CCXXV. A FURTHER STUDY OF THE COMPONENT ACIDS OF OX DEPOT FAT, WITH SPECIAL REFERENCE TO CERTAIN MINOR CONSTITUENTS

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THE investigation in several laboratories of a variety of animal depot fats has revealed certain marked characteristics with respect to the amount of major component acids and their distribution among the complex mixture of glycerides. There is accumulating evidence of the extreme differences between marine or aquatic and land animal depot fats. The former are constructed from a much more complex mixture of fatty acids, largely unsaturated; the latter contain chiefly C_{16} and C_{18} acids with varying but larger amounts of saturated acids (30–60%). Hilditch & Lovern [1936] have called attention to these differences and have indicated the gradual change in the fat depot composition of animals at various stages of evolutionary development. They show a gradual disappearance of polyethenoid C_{18} , C_{20} and C_{22-24} acids and the C_{14} and C_{16} monoethenoid acids (myristoleic and palmitoleic acids). In the present communication the occurrence in beef tallows of small amounts of tetradecenoic and hexadecenoic acids is demonstrated, in addition to the presence of a C_{20} unsaturated acid [cf. Brown & Sheldon, 1934]. These findings are related to the observations of Hilditch & Lovern.

Land animal depot fats appear to contain relatively constant amounts of palmitic acid (25–30 mol. %) and total C_{18} acids (65–70 mol. %). Stearic acid is more variable, occurring in (a) small amounts (5–10 mol. %) in depot fats of the hen [Grossfeld, 1931; Hilditch *et al.* 1934], goose [Bömer & Merten, 1922; Grossfeld, 1931], rat [Banks *et al.* 1933; Spadola & Ellis, 1936], and horse [Heiduschka & Steinruck, 1921]; and (b) in larger amounts (20–30 mol. %) in depot fats of the ox [Armstrong & Allan, 1924; Banks & Hilditch, 1931], sheep [Collin *et al.* 1929], reindeer [Baughman *et al.* 1929] and camel [Armstrong & Allan, 1924]. The stearic acid content of pig depot fat [Bhattacharya & Hilditch, 1931; Banks & Hilditch, 1932; Dean & Hilditch, 1933, 2] is intermediate, as a rule, between those of the two groups. Examination of the glyceride structure has indicated an even distribution of the component fatty acids (resembling that for seed fats of similar composition) in the cases where stearic acid forms only 5–10 mol. % of the total fatty acids [cf. Banks *et al.* 1933]. With larger amounts of stearic acid (20–30 mol. %) a relationship between the molar content of saturated acids and the molar percentage of fully saturated glycerides has been reported [cf. Hilditch & Sleightholme, 1931]. The present work supports these conclusions.

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EXPERIMENTAL

Three samples of beef tallow have been studied. They were obtained through the courtesy of Capt. Golding of the National Institute for Research in Dairying, Shinfield. Tallow I was supplied from an animal of the National Institute's herd; tallows II and III were purchased from Mr John Cumber, Theale, Berks. Approximately 8 lb. of crude fatty tissue were selected from the kidney region of each animal. These tissues were minced and extracted as completely as possible with boiling acetone. The average yield of neutral fat thus obtained was 88% of the original wet weight of the tissue.

Feeding details for each of the three animals from which the tallows were obtained are recorded as fully as possible. It will be seen that tallow I was from an animal on a diet intermediate between winter and summer, tallow II, a summer (pasture) feeding and tallow III, an intermediate between summer and winter diet.

Tallow I. This sample was from a Shorthorn cow (7 years old) slaughtered in May 1936. The animal had been feeding on grass during the winter and from Nov. 1935 had been receiving a supplement consisting of 5 lb. hay, 45 lb. mangels and 15 lb. silage with a concentrate (8 lb.) of 3 parts hominy chop, 3 parts dried grains, 1 part crushed oats, 1 part groundnut cake and 1 part linseed cake. The cow had been dry since Dec. 1935.

Tallow II. The animal (a 4-year-old Shorthorn bullock) from which this tallow was obtained had been on grass continuously from Apr. 1936 until the time of slaughtering, Dec. 1936, and had received no supplementary cake during that time.

Tallow III. A third tallow was taken from a 3-year-old Shorthorn heifer which had been fed on a heavy loam pasture from May 1936 until 23 Nov. 1936; 6 weeks before slaughtering, the grass food was supplemented with 1 gallon per day of a mixture containing equal parts of Shirley's grass nuts and Bibby's fattening cake.

The sap. equiv. and i.v. of the samples were as follows:

Table I

Tallow	Sap. equiv.	i.v. (Wijs)
I	284.4	43.2
II	286.4	44.7
III	284.8	40.4

The methods used in the detailed analysis of these tallows have been described previously on several occasions [Hilditch & Jones, 1929; Hilditch, 1934]. The mixed fatty acids obtained from the saponification of about 200 g. of fat were washed free from mineral acid and weighed. They were separated as lead salts by a modified Twitchell method [Twitchell, 1921; Dean & Hilditch, 1933, 1; Banks *et al.* 1933] involving the use of 10 ml. of alcohol containing 1.5% acetic acid for each g. of mixed acids for the original precipitation of the "solid" lead salts and their recrystallization. The acids from both the soluble and insoluble lead salts were recovered, weighed and esterified with methyl alcohol. The esters from tallow I were fractionally distilled from a Willstätter bulb; those from tallows II and III were analysed using the electrically heated and packed column described by Longenecker [1937]. The "liquid" acids and esters were stored under nitrogen to prevent oxidative changes.

