XXXIV. THE COMPOSITION OF THE MIXED FATTY ACIDS PRESENT IN THE GLYCERIDES OF COD-LIVER AND CERTAIN OTHER FISH-LIVER OILS.

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IN the course of an investigation into the production and medicinal qualities of cod-liver oil obtained in various parts of the British Empire, undertaken in conjunction with Prof. J. C. Drummond of University College, London, on behalf of the Empire Marketing Board and the Medical Research Council, information of a quantitative nature has been collected as regards the composition of the mixed fatty acids present in specimens of Newfoundland, Scottish and Norwegian cod-liver oil, and also of the mixed fatty acids combined in the liver oils of coalfish (saith), hake, ling, skate and dogfish taken from off the north-eastern or north-western coasts of Scotland. The latter group of oils was from fish taken during July–September 1928 during cruises of the Scottish Fishery Board's research steamer "Explorer," and we would express our cordial thanks to the Board, and to Dr A. Bowman of their Aberdeen Marine Biological Station, for the facilities given to one of us on board the "Explorer" and for the particulars of feeding, etc., supplied in respect of the various fish.

Our object has been to collect data of an approximately quantitative kind with reference to the respective proportions of myristic, palmitic, and stearic acids, and of unsaturated acids containing respectively 16, 18, 20 and 22 carbon atoms in the molecule. The analytical methods employed do not, unfortunately, permit of the simultaneous determination of the individual unsaturated acids present, in view of the complex mixtures of these (especially in the C_{20} and C_{22} series) which are generally present in these oils. We have to content ourselves, so far as the present records are concerned, with an approximate expression of the average degree of unsaturation of each group¹, accompanied where possible by qualitative information as to the nature of the components.

¹ This is conveniently given in terms of the average number of gram-atoms of hydrogen lacking as compared with the corresponding saturated acid: thus (–2.0 H) indicates the presence of acids containing only one ethylene linkage, i.e. acids of the monoethylenic (oleic) series.

Whilst, so far as we know, quantitative figures of this kind have not been previously published for any of the liver oils in question, qualitative information has appeared from time to time, the earliest definite contribution in the case of cod-liver oil being that of Bull [1906], who isolated palmitoleic ($\Delta^9:10$ hexadecenoic acid [Armstrong and Hilditch, 1925, 2]) from cod-liver oil; references to the occurrence of highly unsaturated ("clupanodonic") acids of the C_{20} and C_{22} series in cod-liver oil were first made by Tsujimoto [1913] and by Marcusson and Botger [1914]. Other data on the fatty acids of the liver oils now under consideration may be summarised as follows.

Cod-liver oil. Palmitoleic acid [Schmidt-Nielsen, 1922], gadoleic acid [Bull, 1906], "clupanodonic" and "jecoric" acids [Fahrion, 1917]; highly unsaturated acids [Toyama and Tsuchiya, 1925]; qualitative data for the liver oil of *Gadus macrocephalus* [Toyama, 1926]; some mixed glycerides of cod-liver oil [Suzuki and Masuda, 1927, 1928]; relation of composition to vitamin content [Holmes, 1924]; percentage of highly unsaturated acids given (from insoluble polybromides) by various workers as 10–15 %.

Coalfish (saith)-liver oil. Percentage of highly unsaturated acids (from ether-insoluble polybromides) about 17 % [Lewkowitsch-Warburton, 1922].

Ling-liver oil. Percentage of highly unsaturated acids from ether-insoluble polybromides, about 14 % [Lewkowitsch-Warburton, 1922].

Dogfish-liver oil. Unsaponifiable content has been found between the limits of 4 and 32.9 %, both squalene and cholesterol being frequently present; the iodine value of the mixed fatty acids is usually lower than in the preceding cases [cf. Chapman, 1918; Lexow, 1922; Holmes and Pigott, 1925].

The sources of the oils which we have examined are given in Table I, together with additional information in some cases as to the food observed in the fish, the sex or condition, etc., whilst the general analytical characteristics of the oils, together with their comparative vitamin A potencies as indicated by the antimony trichloride colour test, are given in Table II.

In order to secure comparative data for the whole oils, the investigation was conducted in each case on the "crude" (i.e. not refined or chilled) material obtained by rendering the livers for a short time with open steam in a small experimental digester.

The cod- and saith-liver oils were all pale-coloured and of fair to high vitamin potency. The remainder of the specimens were much darker in colour: the ling oil and one of the hake oils were extremely rich in vitamin, but in other samples of hake-liver oil examined (four in all) the vitamin potency was of a comparatively low order, judged by the colorimetric test. The vitamin content of the skate and dogfish oils was not high, but these liver oils are of special interest from the standpoint of their component fatty acids or their content of unsaponifiable matter.

Table I.

Subdivision TELEOSTOMI. Family GADIDAE.							
Species	Fish		Source, etc.				
<i>Gadus morrhua</i> , L.	Cod	Newfoundland	Bay de Verd, 1928 season, cod feeding on squid.				
" "	Cod	Scottish	Moray Firth, end of March, 1928 (after spawning, fish mainly "spent").				
" "	Cod	Norwegian	Lofoten, 1929 season.				
Species	Fish	Position	Depth m.	Date	No.	Size, etc.	Stomach contents
<i>Gadus virens</i>	Coalfish (saith) I	55° 24' N., 4° 59' W.	73	30. viii. 28	82	60-90 cm., mostly adult	Fish remains (2), herring (6), <i>Meganyctiphanes</i> (14), small <i>Squalus acanthias</i> (1), empty (59).
" "	Coalfish (saith) II	56° 54' N., 7° 10' W.	148	22. viii. 28	33	42-75 cm.	Fish remains, mostly herring (21), <i>Gadus esmarkii</i> (1), <i>Gadus merlangus</i> (1), empty (10).
<i>Merluccius merluccius</i>	Hake I	58° 03' N., 6° 13' W.	137	17. viii. 28	25	48-104 cm., mostly adult	All empty
" "	Hake II	56° 54' N., 7° 10' W.	148	22. viii. 28	46	38-78 cm., mostly adult	All empty
" "	Hake III	56° 02' N., 7° 03' W.	78	23. viii. 28		adult	
" "	Hake III	55° 34' N., 5° 24½' W.	88	12. ix. 28	16	59-100 cm., adult (2 males, 14 females)	All empty
<i>Molva molva</i>	Ling	58° 03' N., 6° 12' W.	137	17. viii. 28	1	141 cm., spent female	Empty.
Subdivision ELASMOBRANCHII. Family RAJIDAE.							
<i>Raja maculata</i>	Skate	58° 17' N., 6° 11' W.	15-18	13. viii. 28	17 males 23 females	23-45 cm., 22-53 cm., developing reproductive organs	Chiefly sand-eels (<i>Ammodytes</i>), a few with Annelids and <i>Portunus</i> sp.
Family SQUALIDAE.							
<i>Squalus acanthias</i>	Dogfish	58° 29' N., 7° 35' W.	130	16. viii. 28	154	47-83 cm., developing reproductive organs	Fish remains, mostly herring (110), <i>Moschites</i> (43), empty (30)

Table II. General analytical characteristics of the liver oils.

Fish-liver oil	Saponification equivalent	Iodine value	Free acidity (as % oleic acid)	Unsaponifiable %	Colour of oil*		Vitamin A (colorimetric) blue units
					Yellow	Red	
Cod (Newfoundland)	301.1	163.0	0.46	0.87	3.2	0.1	9.0
Cod (Scottish)	305.1	177.1	0.45	0.91	3.3	0.1	11.0
Cod (Norwegian)	302.6	161.8	0.27	0.63	1.7	0.0	3.6
Coalfish (saith) I	302.2	167.1	0.05	0.80	4.8	0.6	9.5
Coalfish (saith) II	304.8	167.5	0.07	0.60	4.9	0.5	7.8
Hake I	302.7	150.6	0.14	0.91	12.3	0.8	21.7
Hake II	304.2	158.5	0.14	1.65	6.4	0.9	3.4
Hake III	301.3	149.2	0.12	1.21	16.0	2.3	ca. 8
Ling	300.0	150.3	0.22	1.16	8.7	0.8	29.3
Skate	332.8	210.2	0.38	0.31	ca. 30	8.6	3.1
Dogfish	343.5	121.8	0.14	10.5	9.3	0.8	6.5

* Determined in a 1" cell by the Rosenheim-Schuster colorimeter.

GENERAL EXPERIMENTAL PROCEDURE.

Broadly speaking, the method followed in determining the composition of the mixed fatty acids was based on that described for the similar analyses of the fatty acids in the head and blubber oils of the sperm whale [Hilditch and Lovern, 1928], namely, division of the acids into two (or three) groups, mainly saturated and mainly unsaturated, by crystallisation of the less soluble lead salts from alcohol, followed by conversion of each group into methyl esters and systematic fractional distillation and refractionation of the latter under as high a vacuum as possible.

Fish-liver oil acids are amongst the most difficult to handle accurately by this method for the following reasons:

- (i) the unusual number and complexity of the fatty acids present;
- (ii) the readiness with which the very highly unsaturated acids of the C_{20} and C_{22} series oxidise on exposure to air and polymerise when heated;
- (iii) the presence of mono- (and small amounts of di-) ethylenic acids containing 16 and 14 carbon atoms (mainly palmitoleic acid, $C_{16}H_{30}O_2$);
- (iv) the sparing solubility of the lead salts of the monoethylenic acids of the C_{20} and C_{22} series (gadoleic and cetoleic acids).

