Distribution of *trans*-6-Hexadecenoic Acid, 7-Methyl-7-Hexadecenoic Acid and Common Fatty Acids in Lipids of the Ocean Sunfish *Mola mola*

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ABSTRACT

Lipids extracted from various tissues of four individual sunfish have been shown to contain the *trans*-6-hexadecenoic acid previously reported for marine turtles, a metridium and a jelly fish, and also the 7-methyl-7-hexadecenoic acid recently isolated from one sunfish liver oil sample. The other fatty acids present were qualitatively typical of marine lipids in general. Unusual quantitative details included high percentages of 18:0 and 20:4ω6, which are also found in the Atlantic leatherback turtle and presumably linked to a similar diet of jellyfish and to other common factors. In a sample of lipids from intestinal contents *trans*-6-hexadecenoic acid was found to be the predominant C_{16} monoene, and was accompanied by comparatively large amounts of the 7-methyl-7-hexadecenoic acid. This observation and other fatty acid details are compatible with an exogenous origin for these acids and jellyfish, etc., as a predominant dietary material for the ocean sunfish.

INTRODUCTION

Jellyfish have been reported to be dietary factors common to the leatherback turtle *Dermochelys coriacea coriacea* (L) and the ocean sunfish *Mola mola* (L) (1,2). Our interest in marine food chains led us to carry out an analysis of lipids from a single specimen of sunfish caught in the summer of 1969 off the Nova Scotia coast. Three additional sunfish were caught in the summer of 1971 to confirm our early results, because of unnusual features of fatty acids and the dearth of accurate biological data. Thin layer chromatographic (TLC) and gas liquid chromatographic analysis of these lipids for *trans*-6-hexadecenoic acid (*t*-16:1ω10) led to the discovery of 7-methyl-7-hexadecenoic acid (7-M-16:1ω9) as a significant and unusual component, which was isolated for study from liver lipid (3). (The shorthand notation of chain length: number of double bonds and position of the double bond relative to the terminal methyl group is conveniently supplemented with the prefixes *t* for *trans* and 7-M for 7-methyl.)

With the benefit of hindsight, the 7-M-16:1ω9 acid probably also appears as a minor ($<<1\%$) component in the fats of marine turtles (4), a metridium (5), a jellyfish (6) and perhaps in other analyses where it has been reported as tentatively identified 16:2ω6, which would usually fall in about the same position.

In one of these fish the intestinal content was successfully extracted and the presence of both unusual fatty acids lends credence to our belief that their origin is dietary for the sunfish and probably for the leatherback turtle (4).

EXPERIMENTAL PROCEDURES

Samples and Lipid Recovery

Sunfish 1969-1 was caught in a tuna trap off Queensland, N.S., on July 25, 1969, transported to the Halifax Laboratory of the Fisheries Research Board, and frozen the same day. This specimen weighed ca. 60 kg and was male. On August 4th the fish was dissected after being allowed to thaw overnight. Tissue samples were taken as follows: the white collagenous subdermal material (WCSM), 2-5 cm in thickness in our specimens; a thin "fatty" fibrous layer found between this layer and the muscle; white muscle; dark muscle; and a portion of the liver.

All 1971 sunfish were female with no overt gonad development. Details were: Sunfish 1971-1 weighing 190 kg, was caught off Crouchers Island, N.S., on July 2, 1971. It was dissected in the field the same day. Samples of the liver and white muscle were analyzed for fatty acid content. Sunfish 1971-2 was caught off Eastern Passage, N.S., July 29, 1971, and weighed 90 kg. Samples of white muscle and liver were taken. Sunfish 1971-3 was caught in St. Margaret's Bay and was taken to the F.R.B. facilities at Boutiliers Point Biological Station on August 18, 1971. Total weight was ca. 150 kg. Samples obtained included liver and intestinal contents.

