CLXXXVIII. SOME FURTHER OBSERVATIONS ON THE OCCURRENCE OF AN OCTADECADIENOIC ACID IN COW BUTTER FATS.

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THE presence in cow milk fats of an octadecadienoic acid (and, especially, of ordinary linoleic acid ($\Delta^{9:10, 12:13}$ -octadecadienoic acid) in the form in which it occurs in seed fats) has been for some time past a matter of discussion. Hilditch and Jones [1929] examined the acids from pure C_{18} ester fractions obtained in the course of quantitative fractional distillation of the methyl esters from the "liquid" acids of butter fat and failed to obtain any characteristic tetrahydroxystearic acid by oxidation, or any definite brominated acid (other than a trace of ether-insoluble bromo-acids which darkened without definitely melting at $170-180^\circ$). More recently Eckstein [1933] obtained small yields of crystalline bromo-addition products from the unsaturated acids of cow milk fat which however only accounted for about 0.2 % each of linoleic and linolenic acids, whilst Bosworth and Brown [1933], studying fractions obtained by the distillation of the mixed methyl esters of cow butter fat (without recourse to prior separation of the latter into mainly saturated and mainly unsaturated portions), have concluded that neither ordinary linoleic, linolenic nor stearidonic acid is present. The latter authors attribute any polyethenoid unsaturation in cow butter fat to small proportions of arachidonic acid, $C_{20}H_{32}O_2$, a statement which implies that diethylenic C_{18} acids (in any form) are absent. Later, Bosworth and Sisson [1934] have stated that arachidonic acid and small proportions of behenic acid are present in butter, which however contains no arachidic, linoleic or linolenic acid. On the other hand, Bosworth [1934] has examined human milk fat, using the same partial fractionation separation as that of Bosworth and Brown, and obtained ester fractions containing C_{18} unsaturated acids which gave without difficulty the tetrabromostearic acid, m.p. 113° , characteristic of ordinary linoleic acid.

The amount of arachidonic acid present in cow butter fat is insufficient however to account for the unsaturation displayed by the C_{18} ester fractions which are consistently obtained from cow butter fatty acids which have been correctly separated and submitted to the ester fractionation process [Hilditch, 1934]. The ester fractions in question invariably correspond to 15-20 % of the total butter fatty acids and possess an average unsaturation more than 10 % in excess of that of methyl oleate. If, as appears almost certain, stearidonic or linolenic acids, if not absent, are present in only minute proportions, the excess of unsaturation over that demanded by oleic acid remains to be explained. The work now recorded, which has been directed towards this end, includes an examination, by various means, of the acids in these particular ester fractions. The results of the investigation are uniformly consistent with the view that the unsaturation in question is due to the presence of an octadecadienoic acid; and,

although it has unfortunately not proved possible to isolate either the acid itself or any characteristic crystalline derivative thereof, we believe that our results point to the presence of one or more of the geometrically isomeric forms of $\Delta^{9,10,12,13}$ -octadecadienoic acid (other than ordinary linoleic acid).

*Preparation of methyl esters of C₁₈ unsaturated acids
from cow butter fat.*

The butter fat used in the present experiments was from a composite sample of milk from several members of the Shinfield typical herd, taken while the cows were at pasture in the early summer (June) of 1934. About 1350 g. of fat were used to provide the experimental material; this was worked up in batches of 450 g. according to our usual procedure for quantitative determination of the component acids, although a full analysis of the fat was not made in the present instance. The mixed fatty acids from each batch were distilled in steam to remove the acids of lower molecular weight, and the residual fatty acids were submitted to the usual lead salt-alcohol separation. The acids from the alcohol-soluble lead salts were converted into methyl esters and fractionally distilled in the usual way. The results of this distillation in the case of the first batch of butter fat (450 g.) are given in Table I.

Table I. *Fractionation of methyl esters of "liquid" acids
from cow butter fat (450 g.).*

Fraction	g.	B.P.	S.E.	I.V.
L1	61.77	30–139°	240.6	37.0
L2	19.30	139–143	287.2	83.4
L3	17.26	143–146	291.6	88.5
L4	17.69	146–149	293.6	93.5
L5	18.19	149–152	294.7	95.3
L6	19.39	152–157	295.6	96.8
L7	17.46	157–146	297.2	98.7
L8	10.72	146–falling	297.3	101.1
L9	11.62	Residue	346.5	111.4
	193.40			

Fractions L4, L5, L7 and L8 were mixed and redistilled (Table II).