We introduced a number of additional precautions as described below in order to cope with these complications so far as possible, and we believe that the figures given for acids up to and including the C_{18} series are within 1 or 2 units of the true figure, but it is possible that those for the C_{20} and C_{22} acids may not in all cases be of this order of accuracy. Having pointed out, however, that the data obtained are probably not quite of the same order of certainty as in cases of simpler mixtures of acids, we must add that they furnish an approximate guide to the general composition of the acids and serve to reveal certain similarities and dissimilarities between different kinds of marine animal oils which it has not hitherto been possible to detect.

Some details of the analytical methods used are as follows.

Lead salt separations. The mixed fatty acids (1 part) from the liver oil under examination were treated with lead acetate (0.7 part) in 95 % alcohol (10 parts), the acids and the lead acetate being separately dissolved in half of the alcohol to be employed, and the boiling solutions mixed, heated under a reflux condenser for 15–20 minutes and set aside at room temperature overnight. The separated lead salts, which were frequently somewhat amorphous owing to the presence of lead gadoleate and cetoleate, were recrystallised from a volume of 95 % alcohol equal to that used in the first place and containing 1 % of acetic acid. The flasks containing the hot alcoholic solutions of lead salts were tightly stoppered before cooling, thus preventing access of air to the cold solution; liberation of fatty acids from the lead salts (and from the original saponified oil) was always carried out in an atmosphere of carbon dioxide and all collected specimens of fatty acids or the methyl esters were stored under nitrogen.

In a few instances three groups of fatty acids were esterified:

- (i) those from the recrystallised lead salts ("solid" acids *S*);
- (ii) those from the alcohol liquors from the recrystallisation ("liquid" acids *LR*);
- (iii) those from the original alcohol liquors containing dissolved lead salts ("liquid" acids *L*); but it was observed that the acids *LR* and *L* were of much the same composition, and therefore these two groups were more usually united and dealt with as "liquid" acids *L*.

Fractional distillation. The primary distillates were usually collected in about four fractions, each of which of course was far from being a simple mixture; refractionation of each fraction, however, led to mixtures of esters which were apparently sufficiently simple for approximate estimation of their composition from consideration of the saponification equivalent and iodine value.

Myristic acid, palmitic acid, dihydroxypalmitic acid (m.p. 124–125°) and dihydroxystearic acid (m.p. 129–130°) were identified from various fractions of the Scottish cod-liver oil esters after oxidation of the corresponding soaps in alkaline solution with ice-cold permanganate, but formal identifications were not made in the cases of esters from the other oils, the fractionation data indicating similar qualitative composition in each case, and the presence of the individual acids concerned having been definitely established in the majority of the oils by previous workers.

The fractionation of the portions of esters most likely to contain highly unsaturated derivatives was in each case completed as rapidly as possible, and all ester fractions were stored under nitrogen, the iodine values being determined as soon as possible after distillation. To a large extent these precautions eliminated errors due to oxidation or polymerisation at the ordinary temperature, but in the majority of cases appreciable polymerisation of the highly unsaturated components took place during the distillations, as evidenced by the properties of the undistilled residue and progressive decline in iodine value of the higher-boiling fractions. It was therefore decided in such cases to abandon the usual method of estimation, using both iodine values and saponification equivalents, and to obtain an approximate estimate from the latter alone.

It was very noticeable that the unsaturated esters from different oils varied greatly in their tendency to undergo polymerisation during heating; some were hardly affected at all, others quite appreciably. The time and degree of exposure to heat were much the same in all cases, and it remains to be determined whether this behaviour is due to the presence of different isomeric unsaturated acids in different oils, or whether it is conditioned by some external factor such as catalytic action of the walls or internal fittings of the distillation bulb.

Calculation of the results. This was carried out on similar lines to those which we have described at length in earlier papers on this subject [cf. Hilditch and Lovern, 1928; Hilditch and Jones, 1929], with the following additions.

(i) *Composition of low-boiling fractions containing saturated esters.* Owing to the presence of unsaturated acids of the C_{16} (and C_{14}) as well as the C_{18} series, it happened not infrequently that methyl palmitate (or myristate) was accompanied by two unsaturated esters (*e.g.* methyl palmitoleate and oleate), and moreover the latter esters usually contained a certain proportion of diethylenic as well as monoethylenic compounds. This upset the usual procedure by which a mixture of two saturated and one unsaturated ester can be arithmetically analysed from its iodine value and saponification equivalent, and in such cases the ester fractions concerned (1 part) were oxidised in acetone solution (10–15 parts) with sufficient powdered potassium permanganate (2–5 parts, according to the unsaturation of the fraction) to convert all ethylenic esters present into acidic products [cf. Armstrong and Hilditch, 1925, 1]; the residual saturated esters were weighed and their composition estimated from their observed saponification equivalent.

(ii) *Composition of high-boiling fractions containing possibly polymerised C_{20} and C_{22} esters.* In order to estimate these by means of the equivalent alone, as indicated above, it was necessary to deduce the average equivalents of the unsaturated C_{20} and C_{22} esters present, since these were lower by several units than those of the corresponding saturated esters. To do this the assumption was made that mixtures of unsaturated esters of the same molecular carbon content distil together in constant proportions—an assumption which we know from experience to be very nearly, but not rigidly, correct in the case of methyl oleate and methyl linoleate. Then, commencing from the simpler mixtures of lowest boiling point (*e.g.* methyl palmitoleate with methyl oleate and linoleate) it was possible to determine the average unsaturation of the latter esters; and, by selecting suitable fractions corresponding with mixtures of unsaturated C_{18} and C_{20} esters, to repeat the calculation and determine an approximate average iodine value for the C_{20} group; and, again, similarly for the C_{22} group of esters. These approximate iodine values determine within small limits the average molecular weights (equivalents) of the corresponding groups of esters.

It is proposed to give, by way of illustration, the detailed estimations of increments of the various groups of esters in the analysis of the Newfoundland cod-liver oil, but, in order to shorten the tabulated results as much as possible, the experimental data for the remaining ten oils are tabulated and followed by a statement of the final fatty acid compositions arrived at from each analysis.

NEWFOUNDLAND COD-LIVER OIL.

Table III a. *Lead salt separation.*

	g.	%	Saponification equivalent	Iodine value
"Solid" acids <i>S</i>	74.0	21.1	266.2	59.4
"Liquid" acids <i>L</i>	276.0	78.9	286.0	173.4

Table III b. *Fractionation of methyl esters.*

Primary fractions					Refractions					
No.	g.	B.P./l mm.	Sap. eq.	I.V.	No.	g.	B.P./l mm.	Sap. eq.	I.V.	
(i) Esters of "solid" acids <i>S</i> .										
<i>S</i> 1	37.71	110–130°	261.8	19.6	{	<i>S</i> 11	17.62	100–120°	256.1	10.9
<i>S</i> 2	15.73	130–160	287.8	61.4		<i>S</i> 12	8.13	120–125	266.1	17.2
<i>S</i> 3	5.47	160–163	309.0	104.3		<i>S</i> 13	7.36	Residue	284.0	42.3
<i>S</i> 4	11.40	Residue	337.0*	145.7						
(ii) Esters of "liquid" acids <i>L</i> .										
<i>L</i> 1	55.81	122–140°	269.1	86.3	{	<i>L</i> 11	4.98	100–131°	253.4	60.4
						<i>L</i> 12	35.87	131–132	263.5	82.4
						<i>L</i> 13	10.22	Residue	285.5	115.5
<i>L</i> 2	67.90	140–150	291.5	125.5	{	<i>L</i> 21	7.25	115–142	267.7	101.5
						<i>L</i> 22	49.46	142–145	287.1	120.4
						<i>L</i> 23	6.88	Residue	293.0	200.8
<i>L</i> 3	76.36	150–170	319.1	215.1	{	<i>L</i> 31	6.48	135–156	301.6	161.4
						<i>L</i> 32	55.87	156–162	312.2	214.5
						<i>L</i> 33	9.42	Residue	333.2	259.9
<i>L</i> 4	14.33	170–181	338.6	260.7						
<i>L</i> 5	19.96	Residue	377.9*	234.8						

* Saponification equivalents of residual esters, freed from unsaponifiable material, *S* 4: 330.6, *L* 5: 332.4.Table III c. *Determination of saturated components of ester fractions by oxidation.*

No.	Weight taken g.	Saturated ester present	
		g.	Sap. eq.
<i>S</i> 11	13.39	11.59	256.6
<i>S</i> 2	11.71	5.26	275.9
<i>L</i> 12	31.70	7.87	251.3

Table III d. *Estimated composition of fatty acids.*

(i) Details of methyl ester fractions.										
No.	g.	Esters of saturated acids			Esters of unsaturated acids					Unsaponi- fiable matter
		C ₁₄	C ₁₆	C ₁₈	C ₁₄	C ₁₆	C ₁₈	C ₂₀	C ₂₂	
<i>Esters of "solid" acids S.</i>										
Refractionation of primary fraction <i>S</i> 1.										
<i>S</i> 11	17.62	8.15	7.44	—	—	2.03	—	—	—	—
<i>S</i> 12	8.13	0.86	5.79	—	—	1.48	—	—	—	—
<i>S</i> 13	7.36	—	3.25	0.48	—	—	3.63	—	—	—
	33.11	Wts.	9.01	16.48	0.48	—	3.51	3.63	—	—
	% as esters	27.2	49.8	1.4	—	10.6	11.0	—	—	—
Primary fractions.										
<i>S</i> 1	37.71	10.26	18.77	0.55	—	4.00	4.13	—	—	—
<i>S</i> 2	15.73	—	5.46	1.60	—	—	7.87	0.80	—	—
<i>S</i> 3	5.47	—	—	—	—	—	2.62	2.85	—	—
<i>S</i> 4	11.40	—	—	—	—	—	—	7.78	3.62	—
	70.31	Wts.	10.26	24.23	2.15	—	4.00	14.62	11.43	3.62
	% as esters	14.5	34.6	3.0	—	5.7	20.8	16.2	5.2	—
	% as acids	14.4	34.5	3.0	—	5.7	20.9	16.3	5.2	—

Table III *d* (continued).

