XCVI. THE EFFECT OF CERTAIN INGESTED FATTY OILS UPON THE COMPOSITION OF COW MILK FAT.

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THE observations of Drummond *et al.* [1923; 1924] and of Golding *et al.* [1926] that cod liver oil in the diet of lactating cows leads to reduction of the fat content of the milk have been confirmed by several other workers. McCay and Maynard [1935], who also state that ingestion of this oil causes muscular lesions in the animal, found that the effects of shark liver oil or salmon oil were so much less and so inconsistent that it is questionable whether they act like cod liver oil. These workers agreed with Golding [1928] that the specific effect of cod liver oil was not shown when the unsaponifiable fraction of that oil was given in the diet, thus pointing to the injurious or active component occurring as part of the triglycerides. Madsen *et al.* [1935] observed similar harmful results of cod liver oil in the diet of guinea-pigs, rabbits, goats and sheep.

In an attempt to ascertain which of the cod liver oil components were responsible for the diminished fat content of the milk, experiments were conducted at the National Institute for Research in Dairying in the early spring of 1934, in which the effect of cod liver oil was compared with that of rape or linseed oil in the diet. Rape oil contains nearly half its weight of erucic glycerides, *i.e.*, glycerides of an acid with a chain of 22 carbon atoms in the molecule, but monoethenoid as contrasted with the tetra- and penta-ethenoid unsaturation of the cod liver oil C_{20} and C_{22} acids. Linseed oil has a higher mean unsaturation than most cod liver oils, but the unsaturation is due solely to glycerides containing di- and tri-ethenoid acids of the C_{18} series (linoleic and linolenic), each of which forms about 40 % of the total fatty acids in linseed oil. Captain Golding, who informs us that ingestion of neither rape nor linseed oil caused any deficiency in the fat content of the milk, kindly placed at our disposal sufficient of the milk fats from these investigations, with corresponding control samples, to permit full analyses of the component acids by the ester-fractionation method. The present communication records the results of these analyses and of determinations of the proportion of fully-saturated glycerides in each of the fats.

The cows from which these milk fats were obtained received, in addition to the basal winter ration fed to the control animals, 4 oz. of either rape, linseed or cod liver oil twice daily for a week or more before taking the milk samples. (A fuller account of the experimental diets, in which the added oil was given twice daily, first in 2, then in 3 and finally in 4 oz. rations, was given in a recent communication by Dann *et al.* [1935].) The details of the milks, from which were prepared the six specimens of fat which we have studied, are given in Table I.

The general analytical characteristics of the corresponding milk fats are given in Table II.

Table I. *Sources of the six milk fats analysed.*

Reference no.	Cow or cows	Diet	Inclusive dates of collection of milk
1	Gertie 3	Control	p.m. March 14–a.m. March 16, 1934
2	Welcome 7	Control	Feb. 16–Feb. 19, 1934
3	Flora 16 and Flora 20	Control + linseed oil	p.m. March 27–p.m. March 28, 1934
4	Flora 16 and Flora 20	Control + rape oil	p.m. March 13–a.m. March 14, 1934
5	Portia 3	Control + cod liver oil	a.m. March 17–p.m. March 18, 1934
6	Lena 3 and Welcome 7	Control + cod liver oil	March 30–April 3, 1933

Table II. *General analytical characteristics.*

Milk fats from cows no. ...	1	2	3	4	5	6
Oil fed to cow ...	None	None	Linseed	Rape	Cod liver	Cod liver
Reichert-Meissl value	33.6	30.0	28.9	28.5	16.0	15.3
Polenske value	3.3	2.1	2.1	1.8	1.4	1.3
Kirschner value	24.3	23.8	22.4	21.7	13.9	13.7
Saponification equivalent	239.7	244.2	249.0	251.2	264.2	266.0
Iodine value	34.5	34.9	46.0	44.5	51.7	54.1

These figures show quite clearly that intake of fatty oil by the cows has caused considerable modification of the milk fat, but of course they indicate only the broad effects so produced and give little information as to the actual differences in the component acids, except that cod liver oil in the diet has approximately halved the amount of butyric and *n*-hexanoic acids in the milk fat and at the same time caused an increase in the total unsaturation of about 50 %, whilst the average equivalent of the mixed fatty acids has increased by about 25 units. Ingestion of linseed and rape oils apparently had little influence on the content of lower acids, but increased the mean unsaturation and equivalent to values similar to those characteristic of summer pasture butters [*cf.* Hilditch and Sleightholme, 1930; Dean and Hilditch, 1933, 1]. For more definite information as to the relative amounts of specific component acids, recourse to the more elaborate and tedious analytical methods by lead salt separation, ester fractionation *etc.* is essential, and it seemed well worth while to undertake this procedure on these six milk fats in view of the interesting features revealed by the data in Table II.

The methods employed have been described in detail previously [Hilditch and Jones, 1929; Hilditch and Sleightholme, 1930] and may be summed up as follows:

(a) The mixed fatty acids from about 400 g. (when available) of milk fat were first distilled in steam for 4–5 hours. The aqueous distillates were thoroughly extracted with ether and the extract dried over neutral anhydrous sodium sulphate. After removal of the ether, the residual volatile acids were fractionated direct. The acidity of the ether-extracted aqueous liquors, and of the ether *etc.* recovered by distillation up to 160°, was calculated as butyric acid, the compositions of the remaining fractions being calculated from their mean equivalents (with allowance for the small amount of unsaturation present in the final residue).

(b) The acids non-volatile in steam were submitted to the lead salt separation from alcohol and thereby divided into mainly saturated ("solid") and mainly unsaturated ("liquid") acids.

(c) Each group of acids, "solid" and "liquid", was converted into the corresponding neutral methyl esters, which were fractionally distilled in a high vacuum from a Willstätter bulb,¹ the first fraction (25–30 % of the whole) being refractionated whilst subsequent ones were collected in fractions of about 7–12 g. each. In the refractionation of the first primary fractions the distillates were divided into much smaller fractions (2–5 g.).

¹ We desire to emphasise the importance of employing some kind of simple fractionation device in these mixed ester distillations. The use of a Claisen bulb [*cf.* Ault and Brown, 1934] is, in our opinion, insufficient for the present purpose.