Sunfish 1969-1

The lipids were extracted by the method of

Bligh and Dyer (7) and, with the exception of those from the thick white collagenous layer were separated on a scale of 100-500 mg into phospholipids, triglycerides and "other" or on a 25 x 400 mm column of Dow styrene-divinylbenzenecopolymer beads (X_2, 200-400 mesh) eluted with benzene (8,9). Esters were prepared by transesterification of these fractions using 7% BF_3-methanol and refluxing for 30-45 min. The total lipids from the WCSM were saponified following AOCS Method Ca-6b-53. The recovered fatty acids were converted to methyl esters by refluxing briefly with BF_3-methanol.

Sunfish 1971-1,2,3

The lipids from the flesh and liver samples were extracted by the method of Bligh and Dyer (7), and the flesh lipids separated into phospholipid and triglyceride fractions on the gel column. The liver lipids, a clear yellow oil in each case, were found by TLC to be mainly triglyceride. These were purified by gel chromatography, and saponified by AOCS Method Ca-6b-53. The fatty acids were recovered and converted to methyl esters by refluxing with 7% BF_3-methanol.

The intestinal contents were a milky fluid. Lipid was extracted by shaking with petroleum ether. The emulsion formed was centrifuged and the clear upper layer decanted and dried over Na_2SO_4. TLC of the recovered lipid showed small amounts of phospholipids, free sterol, triglycerides and sterol esters, and a large amount of free fatty acid. The sample was saponified as above and the fatty acids purified by preparative scale TLC on Prekotes (Adsorbosil 5, from Applied Science Lab.) eluted by hexanes-diethyl ether-acetic acid 70:30:1. The recovered acids were esterified as described for sunfish 1969-1.

Analyses of samples

GLC analyses of all esters were carried out on 150 ft open-tubular capillary columns coated with butanediolsuccinate polyester (BDS) in Perkin-Elmer Model 226, 900 or 990 GLC apparatus as previously described (4,10). The presence of *t*-16:1ω10 acid was confirmed by preparative scale TLC on silver nitrate-silica plates (Supelcosil 12D) developed with *n*-hexane-benzene 1:1. Analyses of both total esters and the monoene fractions from TLC were carried out on Apiezon columns to confirm the presence of the two unusual acids (11). Quantitation based on Disc Instruments Inc. integrators may have had total errors of the order of ±5% for major (>10%) components, ±10% for moderate scale (1-9%) components, or as much as ±30% for minor (<1%) components. A portion of each ester sample was hydrogenated and analyzed qualitatively and quantitatively as a check on correction factors in the total analysis. The presence of 7-methyl-7-hexadecenoic acid in the 1971 sunfish had previously been demonstrated in detail (3).

RESULTS AND DISCUSSION

The first sunfish examined (1969-1) had a rather lean liver compared to the sunfish livers extracted in 1971 (Table I). However the liver oils of all four fish when fractionated on the gel column were found to be >95% triglyceride. The first fraction to elute, ~2%, was considered to be phospholipids, and TLC showed the remaining 2% to be made up of free fatty acids and sterol esters with the properties of cholesterol esters.

The dark muscle lipid on TLC examination contained a large spot for free fatty acids. It may have been partially hydrolyzed during frozen storage (9), and the lipid fatty acid results are not included in Table I. Similarly the results of the phospholipid analysis of the liver have been omitted as possibly misleading as to their original fatty acid compositions. These two tissues exhibit more enzymatic activity towards lipids than the white muscle. The fatty acids for dark muscle total lipid were basically similar in composition to the white muscle triglyceride results of Table II. The so-called "fibrous layer" was initially thought to be a fatty membrane layer between the WCSM and white muscle, but this proved rather lean (0.3% lipid, of which 38% was recovered as triglycerides and 49% as phospholipid). The WCSM referred to above is the white collagenous layer between 2 and 5 cm in thickness, which surrounds the visceral organs but has no obvious function (12). This material emulsified severely during the Bligh and Dyer extraction and the $CHCl_3$ layer yielded only 0.02% lipid. TLC indicated mostly polar lipids. The low proportion of triglyceride in this lipid (Table I) suggests that the lipid is natural to this tissue and not contamination from adjacent tissue. However the lipid may have been localized in nerves or other specialized inclusions rather than part of the bulk of the tissue. Both chemical and histological analyses confirmed that this material was mainly collagen (D.H. Shaw, P.H. Odense, private communications).