Table II. *Refractionation of ester fractions L4, L5, L7, L8.*

Fraction	g.	B.P.	S.E.	I.V.
L'1	5.11	146–150°	—	93.6
L'2	13.14	152–155	295.0	94.2
L'3	20.23	155	296.5	97.8
L'4	6.97	155–falling	—	100.9
L'5	1.30	Residue	—	—

Of the above, L'2, L'3 and L6 were accepted as C₁₈ unsaturated ester fractions.

The other two batches of butter fat similarly yielded the following primary C₁₈ ester fractions:

	g.	S.E.	I.V.
From batch 2	108.0	295.9	96.2
From batch 3	108.0	295.8	93.5

These two fractions were united and refractionated (Table III).

Table III.

Fraction	g.	B.P.	I.V.
L"1	19.18	138-142°	—
L"2	52.63	142-145	88.1
L"3	48.06	145-146	90.6
L"4	44.02	146-158	95.5
L"5	34.74	158-160	98.7
L"6	9.59	Residue	—
	<hr/> 208.22		

Fractions 2, 3, 4 and 5 (Table III) were used in preparing concentrates of the unsaturated esters in the manner about to be described.

*Further concentration of polyethenoid acids by crystallisation
of lithium salts from alcohol.*

Apart from the fact that these acids do not yield crystalline bromo-derivatives, we felt, for reasons dealt with in the preceding paper, that a chemical method of separation of any octadecadienoic acid present was undesirable. Owing to the greater solubility of linoleic salts as compared with those of oleic acid, separation by purely physical methods is not easy; but we found that by crystallising the lithium salts of the C_{18} unsaturated acids from the above ester fractions from 80 % alcohol, it was possible to increase the i.v. of the acids whose lithium salts remained in solution to about 115, representing (in a mixture of oleic and octadecadienoic acids) a concentration of 25-27 % of the latter. It was not found possible to exceed this figure, and the concentrates thus obtained were used for the remainder of the experiments.

Crystallisation of lithium salts from 95 % acetone. Before undertaking the lithium salt-alcohol separations however an experiment was made in order to ascertain the proportion and i.v. of the acids whose lithium salts were soluble in 95 % acetone. Lithium salts of the polyethenoid acids (stearidonic, arachidonic *etc.*) are freely soluble in this medium [Tsujiimoto, 1920; Ault and Brown, 1934].

A portion of the acids from the ester fraction L6 (Table I) (8.6 g., i.v. 101.5) was dissolved in warm acetone and neutralised with aqueous lithium hydroxide, the solvent being finally adjusted so as to contain 95 % of acetone and 5 % of water. From the lithium salts which crystallised out there were recovered 7.8 g. of fatty acids (i.v. 97.9) while the mother liquors contained lithium salts which yielded 0.6 g. of acids (i.v. 116.8). This result shows that the proportion, if any, of arachidonic or other highly unsaturated acid present in this fraction could only have been exceedingly small (arachidonic acid i.v. 334, stearidonic acid i.v. 368).

Concentration of the polyethenoid acids by crystallisation of lithium salts from 80 % alcohol. The method used was that of Moore [1919]. The fatty acids (*e.g.* 10 g.) were dissolved in 95 % alcohol (40 ml.) and exactly neutralised with approximately 4*N* aqueous lithium hydroxide solution, after which alcohol was added to bring the composition of the solvent to 80 % alcohol, 20 % water. The solution was then cooled to 10°, the crystallised lithium salts were filtered and washed with 80 % alcohol, and the fatty acids were recovered both from the crystallised salts and from those remaining in solution.

The acids recovered from the soluble lithium salts (in which the proportion of polyethenoid acids had been increased, owing to separation mainly of oleic acid in the insoluble salts) were submitted to the same procedure a second and even a third time, with reduction where possible in the ratio of solvent to total

lithium salts; but it was found that, when the acids from the soluble lithium salts had reached i.v. about 113, further increases in i.v. were relatively small, and could only be obtained with concurrent loss of considerable proportions of acids of mean i.v. > 100 in the deposited lithium salts. The results of the successive crystallisations undertaken are summarised in Table IV.