Table III. *Preliminary separation of the mixed fatty acids into (a) steam-volatile and (b) "solid" and "liquid" non-steam-volatile fatty acids.*

Milk fat no. ...	...	1	2	3	4	5	6
Oil fed to cow ...	...	Control	Control	Linseed	Rape	Cod liver	Cod liver
Weight of fat used (g.)		450.9	250.5	450.8	341	208	506
Total fatty acids (g.)		425.3	235.1	429.5	326.3	198.2	481.9
Steam-volatile acids (g.)		32.5	15.1	29.5	18.7	6.3	15.9
Lead salt separation:							
"Solid" acids (g.)		189.2	115.8	177.6	136.8	86.8	220.4
Corresponding methyl esters:							
Equivalent		275.8	275.4	276.0	281.8	276.2	278.6
I.V.		5.2	4.9	11.0	11.1	15.5	22.2
"Liquid" acids (g.)		203.6	106.2	222.4	170.8	105.1	245.6
Corresponding methyl esters:							
Equivalent		268.5	269.3	273.6	276.1	280.8	289.6
I.V.		68.1	71.7	78.6	76.7	81.0	84.4

Table IV. *Analyses of acids volatile in steam.*

Milk fat no. ...	...	1	2	3
Oil fed to cow ...	...	Control	Control	Linseed
Acidity (as butyric acid) in:		g. Equiv.	g. Equiv.	g. Equiv.
Ether-extracted aqueous solution		1.16 —	0.74 —	2.31 —
Recovered ether		0.55 —	0.26 —	0.66 —
Distillate fractions:				
1 (up to 100°)		0.29 —	0.11 —	0.27 —
2 (100–160°)		9.48 —	3.52 —	0.70 —
3		3.70 94.1	1.99 91.3	1.61 90.1
4		4.02 96.7	2.49 91.8	1.99 91.0
5		3.95 100.7	2.04 93.9	3.87 92.0
6		4.95 114.5	1.27 103.3	3.75 93.0
7		— —	1.30 116.2	6.92 96.3
8		— —	— —	2.68 112.5
9		— —	— —	2.15 121.8
10		— —	— —	1.26 139.4
Residue {		4.40 153.8	1.42 155.5	1.29 198.2
I.V.		— 9.9	— 12.2	— 22.3
Milk fat no. ...	...	4	5	6
Oil fed to cow ...	...	Rape	Cod liver	Cod liver
Acidity (as butyric acid) in:		g. Equiv.	g. Equiv.	g. Equiv.
Ether-extracted aqueous solution		0.35 —	0.21 —	0.84 —
Recovered ether		0.30 —	0.16 —	0.38 —
Distillate fractions:				
1 (up to 100°)		0.37 —	0.49 —	0.14 —
2 (100–160°)		1.68 —	1.90 —	1.68 —
3		1.07 91.8	0.87 95.0	1.59 91.4
4		1.08 92.1	0.74 97.3	2.19 91.6
5		2.17 92.2	0.70 108.1	1.65 91.8
6		1.99 92.5	— —	1.46 93.0
7		2.47 93.7	— —	1.27 95.5
8		2.18 95.8	— —	1.17 99.9
9		1.50 101.7	— —	2.13 115.7
10		1.25 114.2	— —	— —
11		0.86 121.5	— —	— —
Residue {		1.40 151.2	1.27 152.9	1.40 177.8
I.V.		— 10.6	— 11.5	— 20.3

(d) In certain cases, the mean equivalent of the saturated esters present in individual fractions was determined, after removal of all unsaturated esters by permanganate-acetone oxidation. This was carried out when it was desired to ascertain the mean unsaturation and equivalent of the unsaturated components also present in the fractions in question.

These detailed analyses show very clearly, especially with the milk fats from cows fed with rape or cod liver oil, the factors which have contributed to the marked changes in general analytical characteristics. In order to discuss the results adequately it is necessary to refer to specific parts of the ester fractionations and therefore also to quote the fractionation data somewhat more fully