Results of the lead salt separations of the mixed acids from each tallow and a set of typical fractionation data are given in Tables II and III. The complete fractionation data from which the respective fatty acid compositions of each tallow were calculated (Table IV) have been omitted only because of their lengthy nature.

Table II. *Lead salt separation of mixed fatty acids*

Tallow	"Solid" acids				"Liquid" acids			
	g.	%	Corresponding methyl esters		g.	%	Corresponding methyl esters	
			Sap. equiv.	I.V.			Sap. equiv.	I.V.
I	379.8	54.4	268.9	9.1	318.4	45.6	278.4	82.8
II	254.8	52.0	269.6	7.4	235.4	48.0	276.0	81.9
III	198.8	59.2	270.7	12.9	137.0	40.8	276.9	84.0

Table III. *Fractional distillation of methyl esters (Tallow II)*
(electrically heated and packed column)

No.	g.	Sap. equiv.	I.V.
(a) Methyl esters of "solid" acids			
S1	2.02	256.1	2.2
S2	2.12	268.0	1.2
S3	3.01	269.5	1.0
S4	14.15	270.6	0.5
S5	4.26	284.0	8.6
S6	11.54	296.9	12.7
S7	12.87	297.3	11.5
S8	4.61	303.5	10.9
	54.58		
(b) Methyl esters of "liquid" acids			
L1	1.14	224.8	16.8
L2	2.58	239.3	18.6
L3	7.08	244.5	15.8
L4	2.64	253.4	20.4
L5	4.65	264.7	40.3
L6	5.60	273.2	52.2
L7	3.06	272.3	46.3
L8	5.26	282.3	63.9
*L9	3.17	271.9	48.9
L10	10.40	289.2	77.3
L11	13.04	291.2	81.3
L12	52.01	295.7	85.4
L13	67.55	295.8	89.2
L14	20.00	295.6	89.8
L15	17.61	295.8	89.8
L16	10.59	322.8	95.0
	227.28	301.2†	96.5†

* Distillation interrupted.

† Residual esters, freed from unsaponifiable.

In arriving at the final figures given in Table IV several assumptions have been made. These were as follows:

(1) Simultaneous equations were used to calculate the amounts of two saturated and two unsaturated acids present in any one ester fraction [cf. Hilditch, 1936; Harper *et al.* 1937]. Where "liquid" ester fractions were not oxidized to obtain the saturated esters, the equivalent of the latter was assumed to be equal to the equivalent of the whole fraction (cf. Table VII for justification of this procedure).

Table IV. *Component acids of beef tallow*

(a) Tallow I (715 g. hydrolysed for analysis; fractionations with Willstätter bulb)

Component acids	"Solid" (54.4%)	"Liquid" (45.6%)	Total	% (wt.)	% (mol.)
Saturated:					
Myristic	1.55	1.41	2.96	3.0	3.5
Palmitic	25.57	3.59	29.16	29.2	31.0
Stearic	20.98	—	20.98	21.0	20.1
As arachidic	0.37	—	0.37	0.4	0.3
Unsaturated:					
$\Delta^{8:10}$ -Tetradecenoic	—	0.58	0.58	0.6	0.7
$\Delta^{8:10}$ -Hexadecenoic	0.11	2.54	2.65	2.7	2.8
Oleic	5.82	35.14	40.96	41.1	39.6
Octadecadienoic	—	1.82	1.82	1.8	1.8
As arachidonic	—	0.21	0.21	0.2	0.2
Unsaponifiable	—	0.31	0.31	—	—

(b) Tallow II (513 g. hydrolysed for analysis; fractionations with electrically heated and packed column)

Component acids	"Solid" (52.0%)	"Liquid" (48.0%)	Total	% (wt.)	% (mol.)
Saturated:					
As lauric	—	0.17	0.17	0.2	0.2
Myristic	1.11	1.95	3.06	3.1	3.7
Palmitic	21.40	3.42	24.82	24.9	26.5
Stearic	24.02	—	24.02	24.1	23.1
As arachidic	0.77	—	0.77	0.8	0.7
Unsaturated:					
$\Delta^{8:10}$ -Tetradecenoic	—	0.43	0.43	0.4	0.5
$\Delta^{8:10}$ -Hexadecenoic	0.41	2.03	2.44	2.4	2.6
Oleic	4.29	37.50	41.79	41.8	40.4
Octadecadienoic	—	1.81	1.81	1.8	1.8
As arachidonic	—	0.53	0.53	0.5	0.5
Unsaponifiable	—	0.16	0.16	—	—

(c) Tallow III (356 g. hydrolysed for analysis; fractionations with electrically heated and packed column)

Component acids	"Solid" (59.2%)	"Liquid" (40.8%)	Total	% (wt.)	% (mol.)
Saturated:					
As lauric	—	0.11	0.11	0.1	0.2
Myristic	0.24	1.73	1.97	2.0	2.3
Palmitic	24.62	2.23	26.85	26.9	28.7
Stearic	26.43	—	26.43	26.5	25.4
As arachidic	1.28	—	1.28	1.3	1.1
Unsaturated:					
$\Delta^{8:10}$ -Tetradecenoic	—	0.37	0.37	0.4	0.5
$\Delta^{8:10}$ -Hexadecenoic	0.22	1.67	1.89	1.9	2.0
Oleic	6.39	32.70	39.09	39.1	38.0
Octadecadienoic	—	1.70	1.70	1.7	1.7
As arachidonic	—	0.12	0.12	0.1	0.1
Unsaponifiable	0.02	0.17	0.19	—	—

(2) The mixture of C_{18} unsaturated esters distilling with hexadecenoic and palmitic acids was arbitrarily given the same composition as that of the pure C_{18} unsaturated ester fraction ultimately obtained [cf. the relative inability to separate a mixture of oleic and linoleic esters by distillation, Longenecker, 1937].