Table IV. *Partial concentration of polyethenoid acids by lithium salt-alcohol crystallisations.*

No.	Acids used			Acids from lithium salts			
	From ester-fractionation	g.	I.V.	Insoluble		Soluble	
				g.	I.V.	g.	I.V.
	First crystallisations.						
(a)	L'2, L'3, L6	10.1	101.5	4.4	96.3	5.6	106.9
(b)	L'2, L'3, L6	36.8	101.5	21.2	93.9	15.5	110.2
(c)	L'2	45.3	92.5	95.8	97.4	11.1	104.7 (x)
(d)	L'3	41.0	95.1			11.0	113.4
(e)	L'4	37.2	100.2			14.5	113.5
(f)	L'5	28.3	103.6			9.4	118.8
	Second crystallisations.						
	From insol. lithium salts						
(g)	(a) and (b)	20.7	109.3	6.0	98.5	14.3	114.8
(h)	(c), (d), (e) and (f)	94.0	97.4	77.6	95.9	15.8	109.4
	Third crystallisations.						
	From lithium salts						
(i)	(h) insol.	77.3	95.9	63.6	93.6	13.2	105.3 (y)
	(x) and (y) sol.	24.0	104.9	11.2	96.3	11.9	109.2

In Table V is a list of the more unsaturated acid fractions finally obtained, some of which we employed in the subsequent experiments.

Table V. *Final acid fractions used for further examination.*

	A	B	C	D	E	F	G	H
Wt. (g.)	5.6	11.9	15.8	15.5	11.0	14.5	14.3	9.4
i.v.	106.9	109.2	109.4	110.2	113.4	113.5	114.8	118.8

The mean mol. wt. of the acids G (i.v. 114.8) was 280.1; since lithium salts of palmitic and myristic acids are almost insoluble in 80 % alcohol, the procedure employed will have concurrently led to the removal of any traces of these acids present in the original ester fractions, and therefore the observed value for the mean mol. wt. indicates that the acids consisted wholly of members of the C₁₈ series.

Bromo-addition products of the unsaturated C₁₈ acids.

The acids G (i.v. 114.8, 5 g.) were dissolved in anhydrous ether (50 ml.) and cooled to -10°. Bromine (2.5 ml.) was slowly added from a burette during a period of 1 hour, the temperature being kept below -5°. After standing at -10° for 2 hours, the solution was filtered and the separated crystalline bromo-addition products were washed with ice-cold ether (50 ml.), dried at 90° for 2 hours and weighed. The ether filtrates were washed with a little aqueous sodium bisulphite, the ether was removed and the residue was dissolved in light petroleum (B.P. 40-60°, 10 ml.); on cooling the solution to 0° a small amount of solid separated and was filtered. After complete removal of the light petroleum from the mother-liquor a pale yellow oil remained.

The weights, bromine contents and M.P. of these three products are recorded in Table VI, which also shows the weights of fatty acids (and their mean I.V.) corresponding with the observed bromine contents.

Table VI. *Bromo-addition products of fatty acids G (5 g., I.V. 114.8).*

Separation	Bromo-addition products			Corresponding unsaturated acids		
	g.	% Br	M.P.	g.	%	I.V.
Insoluble in ether	0.19	65.8	Indefinite, charred at 160°	0.065	1.2	304.9
Soluble in ether, insoluble in light petroleum at 0°	0.06	64.7	165° with charring	0.02	0.4	291.1
Soluble in light petroleum at 0°	8.20	40.0	Liquid	4.92	98.4	105.9

It will be seen that, in agreement with previous observations, no indication of crystalline insoluble tetrabromostearic acid, M.P. 114°, is given by these results, the small amounts of ether- or petroleum-insoluble bromo-addition products melting indefinitely and charring at about 160° and containing about 65 % of bromine. In the light of Bosworth and Sisson's recent study [1934] of the bromo-addition products from arachidonic acid, these products might be derived from the latter; they might equally well, from the data, include a certain amount of the bromo-addition products of linolenic or stearidonic acid (% Br (theory): octabromoarachidic acid 67.8 %, octabromostearic acid 69.9 %, hexabromostearic acid 63.3 %). However this may be, the fatty acids represented by these crystalline bromo-products only form 1.6 % of the whole fraction.

The remaining 98.4 % of fatty acids yield the liquid, petroleum-soluble products, and neither the proportion nor the bromine content of these soluble products appears to have been considered by Eckstein or by Bosworth and Brown. The bromine content, as would be expected, is definitely higher than that of dibromostearic acid (36.2 %), although the calculated mean I.V. for the original mixture of acids (from the last column of Table VI) is only 109.4 as compared with 114.8 (observed); in other words, the total addition of bromine was about 4.5 % short of the theoretical, in terms of the (Wijs) I.V. determinations.

This result establishes definitely that the material, which gives rise in these acids to I.V. in excess of that of oleic acid, also yields bromo-addition products of corresponding bromine content which, to an overwhelming extent, are liquid and soluble in light petroleum at 0°.