Table V. *Methyl esters of "solid" acids.*

Milk fat no. ...	1			2			3		
Oil fed to cow...	Control			Control			Linseed		
Fraction	g.	Sap. eq.	I.V.	g.	Sap. eq.	I.V.	g.	Sap. eq.	I.V.
Primary fractionations.									
S 1	48.75	258.3	1.9	35.60	262.3	1.9	31.40	258.2	3.1
S 2	11.19	270.5	3.0	7.64	271.6	2.9	9.03	268.9	4.9
S 3	13.64	272.9	3.4	9.53	274.0	3.3	9.64	271.6	6.0
S 4	13.71	276.8	4.2	10.38	276.1	3.9	12.12	275.3	7.1
S 5	12.45	279.5	5.4	10.75	278.2	5.2	13.73	277.3	8.7
S 6	13.09	283.2	6.7	9.73	283.9	7.5	15.62	280.7	12.1
S 7	8.85	287.0	8.2	9.93	291.2	11.4	14.80	287.9	18.2
S 8	10.98	291.7	10.8	4.11	296.8	13.0	10.60	295.6	23.1
S 9	8.63	296.1	11.5	—	—	—	8.49	298.4	23.9
S 10	7.19	297.9	12.1	—	—	—	—	—	—
Residue	4.36	311.6	14.1	7.58	305.1	14.5	3.78	307.2	25.6
Refractionations of primary fractions S 1.									
S 11	2.80	237.1	0.6	4.42	248.0	0.9	2.62	241.8	0.6
S 12	2.87	243.1	0.6	4.02	250.5	0.9	2.90	246.4	0.5
S 13	4.89	247.7	0.7	5.38	255.3	0.8	3.74	250.6	0.6
S 14	6.45	251.0	0.7	4.91	261.4	0.9	5.10	256.7	1.3
S 15	8.40	256.7	0.9	4.82	269.7	1.4	5.42	264.6	2.5
S 16	7.80	265.9	1.5	5.57	273.5	2.4	3.72	269.6	4.8
S 17	5.85	273.0	2.9	—	—	—	—	—	—
Residue	4.98	283.1	6.6	2.92	281.5	7.9	3.99	279.4	11.8
Milk fat no. ...	4			5			6		
Oil fed to cow...	Rape			Cod liver			Cod liver		
Fraction	g.	Sap. eq.	I.V.	g.	Sap. eq.	I.V.	g.	Sap. eq.	I.V.
Primary fractionations.									
S 1	43.44	262.8	3.5	31.42	255.8	4.3	45.37	265.6	6.2
S 2	12.71	276.4	6.0	7.72	275.6	7.9	9.54	272.4	11.1
S 3	12.07	279.7	7.7	7.20	276.6	10.2	12.37	277.7	13.1
S 4	12.19	282.8	9.9	7.82	280.3	13.0	13.90	278.9	16.0
S 5	12.91	287.7	14.0	6.31	284.6	19.9	11.68	280.6	20.4
S 6	11.98	295.6	18.4	6.92	292.1	27.4	13.83	284.5	27.0
S 7	8.86	300.8	21.4	—	—	—	11.98	289.4	37.0
S 8	6.00	306.6	23.7	—	—	—	14.98	298.4	50.1
Residue	6.87	330.8	37.2	10.56	304.6	39.6	10.39	315.2	62.8
Refractionations of primary fractions S 1.									
S 11	4.49	243.9	1.1	2.94	250.0	1.4	4.01	247.7	1.2
S 12	4.80	252.2	2.0	2.80	252.2	1.3	4.26	253.3	1.4
S 13	5.92	256.1	1.2	4.81	258.9	1.6	5.10	256.0	2.0
S 14	7.00	263.0	1.9	3.82	267.4	2.6	7.59	263.1	2.8
S 15	7.03	270.0	2.9	4.26	271.5	4.0	5.56	268.1	4.0
S 16	5.45	276.2	5.1	3.06	275.0	7.0	4.55	271.5	6.5
S 17	—	—	—	—	—	—	5.13	273.3	9.7
Residue	4.95	284.8	11.2	2.69	274.7	17.9	4.94	282.4	25.5

than has been the recent custom in communications from this laboratory, where it has been considered sufficient to give only the final percentage compositions obtained for the component acids of a fat. The details of the analyses are therefore summarised in Tables III, IV, V and VI. The weights of the fats used and the corresponding total weights of fatty acids accounted for, were as shown in Table III.

Table VI. *Methyl esters of "liquid" acids.*

Milk fat no. ...	1			2			3		
Oil fed to cow ...	Control			Control			Linseed		
Fraction	g.	Sap. eq.	I.v.	g.	Sap. eq.	I.v.	g.	Sap. eq.	I.v.
Primary fractionations.									
L 1	53.38	216.7	22.6	31.23	227.6	33.5	41.51	232.2	37.4
L 2	12.81	275.5	68.8	6.37	280.3	71.8	11.87	283.5	82.4
L 3	16.56	289.3	87.8	8.99	286.1	80.1	12.14	289.8	88.4
L 4	17.30	292.4	94.7	10.46	291.7	87.1	16.21	291.3	94.0
L 5	10.14	293.2	98.2	11.14	293.4	91.9	22.08	293.0	97.8
L 6	22.62	294.8	101.2	8.85	296.0	94.0	11.96	294.8	98.7
L 7	12.01	299.7	106.5	10.60	297.6	96.3	9.73	295.3	98.9
L 8	—	—	—	6.19	298.7	96.9	8.76	297.2	101.0
Residue	7.13	341.3	112.6	6.46	325.5	99.7	10.00	323.8	106.1
	—	303.9*	109.2*	—	301.8*	100.7*	—	304.8*	103.9*
Refractionations of primary fractions L 1.									
L 11	2.64	163.7	3.2	2.78	169.4	6.7	3.08	170.0	5.8
L 12	2.54	174.0	6.2	3.09	187.5	13.4	2.55	188.8	12.0
L 13	5.48	185.4	9.1	4.10	209.8	11.5	2.35	196.4	11.7
L 14	5.20	193.1	9.1	5.14	232.4	18.1	3.22	212.7	10.6
L 15	6.09	213.3	11.2	3.76	248.9	27.6	4.45	228.0	14.0
L 16	10.53	227.9	11.9	4.11	263.4	48.0	4.90	244.5	23.7
L 17	6.49	244.4	19.5	—	—	—	4.90	254.5	34.8
L 18	6.81	263.3	49.8	—	—	—	3.50	268.0	56.0
L 19	—	—	—	—	—	—	4.03	282.5	80.0
Residue	4.20	295.7	86.2	5.15	290.1	86.8	4.62	292.1	94.2
Milk fat no. ...	4			5			6		
Oil fed to cow ...	Rape			Cod liver			Cod liver		
Fraction	g.	Sap. eq.	I.v.	g.	Sap. eq.	I.v.	g.	Sap. eq.	I.v.
Primary fractionations.									
L 1	48.25	240.5	40.8	35.21	252.0	56.8	45.44	251.7	53.5
L 2	23.78	289.7	87.9	16.08	291.4	90.4	12.00	290.0	89.0
L 3	12.91	293.7	92.1	8.78	294.5	95.3	12.58	290.2	93.7
L 4	15.44	295.5	94.1	9.32	294.2	95.5	13.38	296.4	96.9
L 5	16.35	296.6	95.7	10.09	298.3	98.1	10.99	296.8	99.6
L 6	11.49	297.2	96.5	6.11	302.3	98.4	15.91	298.5	102.3
L 7	11.53	299.7	98.3	5.79	308.1	101.6	7.56	299.7	103.2
L 8	—	—	—	—	—	—	7.46	303.3	105.5
L 9	—	—	—	—	—	—	7.42	306.4	106.9
L 10	—	—	—	—	—	—	6.02	317.3	113.5
Residue	13.35	330.6	99.9	8.16	320.7	99.0	8.16	375.8	118.5
	—	320.1*	—	—	310.0*	—	—	318.0*	110.1*
Refractionations of primary fractions L 1.									
L 11	3.49	172.4	5.8	2.50	186.2	11.7	3.04	182.7	10.5
L 12	3.20	190.6	13.8	4.62	222.3	21.1	4.37	215.5	17.2
L 13	5.05	215.1	12.1	10.17	250.6	42.1	5.45	238.0	33.3
L 14	13.81	244.2	21.9	10.31	283.6	80.7	5.89	252.5	37.1
L 15	15.70	279.5	66.5	—	—	—	7.85	265.9	55.9
L 16	—	—	—	—	—	—	5.36	280.4	77.1
L 17	—	—	—	—	—	—	4.97	288.9	89.7
Residue	3.43	293.4	89.8	4.35	298.2	94.7	4.57	296.9	99.3

* Residual esters, freed from unsaponifiable.