The fatty acid analyses of the lipids from the various sunfish tissues (Table II) include all the major and minor fatty acids found in lipids of other higher marine species that we have analyzed, e.g., cod liver oil (10), and the two unusual fatty acids previously reported (3,4).

TABLE I

Lipid Recoveries from Samples and from Gel Chromatography of Lipid Extracts

Sunfish	Sample	% Lipid	% Triglycerides	% Phospholipids	% "Other"
Sunfish 1969-1	Liver, 4.2% of fish	31.9	96.2	1.8	1.5
	Dark flesh	1.21	42.7	51.2	8.6
	White flesh	0.48	32.8	59.5	8.2
	Fibrous Layer	0.30	38	49.2	12.4
	White collagenous layer	.02	<10	>80	∿10
Sunfish 1971-1	Liver, 3.2% of fish	44.3	>95	>2	∿3
	White flesh	0.73	58	20.8	N.D.
Sunfish 1971-2	Liver, 5.0% of fish	56.0	>95	>2	∿3
	White flesh	0.22	36	60	N.D.
Sunfish 1971-3	Liver	42.0	>95	<2	∿3
	Intestinal content	N.D.	>75% free fatty acids remainder phospholipids, mono-, di- and tri-glycerides.		

The percentages of *t*-16:1ω10 and 7-M-16:1ω9 show a parallel relationship in all samples. For example in the four liver oils (Table II), the ratios *t*-16:1ω10 to 7-M-16:1ω9 were 1.12, 1.20, 1.12 and 1.27. In the intestinal content and WCSM, where both values are unusually high, these ratios are 1.05 and 1.26, respectively. This constant value for the two unusual fatty acids in four different fish indicates that these are both normal components for the ocean sunfish, and as the *t*-16:1ω10 is thought to be exogenous in origin the same origin may be inferred for the 7-M-16:1ω9.

An unusual feature in two of the four individuals (sunfish 1969-1 and sunfish 1971-3) is the ratio in all lipids of 22:1ω11 + 13<22:1ω9. The normal proportion for marine oils containing these acids is 22:1ω11 + 13>22:1ω9. This peculiarity in two animals is observed not only in the liver oil, where these depot-type acids are important, but also in the rest of the lipids, including phospholipids. Other monoethylenic acids do not show a comparable distribution of related isomers. However 20:1ω7 nearly equal to 20:1ω9 in 1969-1 and 1971-3 is also novel, and the proportion of 20:1ω7 in the 20:1 isomers is higher than is found in depot fats of most higher marine animals, e.g., 4,10,13-16, except data in some analyses of marine turtles, where parallels may be found (4). Our jellyfish studies (6,8) provide clear evidence for 20:1ω7 > 20:1ω9 and 22:1ω9 > 22:1ω11 + 13.

The most common fatty acids (Table II) have some interesting features. For example the saturated acids show the minor component distribution pattern usual for marine lipids from higher animals; but 18:0 in the liver oil is undoubtedly at least twice the percentage usually found in marine animal triglycerides, although not exceptional for phospholipids. In the opinion of the authors, 20:0 is also a more obvious component in the sunfish liver oils, perhaps aobut twice the percentage commonly observed in other fish depot fats; but less importance should be attached to this because of the possible errors in GLC quantitation of a component of this magnitude. The saturated acids as percentages of totals in the four liver oils are quite surprisingly uniform at 31, 31, 28 and 30%, whereas the total usually observed in marine oils is 20-25% of fatty acids.