We have found that, when linolenic acid (of linseed oil) is brominated, the ether-soluble bromo-addition products are also soluble to the extent of somewhat less than 50 % in petroleum (representing about 25 % of the total linolenic acid). If the 98.4 % of acids yielding petroleum-soluble bromo-addition products in Table VI consisted of a mixture of oleic and linolenic acids, the proportion of the latter in this fraction would have been about 9 %, that is, about 5–6 times as much as the whole of the unsaturated acids which yielded crystalline addition products containing about 65 % of bromine. It is therefore extremely unlikely that the polyethenoid acid present is linolenic acid, and correspondingly less likely that it is stearidonic or other still less saturated acid. The only alternative is of course an octadecadienoic acid.

The composition of the unsaturated acids (G) of I.V. 114.8 indicated by these bromo-addition products is thus (in round numbers): oleic acid 75 %, octadecadienoic acids 23.5 %, tri- and/or tetra-ethenoid C₁₈ acids 1.5 %.

*Oxidation of the methyl esters of the C₁₈-unsaturated acids by
potassium permanganate in acetone.*

When ordinary methyl linoleate or methyl oleate is oxidised by potassium permanganate in acetone the products are methyl hydrogen azeläate and, respectively, *n*-hexanoic or *n*-nonanoic acid [Armstrong and Hilditch, 1925; Hilditch and Vidyarathi, 1929]. The acids given in Table V, if mixtures of oleic and octadecadienoic acids, must still contain about 75 % of oleic acid, so that the oxidation products of their esters would include considerable proportions of *n*-nonanoic acid. Nevertheless, it was decided to investigate the mixture of acids obtained from their oxidation, since it appeared impracticable to obtain a higher concentration of the polyethenoid acids.

The acids of fractions E and F (Table V, i.v. 113·5) were converted into methyl esters (25 g., i.v. 108·2), which were dissolved in boiling acetone (250 ml.) and gradually oxidised with finely powdered potassium permanganate (100 g.). After refluxing for an hour after the addition of permanganate was completed, the acetone was removed and the residual product powdered and mixed with sodium bisulphite (120 g.). Water (200 ml.) was then cautiously added, followed by 50 % sulphuric acid (240 g.) in order to effect decoloration and solution of manganese oxides. The solution was extracted with ether and the ether washed twice with water (the aqueous solution and washings (A, *v. infra*) were united and reserved). The ethereal solution was then thoroughly extracted with 10 % aqueous potassium carbonate and subsequently washed with water, the alkaline extract and washings (B) being united and reserved. The ether contained 8·2 g. of unchanged or partially oxidised neutral esters (i.v. 63·6).

The aqueous extracts A were distilled in steam, the condensate being thoroughly extracted with ether. The ethereal extract was dried over sodium sulphate for 2 weeks, and the ether was then removed by distillation. A very small amount of residue was left which possessed the characteristic smell of ethyl acetate and distilled almost completely at 78°; the amount of distillate was however too small for further examination.

The potassium carbonate extracts and washings B were made acid with dilute sulphuric acid and were then also distilled in steam for some hours. The residue from the steam-distillation was hydrolysed by aqueous caustic potash and subsequently made acid, somewhat impure azelaic acid (8 g., m.p. 98°) being obtained; we were not able to detect the presence of any other dicarboxylic acid. (The complete oxidation of 16·8 g. of unsaturated ($\Delta^{9,10}$) C₁₈ esters would result in the production of 10·7 g. of azelaic acid.) The condensate (*ca.* 1500 ml.) from the steam distillation was extracted with ether and the ether extract dried as under (A); after removal of the ether the residue was fractionally distilled from a plain Willstätter bulb at atmospheric pressure with the results shown in Table VII.

Table VII.

Fraction	g.	B.P./760 mm.	Equiv. wt.
1	0·35	85–100°	664·0
2	0·69	100–200	137·5
3	0·74	200–210	131·1
4	1·74	210–230	139·2
5	1·77	230–237	148·9
6	1·04	Residue	163·7
	<u>6·33</u>		

Fraction 1 was obviously mainly water with some ether. Fraction 2 also evidently still contained a little water, but the greater part of this fraction came

over at 190–200°, and steady boiling only set in at this point. From this, and the fact that the lowest equiv. wt. observed was 131.1, we feel confident that no acid lower than *n*-hexanoic (mol. wt. 116, B.P. 205°) was present in any quantity. If we assume that the acids in fractions 2–5 were mixtures of *n*-hexanoic and *n*-nonanoic, the total yield of *n*-hexanoic acid would be 1.74 g., corresponding with 4.41 g. of a methyl $\Delta^{9:10, 12:13}$ -octadecadienoate; the amount of the latter ester in the 25 g. of esters oxidised (again supposing this to be a mixture of methyl oleate and octadecadienoate) would have been 6.43 g.