Without entering into the full details of the calculations used in evaluating the complicated series of data in Tables III–VI, the general principles which were employed may be briefly enumerated:

(i) *Esters of unsaturated C_{18} acids.* The unavoidable assumption has been made that in each case the unsaturated esters of the C_{18} acids distil throughout in the same proportions (*e.g.*, in milk fat 1, these esters have been taken throughout as of i.v. 101.2; in milk fat 4, of i.v. 94.1 *etc.*). This assumption, although strictly not correct (di-ethenoid esters distilling slightly more readily than mono-ethenoid esters in the lower-boiling ranges), is not very far from the truth. In previous papers these acids have finally been computed as mixtures of oleic and “linoleic” acids. The occurrence of ordinary linoleic acid in milk fats has recently been discussed at some length [Bosworth and Brown, 1933; Eckstein, 1933; Green and Hilditch, 1935]. The result of these investigations leaves us of the opinion that practically the whole of the unsaturated C_{18} acids are either mono- or di-ethenoid. Consequently, for the purpose of these analyses, we feel justified in making no alteration in the presentation of the results, except that it is more appropriate to term the diethenoid acid or acids “octadecadienoic” and to avoid the use of the specific connotation “linoleic”.

(ii) *C_{20} acids present in normal cow milk fats.* The final fractions of the “solid” esters have almost always possessed equivalents slightly higher than correspond with esters of C_{18} acids and fractional percentages “as arachidic acid” have consequently been recorded. Whether this is in reality arachidic acid, $C_{20}H_{40}O_2$, or traces of still higher saturated acids, we cannot say; it might be a mono-ethenoid acid or acids, but the more unsaturated arachidonic acid should not appear in the “solid” acids. The latter acid or acids, in the small amounts (0.2–0.3 %) recorded by Brown and Sutton [1931], has, in our previous butter fat analyses, been left in the residue from the primary fractionation of the liquid esters [*cf.* Hilditch, 1934, p. 784] and it has not been necessary to include it in our calculations. Curiously (and somewhat unfortunately in view of the fact that some of the present fats are further complicated by the presence of ingested unsaturated C_{20} and C_{22} acids), all the milk fats (including the controls) now studied contain definitely more of these unsaturated C_{20} or C_{22} acids than has previously been observed: so much so, that the equivalents not only of the residual “liquid” esters but also of the fraction or fractions immediately preceding are definitely higher than that of C_{18} esters (*ca.* 295.5). (*Cf.*, for example, Table VI, milk fat no. 2 (control), primary fractions L 7, L 8 and residue, equivalents respectively 297.6, 298.7 and 301.8.) A similar state of affairs has been observed in the case of some pig depot fats [Banks and Hilditch, 1932; Dean and Hilditch, 1933, 2], and the method of approximate calculation used for these pig fat analyses has also been employed in the present work. Apparently the unsaturated C_{20-22} acids in the present group of milk fats amount to nearly 1 % of the component acids, apart from any of these acids derived from fatty oils added to the diet.

(iii) *Rape oil diet.* In this instance abnormally high equivalents characterised the final fractions (S 7, S 8, S 9, L 8) from both “solid” and “liquid” esters. Examination of the saturated esters present in S 7–9 (obtained after oxidation with permanganate in acetone, *v. infra*, Table VIII) led to the conclusion that methyl erucate was present in the unsaturated portion of these fractions, and allowance for this was therefore made in the calculations.

(iv) *Cod liver oil diet.* Nothing very abnormal was noticed in the fractions obtained by the distillation of the “solid” esters from milk fats 5 and 6, but the later fractions from the “liquid” esters (no. 5, L 5–8 and no. 6, L 5–11) were marked by equivalents which rose well over that of C_{18} esters and, at the same

time, by increases in I.V. as compared with that of the C_{18} esters. Clearly, unsaturated acids of the C_{20-22} series were present in much greater quantity than in the other fats and had passed into the milk fat from the ingested cod liver oil.

With the modifications referred to above, the composition of the various groups of acids (steam-volatile, "solid", "liquid") derived from each milk fat was calculated from the data in Tables III-VI by the usual methods, and the final results are given, in the form of weight and also of molar percentages, in Table VII.

Table VII. *Component acids of cow milk fats nos. 1-6.*

Milk fat no.	...	1	2	3	4	5	6
Oil fed to cow	...	Control	Control	Linseed	Rape	Cod liver	Cod liver
Acid		%	%	%	%	%	%
(i) Weight percentages.							
Butyric		4.4	4.4	4.2	3.6	2.0	2.1
<i>n</i> -Hexanoic		2.2	1.4	2.0	1.6	0.6	0.9
<i>n</i> -Octanoic		2.4	1.8	1.3	1.3	0.9	0.5
<i>n</i> -Decanoic		3.8	1.9	2.3	1.6	1.3	1.2
Lauric		4.4	3.1	3.1	2.8	4.1	3.1
Myristic		10.9	9.3	8.4	8.6	6.7	6.4
Palmitic		23.1	27.5	21.8	18.1	23.2	22.7
Stearic		12.6	12.2	9.9	13.8	8.2	6.7
As Arachidic		0.7	1.0	0.6	0.5	0.6	0.6
Oleic		28.9	33.1	39.3	39.7	42.8	43.3
As Octadecadienoic		5.6	3.1	5.9	3.7	4.5	4.8
As C_{20-22} unsaturated		1.0	1.2	1.2	1.0	5.1	7.7
As Erucic		—	—	—	3.7	—	—
(ii) Molar percentages.							
Butyric		11.5	11.7	11.2	9.9	5.7	6.1
<i>n</i> -Hexanoic		4.3	2.8	4.1	3.4	1.2	2.0
<i>n</i> -Octanoic		3.7	2.9	2.1	2.3	1.6	0.8
<i>n</i> -Decanoic		5.0	2.6	3.1	2.2	1.9	1.8
Lauric		5.0	3.6	3.6	3.4	5.1	3.9
Myristic		10.8	9.5	8.6	9.1	7.4	7.1
Palmitic		20.5	25.2	20.0	17.0	22.8	22.4
Stearic		10.1	10.0	8.2	11.7	7.3	6.0
As Arachidic		0.5	0.8	0.4	0.4	0.5	0.5
Oleic		23.3	27.4	32.8	34.0	38.3	38.8
As Octadecadienoic		4.5	2.6	5.0	3.2	4.1	4.4
As C_{20-22} unsaturated		0.8	0.9	0.9	0.8	4.1	6.2
As Erucic		—	—	—	2.6	—	—

