TABLE II

Comparison of Hand and Motor Driven Consistometer Results

Sample	Hand-Operated Consistometer				Motor-Driven Consistometer			
	A	B	C	D	A	B	C	D
Can 1	53	42	....	161	54	40	....	154
	54	42	....	150	57	41	....	149
	56	43	....	150	55	39	....	151
Can 2	53	41	34	162	54	49	42	156
	50	41	37	146	58	46	41	147
	54	40	36	148	54	49	46	164
	52	44	39	151	56	47	46	158
Can 3	55	39	42	139	59	45	42	171
	57	41	43	128	61	47	42	175
	55	41	41	148	59	46	43	169
	53	38	45	140	57	48	43	164
Can 4	55	40	43	143	61	47	43	147
	56	42	42	144	58	47	42	150
	58	43	38	147	58	44	44	155
	57	39	42	156	61	44	45	158
Standard deviation	1.6	1.6	2.0	7.0	1.7	1.5	1.8	5.2

Procedure. Hold the sample at the specified temperature long enough for it to come to equilibrium at that temperature. This is usually a minimum of 24 hours, but it depends on the size of the sample. Grasp the instrument firmly and hold at right angles to the surface of the sample. If there is a light crust on the surface, this may be removed with a spatula. With a constant application of pressure, force the plunger slowly (1½-inch depression in 30 seconds) into the sample until the maximum pointer is practically stationary or until the core of sample forced through the ring of the plunger is about 1½ inches long. The figure on the dial coinciding with the maximum reading hand is the consistency.

The following data are an indication of the performance of the consistometer. These data were obtained by making hand- and motor-driven tests on several different samples representing a range in consistency.

The foregoing data represent various types of shortenings. There was no significant difference between the two instruments. The coefficient of variation calculated for the above data is 3.8%, from which the standard deviation for any range of consistency may be estimated. The coefficient of variation equals $\sigma/\bar{x}$ when σ equals the standard deviation and $\bar{x}$ equals the mean.

The consistometer, which has been described, provides a means of obtaining numerical measurements of the consistency of fats. It is applicable to shortening and other plastic products such as margarine and butter. Results are reliable over a range of from 20 to 170 on the scale. This extends from products which are very soft to those which are very firm. The method is simple and very rapid. An important advantage of this instrument is that it is small and light in weight so that it may be applied directly to the original container whether this container be a 1-lb. package or a 50-lb. drum. In fact, it is a relatively easy matter to go through an entire stock in a short time checking individual packages. The instrument and method are therefore particularly suitable for production control purposes.

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Component Fatty Acids of Indian Shark Liver Oils

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LIVER oils from several varieties of sharks have been studied by a number of investigators, notably from Japan (Tsujimoto and co-workers, for example). The data for most of them are however of semi-quantitative nature, and the fatty acid compositions of only a few are available in some details. The composition of the liver oil of the shark *(Carcharius gangeticus)*, reported by Tsujimoto (9), is of special interest in being unusually rich in saturated (mainly palmitic) acids (nearly 50% of the total fatty acids).

The present investigation was undertaken to study the fatty acid compositions of the liver oils of Indian sharks *(Galeocerdo rayneri)*, one of which was from the Arabian Sea and the other from the Bay of Bengal. These oils proved to belong to Tsujimoto's fourth group (10) of Elasmobranch fish liver oils in having high (ca. 40%) saturated acid contents. Oils of this group are very rare indeed, and the liver oil of this species of sharks appears to be the third instance of this peculiar group. The present study is probably the first detailed analysis of an oil of this group where an efficient modern fractionating column has been employed.

Experimental

A sample of refined oil from the liver of a shark *(Galeocerdo rayneri)*, from the Arabian Sea, was kindly supplied to us by the Director of Fisheries, Bombay, and is referred to as No. 1 shark liver oil.

Another sample of the liver oil of the same species of shark, caught in the Bay of Bengal, was kindly sent to us by U. S. Kini, manager, Government Oil Factory, Kozhikode, Calicut, Madras, and will be named as No. 2 shark liver oil.