(3) No saturated acids higher than palmitic were assumed to be present in the "liquid" esters. It is considered most improbable that more than traces

(0.05–0.10 %) of stearic or higher saturated esters were present in the “liquids” because of the slight solubility of their lead salts in alcohol.

(4) The unsaturated acids present in the “solid” ester fractions have been calculated as hexadecenoic and octadecenoic acids. The amount of the former does not normally exceed 0.1 % of the total fatty acids whereas the occurrence of C_{18} unsaturated acids in the “solids” is 4.0–6.5 % [cf. also Table I, Banks & Hilditch, 1931]. It is unlikely that C_{18} acids more unsaturated than monoethenoid were present in the “solid” acids, since they occurred in only small proportions in the “liquid” acids.

*Further study of the occurrence of minor component acids
(tetradecenoic, hexadecenoic etc.)*

The inclusion, in Table IV, of lauric, arachidic, tetradecenoic, hexadecenoic and arachidonic acids among the component acids of tallows seems to require somewhat more detailed consideration. These acids have not been previously reported in tallows. They form about 4 % of the total mixed acids. Previous investigators reported only myristic, palmitic, stearic, oleic and linoleic acids. Their recalculated results are shown later in Tables XIII and XIV for the purpose of comparison with the data presented in this communication.

The occurrence of these acids was first suspected when we repeatedly obtained low mol. wt. fractions with relatively high i.v. (Wijs) during the normal refractionation of the lower “liquid” esters. For example, 203 g. of tallow I ultimately yielded “liquid” ester fractions as follows:

No.	g.	Sap. equiv.	i.v.
L11	2.84	246.2	30.5
L12	3.01	258.9	40.7
L13	3.51	262.0	56.4

Similar results were obtained by Banks & Hilditch [1931, p. 1169], who attributed them to products of oxidative rancidity because of the long storage period previous to their analyses. Tallow I had been stored for 5 months before analysis. It was considered improbable, however, that this could be offered in the present instance as an explanation of the relatively large amounts of low mol. wt. material.

During a refractionation (Willstätter bulb) of lower “liquid” esters in a second analysis of the same tallow (I) (212 g.) suitable fractions were taken for oxidation by permanganate in acetone. The saturated esters were recovered from these oxidations and their sap. equiv. determined. The fractions and the result of their oxidations are given in Table V.

Table V. *Fractional distillation and oxidation of low mol. wt.
“liquid” esters*

Original ester fractions				Amount oxidized	Saturated esters		Unsaturated esters (calc.) Sap. equiv.
No.	g.	Sap. equiv.	i.v.		g.	Sap. equiv.	
(L11)	2.28	251.1	27.6)	—	—	—	—
L12	8.19	260.2	45.6	5.52	2.21	256.1	262.7
L13	8.29	287.7	69.6	5.41	1.32	268.3	294.6

The evidence obtained in these oxidations indicated the presence of two unsaturated acids of lower mol. wt. than oleic. Subsequently, more efficient fractionation equipment was used for all distillations [Longenecker, 1937]. Two

combinations of "liquid" ester fractions (tallow I) obtained earlier with a Willstätter bulb were fractionally distilled (Table VI) for qualitative examination of these lower mol. wt. acids. These combinations were of ester fractions having the following range of analytical data:

Combination	Sap. equiv.	I.V.
I	248.3-277.8	26.6-59.6
II	284.2-288.0	69.6-74.6

Table VI. *Fractional distillation of two recombinations of "liquid" esters (tallow I) in electrically heated and packed column*

Combination I				Combination II			
No.	g.	Sap. equiv.	I.V.	No.	g.	Sap. equiv.	I.V.
L11	2.82	228.6	20.4	L21	1.25	222.6	27.6
L12	8.03	241.8	18.4	L22	6.05	267.8	45.8
L13	4.47	243.5	10.8	L23	2.99	270.6	43.9
L14	3.90	255.3	13.5	L24	6.16	270.4	37.8
L15	4.20	267.5	45.2	L25	5.13	270.6	32.6
L16	8.36	269.7	46.6	L26	3.13	271.5	36.2
L17	3.04	271.1	34.7	L27	3.52	284.8	56.3
L18	2.77	288.1	73.4	L28	3.77	289.7	73.1
L19	6.51	294.0	89.5	L29	4.48	292.8	81.7
				L210	10.51	294.8	87.2
				L211	10.02	295.7	90.4

The occurrence of a lower saturated acid is indicated in these distillations, although the presence of lauric acid was not established by isolation of a pure specimen. Both L11 and L21 had sap. equiv. which, when corrected for the presence of tetradecenoic acid, indicated a high percentage of saturated acids of lower mol. wt. than myristic. It is quite possible that the low mol. wt. material in these two fractions is some degradation product or products formed from the unsaturated acids either on storage or during the distillations.

In order to establish the presence of hexa- and tetra-decenoic acids more conclusively, fractions L12 and L13 (A) were combined for oxidation (permanganate-acetone) as were L15 and L16 (B) (Table VII). 40 g. of KMnO_4 and 100 ml. of acetone were used for the oxidation of (A) and 58 g. of KMnO_4 and 150 ml. of acetone for that of (B). In each case the manganese oxides were reduced with sulphur dioxide. The oxidation products were extracted with ether. Acidic scission products were washed from the ether with aqueous 10% K_2CO_3 and the saturated esters recovered from the ether after it had been washed until neutral to phenolphthalein. Myristic and palmitic acids were respectively readily identified in these saturated esters. The K_2CO_3 solution and washings were acidified and steam-distilled. The distillate was extracted with ether (sp. gr. 0.720), dried over anhydrous Na_2SO_4 , and the ether recovered. The residue from the steam distillation was saponified to decompose half esters of dibasic acids present and again acidified. The dibasic acids were recovered after titration for mol. wt. Recrystallized and dried, they melted at 104° (mixed with an authentic specimen of azelaic acid, there was no depression).