The general results of the investigation are readily revealed by inspection of Table VII: ingestion of cod liver oil has resulted in each case in reduction of the proportion of butyric-lauric acids in the milk fats to about half of the amount present in normal milk fats, whilst the milk fat component acids include 5-8 % (wt.) of unsaturated C_{20-22} acids, as compared with about 1 % in cases in which the cows received no cod liver oil. On the other hand, ingestion of neither linseed nor rape oil led to any comparable effect (although certain minor effects, almost within the range of experimental error, might possibly be attributed to the rape oil); consequently neither high mean unsaturation of the ingested (linseed) oil, nor the presence therein (rape) of high proportions of the mono-ethenoic C_{22} (erucic) acid, produces the results specific to cod liver oil.

Before discussing the data in further detail, it is desirable at this point to record the results of some experiments which were made in order to ascertain whether palmitoleic acid (which also occurs in quantity in cod liver oil) had passed, like the fish oil C_{20-22} acids, into the milk fat to any extent. If so, this

acid would be present in "liquid" ester fractions immediately preceding the approximately individual C_{18} ester fractions. Some of these fractions were therefore submitted to permanganate-acetone oxidation in order to isolate the saturated esters present and determine the equivalent of the latter. At the same time, similar oxidations were carried out on certain lower fractions the mean equivalent of which indicated a high concentration of esters of C_{14} acids.

The results of these experiments (and also of oxidations of certain "solid" ester fractions undertaken to ascertain whether erucic or gadoleic acids were present in specific cases) are given in Table VIII.

Table VIII. *Saturated esters present in certain ester fractions (Tables V-VI).*

Milk fat no.	Oil fed to cow	Ester fractions				Saturated esters
		No.	g.	Sap. eq.	I.V.	Sap. eq.
		(i) "Liquid" ester fractions.				
4	Rape	L 14	13.81	244.2	21.9	249.0
4	"	L 15	15.70	279.5	66.5	263.4
4	"	L 2	23.78	289.7	87.9	270.4
5	Cod liver	L 13	10.17	250.6	42.1	249.6
5	"	L 14	10.31	283.6	80.7	267.9
5	"	L 2	16.08	291.4	90.4	265.3
6	"	L 15, L 16, L 17	18.18	276.1	71.4	261.8
6	"	L 2, L 3	24.58	290.1	91.4	290.2
(ii) "Solid" ester fractions.						
4	Rape	S 7, S 8	14.86	303.1	22.3	301.2
4	"	S 9	6.87	330.8	37.2	327.2
5	Cod liver	S 7	10.56	304.6	39.6	303.2
6	"	S 8	14.98	298.4	50.1	297.4
6	"	S 9	10.39	315.2	62.8	320.6

The data in Table VIII for fractions of equivalent 280-290 suggest that, in all three milk fats, the equivalent of the unsaturated esters is somewhat lower than 295.5 (methyl esters of C_{18} acids)—in other words, that small amounts of palmitoleic ester are also present. (It should be pointed out that the proportion of saturated ester in fractions such as L 2, with I.V. 85-90, is very small and its isolation and the accurate determination of its equivalent a matter of some difficulty.) In fractions 4, L 14, and 5, L 13, with mean equivalents of 244.2 and 250.6, the proportion of saturated ester was fairly large, and the interesting feature emerges that the mean equivalent of the unsaturated esters here is also low (240-250), indicating the presence of detectable amounts of methyl tetradecenoate. Evidently therefore the small proportions of decenoic and tetradecenoic acids observed in milk fats by Grün *et al.* [1922; 1924] and by Bosworth and Brown [1933] have appeared, as would be expected, in the lower-boiling fractions of the refractionated first primary fraction of the "liquid" esters. We know however that methyl oleate also passes in small quantities into ester fractions of the average equivalents in question, and it is therefore quite beyond the capacity of the fractionation procedure to attempt any precise estimate of the amounts of these minor unsaturated components. Yet, if we assume for the moment that fractions L 11 and L 12 contain only methyl decenoate as unsaturated ester, and that succeeding fractions contain only either tetradecenoate, or the latter with hexadecenoate, or hexadecenoate with C_{18} unsaturated esters, the results so calculated (while probably exaggerating the

amounts of these lower unsaturated acids) will afford at all events a comparison of the hexadecenoic (palmitoleic) contents of milk fats when cod liver oil has or has not been included in the diet. These figures also show the relatively small effect which allowance for these minor components has upon the estimated amounts of the major component acids. Table IX gives the result of such alternative computations for milk fats 4 (rape oil) and 5 (cod liver oil). (Unfortunately the L 11 *etc.* fractions in the two control fats 1 and 2 were not collected in quantities suitable for oxidation experiments, but, since palmitoleic acid is not present in rape oil, the use of milk fat 4 for the present purpose presents no objection.) In Table IX the weight percentages of the component acids are calculated (*a*) without allowance for lower unsaturated acids (as in Table VII) and (*b*) allowing as described above for decenoic, tetra- and hexadecenoic acids in the specified ester fractions of the L 11 *etc.* groups.