Liver Oil of Shark No. 1 (Arabian Sea). The oil deposited 5.8% of phosphatides on keeping in 10 volumes of acetone solution at about 0°C. for 4 weeks. The glyceride portion (220.6 g.) was added in minimum quantity (45.0 g.) of potassium hydroxide in 1,100 c.c. alcohol (95%) and heated for one hour on water bath after which about 700 c.c. of alcohol was distilled off. This precaution was taken to keep the highly unsaturated acids from undergoing isomeric rearrangement. It is preferable to risk the chance of slightly incomplete conversion of the whole fat into fatty acids rather than to incur rearrangement of some of the highly unsaturated components. The unsaponifiable matter was extracted with ether from

the soap solution after dilution with water. The residual soap solution was then decomposed with dilute mineral acid, and finally 203.8 g. of the mixed fatty acids (I. V. 144.6) were obtained in the usual manner. The complex mixture of the mixed acids (195.7 g.) was resolved into simpler groups by the lithium-salt acetone method first applied by Tsujimoto (11) and then by lead salt alcohol (Hilditch's modification [2]) method. The results are summarized in Table I.

TABLE I

Separation of the Acids of Shark Liver Oil No. 1 by the Lithium Salt Acetone and Lead Salt Alcohol Methods

Group	Description	Weight		Iodine value
		g.	%	
A	Lead salt alcohol insoluble	64.4	32.9	8.3
B	Lead salt alcohol soluble but lithium salt acetone insoluble	75.9	38.8	132.3
C	Lithium salt acetone soluble	55.4	28.3	317.3

Each group of the acids was separately converted into methyl esters, taking the precautions suggested by Bjarnson and Meara (1), and fractionated through the Longenecker E.H.P. column (3). The fractions were studied in order of their decreasing unsaturations, i.e., the most unsaturated fraction C was taken up first without much loss of time, next the less unsaturated fraction B, and lastly the least unsaturated fraction A. The ester-fractionation data, along with saponification equivalents and iodine values, are given in Table II.

The composition of each of the ester-fractions was calculated from saponification equivalent and iodine value by the method described by Hilditch (2). As usual, the mean unsaturation, expressed as the fractional number of hydrogen atoms short of saturation, for example, —2.0 (monoethenoid), was determined by interpolation or extrapolation from the respective ester-fractions in each of the groups B and C, from which the mean equivalent of each of the homologous ester groups (C_{16}, C_{18}, C_{20}, etc.) follows. The final fatty acid composition of the original oil was built up from these figures, giving the data in Tables I and II, and is recorded in Table III.

Liver Oil of Shark No. 2 (Bay of Bengal). Some 225.2 g. of the mixed fatty acids (I.V. 124.5) were obtained in the manner already described from 250.0 g. of the liver oil of shark No. 2 (I.V. 119.2) from the Bay of Bengal. A portion of the mixed acids was resolved into simpler groups by the technique of Lovern (4), i.e., the mixed acids were separated into saturated and unsaturated acids by the lead-salt alcohol method and the unsaturated acids so obtained then separated into the mono- and polyethenoid acids by the lithium-salt acetone method. These results are summarized in Table IV in the same pattern as that of Table I.

TABLE II

Fractionation Data of Methyl Esters of Acids of Shark Liver Oil No. 1, Fractions A, B, and C

No.	G	S.E.	I.V.
Methyl Esters of A Acids			
A1	3.05	259.0	1.2
A2	3.89	263.1	1.3
A3	4.77	266.7	1.4
A4	5.44	269.5	1.4
A5	4.79	271.8	1.7
A6	4.39	273.6	2.7
A7	4.14	279.0	3.8
A8	3.82	284.9	5.7
A9	3.71	295.9	7.6
A10	3.21	299.7	8.8
A11	4.25	352.4[a]	44.7
Methyl Esters of B Acids			
B1	2.74	232.9	36.2
B2	3.35	258.8	66.6
B3	5.20	263.8	76.1
B4	4.63	271.7	81.4
B5	4.87	276.9	88.6
B6	4.62	283.4	91.9
B7	4.53	283.9	96.3
B8	4.64	288.9	100.6
B9	4.18	292.4	107.5
B10	4.61	292.2	136.4
B11	3.54	294.6	170.8
B12	4.03	307.7	214.7
B13	3.37	310.2	212.8
B14	4.59	327.7[a]	192.4
Methyl Esters of C Acids			
C1	2.15	269.9	109.6
C2	2.88	270.5	131.1
C3	4.61	292.2	161.6
C4	4.31	307.4	314.5
C5	4.12	310.9	335.3
C6	4.84	317.3	359.9
C7	4.83	323.6	360.4
C8	4.80	325.6	367.1
C9	2.66	327.2	361.9
C10	2.09	327.8	288.9
C11	3.65	346.3[a]	181.7

[a] S.E. of esters, after extracting the unsaponifiable matter with ether, A11, 327.0; B14, 318.1; and C11, 332.7.

