931). *Biochem. J.* **25**, 1954.
Dhingra & Haneef (1939). *J. Soc. chem. Ind., Lond.*, **58**, 292.
—— & Sharma (1938). *J. Soc. chem. Ind., Lond.*, **57**, 369.
Ellis, Rothwell & Pool (1931). *J. biol. Chem.* **92**, 385.
Hilditch (1937). *Analyst*, **62**, 250.
—— (1940). The chemical constitution of natural fats, chapter III. London.
—— & Longenecker (1937). *Biochem. J.* **31**, 1805.
—— & Paul (1938). *Biochem. J.* **32**, 1775.
—— —— (1940). *J. Soc. chem. Ind., Lond.*, **59**, 138.
—— & Pedelty (1940). *Biochem. J.* **34**, 971.
—— & Stainsby (1935). *Biochem. J.* **29**, 90.
Lovern (1932). *Biochem. J.* **26**, 1985.
—— (1936). *Biochem. J.* **30**, 2023.
Schoenheimer & Rittenberg (1937). *J. biol. Chem.* **120**, 155.
Stetten & Schoenheimer (1940). *J. biol. Chem.* **133**, 329.