No.	g.	Esters of saturated acids			Esters of unsaturated acids					Unsaponifiable matter
		C ₁₄	C ₁₆	C ₁₈	C ₁₄	C ₁₆	C ₁₈	C ₂₀	C ₂₂	
Esters of "liquid" acids L.										
Refractionation of primary fractions L 1, L 2, L 3.										
L 11	4.98	1.92	—	—	0.70	2.36	—	—	—	—
L 12	35.87	5.73	3.18	—	—	26.96	—	—	—	—
L 13	10.22	—	—	—	—	3.37	6.85	—	—	—
	51.07 Wts.	7.65	3.18	—	0.70	32.69	6.85	—	—	—
	% as esters	15.0	6.2	—	1.4	64.0	13.4	—	—	—
L 21	7.25	—	—	—	—	5.78	1.47	—	—	—
L 22	49.46	—	—	—	—	13.14	36.32	—	—	—
L 23	6.88	—	—	—	—	—	2.77	4.11	—	—
	63.59 Wts.	—	—	—	—	18.92	40.56	4.11	—	—
	% as esters	—	—	—	—	29.8	63.8	6.4	—	—
L 31	6.48	—	—	—	—	4.65	1.83	—	—	—
L 32	55.87	—	—	—	—	16.46	39.41	—	—	—
L 33	9.42	—	—	—	—	—	4.51	4.91	—	—
	71.77 Wts.	—	—	—	—	21.11	45.75	4.91	—	—
	% as esters	—	—	—	—	29.4	63.8	6.8	—	—
Primary fractions.										
L 1	55.81	8.36	3.47	—	0.76	35.73	7.49	—	—	—
L 2	67.90	—	—	—	—	20.20	43.30	4.40	—	—
L 3	76.36	—	—	—	—	—	22.46	48.67	5.23	—
L 4	14.33	—	—	—	—	—	—	3.46	10.87	—
L 5	19.96	—	—	—	—	—	—	8.52	9.04	2.40
	234.36 Wts.	8.36	3.47	—	0.76	55.93	73.25	65.05	25.14	2.40
	% as esters	3.6	1.4	—	0.3	23.9	31.3	27.8	10.7	1.0
	% as acids	3.5	1.4	—	0.3	23.8	31.3	27.8	10.8	1.1

(ii) Composition of original acids.

Acids	"Solid" acids S (21.1 %)	"Liquid" acids L (78.9 %)	Total %	Fatty acids (mean unsaturation)
Saturated:				%
Myristic	3.0	2.8	5.8	6
Palmitic	7.3	1.1	8.4	8.5
Stearic	0.6	—	0.6	0.5
Unsaturated:				
C ₁₄ group	—	0.2	0.2	Trace
C ₁₆ "	1.2	18.8	20.0	20 (-2.3 H)
C ₁₈ "	4.4	24.7	29.1	29 (-2.8 H)
C ₂₀ "	3.5	21.9	25.4	26 (-6.0 H)
C ₂₂ "	1.1	8.5	9.6	10 (-6.9 H)
Unsaponifiable	—	0.9	0.9	—

The iodine values of the higher-boiling unsaturated ester fractions fell off somewhat on each distillation, and the average unsaturation of the acids of the C₂₀ and C₂₂ groups is therefore actually higher than the values suggested in the final column. The unsaturated C₁₈ acids, however, consisted of about 60 % oleic and 40 % linoleic acids, whilst the corresponding C₁₆ group contained a little hexadecadienoic acid but was mainly composed of palmitoleic acid. A certain amount of gadoleic (monoethylenic C₂₀) acid was present, but, apparently, little cetoleic acid (C₂₂H₄₂O₂).

SCOTTISH COD-LIVER OIL.

Table IV a. *Lead salt separation.*

	g.	%	Sap. eq.	I.V.
"Solid" acids <i>S</i>	200.2	46.5	309.5 (E)	110.3 (E)
"Liquid" acids <i>L</i>	138.6	32.2	297.4 (A)	229.7 (A)
"Liquid" acids <i>LR</i>	91.3	21.3	—	203.8 (E)

(A), analyses on acids; (E), analyses on corresponding methyl esters.

Table IV b. *Fractionation of methyl esters.*

Primary fractions					Refractionations					
No.	g.	B.P./1 mm.	Sap. eq.	I.V.	No.	g.	B.P./1 mm.	Sap. eq.	I.V.	
(i) Esters of "solid" acids <i>S</i> .										
<i>S</i> 1	75.28	125–135°	270.7	40.1	{	<i>S</i> 11	7.69	120–134°	253.0	19.8
						<i>S</i> 12	17.93	134–138	259.8	21.5
						<i>S</i> 13	36.32	138–152	271.7	36.8
						<i>S</i> 14	7.56	Residue	299.3	104.0
<i>S</i> 2	49.70	135–155	297.1	102.3	{	<i>S</i> 21	13.92	145–163	285.3	66.2
						<i>S</i> 22	21.25	163–165	296.6	94.5
						<i>S</i> 23	8.85	Residue	317.8	158.8
<i>S</i> 3	57.28	155–180	329.9	189.0	{	<i>S</i> 31	8.75	165–183	318.4	158.9
						<i>S</i> 32	18.40	183	325.9	181.5
						<i>S</i> 33	15.19	183–195	338.3	203.9
<i>S</i> 4	15.11	Residue	348.7	204.5	<i>S</i> 34	9.69	Residue	340.4	206.3	
Further refractionation of <i>S</i> 22					<i>S</i> 221	3.71	160–163	289.4	76.9	
					<i>S</i> 222	5.45	163–164	291.8	83.1	
					<i>S</i> 223	7.42	Residue	302.1	110.5	
(ii) Esters of "liquid" acids <i>L</i> .										
<i>L</i> 1	26.06	108–140°	272.4	103.4	{	<i>L</i> 11	2.55	110–134°	251.9	66.6
						<i>L</i> 12	7.30	134–140	266.1	82.6
						<i>L</i> 13	2.67	140	272.9	97.2
						<i>L</i> 14	6.10	Residue	278.2	119.9
<i>L</i> 2	31.23	140–155	296.1	166.3	{	<i>L</i> 21	5.19	140–149	280.0	122.7
						<i>L</i> 22	12.86	149	284.3	138.8
						<i>L</i> 23	3.53	149–161	298.3	183.5
						<i>L</i> 24	5.47	Residue	310.1	238.1
<i>L</i> 3	25.31	155–178	—	247.7	{	<i>L</i> 31	6.36	135–163	304.3	208.4
						<i>L</i> 32	10.01	163–164	316.1	257.8
						<i>L</i> 33	4.65	Residue	329.0	269.9
<i>L</i> 4	21.63	178–188	—	—	{	<i>L</i> 41	6.12	160–187	321.0	275.4
						<i>L</i> 42	7.19	187	324.0	276.9
<i>L</i> 5	9.23	Residue	446.5*	159.2	<i>L</i> 43	4.54	Residue	337.0	272.3	

* Saponification equivalent of residual esters, freed from unsaponifiable material, *L* 5: 325.8.

Table IV *b* (continued).(iii) Esters of "liquid" acids *LR*.

No.	Primary fractions				No.	Refractionations			
	g.	B.P./1 mm.	Sap. eq.	I.V.		g.	B.P./1 mm.	Sap. eq.	I.V.
<i>LR</i> 1	14.07	110–146°	—	96.7	{ <i>LR</i> 11	5.30	116–134°	255.8	66.6
					{ <i>LR</i> 12	3.32	134–144	271.0	93.9
					{ <i>LR</i> 13	4.82	Residue	288.7	132.2
<i>LR</i> 2	21.43	146–155	278.2	140.2	{ <i>LR</i> 21	5.70	135–148	273.4	110.3
					{ <i>LR</i> 22	6.13	148–154	287.1	127.6
					{ <i>LR</i> 23	5.49	Residue	301.5	186.7
<i>LR</i> 3	16.34	155–170	—	202.4	{ <i>LR</i> 31	5.91	148–161	295.7	155.3
					{ <i>LR</i> 32	3.67	161–170	308.5	202.8
					{ <i>LR</i> 33	5.29	Residue	321.2	246.9
<i>LR</i> 4	24.92	170–178	309.9	269.8	{ <i>LR</i> 41	3.60	140–162	309.9	226.3
					{ <i>LR</i> 42	8.22	162–164	317.5	258.4
<i>LR</i> 5	10.30	Residue	351.7*	262.5	{ <i>LR</i> 43	8.70	Residue	332.8	275.6

* Saponification equivalent of residual esters, freed from unsaponifiable material, *LR* 5: 328.4.Table IV *c*. Estimated composition of fatty acids.