TABLE IV

Separation of the Acids of Shark Liver Oil No. 2 by the Lead Salt Alcohol and Lithium Salt Acetone Methods

Group	Description	Weight		Iodine value
		g.	%	
A	Lead salt alcohol insoluble	86.6	39.8	1.5
B	Lead salt alcohol soluble but lithium salt acetone insoluble	88.0	40.5	157.5
C	Lithium salt acetone soluble	42.9	19.7	283.8

TABLE III

Component Acids in Groups A, B, and C, and in the Whole Fat (Shark Liver Oil No. 1)

Acids	A (32.91%)	B (38.78%)	C (28.31%)	Total	Fatty acids ex-N-S[a]	
					(% wt.)	(% mol.)
Lauric	.....	0.36	.....	0.36	0.36	0.50
Myristic	1.78	1.46	.....	3.24	3.25	3.92
Palmitic	19.44	5.38	.....	24.82	24.93	26.77
Stearic	7.34	3.75	.....	11.09	11.14	10.78
Arachidic	1.18	.....	.....	1.18	1.18	1.04
Lignoceric	0.04	.....	.....	0.04	0.04	0.03
Unsaturated						
C_{12}	.....	0.13(—2.0)	.....	0.13(—2.0)	0.13	0.18
C_{14}	0.01(—2.0)	1.07(—2.0)	.....	1.08(—2.0)	1.08	1.32
C_{16}	0.42(—2.0)	7.49(—2.6)	3.21(—2.8)	11.12(—2.6)	11.17	12.11
C_{18}	0.64(—2.0)	13.33(—4.0)	5.56(—4.0)	19.53(—3.9)	19.62	19.24
C_{20}	1.62(—2.0)	5.72(—6.0)	14.87(—8.0)	22.21(—7.0)	22.31	20.11
C_{22}	0.20(—2.0)	.....	4.57(—11.0?)	4.77(—10.6?)	4.79	4.00
Non-saponifiables	0.24	0.09	0.10	0.43	.....	.....

[a] ex-N-S = excluding unsaponifiable matter.

TABLE V

Fractionation Data of the Methyl Esters of Acids of Shark Liver Oil No. 2, Fractions A, B, and C

No.	G	S.E.	I.V.
Methyl Esters of A Acids			
A1	2.81	264.1	0.8
A2	2.83	271.6	1.1
A3	2.82	276.4	1.1
A4	2.90	277.0	1.9
A5	3.60	294.8	3.1
A6	1.90	306.2[a]	32.4
Methyl Esters of B Acids			
B1	3.08	263.3	49.6
B2	4.62	273.4	94.0
B3	5.42	275.5	94.6
B4	5.46	279.0	96.2
B5	7.18	286.6	99.8
B6	6.39	297.4	105.0
B7	4.85	297.7	109.7
B8	4.50	297.8	119.1
B9	4.69	299.2	134.3
B10	4.49	315.3	196.1
B11	4.21	329.0	251.4
B12	5.08	343.4[a]	127.5
Methyl Esters of C Acids			
C1	1.80	267.6	105.4
C2	3.32	287.5	139.7
C3	3.46	302.0	215.0
C4	3.45	315.3	287.8
C5	4.54	336.7	335.8
C6	4.22	338.5	348.6
C7	3.82	348.0	312.8
C8	2.66	355.0	215.7
C9	4.10	360.1[a]	109.5

[a] S.E. of esters, after extracting the unsaponifiable matter with ether, A6, 300.3; B12, 339.4; and C9, 355.6.

Each group of the acids was converted into methyl esters and fractioned as described. The fractionation data are given in Table V.

The composition of each of the groups, A, B, and C, and the whole fat are recorded in Table VI.

Discussion

The liver oils of the sharks, belonging to the same species but from different localities (Arabian Sea and the Bay of Bengal), differ in their unsaturations, the former having an iodine value of 138.1, and the latter of 119.2. This difference manifests itself clearly when we compare the unsaturated acid portions of the two oils (Table VII).

