ing it to be the hydroxytolyl product. The ratio of hydroxy to non-hydroxy (ether) product in the mixture is about 3:2.

Addition of Thiols to Oleic Acid. The same procedure used for obtaining the addition products with phenolic nucleophiles was employed (1). The molar ratio of methanesulfonic acid to oleic acid was always the same (6:1). However, because of the limited amount of thiol available, the ratio of nucleophile to oleic acid was reduced from 4:1 to 2:1 in several instances. In all the experiments the excess of thiol was recovered with the reaction product. Titration of the products with ethanolic iodine solution gave negative results, demonstrating the absence of free mercapto groups. The yields and analyses of the resulting thioethers are summarized in Table I.

Results and Discussion

The superiority of methanesulfonic acid over sulfuric acid as catalyst-solvent for addition to oleic acid is demonstrated again by the yields of thioethers resulting from the thiol additions. It was even possible to add polycyclic thiols such as 2-naphthalenethiol. Previous investigators using sulfuric acid for the addition of thiols experienced difficulty in isolating the products. The advantage in using methanesulfonic acid is probably due to the lesser tendency for oxidation and other undesirable side reactions to take place in this medium. The negative ethanolic iodine titrations indicate the products to be thioethers exclusively. Rearrangements of thioethers similar to those of the phenolic addition products would yield titratable free mercapto (–SH) groups.

The inclusion of thioanisole in Table I serves to demonstrate that thioethers do not add to oleic acid. This result is not unexpected since the products isolated from thiol addition were thioethers. It will be recalled that phenolic ethers such as anisole and phenetole could be added successfully under similar conditions.

The yield of thiol addition products seems to parallel those obtained from their phenolic counterparts. Steric influences of substituents already present in the aromatic ring play a similar role in influencing the yields, i.e., lower yields from ortho substituted compounds; higher yields with meta and para derivatives. Without exception, the odor of the thioether is less disagreeable than the thiol starting material. No yield data are listed in Table I for the experiment when isooctyl 3-mercaptopropionate was added to oleic acid. About 20 g of a distillate was obtained from a fractionation of the ester. The saponification equivalent and elemental analysis of one fraction gave results that lie between the theoretical values for a mixed isooctyl methyl ester addition product and the dimethyl ester addition product. It may be inferred that the values obtained are due to a partial alcoholysis of the isooctyl group during the esterification of the substituted stearic acid.

In addition to the thiols listed in Table I, two additional sulfur derivatives were added to oleic acid: a, a' dimercapto p-xylene $HSCH_2\text{-}C_6H_4\text{-}CH_2SH$ and phenylmercaptoacetic acid (carboxythioanisole). The dimercaptoxylene added to give a mixture of high mol wt compounds which were unidentifiable. The phenylmercaptoacetic acid was added via the carboxyl group. This type of addition is to be the subject of a future communication.

Acknowledgment

The authors are indebted to E. T. Donahue and members of the analytical section of this laboratory for the analytical results reported in this paper.

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[Received January 15, 1962]

The Lipid Depressant Activities of Whole Fish and Their Component Oils[1,2]

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Abstract

Hypercholesteremic rats were used to investigate the lipid depressant activities of lyophilized whole fish prepared from menhaden, silver salmon, mullet, and ocean perch. Ingestion of the whole fish supplements promoted a significant reduction in the circulating levels of cholesterol and phospholipids, and in the TC/TP ratios of blood lipids from the rat. The effects of whole fish were duplicated by feeding rats proportionate amounts of the oils found in these fish supplements. These supplements had similar, but less dramatic, effects on the liver lipids of the rats. The nonlipid components, isolated from the fish, had no apparent influence on the distribution of lipids in the blood and liver tissues. The preparations of lipid and nonlipid components of the fish are described, and the GLC analyses of marine oils are discussed.

IN PREVIOUS STUDIES we found whole body oils from tuna and menhaden to be very effective cholesterol depressants for the rat (1), while Ahrens *et al.* reported that menhaden oil lowered the blood lipids of a hyperlipemic patient and a hypercholesterolemic patient (2). Further studies revealed that fractions of menhaden oil fatty acid esters were more effective lipid depressants than corn oil or various other dietary fats (3,4). Although these and other studies (5,6,7) suggest that a variety of marine oils may be of benefit in alleviating a hypercholesterolemic con-

[1] Presented, in part, at the A.O.C.S. Meeting in Chicago, 1961.

