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Phospholipids of Tuna White Muscle

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Abstract

The composition of the lipids from the white muscle of five tuna fish has been determined. Total extractable lipid varied from 0.5%–10.3% of the tissue wet weight; phospholipid content ranged from 0.3%–0.6%. The separation of the phospholipid components was made by column chromatography with activated silicic acid and stepwise elution, with increasing concentrations of methanol in chloroform. The components were identified by chemical tests and infrared (IR) spectra. Tuna white muscle contained an average of 0.5% phospholipid on wet weight basis; 23% was cephalin, 54% lecithin, 8% sphingomyelin, 2% phosphoinositide, with small amounts of unidentified components. Ten to 30% of the lecithin and cephalin fractions were in the form of plasmalogens. The gas-liquid chromatographic analyses of the 12–22 carbon fatty acids of the lecithin, cephalin, and neutral fractions are presented. The cephalins were characteristically high in stearic acid and low in palmitic acid, in contrast to the lecithins.

Introduction

WHOLE LIPID EXTRACTS of fish muscle contain a complex mixture of neutral and phospholipids (1). The fatty acids derived from these components offer a broader mixture of chain lengths and degrees of unsaturation than do those found in the lipids from tissues of land animals. Phospholipids in lipid extracts of tuna muscle have been investigated by Katada (2), who showed that the components of light and dark meat varied in composition and complexity. Improved methods of fractionation and techniques for analysis prompted a reinvestigation of the distribution and composition of phospholipids from tuna white muscle. This paper describes the resolution of the components of whole lipid extracts by silicic acid chromatography, and the composition of the fractions obtained.

TABLE I

Identification of Tuna Samples [a]

Sample	Weight	Fat-free residue	Total lipid	Total phospholipid
	kg.	% wet weight	% wet weight	% wet weight
Albacore I	7	22.6	10.3	0.41
Albacore II	12	27.4	1.2	0.26
Albacore III	7	23.4	8.8	0.62
Albacore IV	5	23.4	9.0	0.37
Yellowfin I	11	22.3	0.5	0.31

[a] The albacore, *Thunnus alalunga*, and yellowfin, *Thunnus albacares*, samples were obtained as follows: Albacore I and III, from San Francisco area, fresh, 2 and 3 days old; Albacore IV, from Oregon area, frozen, 11 wk old; Albacore II and Yellowfin I, from Japanese area, frozen, unknown age. Data on percentage composition refer to white muscle only.

Experimental Procedure

Source of material. Five tuna were investigated. Four were albacore, *Thunnus alalunga,* and the fifth yellowfin, *Thunnus albacares.* The frozen tuna were allowed to thaw partially and then were eviscerated. White muscle was carefully separated from the red meat. The source, weight, and length of storage for each fish is included in Table I.

Extraction. The procedure for extraction of lipid from white muscle was based on the method of Bligh and Dyer (3). The tissue, in 100 g lots, was blended mechanically for 2 min with 3 vol of chloroform/methanol (2:1) solution. 100 ml chloroform and 100 ml distilled water were added to the mixture, and blending was repeated for 1 min. The homogenate was filtered and the filtrate allowed to separate into chloroform and aqueous layers. The tissue residue was re-extracted with 150 ml chloroform, and the mixture again filtered. The chloroform-lipid layers were combined. Further extraction of the residue failed to yield a significant amount of lipid. A few crystals of hydroquinone were added during homogenization, and also to the combined chloroform-lipid phase, to inhibit oxidation. The crude extracts contained 2–29 mg lipid/ml extract. They were stored under nitrogen at −13C. These extraction and storage procedures gave products which had a very low degree of oxidation. The peroxide method of Dam and Granados (4) gave values close to zero when performed on fresh extracts.

Fractionation. Whole lipid extracts were fractionated by silicic acid chromatography. The silicic acid (Mallinckrodt, No. 2847) was first washed successively with methanol, 20% methanol in chloroform, chloroform and methanol. The washed silicic acid was then dried under vacuum at 120–140C to remove residual methanol, excess moisture, and absorbed oxygen. Purified nitrogen was introduced into the flask after 2 days of drying, and the material was stored in this manner. The water content of the silicic acid preparations varied from 8–9% as determined by ignition at ca. 750C.

Trial chromatographic columns were run to determine the approximate phospholipid content of the individual samples of whole lipid. Ca. 100 mg whole lipid was applied to a 10 g silicic acid column. The neutral fraction was separated from the phospholipid components by stripping the non-phosphorus fractions off the column with chloroform followed by methanol to elute the phospholipids. The lipid load of each preparative column was determined by the percentage of phospholipid in each trial run. Large scale separations were carried out with not more than 6 mg phospholipid per g adsorbent. The preparative glass columns were 4.5 cm diam and 70 cm

high, with the silicic acid packing ca. 35.0 cm in height. Each column contained 300–350 g of adsorbent. The column was packed with a slurry of silicic acid in chloroform, and the material was washed with chloroform under a pressure of 5 psi.