Considering the size of the animal, the thickness of the integument and the propensity for basking on the ocean surface, the level of 18:0, etc., in the liver oil might suggest an internal body temperature above ambient such as observed (17) in the leatherback turtle, an animal of comparable size and behavior pattern. The iodine values calculated for the fatty acids of the white muscle phospholipids, 168 and 188 (Table II), are quite low, as 200-220 would be expected for fish muscle phospholipid fatty acids (18). One interpretation would be to associate this also with a higher body temperature, but comparative data for fish muscle are lacking. The presence of 20:4ω6 in lieu of 20:5ω3 or 22:6ω3 accounts for only 5-10 IV units of this difference, which otherwise is due to generally low levels (25-30%) for the total of 20:5ω3 + 22:6ω3. This total is usually 35-45% in Atlantic fish muscle phospholipids, and it would seem possible that the proportions of the ω3 acids in the ocean sunfish phospholipids reflect functional differences, while the ω6 acids reflect dietary influences.

Among the polyunsaturated fatty acids of the liver oils, the ω6 or linoleic group is unusually important for marine fish depot fats, but mostly in the form of 20:4ω6—another

TABLE II

Weight Per Cent Composition[a] of Fatty Acids in Various Lipids Recovered from Four Specimens of Ocean Sunfish *Mola mola* and Jellyfish *Cyanea capillata*[a]

	Mola mola Specimen, tissue and lipid														
	1969-1					1971-1			1971-2			1971-3			
	Liver-isolated	White muscle	Fibrous layer		WCSM	Liver oil	White muscle		Liver oil	White muscle		Liver oil	Intestine contents	Jelly fish	
Fatty acid	TG	TG	TG	PL	Total	Total	TG	PL	Total	TG	PL	Total	Total	TG	Polar
12:0	Trace	Trace	ND	Trace	ND	Trace	Trace	.69	Trace	ND	ND	Trace	ND	0.17	ND
14:0	2.00	.75	1.32	.48	4.55	4.72	.94	1.25	3.59	.15	.74	2.18	.86	1.92	0.91
4,8,12	.07	.03	ND	.03	.16	ND	.04	ND	ND	.03	ND	ND	ND	0.11	ND