Acids	"Solid" acids <i>S</i> (46.5 %)	"Liquid" acids <i>L</i> (32.2 %)	"Liquid" acids <i>LR</i> (21.3 %)	Total %	Fatty acids (mean unsat- uration)
Saturated:					%
Myristic	2.5	0.5	0.5	3.5	3.5
Palmitic	9.9	0.3	Trace	10.2	10
Unsaturated:					
<i>C</i> ₁₄ group	—	0.3	0.2	0.5	0.5
<i>C</i> ₁₆ "	4.5	7.6	3.2	15.3	15.5 (–2.0 H)
<i>C</i> ₁₈ "	10.2	7.7	6.6	24.5	25 (–2.9 H)
<i>C</i> ₂₀ "	10.6	12.2	8.3	31.1	31.5 (–6.1 H)
<i>C</i> ₂₂ "	8.8	2.8	2.3	13.9	14 (–6.4 H)
Unsaponifiable	—	0.8	0.2	1.0	—

The composition is not very different from that of the Newfoundland oil, although distinctly more of the unsaturated *C*₂₀ and *C*₂₂ acids are present, at the expense of unsaturated *C*₁₆ and *C*₁₈ acids. Palmitoleic acid is practically the only unsaturated *C*₁₆ acid present, the *C*₁₈ group consists of oleic and linoleic acids in nearly equal proportions, and, as in the previous analysis, the actual mean unsaturation of the *C*₂₀ and *C*₂₂ groups is higher than the figures indicate; gadoleic and cetoleic acids are both present, probably in definitely higher proportions than in the Newfoundland oil.

NORWEGIAN COD-LIVER OIL.

Table V *a*. Lead salt separation.

	g.	%	Sap. eq.	I.V.
"Solid" acids <i>S</i>	168.8	29.6	278.5	95.7
"Liquid" acids <i>L</i>	401.8	70.4	298.5	170.2

Table V b. *Fractionation of methyl esters.*

Primary fractions					Refractionations					
No.	g.	B.P./1 mm.	Sap. eq.	I.V.	No.	g.	B.P./1 mm.	Sap. eq.	I.V.	
(i) Esters of "solid" acids <i>S</i> .										
<i>S</i> 1	44.59	70-135°	262.2	32.1	{	<i>S</i> 11	11.30	50-124°	249.1	23.9
						<i>S</i> 12	10.53	124-128	262.0	26.6
						<i>S</i> 13	8.38	128-135	268.3	30.4
						<i>S</i> 14	8.03	Residue	277.0	48.5
<i>S</i> 2	25.03	135-155	287.3	65.8	{	<i>S</i> 21	4.54	60-132	272.9	45.2
						<i>S</i> 22	7.04	132-135	283.1	56.9
						<i>S</i> 23	9.02	Residue	295.9	79.2
<i>S</i> 3	35.96	155-168	312.1	95.4	{	<i>S</i> 31	13.69	85-152	302.1	88.4
						<i>S</i> 32	8.17	152-168	312.6	93.0
<i>S</i> 4	10.46	168-169	326.0	110.1	<i>S</i> 33	8.36	Residue	323.7	103.6	
<i>S</i> 5	21.50	Residue	326.5	148.9						
(ii) Esters of "liquid" acids <i>L</i> .										
<i>L</i> 1	49.56	100-135°	269.8	74.5	{	<i>L</i> 11	13.89	70-133°	252.1	53.3
						<i>L</i> 12	12.70	135-141	262.7	73.9
						<i>L</i> 13	13.60	Residue	271.5	95.3
<i>L</i> 2	47.25	135-150	289.0	111.2	{	<i>L</i> 21	21.33	100-141	271.6	92.2
						<i>L</i> 22	23.82	141-151	280.5	105.3
						<i>L</i> 23	23.33	Residue	295.3	135.4
<i>L</i> 3	51.51	150-160	304.4	142.0	{	<i>L</i> 31	15.90	108-145	288.0	121.6
						<i>L</i> 32	13.67	145-155	294.9	131.6
						<i>L</i> 33	16.73	Residue	301.1	161.2
<i>L</i> 4	68.62	160-175	322.1	175.3	{	<i>L</i> 41	22.69	110-150	301.2	149.8
						<i>L</i> 42	24.48	150-160	314.0	168.0
						<i>L</i> 43	15.41	Residue	323.1	195.6
<i>L</i> 5	76.85	175-190	336.5	220.8	{	<i>L</i> 51	8.75	110-180	308.7	178.0
						<i>L</i> 52	34.22	180-190	327.5	201.4
						<i>L</i> 53	19.16	190	340.3	219.9
						<i>L</i> 54	9.20	Residue	349.9	235.5
<i>L</i> 6	26.97	Residue	375.6*	195.1						

* Saponification equivalent of residual esters, freed from unsaponifiable material, L 5: 336.5.

Table V c. *Determination of saturated components of ester fractions by oxidation.*

No.	Weight taken g.	Saturated esters present	
		g.	Sap. eq.
L 11	8.10	3.71	242.0
L 21	12.80	2.50	273.1
L 22	18.93	1.94	274.2

Table V d. *Estimated composition of fatty acids.*

Acids	"Solid" acids S (29.6 %)	"Liquid" acids L (70.4 %)	Total %	Fatty acids (mean unsat- uration) %
Saturated:				
Myristic	2.7	2.1	4.8	5
Palmitic	5.1	1.3	6.4	6.5
Stearic	—	0.2	0.2	Trace
Unsaturated:				
C ₁₄ group	—	0.4	0.4	0.5
C ₁₆ "	3.1	13.1	16.2	16 (-2.0 H)
C ₁₈ "	7.4	23.2	30.6	31 (-2.8 H)
C ₂₀ "	10.5	19.7	30.2	30.5 (-5.1 H)
C ₂₂ "	0.8	9.7	10.5	10.5 (?)
Unsaponifiable	—	0.6	0.6	—

The composition is similar to that of the other cod-liver oils studied, although the content of saturated acids is somewhat less. Palmitoleic acid is again practically the only unsaturated C_{16} acid present, the proportion of oleic to linoleic acid is much the same as in the Newfoundland oil examined; in the C_{20} and C_{22} groups of unsaturated acids, gadoleic acid occurs in some quantity, but the presence of cetoleic acid is doubtful, and polymerisation during heating prevented any reliable estimate being given for the average unsaturation of these groups of fatty acids.

COALFISH (SAITH)-LIVER OIL I.

Table VI a. *Lead salt separation.*

	g.	%	Sap. eq.	I.V.
"Solid" acids <i>S</i>	154.5	33.3	268.7	68.6
"Liquid" acids <i>L</i>	309.5	66.7	291.8	200.9

Table VI b. *Fractionation of methyl esters.*

Primary fractions					Refractations					
No.	g.	B.P./1 mm.	Sap. eq.	I.V.	No.	g.	B.P./1 mm.	Sap. eq.	I.V.	
(i) Esters of "solid" acids <i>S</i> .										
<i>S</i> 1	51.37	120–130°	259.0	14.1	{	<i>S</i> 11	3.27	105–123°	245.7	7.3
						<i>S</i> 12	14.64	123–125	251.1	7.1
						<i>S</i> 13	22.27	125–127	264.0	12.1
						<i>S</i> 14	6.02	Residue	274.3	36.9
<i>S</i> 2	32.38	130–140	275.0	27.7	{	<i>S</i> 21	14.57	130–131	265.9	16.0
						<i>S</i> 22	13.32	Residue	275.7	38.4
<i>S</i> 3	29.99	140–160	295.4	75.0	{	<i>S</i> 31	7.19	130–145	281.8	50.9
						<i>S</i> 32	10.46	145–147	287.9	66.8
						<i>S</i> 33	7.97	Residue	302.8	105.7
<i>S</i> 4	22.32	160–183	329.8	151.3	{	<i>S</i> 41	4.06	155–175	320.0	122.8
						<i>S</i> 42	4.40	175–177	320.2	141.3
						<i>S</i> 43	9.16	Residue	330.2	162.2
<i>S</i> 5	9.83	Residue	342.2*	188.4						
(ii) Esters of "liquid" acids <i>L</i> .										
<i>L</i> 1	60.39	130–150°	271.1	102.3	{	<i>L</i> 11	8.11	120–130°	255.1	58.8
						<i>L</i> 12	36.85	130–132	269.7	90.3
						<i>L</i> 13	7.58	Residue	290.5	141.2
<i>L</i> 2	89.58	150–160	292.7	148.5	{	<i>L</i> 21	14.21	135–141	270.4	107.3
						<i>L</i> 22	31.55	141–145	289.6	123.8
						<i>L</i> 23	25.00	145–146	296.2	139.1
						<i>L</i> 24	11.03	Residue	312.5	198.5
<i>L</i> 3	77.33	160–174	314.7	215.0	{	<i>L</i> 31	3.34	121–150	291.7	146.5
						<i>L</i> 32	34.55	150–151	298.9	173.6
						<i>L</i> 33	18.57	151–162	315.8	200.9
						<i>L</i> 34	15.09	Residue	327.9	229.8
<i>L</i> 4	54.64	174–197	335.9	230.8	{	<i>L</i> 41	3.91	150–173	313.4	222.8
						<i>L</i> 42	24.56	173–174	330.1	249.0
						<i>L</i> 43	11.26	174–178	335.2	256.4
						<i>L</i> 44	9.10	Residue	342.7	261.3
<i>L</i> 5	25.70	Residue	387.1*	217.2						

* Saponification equivalents of residual esters, freed from unsaponifiable material, *S* 5: 329.9, *L* 5: 337.1.