Whole lipid was applied to the column as a 10% solution in chloroform/methanol (200:1 v/v). The non-phospholipid fraction was eluted with the same solvent mixture, usually from 1–3 liters. The rate of elution of the neutral fraction was followed by weight and by the Liebermann-Burchard test. When no more neutral lipid appeared in the eluate, phospholipid components were eluted by stepwise increments in the concentration (v/v) of methanol in chloroform (3,7,12,18,25,33,50,85). 100 ml fractions were collected. The next increment in methanol concentration was applied to the column when no more lipid appeared in the eluate. Each fraction was monitored by weight of nonvolatile substance, IR spectrum (5), the plasmalogen-mercuric chloride spot test (6), and the ninhydrin test. Fractions were collected in screwtop vials (Teflon liner) and stored at −13C after the addition of a hydroquinone crystal as antioxidant.

Analytical Methods. Sample from each phospholipid component were analyzed for total ester by the method of Snyder and Stephens (7), total phosphorus by the method of King (8), and plasmalogen by the method of Rapport and Lerner (9). The plasmalogen method is based on the reaction of iodine with vinyl ether groups in neutralized methanol solution (10). Iodine uptake was measured spectrophotometrically with a Cary Model 11 Spectrophotometer. An indirect measure of the plasmalogen content can be obtained from the deviation of the ester:phosphorus ratio from the theoretical value. In most cases the two methods were in agreement.

Iodine number was determined by the micromethod of Sims and Stone (11). IR spectral measurements were made with Perkin Elmer Model 137 Infracord equipped with NaCl optics. Samples for IR spectra were prepared by evaporating an aliquot of the chloroform-lipid solution on the polished NaCl discs.

Gas Liquid Chromatography (GLC). The fatty acid components of the phospholipid and neutral fractions were determined quantitatively as methyl esters by GLC with an Aerograph A-110-C (Wilkens Instrument Co.). The instrument was equipped with a thermal conductivity detector, Brown recorder and disc integrator. Samples for fatty acid determination were saponified in 1.0 *N* KOH in 90% methanol by the method of James (12). The fatty acids were extracted from the acidified saponification mixture with ethyl ether in the case of phospholipids, and petroleum ether in the case of neutral fats. The fatty acids were methylated with dimethoxypropane by the method of Radin et al. (13). An aliquot of each sample was reduced by catalytic hydrogenation with a PtO_2 catalyst as outlined by Farquhar (14). Columns for fatty acid separation were prepared from fire brick treated with hexamethyldisilazane (15) and coated with diethyleneglycol succinate (Wilkins Instrument Co.) (15% w/w of the inert phase). Stainless steel columns were 5 ft long and ¼ in. O.D. For routine analysis, columns were run under two sets of conditions: a) 180C, 80 ml/min helium, and b) 220C, 110 ml/min helium. Fatty acids with shorter chain lengths are easier to estimate at the lower temperature; the higher acids, C-20 and C-22, are more accurately measured under the second set of conditions.

The fatty acid composition was determined by measuring the resultant areas of the elution peaks. Usually the chromatogram obtained at 220C was used. Ratios of the shorter chain fatty acids obtained at 180C were used to compute the final values. The semi-log plots of retention time vs. carbon number of the tuna methyl esters were compared with those of known mixtures run on the same column under the same conditions. Chain length was also confirmed by a plot of the semi-log plot of retention time vs. carbon number of a completely hydrogenated sample. The percentage of each fatty acid was obtained by dividing the area of the individual peak by the total area of the chromatogram. Corrections were applied where necessary based on recoveries measured with mixtures of purified fatty acid esters. Work done subsequent to the completion of this study casts some doubt on the absolute accuracy of the data. The data for fatty acids present in amounts of 10–20% are possibly correct to ± 10%. Those for fatty acids present in amounts of less than 2% may have much greater error.