Iso 15:0	.10	.05	.27	.03	.32	.33	.05	.10	.06	.05	.05	.22	.15	0.56	0.30
Anteiso 15:0	.03	.01	.05	.02	.16	.02	.02	.06	.02	0.2	.01	.09	.08	0.11	0.06
15:0	.73	.45	.55	.30	1.93	.86	.31	.25	.68	.39	.37	.65	.66	1.07	0.84
Iso 16:0	.08	.08	.05	1.06	.64	.12	.04	.20	.13	.05	.05	.13	.09	0.33	0.12
Pristanic	ND	.10	.05	ND	ND	.04	.17	.10	ND	ND	.13	ND	ND	0.11	1.43
16:0	16.49	15.50	14.47	14.35	22.77	16.52	12.71	15.74	14.42	15.46	13.42	15.82	7.76	13.32	6.86
Iso 17:0	.66	.10	.30	.17	.06	.57	.70	.20	.38	.11	.13	.47	.72	1.10	ND
Anteiso 17:0	.16	.10	.09	.06	Trace	.10	.09	.16	.13	.06	.09	.17	.12	0.99	0.35
17:0	.64	1.14	.75	.87	1.42	1.06	.59	.71	1.08	.67	.63	.76	.88	0.55	0.47
Phytanic	.09	.05	.21	.14	ND	.16	.12	.02	.12	.12	.09	ND	ND		
Iso 18:0	.22	.22	.13	.03	.12	.15	.19	.25	.35	.35	.18	.08	.12	0.44	0.12
18:0	8.88	14.40	14.19	14.53	11.55	6.48	9.97	12.51	6.22	8.97	11.03	9.22	6.59	4.57	4.39
19:0	ND	.54	.44	.46	.49	.14	.35	.41	.28	.41	.48	.21	.25	0.65	0.86
20:0	.35	.44	.65	.14	.55	.22	.30	.32	.15	.28	.20	.31	.10	1.40	2.41
Total[b]	31	34	34	33	45	31	27	33	28	27	28	30	19	27	19
14:1ω9?	—	.26	.66	.09	.81	.21	—	—	.21	.08	.05	—	.06	ND	ND
14:1ω7?	.07	—	—	—	—	—	3.24	.10	.39	.23	.07	.13	.06	ND	ND
15:1	.03	.38	.11	ND	.53	.02	.09	.05	.02	.12	.25	ND	ND	ND	ND
t-16:1ω10	2.74	1.62	1.28	1.16	3.45	2.94	1.05	.71	2.48	.77	.72	2.96	3.84	2.22	0.89
16:1ω7	5.50	2.02	1.63	1.40	3.00	6.87	2.38	2.02	6.16	1.53	1.80	5.08	1.83	1.11	0.15
16:1ω5	.11	.15	ND	.23	ND	.33	.17	.10	.38	.64	.27	.13	.20	0.14	ND
7-Methyl-7-hexadecenoic	2.44	2.12	1.09	2.45	2.81	2.45	1.33	1.01	2.22	1.24	1.35	2.33	3.67	1.20(?)	0.38(?)
17:1ω10	ND	ND	ND	ND	ND	.12	.03	.03	.09	.09	.09	ND	ND	ND	ND
17:1ω8	.95	.50	.32	.17	.40	.49	.43	.33	.50	.32	.27	.42	.70	0.08	0.03
18:1ω9	15.70	10.29	9.65	7.99	7.96	11.39	12.61	14.09	8.83	8.88	10.03	17.64	3.48	5.17	1.04
18:1ω7	3.77	2.38	3.15	2.28	1.73	2.31	2.74	1.79	2.12	1.75	1.16	2.49	1.75	1.94	0.98
18:1ω5	.32	.05	.21	0.05	.06	.16	.27	.20	.54	.19	.20	.57	.36	ND	0.22
19:1	.28	.20	.05	.06	.07	.20	.08	.05	.19	.06	.10	.10	.05	0.05	ND