Great similarities in the component acids of the two oils, from such very different sources, become apparent on perusal of Table VII. The total saturated acids are about 41% and 40% (wt.) with 25% and 24% of palmitic, and 11% and 14.5% stearic acids, as the major components of the saturated acid portions of the shark liver oil No. 1 and shark liver oil No. 2, respectively. Similarly the unsaturated acids are 59% and 60% with about 11% of unsaturated C_{16} in both, 20% and 23% of unsaturated C_{18}, and 27% and 26% of higher (C_{20} and above) unsaturated acids, respectively, in these oils. The mean unsaturations of the various unsaturated acids of the shark liver oil No. 1 are higher than that of the corresponding values for the shark liver oil No. 2, which is in conformity with the iodine values of the two oils.

The authenticity of these analyses has been assured by using a modern and efficient technique of separation as well as fractionating column. The similarity in the two analyses further confirms the authenticity of the present work, carried on independently by two different workers. The relatively small differences in the higher unsaturated acids may possibly be accounted for in the phosphatide portions of the oils.

TABLE VII

Component Acids of the Shark Liver Oils

Acids	Shark liver oil No. 1	Shark liver oil No. 2
Lauric	0.4	.....
Myristic	3.3	1.5
Palmitic	24.9	23.6
Stearic	11.1	14.5
Arachidic	1.2	0.3
Lignoceric	Trace	.....
Unsaturated		
C_{12}	0.1(—2.0)	.....
C_{14}	1.1(—2.0)	0.2(—2.0)
C_{16}	11.2(—2.6)	10.9(—2.0)
C_{18}	19.6(—3.9)	23.3(—2.6)
C_{20}	22.3(—7.0)	11.6(—5.8)
C_{22}	4.8(—10.6?)	12.2(—8.4)
C_{24}	.....	1.9(—11.0?)

The chief interest of the present study however lies in the higher content of saturated acids in these liver oils, which is very high for the normal marine fish oils. It may be remarked that the unsaponifiable matter of the oils is below 2% (e.g., that of the shark liver oil No. 1 is 1.48%). Tsujimoto (10) has classified Elasmobranch fish liver oils into four broad groups. The first group of oils contains little unsaponifiable matter and unusually large proportions of highly unsaturated fatty acids. The second group is of oils containing moderately large amounts (10-30%) of the unsaponifiable matter and mainly monoethylenic fatty acids. The third group of oils is characterized by very high unsaponifiable content (up to 90%) and fatty acids somewhat similar to the previous group but having more of tetracosenoic acids. The fourth group of oils is the most remarkable one, with low percentage of unsaponifiable matter (generally below 10% and only 1-2% for shark liver oils) and unusually high saturated acid content, say 50%, or even more. The present shark liver oils appear to belong to this fourth group of the Elasmobranch fish liver oils. The only two other analyses of oils of this group previously reported are those of the liver oils of the shark, *Carcharias gangeticus*, with 50% of satu-

TABLE VI

Component Acids in Groups A, B, and C, and in the Whole Fat (Shark Liver Oil No. 2)

Acids	A (39.8%)	B (40.5%)	C (19.7%)	Total	Fatty acids ex-N-S[a] (% wt.)	Fatty acids ex-N-S[a] (% mol.)
Myristic	1.27	0.22	.....	1.49	1.5	1.8
Palmitic	22.21	1.32	.....	23.53	23.6	25.8
Stearic	14.11	0.33	.....	14.44	14.5	14.2
Arachidic	0.29	.....	.....	0.29	0.3	0.3
Unsaturated						
C_{14}	Trace	0.16(—2.0)	0.01(—2.0)	0.17(—2.0)	0.2	0.2
C_{16}	0.26(—2.0)	9.01(—2.0)	1.64(—2.3)	10.91(—2.0)	10.9	12.0
C_{18}	1.37(—2.0)	18.60(—2.6)	3.30(—3.0)	23.27(—2.6)	23.3	23.2
C_{20}	0.19(—2.0)	7.29(—5.0)	4.10(—7.5)	11.58(—5.8)	11.6	10.6
C_{22}	.....	3.53(—8.0)	8.66(—8.5?)	12.19(—8.4)	12.2	10.3
C_{24}	.....	.....	1.96(—11.0?)	1.96(—11.0?)	1.9	1.6
Unsaponifiables	0.10	0.04	0.03	0.17	.....	.....