Paper Chromatography and Thin-Layer Chomatography. Chromatography of phospholipid fractions on silicic acid-impregnated paper was carried out according to Marinetti and Stotz (16), using diisobutylketone/acetic acid/water and n-butyl ether/acetic acid/chloroform/water systems. Peak tubes of each lipid component were checked for homogeneity. Lipids were applied in a solution of isoamyl alcohol/benzene (1:1) and separated by ascending chromatography. Lipid spots were detected with Rhodamine 6 G (17), ninhydrin (18), and iodine vapor (19). Thin-layer absorption chromatography on silicic acid according to Stahl (20) was also used to check the resolution of phospholipids. Thin layers (250 μ) of silicic acid which contained 1% plaster of Paris (Silica Gel G, E. Merck A.G., Darmstadt, Germany) were prepared. The apparatus for its application was purchased from C. Desaga G.m.b.H., Heidelberg, Germany. Lipids were applied in a solution of chloroform or chloroform-methanol. Three solvent systems were found satisfactory for the separation of phospholipids: 1) chloroform/methanol/water (65/25/4); 2) chloroform/methanol/water (80/25/3); 3) chloroform/methanol/water/concentrated NH_4OH (70/30/4/1). Lipid spots were detected with iodine vapor (19) and 2′,7′-dichlorofluorescein (21).

Results

Extraction data for the five tuna are summarized in Table I. The percentage of fat-free, dry tissue residue was the same in all tuna, an average of 23.8%. The total lipid content of three albacore from local waters was high, 8.8–10.1%, but the albacore and yellowfin obtained from Japanese waters contained only 0.5–1.2% lipid.

A representative elution curve of tuna lipids is presented in Figure 1. In general, one to two liters of chloroform (containing 0.5% methanol) eluted nonpolar, phosphorus-negative material. This lipid fraction (A) had a strong positive Liebermann-Burchard test and included neutral triglycerides, cholesterol, cholesteryl esters, and free fatty acids. With 3–7% methanol in chloroform there appeared a poorly defined fraction (B) containing pigment and, possibly, oxidized triglyceride. Phospholipid was first observed in the next poorly defined peak (C). These fractions were not positively identified. All ninhydrin-positive material (D) was eluted with

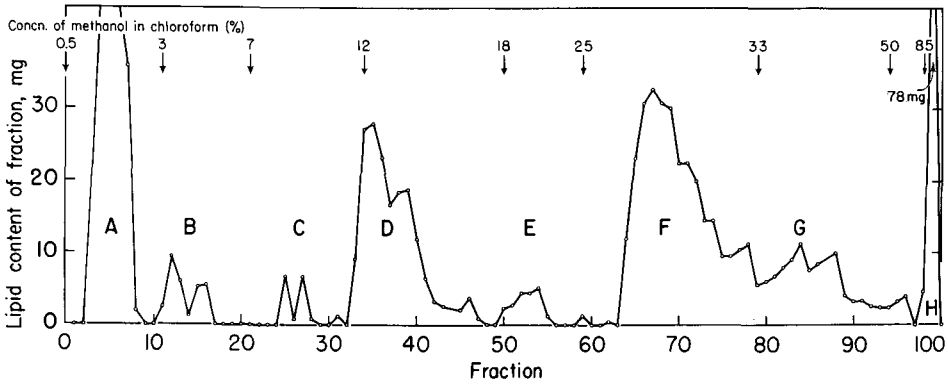

FIG. 1. Representative silicic acid column chromatogram of tuna lipids. Lipid was applied in chloroform onto the column and the neutral fraction was eluted with chloroform. The phospholipids were eluted with increasing concentrations of methanol in chloroform. Each fraction was 100 ml except fraction 99 (500 ml). Lipid applied, 3.437 g; recovered, 3.428 g.

12% methanol. This fraction contained phosphatidyl ethanolamine, phosphatidyl serine, ethanolamine plasmalogen, and serine plasmalogen. Small amounts of phospholipid were eluted with 18% methanol. This material when dried had a white flaky consistency and was insoluble in chloroform. A strong hydroxyl peak was observed at 2.9 μ in the IR spectrum, suggesting the presence of a phosphoinositide. The very small quantities available limited further analyses. The lecithin fraction was eluted with 25% methanol (fraction F). This fraction had a strong adsorption peak at 10.3 μ in the IR spectrum, characteristic of C-N-C bonding, and was a mixture of phosphatidyl choline and choline plasmalogen. The small amount of material (G) which was eluted with 33% methanol never completely separated from the lecithin peak. This fraction was mainly sphingomyelin, although contaminating lecithin was found in every chromatographic separation. No lipid was then obtained until the methanol concentration reached ca. 85%. At this point, a mixture of highly polar material was eluted (H). This fraction was not completely characterized but probably contained lysolecithin and oxidized lecithin. No material was left on the column.