The results of these and other similar oxidations (from ester fractions obtained in the course of the analyses of tallows II and III) are summarized in Table VII.

From the data in Table VII it is obvious that the unsaturated acid in (A) was $\Delta^{9:10}$ -tetradecenoic acid ($\text{C}_5\text{H}_{10}\text{O}_2$, equiv. 102.1; $\text{C}_9\text{H}_{16}\text{O}_4$, equiv. 94.1) and that $\Delta^{9:10}$ -hexadecenoic acid was present in (B) ($\text{C}_7\text{H}_{14}\text{O}_2$, equiv. 130.1). The

Table VII. *The oxidation of "liquid" ester fractions containing tetradecenoic and hexadecenoic acids*

Tallow		Oxidation products											
		Original esters				Acids							
						Saturated esters		Steam-volatile				Non-steam-volatile	
								g.		Sap. equiv.		g.	
No.	g.	Sap. equiv.	I.V.	g.	Sap. equiv.	g.	Sap. equiv.	g.	Sap. equiv.	M.P.			
I	(A)	6.83	242.2	16.7	4.73	242.2	0.43	110.3	0.35	96.3	104°		
	(B)	5.86	269.2	46.3	2.78	269.0	1.04	135.2	0.93	95.1	104°		
II	C ₁₄	4.17	244.5	15.8	3.20	241.0	—	—	0.22	—	105°		
	C ₁₆	4.44	269.0	47.4	2.16	267.8	0.41	135.4	0.52	95.6	101°		
III	C ₁₄	3.56	238.9	14.6	2.64	235.2	—	—	0.20	—	103°		
	C ₁₆	6.32	268.4	46.9	2.97	267.1	—	—	0.75	96.0	101°		

further data confirm the presence of both tetradecenoic (myristoleic) and hexadecenoic (palmitoleic) acids in tallows II and III.

Furthermore, it is clear that in all these fractions (the equivalents of which approximate to those of methyl esters of either C₁₄ or C₁₆ acids) the equivalent of the saturated esters isolated from the oxidations is in each case almost the same as that of the whole original fraction and is, moreover, respectively close to that of methyl myristate (242) or palmitate (270). This is, of course, strong confirmatory evidence that the unsaturated esters present correspond in mol. wt., in these instances, with those of the respective saturated products (i.e. belong respectively to the C₁₄ or C₁₆ groups).

Examination of the C₁₈ unsaturated acids

The occurrence in tallows of acids more unsaturated than oleic is constantly indicated by C₁₈ "liquid" ester fractions with i.v. higher than can be accounted for by oleic acid alone. The usual mean unsaturation of these fractions is -2.1H , corresponding to 5% of diethenoid acids. By successive redistillations of the C₁₈ unsaturated esters from tallow I, however, a concentrate equivalent to about -2.2H was obtained. The corresponding acids of these C₁₈ esters of higher unsaturation were further concentrated by the method of Moore [1919], as modified by Green & Hilditch [1935, 2]. After two recrystallizations of their lithium salts from 80% alcohol, 155.3 g. of C₁₈ acids (i.v. 100.2) yielded (A) 118.3 g. of acids (i.v. 96.2) from the insoluble salts and (B) 36.8 g. of acids (i.v. 112.9) recovered from the soluble lithium salts.

Bromination of the C₁₈ unsaturated acids. 5.48 g. of the acids (B) (i.v. 112.9) were brominated in anhydrous ether (55 ml.). The temperature was maintained

Table VIII. *Bromo-additive products of C₁₈ unsaturated fatty acids (B)*
(5.48 g., i.v. 112.9)

	Bromides			Corresponding unsaturated acids		
	g.	% Br	M.P.	g.	%	I.V.
Insoluble in ether	0.12	65.4	184 (d)	0.04	0.7	306.0
Soluble in ether, insoluble in light petroleum at 0°	0.09	59.4	170 (d)	0.04	0.7	238.2
Soluble in ether and in light petroleum at 0°	8.92	39.8	Liquid	5.37	98.6	105.0

below -5° during the addition of bromine (2.6 ml.). After standing overnight at 0° the solution was filtered and the precipitate washed with cold ether and dried at 90° to constant weight. The filtrate was decolorized with aqueous NaHSO_3 and the ether was recovered. The oily residue was dried under reduced pressure, dissolved in light petroleum (B.P. $40-60^{\circ}$) and kept at 0° for 3 hr. The insoluble bromo-addition products were filtered, dried and weighed. The weights of the bromides, the percentage bromine, M.P. and the calculated I.V. of the unsaturated acids are given in Table VIII.

Permanganate-acetone oxidation of methyl esters of C_{18} unsaturated acids. The acids (B) from the lithium salts soluble in 80% alcohol were re-esterified and oxidized with finely divided KMnO_4 in acetone solution [Armstrong & Hilditch, 1925]. The methyl esters (27.7 g., I.V. 108.9) were dissolved in acetone (750 ml.) to which the permanganate (277 g.) was added gradually. The mixture was refluxed for 4 hr. and, after removal of the acetone, was thoroughly mixed with NaHSO_3 (350 g.). Water was added carefully and decoloration and solution of the manganese oxides was effected by boiling and the addition of dil. H_2SO_4 . The cooled solution was extracted with ether. The water washings (A) were reserved and the ethereal solution was extracted twice with 10% K_2CO_3 and again with water until no alkali was present in the washings. The K_2CO_3 solution and washings were combined (B). The ether contained 1.0 g. of material (I.V. 24.9).