Table IX. *Alternative calculations of component acids in milk fats 4 and 5 (% wt.).*

Milk fat no.	...	4		5	
Oil fed to cow	...	Rape		Cod liver	
Acid		(a) %	(b) %	(a) %	(b) %
Butyric		3.6	3.6	2.0	2.0
<i>n</i> -Hexanoic		1.6	1.6	0.6	0.6
<i>n</i> -Octanoic		1.3	1.0	0.9	0.6
<i>n</i> -Decanoic		1.6	1.5	1.3	1.3
Lauric		2.8	1.8	4.1	0.9
Myristic		8.6	8.1	6.7	8.4
Palmitic		18.1	20.3	23.2	25.4
Stearic		13.8	13.8	8.2	8.2
As Arachidic		0.5	0.5	0.6	0.6
As Decenoic		—	0.2	—	0.5
As Tetradecenoic		—	1.3	—	1.4
As Hexadecenoic		—	2.4	—	3.3
As Oleic		39.7	36.0	42.8	37.8
As Octadecadienoic		3.7	3.3	4.5	3.9
As C ₂₀₋₂₂ unsaturated		1.0	1.0	5.1	5.1
As Erucic		3.7	3.6	—	—

These figures have several points of interest. They show that, with the extreme assumption that unsaturation in the lower ester fractions is due entirely to unsaturated acids of lower molecular weight than oleic, the only notable difference is a drop of about 4% in the estimated content of oleic acid and a rise of about 2% in that of palmitic acid; the rest of the acids are affected either not at all, or to no significant degree. The actual amounts of decenoic and tetradecenoic acids suggested by the calculations accord well with the minimum amounts which Bosworth and Brown [1933] concluded must be present (0.18% decenoic and 0.87% tetradecenoic). If the computation is accepted for these acids, it would be illogical not to apply it to hexadecenoic (palmitoleic) acid, but Bosworth and Brown were unable to confirm Grün's earlier statement that this acid also occurred in butter fat.

We do not consider that the ester-fractionation procedure, as we have used it, can be rightly pushed to this extreme of arithmetical deduction, which in the ordinary way we should not adopt; the case might be otherwise if much larger amounts of fatty acids were employed and it was consequently feasible to perform a number of refractionations of the lower-boiling "liquid" esters. Our

primary purpose in this discussion has been to discover whether palmitoleic acid (present to the extent of about 15% in cod liver oil) has, like the highly unsaturated C_{20} and C_{22} acids of that oil, passed into the milk fats of cows receiving the oil and, whatever may be the limits of accuracy of the alternative calculation adopted, it is clear that the palmitoleic acid content of milk fat 5 is not markedly greater than that of milk fat 4, whereas the unsaturated C_{20-22} acids of fat 5 show a fivefold increase over those of fat 4.

GENERAL DISCUSSION.

A general comparison of the fatty acid compositions in Table VII may now be made.

"Control" milk fats 1 and 2. These are of the usual type for winter-fed cattle, with comparatively low oleic and octadecadienoic contents and the usual proportions of butyric *etc.* acids. The palmitic acid content is rather different in the two fats, that of fat 1 being the more normal, although the higher figure of fat 2 is not extreme; the palmitic and oleic acid contents of normal butters may vary inversely over a range of 5-6%, perhaps depending upon the age of the cow [Dean and Hilditch, 1933, 1].

"Linseed oil" milk fat 3. The only marked effect of the ingestion of linseed oil is an increase in the content of oleic acid. Octadecadienoic (or other polyethenoid C_{18}) acid is present in only slightly, if at all, increased proportion compared with the control fats or other normal milk fats. Although the unsaturated C_{18} acids yielded on oxidation very small amounts (1-2%) of tetrahydroxystearic acid, m.p. 155°, they failed to give either the petroleum-insoluble tetrabromostearic acid or the ether-insoluble hexabromostearic acid characteristic of the linoleic and linolenic acids which are present in large quantity in linseed oil. Thus there is no evidence of passage of the highly unsaturated linseed oil acids into the milk, the lower saturated acids of the milk fat are unaffected and the only notable effect is the increase mentioned in oleic acid. The stearic acid content is a little lower than in the control fats.

"Rape oil" milk fat 4. Here again the chief effect is an increase (quantitatively similar to that with linseed oil) in the proportion of oleic acid. The lower saturated acids are perhaps slightly below the normal amount, as is also palmitic acid, whilst the stearic acid is slightly increased. The reduced proportions mentioned may however be merely the additive result of infiltration of the rape oil acids which, as shown by the small proportion of erucic acid present, have passed into the milk to a small extent. Nevertheless, the large proportion (nearly 50%) of erucic (C_{22}) acid combined in the rape oil has not materially affected the composition of the milk fat, which is very similar to that in the linseed oil feeding experiment.

"Cod liver oil" milk fats 5 and 6. The chief points to be observed in these fats are: (i) the enormous reduction in the lower saturated acids, (ii) a proportionately similar increase in oleic acid, (iii) no great difference from the normal in the amount of octadecadienoic (or other polyethenoid C_{18}) acid, (iv) the presence of about 5% of C_{20-22} unsaturated acids instead of the 1% of these acids found in fats 1-4 and (v) the apparent absence of any palmitoleic (hexadecenoic) acid, although this is almost as prominent a component of the cod liver oil acids as the C_{20-22} acids. These features are seen clearly in Table X, in which the differences between the molar percentages of the component acids are given for the respective mean values of the "control" milk fats 1 and 2 and the "cod liver oil" milk fats 5 and 6.

Table X. *Differences in component acids of milk fats from cows on "control" and "cod liver oil" diets.*

(Mean values of fats 1 and 2, 5 and 6 respectively.)