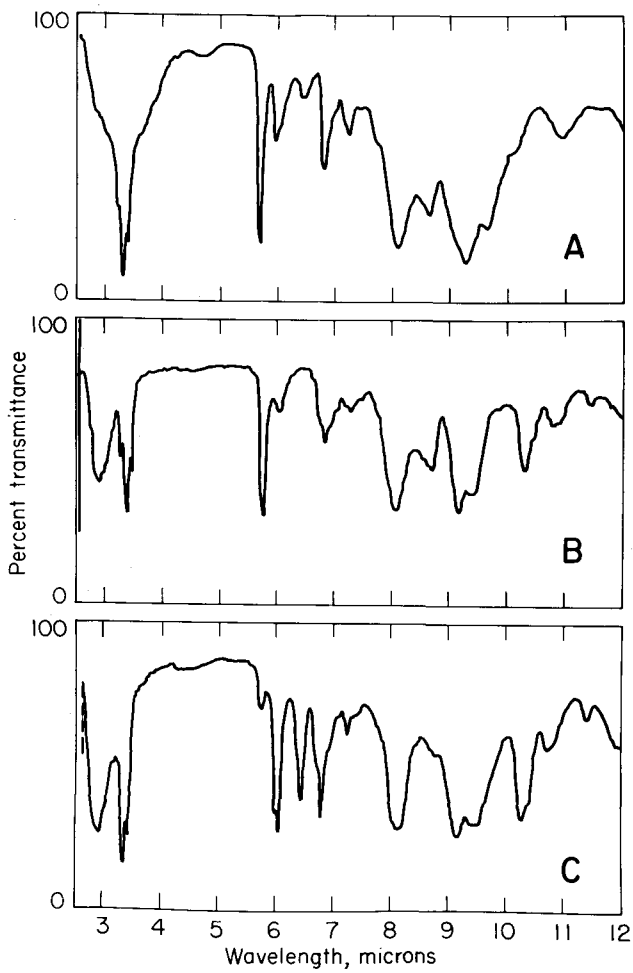

FIG. 2. Representative IR spectra obtained from the peak tubes of chromatographic eluates. Curve A: cephalin fraction, Albacore II; Curve B: lecithin fraction, Albacore III; Curve C: sphingomyelin fraction, Albacore II; these films were run on NaCl discs.

IR spectra were valuable for the identification of the components in various fractions. Representative samples from peak tubes of fractions, D, F, and G are presented in Figure 2. Curve A is from a cephalin peak, B from a lecithin peak, and C from the sphingomyelin region. The over-lap of lecithin into the sphingomyelin region is demonstrated in these curves. The 6.1 μ and the 6.5–6.6 doublet, characteristic of an ester bond, is absent in sphingomyelin. Slight extinction at 5.8 μ is observed in curve C, indicating the degree of lecithin contamination in sphingomyelin. IR adsorption peaks at 5.8, 6.1, 6.5–6.6, 9.0–9.5, 9.8–9.9 μ help to identify the cephalins; at 2.9 μ, phosphoinositide; at 2.9, 5.8, 9.0–9.5, 10.3 μ, lecithins; at 6.1, 6.3–6.8, 10.3 μ, sphingomyelin (5).

Table I shows the amounts of total lipid and phospholipid extracted from the white muscle of each tuna. The total lipid content on a wet weight basis was 0.5%–10.3%, and the phospholipid content, 0.3–0.6%. Olley (1) found that the total phospholipid content for a number of species of fish was in the range of 0.4–0.9%. Phospholipids constituted 67% of the total lipid in the very lean (0.46% lipid) yellowfin muscle. The actual percent of phospholipid in both albacore and yellowfin muscle was in the same range.

The free fatty acids were measured by the method of Hornstein et al. (22), and the values expressed as g methyl ester per 100 g muscle are: albacore II, 0.33; albacore III, 0.23; albacore IV, 0.38; yellowfin I, 0.06.

The distribution of phospholipids in individual tuna is presented in Table II. Lecithin was the major constituent. Sphingomyelin fractions were contaminated with lecithin to some extent. The average distribution of phospholipids (% of total phospholipid) was computed to be: lecithin 54.3%; cephalin 23.2%; sphingomyelin (plus contaminating lecithin) 8.2%; phosphoinositide 2.4%. In early experiments (yellowfin I, albacore I and II), sizeable quantities of material were found in the late eluant fractions, 85% methanol in chloroform. Because of its polar