The washings (B) were acidified and steam-distilled, the distillate extracted with ether (sp. gr. 0.720), dried over anhydrous Na_2SO_4 and the ether recovered; the volatile acids thus obtained were fractionally distilled (cf. Table IX). The residue from the steam distillation was saponified to decompose any half esters of dibasic acids; on acidification, 12.8 g. of crude azelaic acid (M.P. 96° , equiv. 95.2) were obtained. Recrystallization raised the M.P. to 101° . Similar treatment of water washings (A) yielded 0.50 g. of volatile acids (equiv. 118). The 26.7 g. of the original mixture of esters which disappeared during this oxidation, if assumed to be only mono- and di-ethenoid, contained 7.1 g. of octadecadienoic ester and 19.6 g. of octadecenoic ester. The theoretical yields of oxidation products are 2.8 g. of hexanoic acid (mol. wt. 116), 10.5 g. of nonanoic acid (158) and 17.2 g. of azelaic acid (188). The recovery of hexanoic acid has been most complete from this oxidation. It is possible that nonanoic acid was not completely distilled with steam.

Table IX. *Fractional distillation of volatile acids from oxidation of C_{18} unsaturated esters (in electrically heated and packed column)*

Fraction (From recovered ether)	g.	B.P./0.2 mm.	Sap. equiv.	Component acids (g.)	
				C_6	C_9
1	1.55	50-55°	124.0	0.32)	—
2	0.90	83-87°	144.3	1.17	0.38
3	1.06	90-92°	149.9	0.31	0.59
4	1.96	92°	156.6	0.15	0.91
5	1.52	92°	157.8	0.05	1.91
6	2.24	Residue	158.5	—	1.52
	9.23			2.00	7.55
	(0.32)				
	9.55				

Oxidation of C_{18} unsaturated acids by alkaline permanganate. (1) *Esters from tallow I (modified Hazura [1887] process, cf. Green & Hilditch [1935, 2]).* 10.10 g. of esters (sap. equiv. 295.8, I.V. 93.8) were saponified with 200 ml. of alcoholic

KOH (*N*/2). The alcohol was removed as completely as possible and the soaps were taken up in 600 ml. of water and cooled to 0°. 300 ml. of cold 1.5% KMnO_4 were added and the mixture was kept at 0° for 24 hr. Manganese oxides were reduced with sulphur dioxide. A flocculent precipitate formed on addition of 100 ml. of conc. HCl. It was filtered and boiled with water twice to dissolve the tetrahydroxy-compounds. On recrystallization from ethyl acetate 6.32 g. of dihydroxy-acids were obtained, m.p. 131°, sap. equiv. 315.2 (no depression when mixed with dihydroxystearic acid prepared from $\Delta^9:10$ -octadecenoic acid, m.p. 132°, mol. wt. 316.5). No tetrahydroxy-acids could be crystallized from the filtrate on cooling or on evaporation to dryness.

(2) *Esters from tallow II (modified Hazura process)*. The respective acids were concentrated to i.v. 99.6 by the lithium salt-alcohol procedure described above. 5.60 g. were saponified with 3 g. of KOH in 300 ml. of water, cooled to 0° and oxidized with 200 ml. of cold 1.5% KMnO_4 . 2.4 g. of acids, m.p. 131.5°, were ultimately obtained as just described. Again no tetrahydroxy-acids could be identified.

(3) *Esters from tallow II (Lapworth & Mottram [1925] process)*. 12.86 g. of the acids, i.v. 99.6, were heated with 6 g. of KOH in 400 ml. of water. The solution was cooled and added to 3 l. of water (0°). 400 ml. of 1.5% aqueous KMnO_4 (0°) were added. After 5 min. the solution was decolorized with sulphur dioxide and 150 ml. of conc. HCl added. The precipitate, boiled twice with water, yielded 11.95 g. of crude dihydroxy-acids which on recrystallization from ethyl acetate yielded 10.80 g., m.p. 130°. The filtrate from the original oxidation mixture was concentrated to 20 ml. and from it was obtained 0.01 g. of tetrahydroxy-acids, m.p. 165–167°. The combined filtrates from the boiling water extraction of the mixed hydroxy-acids yielded on cooling 0.28 g. of material which on recrystallization from ethyl acetate gave 0.20 g. (m.p. 162–164°) and 0.07 g. (m.p. 130°). The 0.20 g. portion was further resolved by boiling ethyl acetate into two fractions melting at 155 and 173° (mixed with the respective tetrahydroxystearic acids, m.p. 155 and 173°, there was no depression). The yields of di- and tetra-hydroxy-acids, assuming a mixture of oleic and linoleic acids, were 94.2 and 8.8% respectively, of theory.