[a] ex-N-S = excluding unsaponifiable matter.

rated acids (Tsujimoto [9]) and of the ray *Dasyatis akejei* (Chinese fan fish) (Wang and Kan [12]), with 64% of saturated acids. The former has mainly palmitic and the latter mostly stearic acid.

It is worth noting that the *Carcharius gangeticus* shark belongs to the same family of Elasmobranch fishes as the ones whose liver oils have been studied by the present authors (*Carcharhinidae* family). The similar nature of all the three liver oils strongly suggests that such peculiar compositions are characteristic of this particular family of fish. Such specific effects connected with phylogenetic relationships are now very well recognized in the vegetable fats and also in the animal fats.

Lovern has pointed out in the case of eels (7) and of tunny (8) that there is a definite tendency towards the deposition of a less unsaturated fat at higher temperatures. As the present liver oils come from the fish of warmer, almost equatorial waters, their relatively saturated nature is explainable in the light of the above observations. Lovern has also visualized (5) simultaneous hydrogenation of polyethylenic and monoethylenic derivatives in the normal preformed fats of Tsujimoto's fourth group. It is evident that any final conclusions can be drawn only when a few more such reliable and detailed data are available.

Lovern has rightly remarked (6) that "it is unfortunate that out of the great range of Elasmobranch species there are only a few for which any quantitative fat analyses are available. Many qualitative and semi-quantitative investigations, largely by Japanese workers, have served to show that several distinct types of fat may occur in this group." The present analyses are probably the first detailed ones of an oil of this group, and the third illustration of this peculiar group.

Summary

1. Two samples of the liver oil of an Indian species of shark *(Galeocerdo rayneri)*, one from the Arabian Sea, and the other from the Bay of Bengal, have been studied. Their component acids are reported.

2. Tsujimoto's lithium salt acetone method has been adopted for the separation of highly unsaturated acid fraction from the mixed acids in one case while in the other the modified technique of Lovern has been followed. The insoluble acids have been further resolved into two fractions with the help of Hilditch's modified lead-salt alcohol method. The efficient column (E.H.P.) of Longenecker has been employed for fractionation in the present work.

3. The liver oils are found to belong to the fourth group of Tsujimoto's classification of the Elasmobranch fish liver oils. Shark liver oil No. 1 contains 40.9% saturated acids (palmitic 24.9%, stearic 11.1%, also myristic 3.3%, and minor proportions of lauric, arachidic, and lignoceric) and 59.1% unsaturated acids (C_{16} is 11.2%, C_{18} 19.6%, C_{20} and above 27.1%, also some C_{12} and C_{14} monoethenoids).

Shark liver oil No. 2 has the following composition: saturated acid 39.9% (palmitic 23.6%, stearic 14.5%, and myristic 1.5%, together with a minor amount of arachidic acid) and unsaturated acids 60.1% (mainly C_{16} 10.9%, C_{18} 23.3%, and C_{20} and above 25.7%; C_{14} acids are also present).

The abnormal saturated acid content is discussed. These analyses provide the third instance of this peculiar group of Elasmobranch liver oils.

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Studies on Lipolytic Molds.[1] Comparative Study of Lipases Obtained From Molds Grown on Oil Seeds

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CAMUS (1) found that lipase is present in *Aspergillus niger* and *penicillium glaucum*. Gerard (2) observed the presence of lipase in *penicillium glaucum* isolated from the vegetable kingdom. Wehmer (3) detected the presence of lipase in *Aspergillus* species. Haehn (4) had explored the possibility of fat synthesis by fungus and yeast enzymes. David Kirsh (5) had studied the different factors influencing the activity of fungus lipase. Fodor and Chari (6) studied in detail the activity of lipases present in *Aspergillus* and *Penicillium* species.

The literature just cited gives a clue that lipase can be extracted from certain strains of molds like *Aspergillus, penicillium*, etc. Ramakrishnan and Nevgi (7) investigated the various oil seeds, and Ramakrishnan and Banerjee (8) investigated the oil seedcakes for their lipolytic activity with a view to get a cheap and active lipase for fat hydrolysis. In continuation of their search for an active lipase they have investi-

[1] Read before the XIIth International Congress of Pure and Applied Chemistry, September, 1951, New York.