[2] Researches supported by U.S. Department of Interior, Fish & Wildlife Service, American Heart Association, U.S. Public Health Service and the Hormel Foundation.

[3] These studies were completed during the tenure of an Established Investigatorship from the American Heart Association.

dition, a more palatable and practicable method of introducing such oils into the diet would be to make use of fish products which contain these oils. However, it is possible that the lipid depressant activities of the whole fish may differ from that of their component oils. The distribution of lipids in the blood-vascular tissues might be affected by the amino acid composition of ingested proteins (8), as well as by the unsaturated lipids (1–7) or other compounds that may be found in fish.

The experiment reported here describes the comparative effects of four species of fish and proportionate amounts of their lipid and nonlipid components on the blood and liver lipids of hypercholesterolemic rats. An outline of the fractionation procedures used to isolate the components of the fish, and the analytical methods used to characterize these fractions, are also included in this report.

Experimental

Preparation and Properties of Fish Fractions. Whole fish supplements were prepared from menhaden *(Brevoortia tyrannus)*, silver salmon *(Oncorhynchus kisutch)*, mullet *(Mugil cephalus)*, and ocean perch *(Sebastes marinus)* as macerated and freeze-dried products which had been flushed with pure nitrogen and sealed in vacuum containers. (The whole macerated fish were lyophilized at the Department of Food Science and Technology, University of California, Davis.) Approximately one-half of these preparations were subjected to the following extraction procedures: Batches of 800 to 900 g of the fish solids were dispersed into 3.7 l of 98% methanol in a carboy. This dispersed mixture was heated on a water bath until it had boiled for approximately 5 min and cooled to room temperature. The fish oils were extracted from this mixture with 12 l of petroleum ether (bp 30–60C). The residual solids were re-extracted three times with 5 vol of p.e., twice with 5 vol of absolute methanol, and once with 2 vol of absolute ethanol. The extracts were filtered through a Whatman #1 filter paper on a 24 cm Buchner funnel, using slight negative pressure to aid the filtration process. The bulk of the solvents from the combined extracts was distilled off at atmospheric pressure, and the remaining alcoholic concentrate of lipids was further reduced in volume *in vacuo*. The lipids were redispersed in petroleum ether, dried with anhydrous sodium sulfate, and the solvents completely removed *in vacuo*. All operations were blanketed under nitrogen and, except for the hot alcohol treatment, all extractions were made at 40C or less. The initial treatment of the fish solids with hot aqueous alcohol was required to prevent clogging of the filters with muscilageneous materials found in fish.

Analyses of the extracts for lipid phosphorus and Liebermann-Burchard positive materials, and TLC analyses of microchromatoplates (9,10), showed that most of the fish lipids had been removed after the third extraction with p.e. Completeness of extraction was indicated by inability to extract any detectable amounts of lipids from a portion of the final residues with 10 vol of $CHCl_3$-MeOH (3:1).

The isolated marine oils were flushed with pure nitrogen and stored under vacuum in a deep-freeze. The lipid-free residues were air dried, ground in a ball mill to pass aluminum window screening, and stored in glass jars.

The 4 species of fish were quite different in their relative contents of lipid and nonlipid components (Table I). The marine oils represented nearly half of the total solids found in menhaden, but only 11% of the total solids in ocean perch. The nonlipid components were primarily proteins, while triglycerides and phospholipids represented the major lipids found in the marine oils (Fig. 1). However, all of the oils contained small amounts of free sterols and free fatty acids. The oils from these species contained appreciable amounts of docosahexaenoic, eicosapentaenoic, palmitic and, except for mullet oil, oleic acids (Table II). Mullet oil was unique in its comparatively high content of odd carbon fatty acids (i.e., 15:0, 17:0, 19:0, etc.) and it contained more linoleic and arachidonic, but less oleic, acids than the other three oils. Each oil appeared to have its own characteristic distribution of saturated and unsaturated acids.