Acid	"Control" 1 and 2 % (mol.)	"Cod liver oil" 5 and 6 % (mol.)	Difference	Percentage alteration
Butyric	11.6	5.9	- 5.7	- 49
<i>n</i> -Hexanoic	3.6	1.6	- 2.0	- 55
<i>n</i> -Octanoic	3.3	1.2	- 2.1	- 64
<i>n</i> -Decanoic	3.8	1.9	- 1.9	- 50
Lauric	4.3	4.5	+ 0.2	—
Myristic	10.2	7.2	- 3.0	- 29
Palmitic	22.8	22.6	- 0.2	—
Stearic	10.0	6.7	- 3.3	- 33
As Arachidic	0.6	0.5	- 0.1	—
Oleic	25.4	38.5	+ 13.1	+ 52
As Octadecadienoic	3.6	4.2	+ 0.6	—
C ₂₀₋₂₂ unsaturated	0.8	5.2	+ 4.4	+ 550

It should be noted that, even if the increase in C₂₀₋₂₂ acids were interpreted as the result of infiltration of cod liver oil glycerides as a whole (the absence of increase in palmitoleic acid of course shows this is not the case), this would correspond with the admixture of at most 20 % of cod liver oil in the milk fat; and would by no means account for the disproportionate diminution in the saturated acids (other than palmitic). The analytical data disclose definitely an influence on the milk fat metabolism quite beyond the limits of any additive or dilution effect.

The palmitic acid figures in Table VII are also interesting. Cod liver oil has not affected the normal content of this acid in the milk fat, linseed oil has perhaps caused a slight fall, and rape oil has produced a definite fall to below any figure which we have previously observed in a butter fat. Rape oil contains only 2 % of palmitic acid, linseed oil about 6 % and cod liver oil 15-18 %; and the above data might be interpreted in favour of the view that a part, at least, of animal palmitic acid (which forms about 25 % of all depot and milk fats yet examined) is derived by assimilation of preformed vegetable palmitic acid.

Fully-saturated glyceride content of milk fats 1-6. Since the content of fully-saturated glycerides in milk fats and in cow, sheep and pig depot fats has a quite different relation to the total proportion of saturated to unsaturated acids in the whole fats from that which obtains in seed fats or fish oils, and since this relation, when plotted graphically, gives a series of points which, for the group of fats mentioned, lie about a smooth curve, it was felt desirable to determine the content and mean equivalent of the fully-saturated glycerides in each fat of the present series, in order to ascertain whether the cod liver oil diet had also produced an anomalous effect on the glyceride structure of the milk fats. The experimental data obtained are summarised in Table XI and have also been plotted (Fig. 1) together with all the data obtained previously for cow milk fats, depot fats and pig depot fats.

Insufficient material was available for detailed analyses of the component acids in the fully-saturated glycerides, but their saponification equivalents point to a similar composition in each of the fats 1-4; Hilditch and Sleightholme [1931] observed close similarity in the compositions of the fully-saturated glycerides in milk fats (of varying total unsaturation) from cows fed in summer or winter conditions. The "cod liver oil" milk fats 5 and 6 however evidently contain fully-saturated glycerides which have less of the lower saturated acids and more

Table XI. *Fully-saturated glyceride contents of milk fats 1-6.*

Milk fat no.	Oil fed to cow	Whole fat			Fully-saturated glycerides		
		I.v.	Sap. eq.	Saturated acids % (mol.)	% (wt.)	% (mol.)	Sap. eq.
1	Control	34.5	239.7	71.4	37.1	40.4	221.0
2	"	34.9	244.2	69.1	31.1	34.2	224.5
3	Linseed	46.0	249.0	61.3	22.2	24.8	222.4
4	Rape	44.5	251.2	59.4	22.4	25.3	225.0
5	Cod liver	51.7	264.2	53.5	15.3	17.2	235.7
6	"	54.1	266.0	50.6	12.8	14.6	235.1

Fig. 1. Data from previous work: B, cow milk fats; U, Buffalo milk fat; S, sheep depot fat; O, ox depot fat; P, pig depot fat. Data from present work: 1, 2, cow milk fat (control); 3, cow milk fat (linseed oil); 4, cow milk fat (rape oil); 5, 6, cow milk fat (cod liver oil).

palmitic (and probably myristic), as is not unnatural in view of the deficiency of the former acids in the fats as a whole.

At the same time, as will be seen from Fig. 1, the proportion of fully-saturated glycerides in these "cod liver oil" milk fats has exactly the same relation to the total saturated acids in the fat as in the remainder of the butter fat and the corresponding depot fat series. The whole group lies about a regular curve which would intersect the horizontal axis at a point corresponding with about 30 % of total saturated acids (*i.e.* the approximate content of palmitic (with myristic) acid characteristic of all animal depot fats). It forms a series quite distinct from most vegetable and some other animal fats, in which the glycerides are assembled on "evenly distributed" lines, having negligible contents of fully-saturated glycerides unless their total saturated acids amount to more than about 60 %

of the whole, and thereafter giving a relation expressed by the broken line in Fig. 1. These special relationships in the milk and depot fats of the animals concerned are explicable [cf. Hilditch and Sleightholme, 1931; Banks and Hilditch, 1931; 1932; Hilditch and Stainsby, 1935] on the hypothesis that the unusual amounts of fully-saturated components are the result of formation of stearic glycerides or glycerides of lower saturated acids from oleic glycerides (which would have existed primarily mainly in the form of "evenly distributed" mixed palmito-oleo-glycerides). In spite of the marked differences in the relative amounts of their component acids, the milk fats from cows receiving cod liver oil in the diet still conform to this typical relationship with regard to their fully-saturated components. Indeed their positions on the curve in Fig. 1 emphasise the fact that, from this aspect of glyceride structure, there is complete uniformity in type between cow milk fats and ox, sheep or pig depot fats.

The present work has enabled us to define more closely the factor in cod liver oil which is responsible for the marked alteration in the milk fat. Previous investigators [Golding, 1928; McCay and Maynard, 1935] have agreed that the triglycerides of the oil contained the injurious or active component; our analyses show that, of the specific cod liver oil acids not present in the other oils used in the feeding trials, palmitoleic acid has not passed appreciably into the milk fat, whereas the highly-unsaturated C_{20-22} acids appear therein in some quantity. There is thus evidence of specific retention by the mammary tissues of certain fatty acids and not others: whilst these highly-unsaturated C_{20-22} acids are comparatively readily absorbed, linolenic and (very largely) linoleic acids of linseed oil cannot be detected, although erucic acid, $C_{22}H_{42}O_2$, of rape oil is probably accepted to a minor extent. Lovern [1932; 1934] has found evidence for similar specificity in fatty acid absorption in the adipose tissues of fish and marine mammalia. Retention of C_{20-22} glycerides of cod liver oil by the mammary gland does not of course imply in itself that this is the causative factor of the abnormal milk fat composition; nevertheless, we consider that, if the above general hypothesis of the elaboration of the characteristic milk fat structure may be provisionally accepted, there is a simple and logical reason why this should in fact be the case.