An alternative supposition consistent with the above observations (Table VII) is that *n*-heptanoic acid was the member of lower mol. wt.; in this case the acid from which it was derived would have been the conjugated $\Delta^{9:10, 11:12}$ -octadecadienoic acid. The quantity and ease of isolation of the recovered azelaic acid were quite similar to those in many cases we have had occasion to examine in which ordinary methyl oleate-linoleate mixtures have been submitted to this oxidation. Our present results therefore seem to fix the unsaturation in the polyethenoid acids as lying between the ninth and the twelfth or thirteenth atoms in the carbon chain (counting from the carboxyl carbon). This definitely limits the acid or acids in question to the diethylenic series.

We felt however that the detection of small amounts of ethyl acetate merited further attention, since, if the acetic acid had resulted from the decomposition of malonic acid formed during the oxidation, this would be evidence of the original presence of the grouping—CH:CH.CH₂.CH:CH—. (The occurrence of ethyl acetate, rather than free acetic acid, is readily to be accounted for by esterification of the latter, by alcohol present in the ether¹, during the prolonged desiccation of the ether solutions by anhydrous sodium sulphate.) The following additional experiments were therefore undertaken in order to ascertain the origin of the acetic acid.

“Blank” oxidations with potassium permanganate and acetone. In two separate experiments, the acetone as used in the previous oxidation (250 ml.) was refluxed with finely powdered potassium permanganate (10 g.) for 2 hours, after which the acetone was distilled and the residues were worked up exactly as when the butter ester-fractions had been oxidised. In neither case was any distillate boiling below 100° obtained from the dried ether extracts of the respective steam-distillates, nor was any odour of ethyl acetate or acetic acid detectable; the only products obtained were very small amounts of liquid (B.P. > 140°), which from their odour appeared to consist of mesityl oxide or similar condensation products.

Control oxidation with pure methyl oleate. A specimen of pure methyl oleate (25 g.), from oleic acid which had been freed from linoleic acid by crystallisation of its lithium salt, was oxidised, and the products were worked up, exactly as in the case of the above experiment, 6.3 g. of unchanged or semi-oxidised neutral ester (i.v. 62.4) being recovered. No distillable products were obtained from the steam distillation of the aqueous washings (A, p. 1569) and no odour of ethyl acetate or acetic acid could be detected, but the minute amount of liquid residue had the characteristic odour of ethyl nonanoate. Similarly, no acetic acid or ester could be detected in the extract from the steam-distillation of the acids produced after hydrolysing the alkali-soluble products of oxidation (B, p. 1569). The distillation of these products is summarised in Table VIII.

¹ It is our practice to add alcohol, during the extractions with alkali, in order to diminish emulsification.

Table VIII.

No.	g.	B.P.	Equiv. wt.
1	(2 drops)	100–200°/760 mm.	—
2	1.53	200–225°/760 mm.	142.4
3	1.09	225–230°/760 mm.	151.8
4	1.38	79–84°/15 mm.	154.4
5	1.27	84–90°/15 mm.	151.8
6	1.64	90–88°/15 mm.	153.3
7	1.43	Falling / 15 mm.	155.3

Oxidation of C₁₈ unsaturated ester-fractions from ground-nut oil acids. Finally, the unsaturated acids of ground-nut oil were isolated by the lead salt-alcohol separation, and their methyl esters fractionally distilled. The C₁₈ ester fractions so obtained had i.v. 111.7, *i.e.* their mean unsaturation was comparable with that of the butter ester-fraction which had been oxidised, whilst in this instance it was known that the polyethenoid derivative present was the ester of ordinary or seed fat $\Delta^{9:10, 12:13}$ -octadecadienoic acid. On oxidation exactly as in the previous instances, 11.5 g. of unchanged or semi-oxidised neutral esters (i.v. 78.2) were recovered from the 25 g. of esters taken. The extract (A) from the steam-distillate of the aqueous washings yielded 3.4 g. of a liquid, B.P. 78–82°, which was neither acid nor ester and appeared to consist mainly of alcohol; in this case no smell of ethyl acetate or of acetic acid (other than a slight “pyroligneous acid” odour) was observed in this fraction.

The result of the corresponding distillation of the extract (B) from the steam-distillate of the acids obtained after hydrolysis of the alkaline washings is shown in Table IX.

Table IX.