Fully saturated glycerides present in each beef tallow

Each fat was oxidized by KMnO_4 in acetone solution (cf. other communications from this laboratory). The crude material from the oxidation of about

Table X. *Fully saturated glycerides in tallows I, II and III*

(i) Proportions of fully saturated glycerides							
Original data on whole fat			Acids in whole fat		Fully saturated glycerides		
No.	I.V.	Sap. equiv.	Sat-urated % (molar)	Un-saturated	Sap. equiv.	Weight %	Mol. %
I	43.2	284.4	54.9	45.1	278.3	18.0	18.4
II	44.7	286.4	54.2	45.8	279.5	15.5	15.8
III	40.4	284.8	57.7	42.3	278.0	18.1	18.6

(ii) Component acids of the fully saturated glycerides						
	% (weight)			% (molar)		
Tallow	I	II	III	I	II	III
Myristic acid	10.8	14.7	8.3	12.5	17.1	9.6
Palmitic acid	44.0	33.6	45.9	45.4	34.7	47.6
Stearic acid	45.2	51.7	45.8	42.1	48.2	42.8

100 g. of fat, using four times the weight of permanganate, usually contained unoxidized or partially oxidized glycerides. It was combined with the similar product from a second oxidation for reoxidation. The crude fully saturated product obtained after reoxidation had i.v. less than unity in each case. It was convenient to crystallize it from ether at 0° before washing the ethereal solution of the oxidation products with K_2CO_3 to remove acidic products. Final removal of acidic products was effected by boiling the crude fully saturated glycerides with dilute K_2CO_3 [cf. Hilditch & Sleightholme, 1931]. Results of these oxidations are given in Table X, together with the component acids in the purified fully saturated glycerides as determined by the ester fractionation procedure.

DISCUSSION

Component acids in the whole tallows

The qualitative examination of various ester fractions handled during the analyses of these tallows has revealed the presence of several acids hitherto unreported in similar animal depot fats. Perhaps the most interesting of these are the C_{14} and C_{16} unsaturated acids, tetradecenoic and hexadecenoic, both of which have the ethenoid linkage in the 9:10-position (cf. Table VII). Their constitution is thus identical with that reported by Hilditch & Paul [1936] for the C_{14} and C_{16} unsaturated acids in milk fat¹ [Bosworth & Brown, 1933; Riemenschneider & Ellis, 1936]. Tetradecenoic acid has not previously been defined as a component of land animal depot fats although it is a recognized component of several aquatic animal fat depots. Hexadecenoic acid, also a usual component of fish oils, has been recognized in few depot fats of higher types of animals. It is found in rat body fat [Banks *et al.* 1933; Spadola & Ellis, 1936; Channon *et al.* 1937] and hen fat [Hilditch *et al.* 1934]; and has been recently reported to be present in small proportions in pig depot fat [Hilditch & Shorland, 1937]. The proportions of these two acids in ox depot fat are very small, namely, about 0.5% of tetradecenoic acid and 2.0–2.8% of hexadecenoic acid. The occurrence of these two lower mol. wt. unsaturated acids in the depot fat of oxen in less amounts than in depot fat of amphibians or reptiles [Klenk, 1933; Klenk *et al.* 1935] appears to follow the general trend of simplification of the mixed fatty acids paralleling the scale of evolutionary development [cf. Hilditch & Lovern, 1936].

Confirmation of the constitution of the C_{18} unsaturated acids has been obtained by analysis of both their mild and disruptive oxidation products and the bromo-additive products. A similarity in structure of the C_{14} and C_{16} unsaturated acids with the C_{18} group is thus observed:

The formation of both dihydroxystearic (M.P. 131°) and tetrahydroxystearic acids (M.P. 155 and 173°) by oxidation with alkaline permanganate was better accomplished when the Lapworth & Mottram [1925] and not the modified Hazura [1887; Green & Hilditch, 1935, 1] procedure was used. Green & Hilditch point out that the greater dilutions advised by Lapworth & Mottram minimize

¹ We have independently confirmed the presence and the constitution of these acids in cow milk fat. A complete report will be published shortly.

the further oxidation of hydroxystearic acids. We have not experienced appreciable loss of tetrahydroxystearic acids due to their solubility in the large volume of water present after an oxidation by the Lapworth & Mottram method. The latter procedure appears suitable, therefore, for the identification of linoleic acid, in the forms of the tetrahydroxystearic acids, m.p. 173° (insoluble in boiling ethyl acetate) and m.p. 155° (soluble in boiling, insoluble in cold ethyl acetate), in mixtures containing relatively small amounts of linoleic acid. (The tetrahydroxy-acids may be separated from the dihydroxy-acids by means of their solubility in boiling water.)

It should be pointed out, however, that the best yield of tetrahydroxystearic acids obtained did not exceed 9% of the theoretical (assuming that the C_{18} unsaturation was due solely to octadecenoic and octadecadienoic acids); whereas, with the ordinary linoleic acid of seed fats, yields of about 40% of theory may be expected [Green & Hilditch, 1935, 1]. The octadecadienoic acids of ox depot fat thus resemble those of cow milk fat [Green & Hilditch, 1935, 2] in their behaviour to oxidation with alkaline $KMnO_4$.

The presence of an octadecadienoic acid, originally indicated by higher i.v. than could be accounted for by oleic acid alone, is, however, further confirmed by the analysis of the volatile acids produced by disruptive oxidation of an ester mixture of high i.v. (cf. Table IX). On the other hand, bromination of a mixture of C_{18} unsaturated acids of i.v. 112.9 yielded none of the characteristic tetrabromide, m.p. 114°, soluble in ether and insoluble in light petroleum. The absence of this product, and the fact that 98.6% of the mixed bromides are liquid at room temperature, soluble in both ether and light petroleum, with a higher bromine content (39.8%) than can be attributed to dibromostearic acid (36.2% Br) appear significant. Also, the resemblance of these data to those of Green & Hilditch [1935, 2] for the C_{18} unsaturated acids of milk fats and of Smith & Chibnall [1932] for the unsaturated acids of cocksfoot grass is striking. In each case there is sufficient evidence for the presence of an octadecadienoic acid; that it is geometrically identical, however, with the ordinary seed fat linoleic acid is improbable.¹