TABLE I
Composition of Lyophilized Fish Preparations

Fish[a]	Total lipids	Sterols[b, c]	Phospholipids[c]	Proteins[d]
	g/100 g solids	g/100 g solids	g/100 g solids	g/100 g solids
Menhaden	48.7	.24(.48)	2.4(7.0)	50.0
Silver salmon	21.2	.12(.81)	2.8(13.5)	80.0
Mullet	15.8	.43(2.4)	2.7(16.8)	82.5
Ocean perch	11.0	.35(2.6)	2.7(24.6)	88.5

[a] See text for the names of the species.
[b] Based on the analyses for Lieberman-Burchard positive materials.
[c] Figures in parentheses represent relative concentrations of sterols and phospholipids found in the extracted oils (g/100 g oil).
[d] Based on Kjeldahl nitrogen analyses.

Analytical Methods. For the gas liquid chromatographic (GLC) analyses, the fatty acids were isolated from the marine oils and converted into methyl esters, using the methanolic-BF_3 reagent and procedures described by Metcalfe and Schmitz (11). The GLC analyses were performed on both hydrogenated and unhydrogenated samples of the esters. Chain lengths of the fatty acids were determined by fractionation

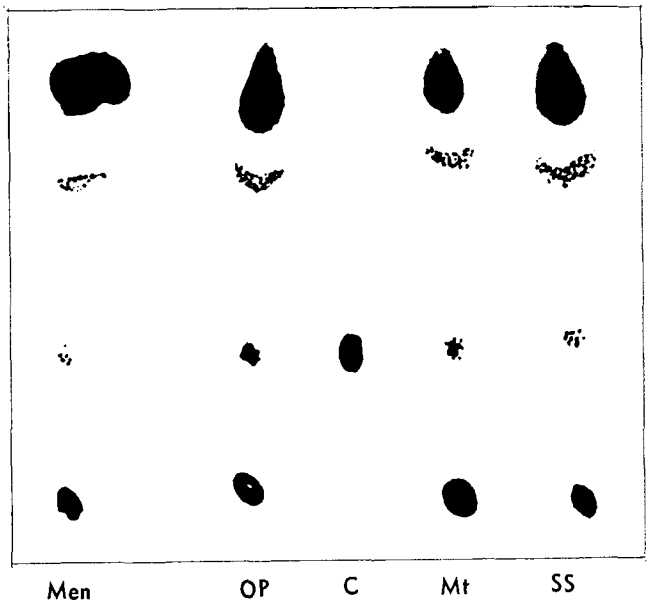

FIG. 1. Thin layer chromatographic (TLC) analyses of marine oil supplements. The quantities of oils fractionated were 67 μg menhaden (Men), 61 μg ocean perch (OP), a cholesterol standard (C), 54 μg mullet (Mt), and 59 μg silver salmon (SS). The components on the chromatograms, from origin to front, were phospholipids, free sterols, fatty acids, and triglycerides. The stippled areas were minor components which could be seen on the microchromatoplates, but which did not show up on the photocopies made directly from the chromatograms. The microchromatoplates were 3¼ x 4 inch lantern slides coated with silica gel, and chromatograms were developed with hexane-ethyl ether-acetic acid (60:40:2) according to the method of Peifer, *et al.* (9,10).

TABLE II
GLC Analyses of Fish Oil Acid Esters [a]

Esters	Menhaden	Silver salmon	Mullet	Ocean perch
14:0	7.7	3.7	3.9	5.5
15:0	.....	.....	7.4	.....
16:0	25.3	22.5	29.6	23.0
16:1	6.7	5.0	6.3	3.8
16:2 & 3	0.5	0.5	0.7	0.5
17:0	.....	.....	5.5	.....
18:0	3.1	3.9	4.8	3.9
18:1	15.4	23.6	5.8	18.5
18:2	.....	0.9	2.6	1.6
18:3	1.6	1.0	1.5	.....
18:4	4.0	.....	.....	.....
19:0	.....	.....	3.4	.....
20:0	2.3	.....	1.6	.....
20:1	.....	5.3	.....	7.2
20.2 s [b]	1.6	2.0	.....	.....
20:4 arachid.	0.7	0.5	2.2	0.6
20:4 ? [c]	1.3	0.9	1.4	0.6
20:5	12.9	11.0	4.7	7.9
21:0	.....	.....	2.3	5.7
22:0	.....	3.8	.....	8.5
22:5	2.3	3.3	3.1	trace
22:6	14.0	12.9	10.3	13.6