No.	g.	B.P.	Equiv. wt.
1	0.24	80–130°/760 mm.	136.3
2	0.63	130–178°/760 mm.	120.1
3	0.74	178–190°/760 mm.	125.5
4	1.28	190–208°/760 mm.	130.6
5	1.00	208–210°/760 mm.	135.3
6	0.75	63–84°/15 mm.	143.0
7	0.42	84–86°/15 mm.	151.1
8	0.69	Residue	154.4*

* Determined after extraction of acidic matter from the charred residue by aqueous potassium carbonate, followed by recovery of the extracted acids.

Fraction 1 smelled strongly of ethyl acetate and acetic acid and fractions 2–3 of the latter. The boiling-points and equiv. wt. of fractions 2 and 3, considered together, also suggest that a mixture of acetic with acids of much higher mol. wt. was coming over at this stage. These fractions were united, dissolved in ether and extracted with water to effect as much separation as possible of any acetic acid present; the recovered acids (0.94 g.) had equiv. wt. 125.8. In this experiment the acetic acid thus appeared in fraction “B” rather than “A”. Apart from this, the character of the acids in fractions 4–7 closely resembles that of those obtained in the corresponding distillation (Table VII) of the monobasic acids from the oxidation of the butter ester-fraction.

Calculated on a mixture of *n*-hexanoic and *n*-nonanoic acids, the fractions 4–7 (with the recovered 0.94 g. of acids from fractions 2 and 3) contained 2.14 g. of *n*-hexanoic acid, corresponding with 5.42 g. of methyl $\Delta^{9:10, 12:13}$ -octadecadienoate. The amount of the latter ester in the 25 g. of ground-nut oil esters oxidised (i.v. 111.7) is 7.44 g.

Comparing the oxidation of the ground-nut oil esters (Table IX) with those of the butter esters of similar mean unsaturation (Table V), and assuming that the latter were also mixtures of methyl oleate with a methyl $\Delta^{9:10, 12:13}$ -octadecadienoate, the respective proportions of octadecadienoic ester accounted for as *n*-hexanoic acid are 73 % (ground-nut oil esters) and 69 % (butter esters). This similarity is accompanied by a general parallelism in the mol. wt. of the distilled acid fractions of corresponding boiling-point in the two series. In these respects, and in the appearance of acetic acid (or ester), the two experiments are remarkably comparable and the results point to the conclusion that an octadecadienoic acid of the same structure (but not necessarily the same configuration) is present in cow butter fat and in ground-nut oil.

Little significance would be attached to the appearance of small amounts of acetic acid in the oxidation products were it not for the fact that, in the products from the corresponding experiments with pure methyl oleate or with acetone alone, acetic acid was not detected in any instance. These negative observations, on the other hand, justify the conclusion that, in the ground-nut oil esters, the acetic acid results from the $\Delta^{9:10, 12:13}$ -octadecadienoate present (by decomposition of malonic acid formed in the course of the oxidation). The similar appearance of acetic acid in the oxidation products of the butter esters must therefore be ascribed to the same cause.

The potassium permanganate-acetone oxidations of the C_{18} unsaturated butter esters from the acid fractions in which the polyethenoid acids had been concentrated as far as possible by the lithium salt separation thus support the view that a $\Delta^{9:10, 12:13}$ -octadecadienoic acid is present therein in some quantity.

*Oxidation of the unsaturated acids of cow butter fat by
aqueous alkaline potassium permanganate.*

We have found, in the course of oxidations with aqueous alkaline permanganate carried out according to the modified Hazura procedure described in the preceding paper [1935], that the acids from the unsaturated C_{18} ester fractions with which we have been dealing give rise to small quantities of the crystalline tetrahydroxystearic acids which correspond with seed fat linoleic acid. In common with other workers, we had formerly been unable to isolate any of these acids from the alkaline oxidation of butter acids. The quantities now obtained are but small and only represent a fraction of the total octadecadienoic acids which we believe to be present; but they have been detected in each case in which the alkaline permanganate oxidation described in the preceding paper has been applied either to the acids from the unsaturated C_{18} ester fractions or to acids obtained from the latter in which the polyethenoid members had been concentrated by the lithium salt process. Moreover, study of these fractions from butters taken at various seasons over a number of years has shown that production of the typical crystalline tetrahydroxystearic acids is at a minimum in summer pasture butters and at a maximum at the end of the winter season.