TABLE II
Distribution of Tuna Phospholipids

Fraction	Sample				
	Albacore I	Albacore II	Albacore III	Albacore IV	Yellowfin I
Total Phospholipid %					
Cephalin	22.6	24.7	27.2	23.0	18.2
Phosphoinositide	3.4	4.2	1.2	> 1.0	1.4
Lecithin	61.0	40.6	66.2	45.7	58.1
Sphingomyelin	7.5	16.6	5.4	4.2	7.2
Unidentified and end products	5.8	14.0	< 1	22.6	15.2

nature, this material was considered to be a mixture of highly oxidized compounds. The addition of hydroquinone to the whole lipid extract decreased the amount of this fraction (albacore III). Absence of this peak may be ascribed either to the freshness of the tuna, its quick freezing and immediate preparation for analysis, or to the addition of hydroquinone throughout the extraction and fractionation. However, a high percentage of end-products was found in the analyses of albacore IV, despite the use of very fresh material and the addition of hydroquinone throughout fractionation. These "oxidized" fractions often accounted for 3%–7% of the total lipid in earlier fractionations; the end fraction of albacore IV fractionation was only 0.8% of the total lipid, but 18.8% of the phospholipid content. The fraction may have been lysolecithin, contaminated with oxidized lecithin.

The analytical data for ester, phosphorus and plasmalogen content of the cephalin, lecithin and sphingomyelin fractions are presented in Table III. These data represent only the values from peak tubes of each fraction. The plasmalogen content in particular was found to be of wide range within a single phospholipid fraction. As the eluant containing the yellowfin cephalin fraction developed, the plasmalogen content varied from 13%–28%. The peak tube, with the highest concentration of cephalin, contained 21% plasmalogen. The plasmalogen content of an individual lipid class increased as the elution of the component progressed, indicating partial resolution. In general, the cephalin fractions contained more plasmalogen than did lecithin. Both cephalin and lecithin from yellowfin had less plasmalogen than analogous fractions from albacore. No plasmalogens were detected in the neutral fraction.

TABLE III
Composition of Lipid Fractions

Phospholipid fraction	Plasmalogen	Phosphorus		Ester	Ester/Phosphorus ratio
	%	%	μM P/100 mg	μeq/100 mg	
Cephalin					
Albacore I	17.7	3.62	1.17	2.00	1.71
Albacore II	34.3	3.68	1.18	1.90	1.61
Albacore III	28.9	3.92	1.26	2.15	1.70
Albacore IV	20.5	3.74	1.21	1.80	1.49
Yellowfin I	21.2	3.65	1.18	1.95	1.66
Lecithin					
Albacore I	2.5	4.04	1.31	2.30	1.75
Albacore II	17.5	3.89	1.20	2.10	1.74
Albacore III	15.3	3.94	1.27	2.22	1.75
Albacore IV	13.7	3.42	1.1	2.29	2.08
Yellowfin I	13.9	3.66	1.16	2.03	1.77
Sphingomyelin					
Albacore I	0	3.35	1.09	0	0
Albacore II	0.9	2.91	0.94	0.11	.....
Albacore III	13.6	3.24	1.045	0.51	0.48
Albacore IV	.....	.....	.....	.....	.....
Yellowfin I	0	3.33	1.07	0.58	0.54

All eluant fractions were tested for homogeneity by ascending paper chromatography. The lecithin fractions were uniform, traveling as single spots in several solvents. On the other hand, cephalin usually separated into three constituents. Chromatograms of the sphingomyelin fractions always showed traces of contaminating lecithin. Thin layer chromatography also proved to be a convenient method for testing the purity of fractionated phospholipids. Fish lipids stain very dark in iodine vapor, denoting the high degree of unsaturation. Synthetic lecithin and phosphatidyl ethanolamine (California Corporation for Biochemical Research) containing no unsaturated bonds also stain to a slight degree. These synthetic phospholipids developed with slightly lower R_f values than the corresponding lipid fractions in all solvent systems. With the thin layer method, tuna lecithin developed as a single well-defined spot in all solvent systems tested. Cephalin fractions showed one major component with two or three minor constituents developing with solvent 2. The minor spots migrated with lower R_f values than the major component and could be visualized by iodine staining. However, the minor components did not show fluorescence with dichlorofluorescein. Thus far, these methods have not been successful in separating the cephalin fraction into phosphatidyl serine and phosphatidyl ethanola-

TABLE IV
Fatty Acid Composition [a] of Neutral (N), Cephalin (C), and Lecithin (L) Fractions of Individual Tuna