Table VI c. *Determination of saturated components of ester fractions by oxidation.*

No.	Weight taken g.	Saturated esters present	
		g.	Sap. eq.
S 12	10.66	8.37	250.6
S 21	10.71	9.00	266.0
S 32	6.72	2.91	283.6
L 11	3.86	1.67	242.0
L 12	32.49	6.77	255.8
L 23	18.96	nil	—

Table VI d. *Estimated composition of fatty acids.*

Acids	"Solid" acids S (33.3 %)	"Liquid" acids L (66.7 %)	Total %	Fatty acids %
Saturated:				
Myristic	4.6	1.9	6.5	6.5
Palmitic	12.0	0.9	12.9	13
Stearic	0.6	—	0.6	0.5
Unsaturated:				
C ₁₈ group	3.1	11.3	14.4	14.5
C ₁₈ "	5.2	25.1	30.3	31
C ₂₀ "	6.3	18.1	24.4	24.5
C ₂₂ "	1.4	8.7	10.1	10
Unsaponifiable	0.1	0.7	0.8	—

The highly unsaturated esters from this oil were extremely susceptible to polymerisation, and the iodine values fell continuously at every operation involving heat; the cause of this behaviour is uncertain, but in consequence it is not possible to give estimates for the average unsaturation of the various groups. The C₁₈ acids appear to be made up of approximately equal proportions of oleic and linoleic acids, whilst palmitoleic acid forms the greater part of the C₁₆ group. On the whole the fatty acids are very similar in type and proportion to those of the cod-liver oils (especially, perhaps, the Newfoundland oil).

COALFISH (SAITH)-LIVER OIL II.

Table VII a. *Lead salt separation.*

	g.	%	I.V. (of corresponding methyl esters)
"Solid" acids S	92.0	31.5	57.5
"Liquid" acids L	200.0	68.5	203.1

Table VII b. *Fractionation of methyl esters.*

(i) Esters of "solid" acids S.

No.	Primary fractions			
	g.	B.P./1 mm.	Sap. eq.	I.V.
S 1	57.31	100–133°	268.7	24.42
S 2	14.92	133–155	311.0	101.6
S 3	6.18	155–165	333.3	140.5
S 4	8.00	Residue	339.7	146.3

Table VII *b* (continued).(ii) Esters of "liquid" acids *L*.

Primary fractions					Refractionations				
No.	g.	B.P./1 mm.	Sap. eq.	I.V.	No.	g.	B.P./1 mm.	Sap. eq.	I.V.
<i>L</i> 1	56.51	120–143°	282.1	116.6	<i>L</i> 11	11.27	100–123°	262.3	77.1
					<i>L</i> 12	31.22	123–130	282.4	115.5
					<i>L</i> 13	8.74	Residue	291.8	177.1
<i>L</i> 2	73.31	143–163	309.4	196.4	<i>L</i> 21	7.07	120–145	290.5	140.6
					<i>L</i> 22	47.84	145–157	301.6	184.9
					<i>L</i> 23	13.45	Residue	326.8	271.8
<i>L</i> 3	51.23	163–180	336.7	277.4	<i>L</i> 31	4.72	145–170	322.5	260.1
					<i>L</i> 32	31.11	170–175	334.0	280.0
<i>L</i> 4	14.62	Residue	372.1*	234.3	<i>L</i> 33	11.03	Residue	340.9	288.5

* Saponification equivalent of residual esters, freed from unsaponifiable material, *L* 4: 327.6.

Table VII *c*. Determination of saturated components of ester fractions by oxidation.

No.	Weight taken g.	Saturated esters present	
		g.	Sap. eq.
<i>S</i> 1	18.45	14.03	260.4
<i>S</i> 2	11.52	3.50	288.3

(I.V. still 13.7)

Table VII *d*. Estimated composition of fatty acids.

Acids	"Solid" acids <i>S</i> (31.5 %)	"Liquid" acids <i>L</i> (68.5 %)	Total %	Fatty acids (mean unsat- uration)
Saturated:				
Myristic	5.0	0.8	5.8	6
Palmitic	12.0	—	12.0	12
Stearic	0.2	—	0.2	Trace
Unsaturated:				
<i>C</i> ₁₄ group	—	0.1	0.1	Trace
<i>C</i> ₁₆ "	—	9.2	9.2	9.5 (–2.0 H)
<i>C</i> ₁₈ "	4.5	24.8	29.3	29.5 (–3.2 H)
<i>C</i> ₂₀ "	7.0	19.5	26.5	26.5 (–5.5 H)
<i>C</i> ₂₂ "	2.8	13.5	16.3	16.5 (–7.3 H)
Unsaponifiable	—	0.6	0.6	—

In contrast to the experience in the previous analysis (saith oil I), little or no tendency to polymerisation was found when the methyl esters of the highly unsaturated *C*₂₀ and *C*₂₂ acids were distilled; the cause of this difference in behaviour is not clear. The total composition of the fatty acids differs but little from that observed in the first-named case, except that there is less palmitoleic acid present than usual, and correspondingly more of the acids of the *C*₂₂ group—a feature which is in keeping with the saponification equivalents of the respective oils.

HAKE-LIVER OIL I.

Table VIII *a*. Lead salt separation.

	g.	%	I.V. (of corre- sponding methyl esters)
"Solid" acids <i>S</i>	50.0	30.0	46.3
"Liquid" acids <i>L</i>	117.0	70.0	182.0

Table VIII b. *Fractionation of methyl esters.*(i) Esters of "solid" acids *S*.

No.	Primary fractions			
	g.	B.P./1 mm.	Sap. eq.	I.V.
<i>S</i> 1	17.82	110–130°	280.1	8.5
<i>S</i> 2	14.35	130–145	277.1	23.1
<i>S</i> 3	9.56	145–168	316.7	84.0
<i>S</i> 4	8.91	Residue	337.3	118.4

(ii) Esters of "liquid" acids *L*.

No.	Primary fractions				No.	Refractionations			
	g.	B.P./1 mm.	Sap. eq.	I.V.		g.	B.P./1 mm.	Sap. eq.	I.V.
<i>L</i> 1	19.31	117–137°	267.7	89.0	{ <i>L</i> 11	6.94	100–120°	254.6	57.2
					{ <i>L</i> 12	8.23	Residue	270.5	116.1
<i>L</i> 2	39.66	137–155	290.9	150.4	{ <i>L</i> 21	7.77	110–134	273.9	112.3
					{ <i>L</i> 22	19.14	134–139	286.6	141.5
					{ <i>L</i> 23	8.71	Residue	312.5	203.0
<i>L</i> 3	40.84	155–175	318.4	224.2	{ <i>L</i> 31	4.13	120–163	305.0	185.5
					{ <i>L</i> 32	21.89	163–167	316.3	215.0
<i>L</i> 4	14.79	Residue	372.1*	235.4	{ <i>L</i> 33	10.31	Residue	334.6	231.7

* Saponification equivalent of residual esters, freed from unsaponifiable material, *L* 4: 336.8.Table VIII c. *Determination of saturated components of ester fractions by oxidation.*

No.	Weight taken g.	Saturated esters present	
		g.	Sap. eq.
<i>S</i> 1	14.35	12.79	257.3
<i>S</i> 2	10.88	7.88	268.3
<i>S</i> 3	6.31	1.60	268.3

Table VIII d. *Estimated composition of fatty acids.*

Acids	"Solid" acids <i>S</i> (30.0 %)	"Liquid" acids <i>L</i> (70.0 %)	Total %	Fatty acids (mean unsat- uration)
Saturated:				%
Myristic	4.3	2.2	6.5	7
Palmitic	12.7	—	12.7	13
Unsaturated:				
<i>C</i> ₁₄ group	—	0.3	0.3	Trace
<i>C</i> ₁₆ "	1.0	16.2	17.2	17 (–2.0 H)
<i>C</i> ₁₈ "	1.7	16.3	18.0	18 (–3.5 H)
<i>C</i> ₂₀ "	5.4	24.9	30.3	31 (–4.9 H)
<i>C</i> ₂₂ "	4.9	9.2	14.1	14 (?)
Unsaponifiable	—	0.9	0.9	—

HAKE-LIVER OIL II.

Table IX a. *Lead salt separation.*

	g.	%	Sap. eq. (of corresponding methyl esters)	I.V.
"Solid" acids <i>S</i>	65.0	38.9	292.1	70.6
"Liquid" acids <i>L</i>	102.0	61.1	308.9	167.5

Table IX *b*. Fractionation of methyl esters.(i) Esters of "solid" acids *S*.

No.	Primary fractions			
	g.	B.P./1 mm.	Sap. eq.	I.V.
<i>S</i> 1	9.88	100–130°	259.1	12.6
<i>S</i> 2	10.93	130–140	270.1	22.2
<i>S</i> 3	10.60	140–150	284.4	45.6
<i>S</i> 4	9.39	150–165	311.8	96.1
<i>S</i> 5	8.57	165–170	334.0	122.9
<i>S</i> 6	7.76	Residue	334.1	111.3

(ii) Esters of "liquid" acids *L*.