A summary of these experiments is given in Table X. As far as possible, the same weight of unsaturated acids was oxidised in each instance, so that the weights of tetrahydroxystearic (water-soluble) acids isolated give a direct comparison of the respective yields (except for unavoidable differences in i.v., or rather, in the proportions of mono- and di-ethenoid acids, of the original mixture). For further illustration, the final column of relative yield percentages has been added: this gives the amount of linoleic acid, corresponding to the tetrahydroxystearic acids obtained, expressed as a percentage of the total

Table X. *Crystalline tetrahydroxystearic acids obtained by alkaline permanganate oxidation of unsaturated C₁₈ ester fractions from summer and winter butters.*

Butter	Unsat- rated ester fractions	Corresponding unsaturated C ₁₈ acids oxidised		Tetrahydroxystearic acids (water-soluble) isolated		% yield (calcu- lated as stated above)
		I.V.	g.	g.	M.P.	
Early summer, May 1931	96.7	101.5	4.8	0.0702	140–153°	9.3
„ June 1934	95.0	99.7	4.8	0.059	144–156	9.3
„ June 1934	—*	114.8	4.0	0.080	145–157	5.9
Late summer, Sept. 1932	95.0	99.7	4.8	0.060	143–156	9.5
„ Oct. 1930	98.8	103.7	4.8	0.073	142–157	8.1
Winter feeding, Feb. 1930	93.6	98.3	4.8	0.076	139–154	14.0
„ Feb. 1934	96.3	101.1	4.8	0.097	143–157	13.3
„ Mar. 1934	101.2	106.2	4.2	0.105	153–154	11.3†

* Acids G (p. 1567) from lithium salt process.

† Oxidation by Lapworth-Mottram procedure.

octadecadienoic acid present (the i.v. of the original acid fractions being assumed, for this purpose, to be due only to oleic and octadecadienoic acids).

The water-soluble acids isolated were shown to be mixtures of the two characteristic tetrahydroxystearic acids, M.P. 155° and 172°. Thus, the acids, M.P. 143–157°, from the winter butter of February 1934 (0.091 g.), when heated with ethyl acetate, yielded an insoluble portion (0.027 g., M.P. 170–172°), whilst the filtrates gave two successive crops of crystals: 0.033 g., M.P. 154°, and 0.013 g., M.P. 114–117°.

Since, as shown by the study of their bromo-addition products (p. 1568), the unsaturated acids in Table X include a very small proportion (not more than 1 %) of acids with more than two ethenoid linkages, the true yield of the two crystalline tetrahydroxystearic acids in terms of octadecadienoic acid will be slightly greater than shown in the final column, but it is still far below the figure of about 40 % of theory given alike by seed fat linoleic acid or the related “ α ”-linoleic acid (*cf.* preceding paper, p. 1555). From these figures, ordinary linoleic acid cannot form more than about one-fifth of the octadecadienoic acids in the summer pasture butters, but in the winter butters it rises to about a third of the total octadecadienoic acids. The definitely higher proportions in the winter butters are probably significant in relation to the diet of the cows at this period, which of course includes roots and a much higher ratio of concentrates (grain) than when the cows are at pasture.

Smith and Chibnall [1932], in their investigation of the glyceride fatty acids of cocksfoot grass, found that the unsaturated acids (i.v. 185.3) did not yield the crystalline tetrabromostearic acid, M.P. 114°, although 8.9 g. of oily tetrabromo-derivatives insoluble in cold light petroleum (B.P. 40–60°) resulted from 10.4 g. of the unsaturated acids. They inferred the presence of a mixture of isomeric tetrabromostearic acids. Again, they obtained only 0.45 g. of mixed crystalline tetrahydroxystearic acids when 11 g. of the unsaturated acids were oxidised with alkaline permanganate according to the Lapworth-Mottram procedure, corresponding to an apparent content of only 3.3 % of ordinary linoleic acid. Their experiences thus resemble ours with the pasture butter unsaturated fatty acids to a remarkable degree, and we consider that the peculiarities of the

butter octadecadienoic acids are the consequence of their derivation from grass glycerides by direct assimilation. This opinion is reinforced by the recent observations of Bosworth [1934] on human milk fat: he obtained fractions of unsaturated C_{18} acids which yielded without difficulty the tetrabromostearic acid, m.p. 113° , insoluble in light petroleum. The consensus of all these observations is clearly that absence of, or diminution in the proportion of, ordinary linoleic acid in milk fats is paralleled by the extent to which grass has formed a component of the diet, while the grass glycerides themselves have been found by Smith and Chibnall to exhibit similar anomalies as regards the type of octadecadienoic acid present.