20:1ω11	.21	.10	.13	0.03	.12	.36	.34	.39	.26	.43	.65	.21	.04	2.56	1.33
20:1ω9	2.42	2.01	3.27	.80	1.22	4.17	4.58	2.90	7.54	4.61	3.27	3.80	.93		
20:1ω7	1.08	.71	1.32	.23	.98	1.55	1.15	.49	.86	.34	.52	2.36	.66	3.28	4.70
20:1ω5	.11	.02	.05	.06	.12	.08	.08	.05	.12	.05	.04	.26	.05	0.64	1.14
22:1ω13+11	.45	.92	1.41	ND	.61	2.32	4.36	.97	5.61	7.35	2.29	1.42	.09	0.53	0.06
22:1ω9	.58	1.19	1.93	ND	.30	1.18	2.01	.15	1.40	.98	.34	2.29	.36	2.90	0.68
22:1ω7	.08	.10	.05	ND	ND	.04	.07	ND	.30	.06	ND	.18	.01	0.42	0.14
22:1ω5	ND	ND	ND	ND	ND	.13	ND	ND	ND	ND	ND	ND	ND	0.08	ND
24:1	.05	.12	.20	ND	ND	.02	.50	ND	ND	.33	.09	ND	.11	ND	ND
Total[c]	37	25	26	18	25	37	38	25	40	29	22	42	18	22	12
16:2	ND	ND	ND	ND	ND	ND	.07	ND	.25	.02	ND	ND	ND	0.11	ND
18:2ω6	.83	.94	.57	.74	.93	.80	.82	1.09	1.12	1.14	1.36	.93	1.99	0.81	0.17
20:2ω6	.51	.22	.41	.26	.12	.24	.20	.17	.49	.21	.26	.97	.91	1.33	1.08
22:2ω6	.13	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.42	0.22
Total	1.5	1.2	1.0	1.0	1.0	1.0	1.1	1.3	1.9	1.4	1.6	1.9	2.9		
18:3ω6	.09	.15	ND	.09	.03	.23	.28	ND	.20	ND	ND	ND	ND	0.08	ND
18:3ω3	.60	.05	.16	.38	.25	ND	.31	.06	.11	.50	.31	.75	.72	0.51	0.12
20:3ω6	ND	.05	.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	.30	ND	ND
20:3ω3	.19	.08	.06	ND	ND	.04	.06	ND	.15	ND	.08	.29	.40	0.65	0.28
Total	.88	.33	.27	.47	.28	.27	.65	.06	.46	.50	.39	1.04	1.42	1.24	.40
16:4ω1	ND	ND	ND	.31	ND	.04	.07	.40	.06	ND	ND	ND	ND	0.11	ND
18:4ω3	.61	.27	.29	.03	.19	2.96	.48	.69	3.09	.78	.62	1.09	.48	0.84	0.13
20:4ω6	3.13	7.73	5.93	10.62	10.30	1.35	3.80	6.21	1.74	5.89	7.35	1.79	9.26	3.27	11.10
20:4ω3	.57	.15	.12	.03	.25	.51	.21	.21	.52	.13	.23	.62	.45	0.92	0.61
22:4ω6	.65	.61	.18	.54	.14	.11	.24	.42	.10	.17	.42	.26	.60	0.46	1.64
Total	5.0	8.8	6.5	11.5	10.9	5.0	4.8	7.9	5.5	7.0	8.6	3.8	10.8	5.6	13.5
20:5ω3	7.24	8.67	5.70	6.90	3.77	7.70	8.34	10.47	7.55	9.31	11.01	7.12	17.41	12.38	24.46
21:5ω2?	.15	ND	ND	ND	ND	ND	ND	ND	.25	.07	ND	ND	.49	0.16	0.53
22:5ω6	.65	1.08	1.24	2.11	1.53	.26	.63	.76	.82	2.05	2.31	.64	1.40	1.34	2.15
22:5ω3	4.17	1.62	2.69	1.69	.72	5.02	3.81	2.52	3.06	1.37	1.15	3.90	2.81	3.99	5.51
Total	12.2	11.4	9.6	10.3	6.0	13.0	12.8	13.8	11.7	12.8	14.5	11.7	22.1	17.9	32.6
22:6ω3	10.87	16.97	21.60	21.55	8.22	10.72	16.16	14.70	11.16	18.75	18.30	8.75	24.48	21.15	17.56
Unknowns[d]	1.76	2.19	.99	5.18	3.75	1.17	.41	3.89	1.44	2.40	5.43	.21	2.62	2.00	3.50
Calculated IV	153	175	180	194	122	147	171	168	150	183	188	136	251	208	269