[a] See text for details of these analyses.
[b] These values represent the sum of two eicosadienoic acids identified on the basis of the relative retention times reported by Farquhar *et al.* (12).
[c] This component appears between the arachidonic (arachid.) and eicosapentaenoic acid ester peaks in the GLC chromatograms.

of the hydrogenated esters; there were chromatographed on a 6 x ¼ inch polyester-packed aluminum column (temp, 185C; helium pressure, 18 psi) using an instrument equipped with a thermal conductivity detector. (Aerograph, model A-90-C, and GLC column packing material from Wilkens Instrument & Research Co., Walnut Creek, Calif.) The relative amounts of saturated and unsaturated esters within each chain length were estimated from GLC analyses of the unhydrogenated samples. One μl of hexane solution containing 40–70 μg of unhydrogenated esters, was injected into 4 ft. x ¼ inch silicone rubber stoppered glass columns packed with polyester, and the eluted fractions assayed through the use of a β-ionization detector: temp, 176C; argon, 15 psi; detector voltage, 1250). (Instrument with 10 mc sealed source of Sr-90. Pye Argon, Jarre-Ash Co., Nashville, Mass.) Identification of components in chromatograms was based on relative retention times compared with those obtained with pure esters and on those values reported by Farquhar *et al.* (12) for the acids of menhaden oil. [Reference standards for GLC analyses were pure methyl esters of saturated (8:0 to 22:0) and unsaturated (18:1, 18:2, 18:3, 20:1, 20:4, and partially purified 20:5 and 22:6) acids. Mixtures containing different proportions of these methyl esters were used in calculating the needed corrections for the nonlinear response of the β-ionization detector.] Both the hydrogenated and unhydrogenated samples were fractionated on columns packed with 25% (w/w) of diethylene glycol adipate polyester (LAC-2R-446) adsorbed on Chromosorb W (80–100 mesh) and the mixture was treated with 2% phosphoric acid according to the method of Metcalfe (13). The areas under the GLC curves were estimated on the basis of retention times (T) and peak heights (H), these TH values being directly proportional to the absolute areas under the curves (14). A correction factor, based on the GLC analyses of pure methyl esters, was introduced in these analyses for the assay of the linolenate component of the marine oils. The use of TH values and the derivation of correction factors were procedures similar to those described by Tandy *et al.* (14).

Cholesterol and the marine oil sterols were analyzed according to the method of Abell *et al.* (15). Our other studies with tuna and menhaden oils (3,4) showed that the values obtained by this method were equivalent to 90% of those obtained by gravimetric analyses of the unsaponifiables from these two marine oils; nevertheless, this method would allow only a crude measure of sterols other than cholesterol. The relative R_f values and transient colors developed on microchromatoplates (9) suggest that the major sterol component of the marine oil was similar to, or identical with, cholesterol (Fig. 1).

Phospholipids were analyzed by a modification of the method of King (16). Fish proteins were calculated on the basis of their protein-N x 6.25 (this factor is based on the assumption that the protein contained 16% nitrogen).

Experimental Design. Adult male Sprague Dawley rats were made hypercholesterolemic by feeding them the control diet which contained 18% casein, 10% beef tallow, 0.5% cholesterol, and 0.5% ox bile (Table III). Following two months of preconditioning with this diet, the rats were distributed into groups having similar mean plasma cholesterol levels (1). It was not feasible to feed the supplements of fish in amounts that would provide equal quantities of marine oils to all groups of rats, because of the widely different contents of the oils in the four species (Table I). However, the dietary adjustments, shown in Table III, made it possible for two of the groups, those fed menhaden and silver salmon, to receive approximately 50% of their dietary lipids from the marine oils. Furthermore, the groups fed salmon, mullet, and ocean perch obtained 80% of their dietary protein from the fish. The protein contents of the diets were increased from 18% (used in the preconditioning diet) to 25% during the supplementation period to allow the inclusion of fish supplements in amounts that would provide an appreciable quantity of their component oils in the diets; this was particularly necessary for the groups receiving mullet and ocean perch. The lipid and nonlipid fractions were fed at levels proportionate to the concentrations of these components found in the whole fish supplemented diets. In all cases, the oil and protein components of the fish were substituted isocalorically for the protein and tallow found in the 25% casein control diet.