Primary fractions					Refractionations				
No.	g.	B.P./1 mm.	Sap. eq.	I.V.	No.	g.	B.P./1 mm.	Sap. eq.	I.V.
<i>L</i> 1	18.94	114–142°	274.8	88.8	<i>L</i> 11	5.70	110–125°	262.7	60.5
					<i>L</i> 12	3.29	125–130	265.3	83.1
					<i>L</i> 13	5.28	Residue	285.5	124.9
<i>L</i> 2	20.51	142–155	290.7	143.2	<i>L</i> 21	6.06	120–135	281.8	119.9
					<i>L</i> 22	4.81	135–144	290.3	142.5
					<i>L</i> 23	5.18	Residue	294.8	168.6
<i>L</i> 3	22.19	155–165	305.2	184.5	<i>L</i> 31	5.94	130–152	303.2	164.9
					<i>L</i> 32	6.05	152–157	315.2	182.2
<i>L</i> 4	15.50	165–175	333.2	208.5	<i>L</i> 33	5.87	Residue	324.8	202.0
<i>L</i> 5	15.00	Residue	396.8*	201.7					

* Saponification equivalent of residual esters, freed from unsaponifiable material, *L* 5: 334.8.Table IX *c*. Determination of saturated components of ester fractions by oxidation.

No.	Weight taken g.	Saturated esters present	
		g.	Sap. eq.
<i>S</i> 2	7.21	5.53	262.8
<i>S</i> 3	6.90	3.32	273.6

Table IX *d*. Estimated composition of fatty acids.

Acids	"Solid" acids <i>S</i> (38.9 %)	"Liquid" acids <i>L</i> (61.1 %)	Total %	%	Fatty acids (mean unsat- uration)
Saturated:					
Myristic	3.6	1.0	4.6	4.5	
Palmitic	10.8	1.2	12.0	12	
Stearic	0.7	—	0.7	0.5	
Unsaturated:					
<i>C</i> ₁₆ group	0.9	10.6	11.5	12	(–2.0 H)
<i>C</i> ₁₈ "	8.1	18.3	26.4	27	(–3.0 H)
<i>C</i> ₂₀ "	9.7	19.6	29.3	30	(–4.1 H)
<i>C</i> ₂₂ "	5.1	8.8	13.9	14	(–5.4 H)
Unsaponifiable	—	1.6	1.6	—	

HAKE-LIVER OIL III.

Table X *a*. Lead salt separation.

	g.	%	Sap. eq.	I.V. (of corre- sponding methyl esters)
"Solid" acids <i>S</i>	53.0	28.8	287.2	42.5
"Liquid" acids <i>L</i>	131.4	71.2	310.5	146.5

Table X b. *Fractionation of methyl esters.*(i) Esters of "solid" acids *S*.

No.	Primary fractions			
	g.	B.P./1 mm.	Sap. eq.	I.V.
<i>S</i> 1	5.82	100–130°	255.5	9.7
<i>S</i> 2	8.57	130–133	264.2	11.6
<i>S</i> 3	11.82	133–155	278.5	22.1
<i>S</i> 4	12.75	155–175	310.9	66.4
<i>S</i> 5	4.95	Residue	338.5	88.3

(ii) Esters of "liquid" acids *L*.

Primary fractions					Refractionations					
No.	g.	B.P./1 mm.	Sap. eq.	I.V.	No.	g.	B.P./1 mm.	Sap. eq.	I.V.	
<i>L</i> 1	23.36	100–150°	273.0	86.8	{	<i>L</i> 11	6.18	80–115°	255.0	45.9
						<i>L</i> 12	5.88	115–121	267.0	77.9
						<i>L</i> 13	7.42	Residue	292.7	126.7
<i>L</i> 2	17.54	150–160	291.0	128.0						
<i>L</i> 3	37.24	160–170	312.7	159.3	{	<i>L</i> 31	11.29	110–150	297.5	142.6
						<i>L</i> 32	12.04	150–160	303.8	158.2
						<i>L</i> 33	9.49	Residue	332.7	168.1
<i>L</i> 4	20.15	170–180	335.2	195.4	{	<i>L</i> 41	6.61	140–156	322.9	172.1
<i>L</i> 5	16.30	Residue	385.0*	169.1		<i>L</i> 42	9.15	Residue	340.0	184.1

* Saponification equivalent of residual esters, freed from unsaponifiable material, *L* 5: 341.6.Table X c. *Determination of saturated components of ester fractions by oxidation.*

No.	Weight taken g.	Saturated esters present	
		g.	Sap. eq.
<i>S</i> 2	4.04	3.10	262.1
<i>S</i> 3	6.02	4.01	272.4
<i>L</i> 2	13.14	1.20	262.2

Table X d. *Estimated composition of fatty acids.*

Acids	"Solid" acids <i>S</i> (28.8 %)	"Liquid" acids <i>L</i> (71.2 %)	Total	Fatty acids %
Saturated:				
Myristic	2.9	2.6	5.5	5.5
Palmitic	9.4	1.6	11.0	11
Stearic	0.5	—	0.5	0.5
Unsaturated:				
<i>C</i> ₁₆ group	1.7	6.3	8.0	8
<i>C</i> ₁₈ "	6.3	27.1	33.4	34
<i>C</i> ₂₀ "	6.0	18.4	24.4	25
<i>C</i> ₂₂ "	2.0	14.0	16.0	16
Unsaponifiable	—	1.2	1.2	—

The highly unsaturated esters of the *C*₂₀ and *C*₂₂ groups showed considerable tendency to polymerise during distillation in the case of all three hake-liver oils, and the mean unsaturation of the corresponding fatty acids is probably somewhat greater than that suggested in the analysis of hake-liver oil II. The other acids present were similar in type to those of the cod- and saith-liver oils, and the proportions of all the acids were for the most part of the same order as those in the last-mentioned liver oils.

LING-LIVER OIL.

Table XI a. *Lead salt separation.*

	g.	%	I.V. (of corresponding methyl esters)
"Solid" acids <i>S</i>	41.5	22.5	27.3
"Liquid" acids <i>L</i>	142.5	77.5	164.6

Table XI b. *Fractionation of methyl esters.*(i) Esters of "solid" acids *S*.

No.	Primary fractions			I.V.
	g.	B.P./1 mm.	Sap. eq.	
<i>S</i> 1	24.21	107–123°	262.1	7.9
<i>S</i> 2	8.60	123–142	280.5	32.2
<i>S</i> 3	8.61	Residue	317.1	84.8

(ii) Esters of "liquid" acids *L*.

No.	Primary fractions				No.	Refractations			
	g.	B.P./1 mm.	Sap. eq.	I.V.		g.	B.P./1 mm.	Sap. eq.	I.V.
<i>L</i> 1	29.51	113–139°	266.2	90.6	{ <i>L</i> 11	5.29	100–124°	256.1	58.0
					{ <i>L</i> 12	10.27	124–125	268.1	82.5
					{ <i>L</i> 13	10.50	Residue	288.5	118.9
<i>L</i> 2	47.70	139–153	291.5	131.3	{ <i>L</i> 21	5.62	126–139	278.3	106.2
					{ <i>L</i> 22	31.05	139–140	292.5	124.8
					{ <i>L</i> 23	6.48	Residue	312.0	196.4
<i>L</i> 3	44.72	153–175	315.4	210.7	{ <i>L</i> 31	5.05	140–155	299.0	154.8
					{ <i>L</i> 32	22.12	155–160	315.9	198.2
<i>L</i> 4	5.83	175–182	337.0	254.6	{ <i>L</i> 33	14.22	Residue	335.7	249.8
<i>L</i> 5	11.64	Residue	403.4*	225.2					

* Saponification equivalent of residual esters, freed from unsaponifiable material, *L* 5: 335.3.

Table XI c. *Determination of saturated components of ester fractions by oxidation.*

No.	Weight taken g.	Saturated esters present	
		g.	Sap. eq.
<i>S</i> 1	19.73	18.03	260.9
<i>S</i> 2	5.05	3.46	271.9
<i>S</i> 3	3.01	0.70	283.2

Table XI d. *Estimated composition of fatty acids.*

Acids	"Solid" acids <i>S</i> (22.5 %)	"Liquid" acids <i>L</i> (77.5 %)	Total %	Fatty acids (mean unsaturation)
Saturated:				%
Myristic	3.6	1.2	4.8	5
Palmitic	11.9	0.9	12.8	13
Stearic	0.8	—	0.8	1
Unsaturated:				
C_{14} group	—	0.2	0.2	Trace
C_{16} "	0.8	11.8	12.6	13 (–2.0 H)
C_{18} "	1.5	30.9	32.4	32.5 (–2.8 H)
C_{20} "	3.1	20.6	23.7	24 (–5.7 H)
C_{22} "	0.8	10.8	11.6	11.5 (–7.0 H)
Unsaponifiable	—	1.1	1.1	—

The ling-liver fatty acids, like those from hake, bear a general similarity to those of cod-liver oil. Palmitoleic is substantially the only unsaturated C_{16} acid present, whilst the C_{18} acids are a mixture of about 60 % oleic and 40 % linoleic acid; little polymerisation, as evidenced by falling iodine values, took place during distillation of the ling unsaturated esters and the figures given for the average unsaturation of the C_{20} and C_{22} groups are probably fairly accurate in this instance. Some monoethylenic C_{20} acid was present, but only very small quantities of the corresponding C_{22} acid.

SKATE-LIVER OIL.