At this point we would make a more general observation with reference to the appearance of linoleic or other octadecadienoic acids in the fats of most of the land animals, namely, that recent work suggests that these acids are probably invariably derived by assimilation and not by synthesis in the animal from non-fatty compounds. The results obtained in the past few years in this laboratory on various land animals have led to the conclusion that, so far as concerns palmitic and oleic acids (and, at least in the herbivora, the approximately constant stearic-oleic acid balance), these glyceride components can be produced by the animal, and in such manner that the palmitic content is closely similar (25–30 %) for all animals, whilst the stearic and oleic contents vary with the animal, or even with the site in which the fat is deposited. On the other hand, the following observations accord with the hypothesis that in land animal glycerides octadecadienoic acids are the result of assimilation:

(a) *Cow and human milk-fats*. The relevant data in these cases have been presented above.

(b) *Pig depot fats*. Young pigs (8–10 months old) on fat-free or low-fat diets yield body fats with only 1–5 % of combined linoleic acid [Ellis and Zeller, 1930; Bhattacharya and Hilditch, 1931]; the corresponding fats of older animals (4–8 years) contain as much as 14–15 % of linoleic acid, and this proportion is unaltered in fats from different parts of the same animal which differ considerably in their relative contents of oleic and stearic acids [Banks and Hilditch, 1932; Dean and Hilditch, 1933]. (The linoleic acid of pig fats gives the ordinary tests as regards tetrabromo- and tetrahydroxy-stearic acids [Hilditch and Stainsby, 1935]).

Further, young pigs fed on rations high in fatty oils such as those of cottonseed, ground-nut or soya bean yield depot fats with abnormally high contents of linoleic acid [Ellis *et al.*, 1925; 1926; 1931].

(c) *Rat depot fats*. With fat-free diets, the depot fats of rats are devoid of linoleic acid, but ingestion of cod-liver oil leads to the appearance of linoleic glycerides in the depot fats [Burr and Burr, 1930; Gregory and Drummond, 1932; Banks *et al.*, 1933].

We hope to make a similar examination in the near future of the polyethenoid C_{18} acids present in small quantities in the depot fats of oxen which have been reared under known dietary conditions.

SUMMARY.

A further examination has been made of the polyethenoid C_{18} acids in cow butter fat. The acids were prepared by refractionation of the esters of the butter "liquid" acids, followed by separation of the more soluble lithium salts from alcohol; C_{18} acids with i.v. 113.4–114.8 were thus obtained and used in the experiments described.

Addition of bromine to the acids of i.v. 114.8 led to a mixture of bromo-addition products, 1.6 % of the original acids giving products insoluble in cold ether or light petroleum, whilst the remaining 98.4 % yielded liquid bromo-addition products (Br 40.0 %) soluble in cold light petroleum. It is shown that these are unlikely to include any appreciable amount of hexa- or octa-bromostearic acids and that, accordingly, the original acids contained about 75% oleic, 1.5 % tri- or tetra-ethenoid C₁₈ acids, and 23.5 % octadecadienoic acid.

Examination of the acids formed as a result of oxidation of the methyl esters of acids of i.v. 113.5 by permanganate in acetone showed that the monobasic acids (*n*-hexanoic or *n*-heptanoic, *n*-nonanoic and small quantities of acetic) resembled, qualitatively and quantitatively, those obtained by similar treatment of a fraction of the unsaturated esters from ground-nut oil (of similar i.v.); while the amount of azelaic acid recovered showed that unsaturation did not commence before the ninth carbon atom from the carboxyl group. Control experiments with pure methyl oleate in acetone, or with acetone alone, failed to yield any acetic acid. These experiments therefore suggest that the octadecadienoic acids of ground-nut oil and cow butter fat are structurally similar, and that the latter is a different geometrical isomeride (or mixture of geometrical isomerides) of the $\Delta^{9:10, 12:13}$ -octadecadienoic acid of seed fats.

Oxidation of unsaturated C₁₈ acid fractions from a number of butters by means of aqueous alkaline permanganate (*cf.* preceding paper) led in all cases to the isolation of small proportions of the same mixture of tetrahydroxystearic acids as that which is thus obtained in a yield of about 40 % from seed fat linoleic acid. The amount of these acids obtained from butters from cows on winter diet was considerably (*ca.* 50 %) greater than that from summer pasture butters.

It is concluded that the kind of octadecadienoic acid present in butter fat depends upon the diet of the cows and, since the bromination and alkaline oxidation results in this investigation are closely parallel with those of Smith and Chibnall on the glyceride fatty acids of grasses, that the peculiarities of butter fat octadecadienoic acid are the consequence of assimilation of the same acid from the grass glycerides.

Study of the data now available in the cases of cow and human milk fats and of the depot fats of pigs and rats has led us to the general conclusion that linoleic acid, when present in the glycerides of land animals, is a product of assimilation and not of synthesis by the animal.

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