The production of stearic from preformed oleic glycerides (in depot fats and to some extent in milk fats) involves simple reduction or hydrogenation; that of the lower saturated glycerides of the milk fats is a much more complex process, the mechanism of which cannot yet be surmised but obviously would involve oxidation as well as reduction. It is well known that the highly unsaturated C_{20} and C_{22} acids of the "clupanodonic" group (or their esters) are avid acceptors of both hydrogen and oxygen; for example, they absorb atmospheric oxygen, or undergo catalytic hydrogenation, far more rapidly than monoethenoid fatty acids or esters. Their presence in a biological oxidation-reduction system, such as must be conceived necessary for the production of glycerides of butyric-decanoic and stearic acids, where otherwise glycerides of oleic acid would appear, would therefore be expected to retard this part of the metabolic process. It is unnecessary to postulate that in such circumstances alternative products of metabolism should be observed. Selective adsorption alone by the enzyme system concerned of the highly-unsaturated glycerides would disturb and hinder its normal action. In other words, the function of the highly-unsaturated glycerides would be that of a "catalyst poison", entirely analogous, for example, to the prevention of atmospheric oxidation of an unsaturated fat by an "anti-oxidant", or to the retardation of the union of ethylene and hydrogen at a nickel or other catalytic surface in presence of carbon monoxide, when the

retarding agent may be chemically unaltered, or much more slowly altered, by concurrent catalytic action upon itself.

This conception of the effect of the presence of highly-unsaturated glycerides in the mammary gland accounts for the nature of the component acids observed in milk fats 5 and 6, and also for the fact that the general relationship of fully-saturated glycerides to total saturated acids therein still remains unaltered as compared with other milk and depot fats. Table X shows that, after allowing for the influx of C_{20-22} acids, all that has happened is a substantial increase above normal in the proportion of oleic acid while the formation of butyric-decanoic acids has been greatly retarded and also, to a smaller extent, that of stearic and myristic acids. The explanation offered is also in harmony with the results of the other feeding experiments. With linseed oil, which also possesses high mean unsaturation, no evidence was found of absorption of tri- and di-ethenoid acids into the milk fat, and it therefore seems that the mammary tissue is not permeable to these particular glycerides. The metabolic processes are consequently undisturbed except that the abnormal intake of oleic acid (also present in the linseed oil) is reflected in a higher percentage of that acid at the expense of slight reductions in stearic and one or two of the other saturated acids. A similar state of affairs is seen in the rape oil experiment, although erucic glycerides have passed to some extent into the milk fat; but erucic is a monoethenoid acid and would have no more intense effect than oleic as regards selective adsorption by the metabolic enzymes.

Similarly, shark liver oil which, according to McCay and Maynard [1935], is innocuous, is an Elasmobranch oil; the glycerides of these contain unsaturated acids of the C_{16} , C_{18} , C_{20} , C_{22} and C_{24} series which however are almost wholly monoethenoid in contrast to the C_{20} and C_{22} acids of Teleostean fish [Hilditch and Houlbrooke, 1928]: so that, on the present hypothesis, the results observed are those which would be expected. Salmon oil, on the other hand, contains highly-unsaturated C_{20-22} acids, but McCay and Maynard found no abnormal effect in its ingestion, this observation being the only one not in harmony with our suggested explanation. McCay and Maynard's studies were focussed on changes in the muscular tissues, another instance in which oxidation-reduction processes are involved, and the influence of highly-unsaturated C_{20-22} glycerides here may perhaps be similar in character to that suggested in the mammary gland.

Finally, the rapidity with which the milk fat (and its content in the milk) reverts to normal when the addition of cod liver oil to the diet is abandoned [Golding, 1928; Dann *et al.*, 1935] is wholly in accord with the view that the enzyme system concerned in producing the characteristic butter-glycerides has been temporarily and partially put out of action owing to selective adsorption of the highly-unsaturated C_{20-22} glycerides.

SUMMARY.

Detailed studies were made of the component acids in milk fats from cows receiving (a) a normal winter ration and (b) the same ration with additions of cod liver, linseed or rape oils. Cod liver oil had a marked effect, the lower saturated acids of the milk fats being reduced to half the normal content, while the proportion of oleic acid was much increased, and a certain amount (5-7 %) of highly-unsaturated C_{20-22} acids was present. Polyethenoid unsaturation in the C_{18} acids was not more than normal and palmitoleic acid had not been appreciably absorbed from the cod liver oil. The effect of linseed oil was merely to increase the proportion of oleic acid; linolenic acid was not detected in the milk

fat and linoleic acid only in very small amounts, the mean unsaturation of the C_{18} acids and the amount of lower saturated acids were normal. Rape oil had a similar effect to linseed oil, but small amounts of erucic glycerides were present in the milk fats. The relationship between fully-saturated glyceride content and proportion of total saturated acids in the milk fats was normal (*i.e.* of the type characteristic of cow milk and depot fats) in all cases.

The investigation shows that some of the highly-unsaturated C_{20-22} glycerides of cod liver oil (but not its palmitoleic acid or the linolenic and linoleic acids of linseed oil) pass into the milk fats; and it is suggested that selective adsorption of these highly-unsaturated compounds by the enzymes responsible for the elaboration of typical cow milk fats retards their normal function and causes the observed effects. This hypothesis is a corollary to that previously suggested to explain the abnormal production of fully-saturated glycerides in milk and certain animal depot fats as compared with vegetable fats and some other animal depot fats, and is also in harmony with the results obtained on adding linseed, rape or shark liver oils to the diet of lactating cows.

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