Table XII a. *Lead salt separation.*

	g.	%	I.V. (of corresponding methyl esters)
"Solid" acids <i>S</i>	38.5	21.9	20.9
"Liquid" acids <i>L</i>	137.0	78.1	249.4

Table XII b. *Fractionation of methyl esters.*(i) Esters of "solid" acids *S*.

No.	Primary fractions				I.V.
	g.	B.P./l mm.	Sap. eq.		
<i>S</i> 1	30.47	110–133°	268.1		7.5
<i>S</i> 2	6.55	Residue	321.2		80.6

(Fraction *S* 1 (25.72 g.) gave 24.79 g. of saturated esters (sap. eq. 263.8) on oxidation.)

(ii) Esters of "liquid" acids *L*.

No.	Primary fractions				No.	Refractionations				
	g.	B.P./l mm.	Sap. eq.	I.V.		g.	B.P./l mm.	Sap. eq.	I.V.	
<i>L</i> 1	46.98	130–160°	283.9	not done	<i>L</i> 11	4.71	103–124°	265.5	83.0	
					<i>L</i> 12	24.11	124–134	280.5	120.6	
					<i>L</i> 13	8.68	134–136	303.3	186.7	
					<i>L</i> 14	5.87	Residue	323.2	265.9	
<i>L</i> 2	46.30	160–190	329.0	,,	<i>L</i> 21	7.65	130–155	296.6	203.0	
					<i>L</i> 22	29.29	155–162	312.6	272.7	
					<i>L</i> 23	6.43	Residue	336.0	308.0	
<i>L</i> 3	31.11	190–196	333.4	,,	<i>L</i> 31	5.14	145–175	325.7	307.0	
					<i>L</i> 32	14.05	175–178	335.4	335.4	
<i>L</i> 4	10.60	Residue	347.8*	,,	<i>L</i> 33	7.65	Residue	341.7	339.9	

* Saponification equivalent of residual esters, freed from unsaponifiable material, *L* 4: 331.6.

Table XII c. *Estimated composition of fatty acids.*

Acids	"Solid" acids <i>S</i> (21.9 %)	"Liquid" acids <i>L</i> (78.1 %)	Total %	Fatty acids (mean unsaturation)
Saturated:				%
Myristic	3.5	0.2	3.7	4
Palmitic	13.8	—	13.8	14
Unsaturated:				
C_{14} group	—	Trace	Trace	Trace
C_{16} "	—	10.5	10.5	10.5 (–2.0 H)
C_{18} "	1.0	19.7	20.7	20.5 (–3.3 H)
C_{20} "	3.6	29.0	32.6	32.5 (–7.3 H)
C_{22} "	—	18.4	18.4	18.5 (–9.5 H)
Unsaponifiable	—	0.3	0.3	—

The skate-liver fatty acids stand out in the present series by reason of the high average unsaturation of the C_{22} and C_{20} groups, and equally by the absence of polymerisation during distillation of the corresponding esters. The oil also contains somewhat more of the C_{22} acids than the previous cases, and cetoleic acid is apparently absent; gadoleic acid is present, the C_{18} unsaturated acids are made up of about one-third oleic and two-thirds linoleic acid, and palmitoleic acid is substantially the only C_{16} unsaturated acid present.

DOGFISH-LIVER OIL.

Table XIII a. *Lead salt separation.*

	g.	%	Sap. eq.	I.V.
"Solid" acids <i>S</i>	110.0	33.2	286.2	56.0
"Liquid" acids <i>L</i>	221.0	66.8	361.0	139.0

Table XIII b. *Fractionation of methyl esters.*

Primary fractions					Refractations					
No.	g.	B.P./1 mm.	Sap. eq.	I.V.	No.	g.	B.P./1 mm.	Sap. eq.	I.V.	
(i) Esters of "solid" acids <i>S</i> .										
<i>S</i> 1	13.46	115–134°	252.5	11.3	{	<i>S</i> 21	8.04	115–134°	8.6	
<i>S</i> 2	21.27	134–144	270.2	16.1		<i>S</i> 22	4.18	134	262.7	11.6
<i>S</i> 3	13.65	144–154	284.1	32.3		<i>S</i> 23	6.38	Residue	274.2	26.7
<i>S</i> 4	8.88	154–165	299.8	54.5	{	<i>S</i> 51	5.92	154–174	318.7	67.3
<i>S</i> 5	26.25	165–184	334.1	72.1		<i>S</i> 52	7.51	174–175	326.4	71.1
						<i>S</i> 53	2.21	175	337.3	72.7
<i>S</i> 6	3.96	184–187	344.2	74.6		<i>S</i> 54	6.65	Residue	340.5	71.1
<i>S</i> 7	9.01	Residue	332.3	84.4						
(ii) Esters of "liquid" acids <i>L</i> .										
<i>L</i> 1	26.15	126–154°	276.0	84.1	{	<i>L</i> 11	7.09	108–130°	256.9	49.3
						<i>L</i> 12	6.40	130–138	267.8	68.7
						<i>L</i> 13	3.11	138–140	279.4	81.7
						<i>L</i> 14	5.91	Residue	296.0	101.3
<i>L</i> 2	32.60	154–165	300.0	122.1	{	<i>L</i> 21	13.94	120–145	285.0	99.7
						<i>L</i> 22	6.56	145–155	298.1	117.0
						<i>L</i> 23	2.05	155	305.0	123.9
						<i>L</i> 24	6.53	Residue	313.4	136.6
<i>L</i> 3	57.22	165–187	351.4*	153.0	{	<i>L</i> 31†	6.52	120–155	288.2	121.5
						<i>L</i> 32	11.20	155–162	304.1	130.0
						<i>L</i> 33	9.18	165–175	315.3	132.6
						<i>L</i> 34	10.50	Residue	338.3	147.4
<i>L</i> 4	66.82	Residue	532.5*	138.4	{	<i>L</i> 41†	3.44	80–145	312.7	93.0
						<i>L</i> 42	10.33	145–170	324.5	98.2
						<i>L</i> 43	16.66	Residue	426.0*	141.2

* Saponification equivalents of these fractions, freed from unsaponifiable material, *L* 3: 319.4; *L* 4: 381.6; *L* 43 367.5.

† Unsaponifiable constituents removed as far as possible by ether-extraction of hydrolysed fractions *L* 3 and *L* 4, recovered fatty acids reconverted to methyl esters which were refracted (L 31–34 and L 41–43).

Table XIII c. *Estimated composition of fatty acids.*

Acids	"Solid" acids <i>S</i> (33.2 %)	"Liquid" acids <i>L</i> (66.8 %)	Total %	%	Fatty acids (mean unsat- uration)
Saturated:					
Myristic	4.0	1.2	5.2	6	
Palmitic	8.2	1.2	9.4	10.5	
Stearic	2.5	—	2.5	3	
Arachidic	0.2	—	0.2	Trace	
Unsaturated					
C ₁₆ group	1.3	6.9	8.2	9	(-2.0 H)
C ₁₈ "	3.4	18.5	21.9	24.5	(-2.3 H)
C ₂₀ "	9.2	16.5	25.7	29	(-3.3 H)
C ₂₂ "	4.4	6.7	11.1	12	(-4.0 H)
C ₂₄ "	—	5.3	5.3	6	(-2.0 H)
Unsaponifiable	—	10.5	10.5	—	

In some ways the fatty acid composition of this oil is the most interesting of the whole series. The oil contained much more unsaponifiable matter than the rest, and squalene formed a considerable proportion of the non-fatty matter, but the oil was not by any means so rich in this hydrocarbon as many shark- and ray-liver oils which have been examined by other workers. Nevertheless the character of the fatty acids is different from those of the oils previously dealt with in this paper, and in many ways (as appears from the general summary on p. 288) the acids of this dogfish-liver oil are intermediate in type between those of the Gadidae and of the skate-liver oil on the one hand, and those of Elasmobranch oils of high squalene content on the other. Palmitoleic acid is less prominent than usual, the average unsaturation of the C₂₀ and C₂₂ acids is much lower than usual, and especially noteworthy is the appearance of a small amount of monoethylenic acid, C₂₄H₄₆O₂.

SUMMARY.

Comparison of the fatty acid compositions of various fish oils.

It may be said at once that no apparent correlation between the vitamin potencies of the oils studied and the composition of their fatty acids is revealed as a result of the foregoing analyses; the most that can be stated is that, in the cases examined, the highest vitamin potency was observed in liver oils from the family Gadidae, but this may be a more or less fortuitous observation.

It is interesting, however, to consider the analyses which have now been made, in conjunction with a few other results of a similar nature which are already available, when classified from a biological standpoint. This has been done in Table XIV: the data for the whale oils (*Balaena* sp.), menhaden oil and Japanese sardine oil are taken from a communication by Armstrong and Allan [1924], those for the liver oils of *Scymnorhinus* and allied genera were given by Hilditch and Houlbrooke [1928], those for the head and blubber oils of the sperm whale by Hilditch and Lovern [1928], and for sperm-liver oil by Tsujimoto and Kimura [1928].

The figures in Table XIV are perhaps chiefly valuable at the moment in suggesting the desirability of adding as rapidly as possible to the meagre number of quantitative data of the kind which have so far been collected. Investigations of this kind are necessarily lengthy and tedious, but the results so far obtained indicate the possibility that they may lead to useful correlations as well as differentiations between the build of the fats occurring in different classes of marine animals or, for example, present in different parts of the same animal.