[a]TG = triglyceride. PL = polar lipid. ND = not determined usually for technical reasons associated with gas liquid chromatographic conditions. Two decimal places are for minor component comparison purposes only.

[b]21:1 and 22:0 were not determined, but 22:0 was not important.

[c]Two isomers were sometimes observed for 15:1, three for 17:1 and three for 19:1.

[d]Mostly a few corresponding to acetal breakdown products.

TABLE III

Chain Length Percentages of Triglyceride Fatty Acids as Determined on Hydrogenated Samples

	1969-1			1971-1		1971-2		1971-3
Fatty acid	Liver TG	White muscle TG	Fibrous layer TG	Liver oil TG	White muscle TG	Liver TG	White muscle TG	Liver TG
14:0	1.8	.5	.8	5.7	.9	5.1	1.4	2.3
16:0	26.5	25.9	17.8	24.4	20.0	26.2	19.9	25.6
18:0	28.6	23.9	28.1	23.7	27.1	23.5	23.8	30.8
20:0	11.7	17.9	17.5	20.7	17.6	19.3	24.7	16.6
22:0	18.2	23.1	29.6	25.9	29.5	21.0	21.8	17.5

feature shared with leatherback turtle oils (4) and also potentially linked to jellyfish (6,8). (In Table I of Reference 6, for 20:1ω6 read 20:4ω6.) The 22:5ω6 is also unusually high in these oils, but may be linked to the fact that 22:5ω3 is nearly half of 22:6ω3 rather than to the accumulation of 20:4ω6. In our experience with fish liver and depot oils, a 1:10 or 1:5 ratio for 22:5ω3 and 22:6ω3 would be normal. The possible biochemistry of higher proportions of 22:5ω3 in marine mammal oils has been discussed elsewhere (15). Thus, in the $C_{22} > C_{20}$ relationship for total chain lengths (Table III), there is an indication of conditions favoring chain extension to the C_{22} polyethylenic acids, but not necessarily 22:6ω3.

Table III summarizes the overall compositional relationships among the four samples of liver triglycerides and also suggests that the white muscle triglycerides, with the exception of the C_{14} acids, resemble the corresponding liver triglyceride in fatty acid composition. In some fatty acids (1971-1, 1971-2) there are indications that the muscle triglycerides are also related to the phospholipids in composition. For example percentages for 18:0, 20:4ω6, 20:5ω3 and 22:5ω3 are intermediate (Table II). This type of organ or tissue specific association has been noted elsewhere, i.e., in seal lipids (19).

The white muscle phospholipids had fatty acid compositions that were slightly abnormal. The total for the linolenic series acids 20:5ω3 + 22:5ω3 + 22:6ω3 is usually 35-45% in comparable muscle phospholipids recovered from freshly killed marine fish or from those samples kept under ideal frozen storage conditions for short periods (9,14,16,18,20). The totals of these acids for sunfish 1971-1 and 1971-2 were 27.9% and 30.7%, but inclusion of the linoleic series acids 20:4ω6 and 22:5ω6 raises these totals to 35.3% and 40.8%, respectively, acceptable as normal values. This indicates that the two linoleic acids replace the acids of the linolenic acid series and are not extra to a normal percentage of the latter. Confirmation that these analyses are valid representations of original compositions is provided by an analysis carried out by J.C. Nevenzel (see also below), which gave 43% of C_{20} and C_{22} polyunsaturated fatty acids from total muscle phospholipids. From the total lipid these phospholipids were estimated as 22.6 ± 4.7% phosphatidylcholine and 9.5 ± 0.1% phosphatidylethanolamine. These proportions are generally observed in fish muscle (20). The four sunfish studied were all dead when examined by laboratory staff and adverse sample handling, including prolonged thawing of a whole fish (1969-1) or frozen storage of other samples (1971), would favor lipid hydrolysis (9,21,22). This process especially degrades phospholipids, not always equally (23), although this may not be obvious from liberated fatty acids (21,22). Triglycerides are also affected and may account for the low lipid recovery for white muscle sample 1971-2 (Table I). We believe that the fatty acid compositions of Table II for phospholipids from white muscle are possibly from mildly hydrolyzed phospholipids but are substantially correct.

The respective fatty acid compositions of the two lipids from the "fibrous layer" (Table II) do not show features that could be paralleled exactly in other isolated lipids. This type of layer may be a fascia layer, as indicated by the tough fibres observed and by the low lipid content. The superficial appearance of fat was evidently misleading, and other types of soft lubricious tissue may have been present. The WCSM, with virtually no recoverable lipid, cannot be discussed in detail, but the low proportions of 20:1 and 22:1 acids indicate that the lipid is cellular or membrane in origin (compare phospholipids). The IV of 122 calculated from the GLC analysis of total lipid methyl esters of fatty acids was the lowest of any tissue examined (Table II). Proportions of *t*-16:1ω10 and 7-M-16:1ω9 acid were unusually high, with *t*-16:1ω10 the predominant C_{16} monoene. These acids may have been associated with similar high proportions of 14:0 and 16:0

for reasons of similarity of chain length and physical behavior.