The presence of C_{20} saturated and unsaturated acids was inferred from the fact that esters of higher mol. wt. than those of C_{18} acids were regularly found in both "solid" and "liquid" ester fractionations. No specimen of arachidic acid was identified from any of the original tallows. It was, however, easily obtained from a fully hydrogenated sample (tallow II). The complete analysis of this sample (cf. Table XI) provides verification of the accuracy of the methods and

Table XI. *Molar percentages of fatty acid groups (tallow II)*

Acids	Original fat	Hydrogenated fat
C_{12}	0.2	—
C_{14}	4.2	4.5
C_{16}	29.1	28.7
C_{18}	65.3	65.6
C_{20}	1.2	1.2

¹ Hilditch & Shorland [1937], experiencing similar difficulties in obtaining the 114° tetrabromostearic acid from ox and other liver fatty acids alone, were able to recover it quantitatively when groundnut oil C_{18} unsaturated acids were added [cf. also Eckstein, 1933]. Thus the difficulty does not appear to be due to interference by other products, but rather to the inherent nature of the dienoic acid itself.

It may be mentioned here that Parry & Smith [1936] succeeded in isolating the tetrabromide, m.p. 114°, from the mixed acids of ox blood fat.

assumptions employed in these analyses and calculations, the hydrogenated sample yielding nearly the same total molar contents of the various acids as those calculated for the original tallow.

For convenience, the component fatty acids of the three tallows we have studied are grouped together in Table XII. Perusal of the molar proportions of the major components, taken in conjunction with the earlier analyses of ox and sheep depot fats which are available, is of some interest.

Table XII. *Component acids of ox depot glycerides*

Acid	Weight %			Molar %		
	I	II	III	I	II	III
As lauric	—	0.2	0.1	—	0.2	0.2
Myristic	3.0	3.1	2.0	3.5	3.7	2.3
Palmitic	29.2	24.9	26.9	31.0	26.5	28.7
Stearic	21.0	24.1	26.5	20.1	23.1	25.4
As arachidic	0.4	0.8	1.3	0.3	0.7	1.1
$\Delta^{9:10}$ -Tetradecenoic	0.6	0.4	0.4	0.7	0.5	0.5
$\Delta^{9:10}$ -Hexadecenoic	2.7	2.4	1.9	2.8	2.6	2.0
Oleic	41.1	41.8	39.1	39.6	40.4	38.0
Octadecadienoic	1.8	1.8	1.7	1.8	1.8	1.7
As arachidonic	0.2	0.5	0.1	0.2	0.5	0.1
	Total C_{18} acids			61.5	65.3	65.1

In the earlier paper by Banks & Hilditch [1931] comparisons were made of the combined contents of palmitic and myristic acids on the one hand, and of the total C_{18} acids on the other. A tendency towards some sort of constancy in the amounts of each of these groups was apparent. In the light of our present work, which has shown the presence of measurable, though small, proportions of acids other than those mentioned, it is necessary to consider how far, if at all, the earlier conclusions require modification. For the present, we propose to restrict comparisons to the major component acids, namely, palmitic, stearic and C_{18} unsaturated; in the three tallows now studied these amount together to 92.5, 91.8 and 93.8% of the total fatty acids, leaving 6–8% of the latter as minor components (of which myristic accounts for 2.3–3.7% and hexa- and tetra-decenoic for 2.5–3.5%).

The palmitic acid contents of the three fats (26.5–31.0% mol.) fall within the usual fairly constant limits which, as mentioned, have already been observed to hold in the case of animal depot fats. Similarly, the total C_{18} acids (saturated and unsaturated) form 61.5–65.3% of the whole, figures which, again, are close to those which Banks & Hilditch considered to be characteristic for ox and sheep depot fats. From the data then available, these authors found that in five instances the combined C_{18} acids formed about 70% of the whole, and in four other cases the total C_{18} acid content was about 62%.

The analyses employed by Banks & Hilditch in their generalization included their own and also some earlier figures published by Armstrong & Allan [1924] for a group of beef and mutton tallows. The latter, in the form of molar %, are reproduced in Table XIII.

The full fractionation data for these analyses were not given, but it is likely that, at the time of this work, only the "solid" acids would have been fractionated, the "liquid" acids being taken wholly as oleic and linoleic. These would therefore include not only oleic and linoleic acids, but also small amounts (1–2%) of myristic and palmitic acids, and, of course, any hexa- or tetra-decenoic, or C_{20} unsaturated acids present. The actual palmitic acid contents of these tallows are therefore probably 1–2% higher than those recorded in Table XIII,

Table XIII. *Component acids of beef and mutton tallows (mol. %).*
 [From Armstrong & Allan, 1924]

Acid	Beef			Mutton	
	North American	South American	Australian	South American	Australian
Myristic	2.4	3.0	2.4	1.8	2.4
Palmitic	34.5	26.7	28.3	22.5	26.8
Stearic	13.9	19.3	21.7	29.0	22.2
Oleic and linoleic	49.2	51.0	47.6	46.7	48.6
Total C ₁₈ acids	63.1	70.3	69.3	75.7	70.8

whilst the oleic and linoleic acids, and therefore the total C₁₈ acids, will be in reality about 4–5 units % lower than in Table XIII. With these corrections, three of the tallows studied by Armstrong & Allan (the South American beef, and the Australian beef and mutton) come very closely into line with our present results in respect of the contents of palmitic acid and of total C₁₈ acids.