Tentative Identification of Fatty Acids [b]	Albacore I			Albacore II			Albacore III			Albacore IV			Yellowfin I		
	N	C	L	N	C	L	N	C	L	N	C	L	N	C	L
12:0	.....	1.3	0.3	4.3	.....	tr	tr	tr	.....	.....	0.5	.....	1.4	6.0	.....
13:br	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.0	.....	.....	.....	.....
14:0	3.4	3.6	1.0	1.6	.....	9.4	2.3	1.3	0.3	2.2	tr	.....	1.0	0.6	tr
14:1 or br	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.5	.....	.....	.....	.....
15:0	0.7	.....	.....	.....	.....	.....	0.5	.....	tr	.....	tr	.....	.....	.....	2.7
15:br	.....	.....	.....	.....	.....	.....	.....	1.9	.....	.....	1.0	.....	.....	.....	.....
15 or 16 [c]	.....	.....	.....	.....	30.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
16:0	23.6	6.1	26.3	44.6	9.2	34.0	21.7	13.8	34.0	24.9	4.6	22.1	48	4.4	32.1
16:1	4.1	3.2	4.9	1.3	tr	tr	2.7	1.7	.....	5.1	.....	.....	tr	9.3	tr
17:0	1.3	1.3	0.5	0.9	5.3	.....	1.1	tr	0.4	.....	.....	.....	tra	2.0	.....
17:br	.....	.....	.....	.....	.....	.....	.....	1.0	.....	.....	1.5	.....	.....	.....	.....
18:0	3.4	19.6	5.4	7.5	28.5	4.6	4.6	14.8	4.7	5.7	23.8	3.7	12.6	36.8	1.5
18:1	22.8	9.2	9.8	19.5	17.5	29.9	21.0	9.5	10.2	19.4	11.4	10.9	13.2	12.9	20.6
18:2	1.0	1.1	1.8	.....	tr	tr	4.5	1.5	0.6	.....	tr	.....	0.9	tr	tr
18:3	3.5	0.6	.....	2.4	tr	tr	5.1	1.9	1.0	5.2	tr	.....	.....	tr	.....
20:0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.2	.....	.....	.....
20:2	.....	.....	.....	.....	.....	.....	1.8	tr	tr	.....	.....	.....	.....	.....	.....
20:4	0.8	3.1	2.5	1.7	2.9	5.6	4.5	4.1	4.1	4.8	4.1	4.1	7.0	6.1	8.9
20:5	12.5	6.2	11.6	2.5	.....	2.3	5.6	6.7	8.2	6.7	4.2	7.5	2.6	1.9	3.3
22:4	tr	.....	.....	.....	.....	.....	.....	tr	tr	.....	.....	.....	tr	1.5	1.7
22:6	22.8	44.7	35.7	13.5	6.1	14.0	24.6	42.0	36.3	25.8	47.4	50.4	11.5	20.5	30.4
Iodine No. exp. [d]	171	208	178	133		145							96.8	132	206
Iodine No. calc. [d]	169	198	174	102.3		117.7							99.0	146.6	205.7
Unsaturated fatty acids, %	67.5	68.1	66.3	40.9	26.5	39.2	69.9	67.4	60.4	67	67.1	72.9	35.2	52.2	64.9

[a] Wt % of total methyl ester sample.
[b] Chain length: number of double bonds.
[c] Unidentified.
[d] Where figures are lacking the determination was not run.