The concentration of fat in the liver of the ocean sunfish is not unusual. The common cod, *Gaduas morhua*, also has mostly white muscle containing ca. 0.75% lipid and the fat reserves are concentrated in the liver, which is 2-4% of the weight of the fish and 30-60% fat. Moreover the normal IV of 150-160 for cod liver oil is comparable to that of the *Mola mola* liver oils. Unlike the cod, the ocean sunfish has no swim bladder (24), but there is no evidence from the type of lipid in the liver that lipid is a factor in controlling buoyancy.

The habit of the ocean sunfish of basking on the ocean surface and some folklore of therapeutic properties of the liver oil in topical administration to man and animals led us to consider the vitamins in this oil. The Laboratory of the Government Chemist, London, U.K., examined the liver oil from specimen 1972-2 and found 180 μg/g of α-tocopherol but only traces of vitamin D. We are therefore inclined to discount that any such properties for this oil could be due to fatty acids or oil-soluble vitamins. However relatively short chain monoethylenic fatty acids are alleged to have therapeutic properties (25), and unknown properties might be conferred by 2-3% of *t*-16:1ω10 or 7-M-16:1ω9. Our iodine values for liver oil of 136-153 compare with 151.8, and unsaponifiable content of 1-3% with 3.2%, for oil from a Pacific specimen (26).

Gel chromatography of the lipid from the white muscle of fish 1969-1 yielded 8.2% of "other" lipids. There were not investigated in detail but the refractometer trace indicates that free sterol and sterol ester were important components. An older report (26) gives 24% unsaponifiable material in muscle lipid of iodine value 102.7 (presumably badly oxidized). This report led J.C. Nevenzel (private communication) to examine the unsaponifiables in the muscle lipid from a small Pacific *Mola mola*. The lipid included 14% free sterols (92% cholesterol by GLC), traces of hydrocarbons and a few per cent of probable wax esters. Otherwise the lipid sample composition was 30-40% phospholipid, 8% triglycerides and 23% free fatty acid (see Table I and discussion above).

The diet of the ocean sunfish includes shallow water bottom invertebrates, (27) but otherwise is fairly obscure. In addition to jellyfish (1,2,27), deep sea fish (of which some probably move near the surface at night) and seaweeds have been mentioned. Our examination of two fish showed empty stomachs and only a milky, oily looking fluid in the intestine. The latter, however, contained very little lipid. This lipid contained fatty acids (Table II) with very low levels of 16:1 (compare jellyfish [8] at 2.2-5.2%), and 18:1 (compare 4.5-7.2%); and high levels of 20:4ω6 (compare 6.2-9.1%), 20:5ω3 (compare 9.8-18.4%) and 22:6ω3 (compare 10.4-19.4%). We therefore feel that this analysis further supports our views linking jellyfish with the diet of the ocean sunfish. Observations by fishermen in Nova Scotian waters have indicated that the fish are active on the surface. Many are caught in net traps set for mackerel, herring or cod, suggesting a considerable degree of activity necessary to follow the net leader wall to the trap. The net may in fact, at the time of year usual for sunfish, also have large jellyfish entangled in it. Otherwise, sea gooseberries (*ctenophores*) are often common in the area near Halifax where ocean sunfish are observed. The suggestion that captures such as ours were moribund and therefore abnormal animals (24) may be discounted on grounds of the availability of food, the relatively warm water, and the apparently good liver condition in three fish.

Our study of fatty acids suggests that the ocean sunfish obtains a major part of its sustenance from jellyfish and similar pelagic invertebrates, which concentrate fatty acids of the linoleic acid type. Two unusual fatty acids may be of exogenous origin. There is a possibility that a fatty acid composition normal for marine fish muscle is modified by above-ambient internal body temperatures.

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