In the analyses of Banks & Hilditch [1931], both “solid” and “liquid” esters were fractionated, but no allowance was made for the presence of lower unsaturated acids. From the details of the fractionations filed in this laboratory, we have recalculated the data for the “liquid” esters, including hexa- and tetradecenoic esters; but, unfortunately, the rancidity known to have developed before some of the specimens could be analysed interferes here and the calculated figures for hexa- and tetra-decenoic acids are in several cases, when judged by the data in this paper, much too high. The original and the recalculated figures for the four beef tallows of Banks & Hilditch, and a mutton tallow [Collin *et al.* 1929] are, however, listed in Table XIV.

Table XIV. *Component acids of beef and mutton tallows (mol. %).*
 [Banks & Hilditch, 1931; Collin *et al.* 1929]

Acid	Beef tallows				Mutton tallow
	N	SA	SB	SC	
(i) Without allowance for hexadecenoic acid etc.					
Myristic	7.5	5.3	9.5	6.9	5.5
Palmitic	29.1	32.9	29.2	25.5	26.2
Stearic	13.4	18.2	23.2	27.4	29.3
Oleic	47.6	40.7	37.1	40.2	34.8
Linoleic	2.4	2.9	1.0	—	4.2
Total C ₁₈ acids	63.4	61.8	61.3	67.6	68.3
(ii) Recalculated, including hexadecenoic acid etc.					
As lauric	—	—	—	—	0.6
Myristic	3.3	1.3	6.7	4.4	1.9
Palmitic	33.2	36.5	32.0	29.2	29.2
Stearic	13.4	18.2	23.1	26.9	29.3
As tetradecenoic	1.4	0.5	—	1.7	0.5
As hexadecenoic	7.4	6.6	6.4	4.5	3.6
Oleic	39.4	34.8	30.8	33.3	31.3
As octadecadienoic	1.9	2.1	1.0	—	3.6
Total C ₁₈ acids	54.7	55.1	54.9	61.2	64.2

The recalculated palmitic acid contents in Table XIV are, with one exception, close to those observed in the present analyses. The recalculated totals for the C₁₈ acids are low in several instances, obviously because rancidity products of low mol. wt. have now been reckoned as lower unsaturated acids, with the consequence that the C₁₈ unsaturated acids are now unduly depreciated. From

our present results, it appears that the combined amount of hexa- and tetradecenoic acids in tallow does not exceed 3.5%; if this figure is employed in Table XIV, and the balance of the unsaturated acids credited to C_{18} acids, the total C_{18} acid figures for the five specimens become respectively 60.0, 58.7, 57.8, 63.9 and 64.8%. It is unfortunate that, for the different reasons mentioned, more precise readjustment of the earlier data considered in Tables XIII and XIV cannot be made. Nevertheless, from the above considerations, it is evident that the final corrected figure for the total C_{18} acids in the ten instances considered tends towards the limits of about 60–65%.

From our present results and those of the earlier analyses, therefore, we incline to the view that the constancy of the C_{18} acids at about 60–65% (mol.) of the total fatty acids of tallow is even more marked than was at first thought, any increase in stearic acid being closely balanced by diminution of oleic acid. At the same time, and largely independently of the amount of unsaturated acids present, the palmitic acid content of nearly all tallows which have been analysed lies within the relatively constant limits of 30 (± 3)% (mol.).

Distribution of fatty acids in the glycerides

The determination of the fully saturated glyceride content of these tallows (Table X) adds further evidence to earlier work which has shown the relationship between the molar % of fully saturated glycerides and the molar % of saturated acids in the whole fat. Our figures fit the general curve for animal depot fats [Hilditch, 1937, p. 255], representing a higher percentage of fully saturated glyceride than has been obtained for (a) seed fats or (b) animal depot fats containing little stearic acid.

The compositions of the fully saturated glycerides in tallows I and III, in which the stearic acid content is 42–43%, resemble those of four of the fats studied by Banks & Hilditch [1931], in which the corresponding figure was 37–41%. A higher proportion (48%) of this acid was, however, found in tallow II and this result, coupled with one of the former series (33% stearic acid), suggests that the composition of ox depot fat fully saturated glycerides may not tend so closely to constancy as Banks & Hilditch were led to believe. It may be recalled that these authors [1932] found less constancy in the fully saturated glycerides of pig depot fat, in which the stearic acid content varied from 37 to 49% (mol.), although here again, in the majority of the cases examined, it lay between 41 and 45%.

SUMMARY

A detailed study of three beef tallows has indicated certain general features of land animal depot fat. The major components are oleic (38.0–40.4%), palmitic (26.5–31.0%) and stearic (20.1–25.4%) acids. Palmitic acid occurs with considerable regularity as about 30 mol. % while the C_{18} acids are present as 60–65 mol. % of the total mixed acids.

Among the minor component acids are two unsaturated members of the oleic acid series, myristoleic ($\Delta^{9:10}$ -tetradecenoic acid) and palmitoleic ($\Delta^{9:10}$ -hexadecenoic acid). The presence of these acids has been proved and their constitution determined. They have not previously been reported as components of the fat stores of higher land animals. A study of the constitution of the C_{18} unsaturated acids has shown that they are a mixture consisting chiefly of oleic acid ($\Delta^{9:10}$ -octadecenoic acid) and a small amount of a geometrically isomeric linoleic acid ($\Delta^{9:10, 12:13}$ -octadecadienoic acid). The presence of small amounts of myristic, arachidic, arachidonic and possibly lauric acids is also discussed.

Previous analyses of ox and sheep depot fats have been reviewed in the light of the present results. The increase in fully saturated glyceride content of these fats with increasing stearic acid content has been further confirmed.

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Note. In the paper by Hilditch & Shorland [1937] on liver lipoids, the “observed palmitic acid content in cow liver glyceride” (p. 1507, l. 3 and p. 1514, l. 16 from bottom) should read 31.3% (mol.) and not 29.8% (mol.).