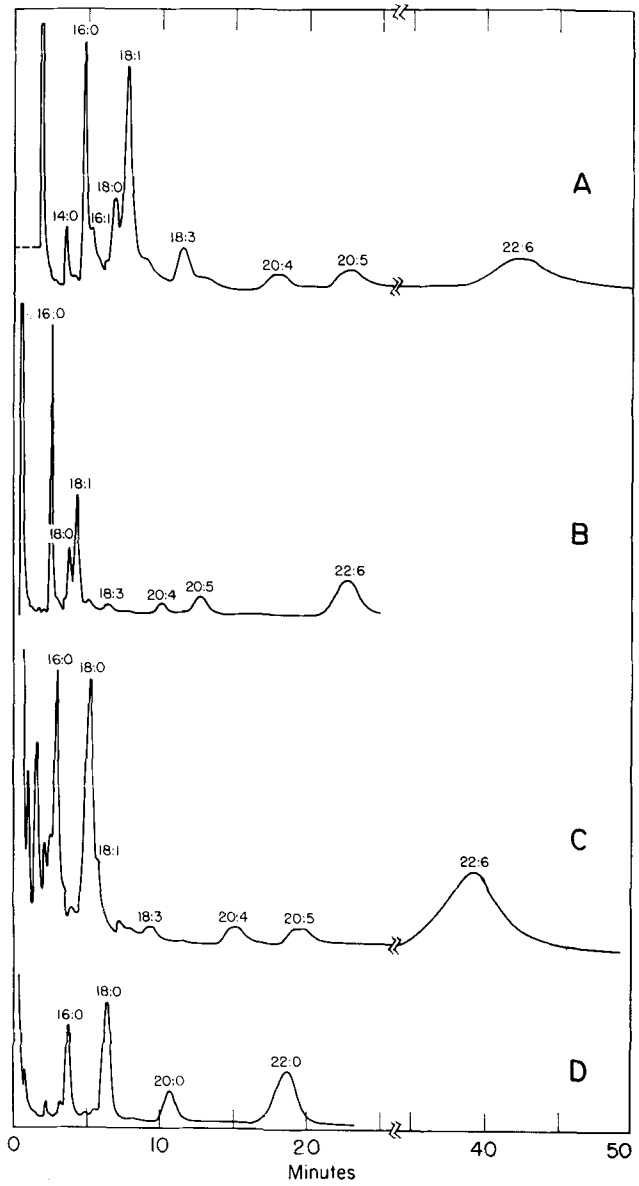

FIG. 3. Typical GLC obtained for fatty acid esters. A: Neutral fraction, Albacore III (flow rate, 83.4 ml/min. Column temperature 206C); B: Lecithin fraction, Albacore III (flow rate, 120 ml/min. Column temperature, 227C); C: Cephalin fraction, Albacore III (flow rate, 87 cc/min. Column temperature, 208C); D: Cephalin fraction, hydrogenated esters, Albacore III (flow rate, 85 cc/min. Column temperature, 202C). Fatty esters are designated by chain length and number of double bonds in the fatty acid. The stationary phase was the polyester of diethylene glycol succinate. Samples were injected in a solution of cyclohexane which produced the initial solvent peak.

mine and their corresponding plasmalogen derivatives. Thin layer chromatography demonstrated that the sphingomyelin fraction contained, in addition to small quantities of lecithin, a third contaminant which remained at the origin in the solvent systems tested.

The fatty acid composition of the neutral, cephalin, and lecithin fractions from five tuna is presented in Table IV. The fatty acid patterns of these phospholipids show characteristic differences. The neutral lipid fraction is characterized by high oleic and unsaturated C_{22} acid content. Palmitic acid is particularly abundant in the neutral fraction whereas the level of stearic acid is comparatively low. By contrast, cephalin fractions contain higher concentrations of stearic acid and are relatively deficient in palmitic acid and oleic acid. Highly unsaturated C_{22} acids are especially abundant in the cephalin fractions. These unsaturated C_{22} acids are also found in the lecithin fractions which contain very low stearic acid values. No single relationship between the percentage of saturated and unsaturated fatty acids of each fraction is apparent. However, in albacore I, III, and IV the unsaturated fatty acids account for ca. two-thirds of the fatty acid content of the lipids. The two tuna obtained from Japanese waters contained less unsaturated acid in all fractions.

TABLE V
Ratio of Palmitic to Stearic Acid in Fractions from Tuna Lipids

Tuna	Neutral	Cephalin	Lecithin
Albacore I	6.9	0.31	4.9
Albacore II	5.9	0.32	7.4
Albacore III	4.8	0.93	7.2
Albacore IV	4.4	0.19	6.0
Yellowfin I	3.8	0.12	21.4

Several fatty acid constituents with chain lengths shorter than C_{12} were found in trace quantities, particularly in the cephalin fractions. These comprised less than 1% of the total fatty acid content and have been omitted from Table IV. Traces from GLC, representing typical analyses of methylated fatty acids from lecithin, cephalin, hydrogenated cephalin and neutral fractions, are presented in Figure 3.

The characteristic variation in the fatty acid composition of lipid fractions is best visualized by the ratio of palmitic/stearic acid, Table V. The fatty acids obtained from cephalin differ quantitatively from those of neutral and lecithin fractions. The determinations indicate that palmitic acid accounts for most of the saturated fatty acid in neutral fat and lecithin, and that stearic acid is predominant in cephalin.

Discussion

A detailed study of the lipids from a tuna species, *Thunnus orientalis*, has been reported by Katada et al. (2). Phospholipid components were separated by solvent fractionation and the results were calculated from analyses for sphingosine, choline, serine, ethanolamine, aldehyde, and sugar. Total lipid was 6.7% and the phospholipid content 0.93%, based on wet weight. Their data showed greater amounts of cephalin, sphingomyelin, and cerebroside relative to lecithin than were obtained in this study. Lecithin accounted for 37%, cephalin 32%, and sphingomyelin 18%—compared to 54%, 23%, and 8% in this study.

Other results on fish lipids from muscle fall within the same range of lipid and phospholipid content. Based on wet weight calculations, herring contained 0.94% phospholipids (23), salmon 1.3% (24), and cod 0.85% (24); haddock (25) and trout (26) phospholipid contents are in the range of 0.6%–0.7%. Gray and Macfarlane (26) reported average lipid content of trout muscle as 4.4%; of this, 16% was phospholipid (0.7%). The components of trout phospholipid were 66% lecithin, 25% cephalin, 2% sphingomyelin, and 2% phosphoinositide. Katada et al. (23) found that cod phospholipid contained 67% lecithin and 25% cephalin. The phospholipid components of the various fish species thus all seem to be quite similar (1).