Inspection of Table XIV will show that, leaving apart minor differences in the percentages of the fatty acids concerned, the liver oils of the Gadidae and the flesh oils of the two members of the herring family stand apart from the rest in the approximate constancy of their general composition, the range of which is as follows:

	%	Mean unsaturation
Myristic acid	3.5-7	—
Palmitic acid	6.5-13	—
Stearic acid	nil-1	—
Myristoleic acid	Trace-0.5	(-2.0 H)
Palmitoleic acid	13-20	(-2.0 to -3.0 H)
Oleic and linoleic acids	18-32.5	(-2.8 to -4.0 H)
*Unsaturated C_{20} acids	19-31.5	(-4.9 to -7.3 H)
*Unsaturated C_{22} acids	10-19	(-5.4 to -10.0 H)

The blubber fats of the ordinary whales approach most closely in composition to the foregoing, but on the whole appear to contain greater amounts of oleic and linoleic acids, with corresponding reduction in the total proportions of polyethylenic C_{20} and C_{22} acids, the total percentage of saturated acids being little different in either group. The interesting, but only partially quantitative, study by Tsujimoto and Kimura [1928] of the fatty acids of the liver of the sperm whale suggests that here also the composition is much the same as in the body fats of the Balaenidae and Clupeidae and the liver fats of the Gadidae, and these authors also state that the fat as obtained contains only 16 % of unsaponifiable matter, of which 8 % is cholesterol, higher fatty alcohols being probably absent; sperm head- and blubber-oils, on the contrary, contain 33-40 % of combined higher fatty alcohols, whilst the fatty acids also present are entirely different in character from those of any other marine animal [Hilditch and Lovern, 1928].

Whilst differences of the latter kind point clearly to synthesis of particular varieties of fatty compounds by specific classes of animals, the general similarity in composition, previously referred to, of the fatty acids of the livers and

* The relative amounts of monoethylenic acids $C_{20}H_{38}O_2$ and $C_{22}H_{42}O_2$ appear to vary somewhat widely from one species to another, and it is not certain that all the highly unsaturated acids of these groups are the same, even in liver oils of the same species of fish. At all events, it was noticeable that in certain cases (for example, Norwegian cod, saith (1), and hake) the esters of these acids polymerised exceedingly readily, whilst in others (especially saith (2), ling and skate) there was practically no tendency to polymerisation and fall in iodine value. This may be due (cf. p. 270) to some accidental catalytic acceleration of the polymerisation process from an unrecognised source, or may be an indication of structural differences in the constitution of the polyethylenic acids.

Table XIV. *Percentage composition of fatty acids of marine animal oils.*

Oil	Species	Saturated acids			Unsaturated acids						
		C ₁₄	C ₁₆	C ₁₈	C ₁₄	C ₁₆	C ₁₈	C ₂₀	C ₂₂	C ₂₄	
		Sub-division TELEOSTOMI. Order TELEOSTEI.									
Family GADIDAE.											
Cod-liver (Newfoundland)	<i>Gadus morrhua</i>	6	8.5	0.5	Trace	20	29	26	10	—	
Cod-liver (Scottish)	" "	3.5	10	—	0.5	15.5	25	31.5	14	—	
Cod-liver (Norwegian)	" "	5	6.5	Trace	0.5	16	31	30.5	10.5	—	
Coalfish (saith)-liver I	<i>G. virens</i>	6.5	13	0.5	—	14.5	31	24.5	10	—	
" " II	" "	6	12	Trace	Trace	9.5	29.5	26.5	16.5	—	
Hake-liver I	<i>Merluccius merluccius</i>	7	13	—	Trace	17	18	31	14	—	
" " II	" "	4.5	12	0.5	—	12	27	30	14	—	
" " III	" "	5.5	11	0.5	—	8	34	25	16	—	
Ling-liver	<i>Molva molva</i>	5	13	1	Trace	13	32.5	24	11.5	—	
Family CLUPEIDAE.											
Japanese sardine (whole fish)	<i>Clupanodon melanostica</i>	6	10	2	—	13	24	26	19	—	
Menhaden (whole fish)	<i>Alosa menhaden</i>	6	16	1.5	—	15.5	30	19	12	—	
Sub-division ELASMOBRANCHII. Order SELACHII.											
Family SQUALIDAE.											
Dogfish-liver	<i>Squalus acanthias</i>	6	10.5	3	—	9	24.5	29	12	6*	
Mixed liver oils from	<i>Centrophorus, Lepidorhinus, etc. sp.</i>	1	13	2.5	Trace	3.5	35.5	16.5*	16*	12*	
Family DALATIIDAE.											
Liver oil of	<i>Scymnorhinus licha</i>	1	14.5	3.5	0.5	4	29	10.5*	26*	10*	
Family RAJIDAE.											
Skate-liver	<i>Raja maculata</i>	4	14	—	Trace	10.5	20.5	32.5†	18.5†	—	
Division MAMMALIA. Order CETACEA.											
Family BALAENIDAE.											
Whale oil (blubber):											
Arctic	<i>Balaena mysticetus</i>	4	10.5	3.5	—	18	33	20	11	—	
Newfoundland	" "	7.5	10	3	1.5	18	44	—	16	—	
South Sea	<i>Balaena australis</i>	8	12	2	1.5	15	43	8	10.5	—	
Family PHYSETERIDAE.											
Sperm whale:											
Head†	<i>Physeter macrocephalus</i>	14	8	2	14	15	17*	6.5*	—	—	
Blubber	" "	5	6.5	—	4	26.5	37*	19*	—	—	
Liver	" "	(24.6 % saturated)				(42 % monoethylenic and 23.4 % polyethylenic)					

* Practically only monoethylenic acids present.

† Particularly high average unsaturation.

‡ The head acids also contained 3.5 % capric, 16 % lauric and 4 % lauroleic acids; the blubber acids 1-2 % of lauric acid.

in some cases the body-oils of many marine animals of diverse biological orders suggests that fish and marine mammalia may derive a considerable proportion of their fats by direct assimilation. The polyethylenic acids of the C₂₀ and C₂₂ series, which at one time were known only as characteristic of fishes and marine mammals, have been detected by Tsujimoto [1925] in Algae of different kinds in every instance tested, although it is true that the proportion present is much lower than in the fish-liver oils which are the subject of this paper; furthermore, it is stated by Grün and Halden [1929] that the fat of

birds which feed on fish also contains these highly-unsaturated acids, which are not present in the fat of birds whose diet is confined to insects, grain, etc. from non-marine sources. The determination of the origin (evidently marine in nature) of these unique highly unsaturated fatty acids is thus a problem of great interest which remains to be solved.

Finally, the detailed composition of the fatty acids of the Elasmobranchii deserves further attention, in view of the interesting results so far obtained.

It will be noticed that, in this group, the acids of skate-liver oil alone resemble those of the more frequently occurring type (but possess distinctly greater average unsaturation and amount of polyethylenic C_{20} and C_{22} acids, whilst the monoethylenic acids of these series are only present in minimal quantities); this skate-liver oil, however, contains, for an Elasmobranch oil, a very low proportion of unsaponifiable matter—lower, indeed, than any of the Gadidae oils.

At the other extreme are the fats of *Centrophorus*, *Lepidorhinus* and *Scymnorhinus* species, which were the complement of large proportions of unsaponifiable matter composed mainly of the hydrocarbon squalene; the fatty acids from these sources contained no highly unsaturated C_{20} and C_{22} derivatives, but were mainly ethylenic in character and notable amounts of the monoethylenic acid containing 24 carbon atoms (selacholeic acid [Tsujiyama 1927]) were also present.

The dogfish-liver oil reported upon in this paper was intermediate in character between these varieties of Elasmobranch oil: it contained 10.5 % of unsaponifiable matter, which consisted partly of liquid hydrocarbons (squalene) and partly of solid crystalline compounds (cholesterol?). The fatty acids present were also intermediate in composition between those of the two extreme types, for selacholeic acid was present in small quantity, whilst the mean unsaturation of the C_{20} and C_{22} acids (about -4.0 H) was higher than monoethylenic but less marked than in the cod- and other fish-liver oils which were first discussed.

So far as the present data go, therefore, there is a definite and close relation in the Elasmobranch group between the character of the fat present and the amount and nature of the unsaponifiable matter. We are not inclined at this juncture to do more than point out that our recent results strengthen the conclusion of Hilditch and Houlbrooke [1928] that there is some connection between the absence of the polyethylenic C_{20} and C_{22} acids so characteristic of most animal oils and the presence of large proportions of squalene. We believe that at present no suggestion can be usefully offered to explain the genesis, much less a hypothetical transmutation into one another, of either the polyethylenic acids, of squalene, or of cholesterol or other sterols which are frequent components of the non-fatty matter. Bearing in mind what is already known of the fundamental differences in chemical structure of these compounds (the normal aliphatic carbon chain present in the polyethenoid C_{20} and C_{22} acids; the aliphatic branched chain system of squalene, $C_{30}H_{50}$, built up

from isoprene units; and the complex condensed ring-systems present in cholesterol), it is very difficult to imagine that they are mutually interconvertible by ordinary biochemical processes. It is perhaps more opportune, for the moment, to speculate whether different metabolic processes characteristic of specific organisms may not lead, according to circumstances, to the accumulation of differing kinds of organic compounds and the disappearance of other forms which are further utilisable by the organism concerned.

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