Fish lipids are remarkable for the complex mixture of their component fatty acids which range from chain lengths of less than 14 to 22 carbon atoms, and include numerous highly unsaturated compounds. The most substantial differences in fatty acid composition appear to be between the different kinds of phospholipids irrespective of tissue or species. Probably the most noteworthy is the preponderance of stearic

acid as the saturated acid in cephalin and cephalin plasmalogen, and palmitic acid in lecithin and lecithin plasmalogen, as pointed out by Gray and Macfarlane (26). This distribution of saturated C_{16} and C_{18} acids seems to be a characteristic pattern in the phospholipids from a number of tissues. Although not as marked as in the tuna lipids, it has also been observed in human serum lipoprotein fractions (27) and phospholipids (28), rat liver subcellular particles (29,30), and different tissues from pig (26) and ox (31).

The relative contents of these two saturated acids may be expressed as their ratios. In this study, the average values for the palmitic/stearic acid ratios in tuna fractions were: neutral 5.2; cephalin 0.37; lecithin 9.4. The results from Nelson's study (32) on normal mouse liver were similar: neutral 8.8; cephalin 0.14; lecithin 17.6. The data of Hornstein et al. (33) also show such trends. Beef muscle lipids had a ratio of 0.1 for cephalin and 3.2 for lecithin (plus sphingomyelin); pork muscle had a ratio of 0.2 for cephalin and 6.6 for lecithin (plus sphingomyelin). High palmitic acid/stearic acid ratios have also been reported in lecithins from other fish oils. The results by Zama et al. (34) on the lecithin of salmon liver showed a ratio of 5.7. Trout lecithin (26) had a ratio of 8.7. Recent work in this laboratory on oil from menhaden muscle showed a ratio of 18.4 for lecithin.

Exceptionally large amounts of stearic acid in phosphoinositide of rat liver has been reported by Brockerhoff (35) and Getz et al. (36) and of beef heart by Dittmer and Hanahan (37). Contamination by inositides cannot account for the high stearic acid content in the tuna cephalin fractions. These combined observations are difficult to reconcile with the theory that the lecithins and cephalins have a common diglyceride precursor (38).

Saturated fatty acids with odd-numbered carbon atoms, and branch-chained fatty acid with even or odd carbon atoms, were minor components in many fractions. In all tuna studies, cephalin had trace amounts of a number of minor components of molecular weight lower than C_{12}. The occurrence of odd numbered mono-ethylenic fatty acids in liver oil of the shark has been reported (39); also C_{15}, C_{17} and C_{19} saturated fatty acids were detected in amounts less than 0.1%. These saturated acids had not been previously found in fish oils, but were isolated earlier from depot fat. The nature of the C_{15} or C_{16} acid in cephalin from albacore II, which amounted to 30.5%, was unsettled. Hydrogenation did not reduce the peak.

The data in this study indicate that tuna lecithin cannot have all its unsaturated fatty acid residues attached to a single position in the molecule, as was once suggested (40,41), since so many of the fractions contain more than 50% unsaturated fatty acid (see Table V).

Eichberg et al. (42) reported the occurrence of plasmalogens in neutral triglycerides isolated from the digestive glands of the starfish. Rapport and Alonzo (43) studied the total extracts of tissues from eleven marine invertebrate species for the presence of the α-β unsaturated ether content. Plasmalogen was detected in the neutral fraction but was found to be associated predominantly with the ninhydrin-positive (cephalin) fraction. It appeared probable that the non-phosphatide plasmalogens did not constitute more than a small part (<20%) of the plasmalogen content. The present study of tuna lipids showed no plasmalogen in the neutral fraction.

Igarashi et al. (44) reported the presence of a threonine-containing phospholipid in the "cephalin" fraction of a tuna, *Thunnus orientalis*. Analyses of the bases of tuna cephalin in the present study by paper chromatography revealed spots corresponding to serine and ethanolamine only. The absence of threonine possibly reflects a difference in species.

Hornstein (33) et al. reported that phospholipid (cephalin, lecithin and sphingomyelin) from beef muscle was associated with protein. Their cephalin fraction contained ca. 20% of this "proteolipid" material. No protein associated with tuna fractions has been observed during this study. Carbohydrate such as galactose (24) has been found in fish lipids, but no sugars were observed in tuna muscle lipid fractions.

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