The macerated fish preparations were combined with the other dietary ingredients, pulverized in a food grinder, and thoroughly mixed in a food mixer. The lipid and nonlipid fractions from the fish were mixed directly with the other dietary ingredients in a food mixer. The diets were prepared at weekly intervals and stored in glass jars in a deep freezer filled with CO_2 from a reservoir of dry ice. Weighed amounts of fresh diets were fed to the rats three times each week. At the termination of the experi-

TABLE III
Percentage Composition of Experimental Diets [a]

Suppl.[b] fed	Casein	Whole fish	Fish oil	Nonlipid (NL) solids	Beef tallow
Menhaden	20.0	10.0			5.0
Whole	25.0		4.9		5.0
Lipids	20.0			5.0	10.0
NL-solids					
Silver salmon	5.0	25.0			4.6
Whole	25.0		5.3		4.6
Lipids	5.0			20.5	10.0
NL-solids					
Mullet	5.0	24.2			6.1
Whole	25.0		3.8		6.1
Lipids	5.0			21.1	10.0
NL-solids					
Ocean perch	5.0	22.6			7.4
Whole	25.0		2.5		7.4
Lipids	5.0			21.0	10.0
NL-solids					
Controls	25.0				10.0

[a] All diets contained .5% cholesterol, .5% ox bile and the salts, vitamins, and other ingredients (totaling 65 g).
[b] Whole refers to the macerated lyophilized fish product; Lipids, the fish oil fraction; NL-solids, the non-lipid fraction isolated from the fish.

ment, the rats were exanguinated and their plasmas and tissues isolated for analyses. Further details of the methods, dietary ingredients, and experimental procedures used are in previous reports by Peifer *et al.* (1,4).

Experimental Results. The comparative effects of the dietary supplements of the fish on the circulating lipids and food intakes of hypercholesterolemic rats are summarized in Table IV. Ingestion of the whole fish preparations promoted a significant lowering of both cholesterol and phospholipids in the blood. The rats fed salmon had 48.5% less of these lipids in their plasmas than did the controls fed the 25% casein diet. The whole fish supplements had their greatest effects on the circulating cholesterol of the rats; salmon promoted a 62% reduction of the cholesterol levels, but only a 32% reduction in plasma phospholipids. For this reason, these changes in circulating lipids were accompanied by a significant reduction in the cholesterol-phospholipid ratios, TC/TP, found in the blood lipids. The four groups had an average TC/TP ratio of 1.3, this value being significantly below that of the 2.2 value found in the blood lipids of the controls. Although these rats received different amounts of the total ingredients of the fish and their component oils (Tables III and IV), all four groups had surprisingly similar lipid patterns in their plasmas at the termination of the experiment.

The lipid depressant activities of the whole fish were duplicated by feeding rats proportionate amounts of the oils found in the fish-supplemented diets. The patterns of circulating lipids found in these two groups were nearly identical. However, salmon oil appeared to have a somewhat greater depressant activity than whole salmon and this was more than could be explained by the 5% increased food intakes by the oil-fed groups (Table IV).

The nonlipid components had no apparent effect on the blood or liver lipids (Table V) of these hypercholesteremic rats. The mean body weights and tissue lipids of these groups were essentially the same as those found in rats fed the control diets. These results demonstrate that fish protein could be substituted for casein in the adult rats without apparent effects on the growth or tissue lipids during such short term studies.

The observed changes in blood lipids were not due to any apparent differences in the palatability of the dietary supplements. The small variations in the dietary intakes (i.e., 14 to 17 g/rat/day) could not be correlated with an obvious aversion by the rats for the marine oils (note the food intakes of the controls, menhaden, and mullet supplemented groups) or other components found in the fish.

The cholesterol and bile acids present in these experimental diets promote an accumulation of lipids, especially cholesterol esters, in the livers of rats (1). However, ingestion of the fish promoted a significant reduction in this accumulation of liver lipids (Table V). Although whole fish and fish oils had equivalent depressant activities for the plasma lipids, the marine oils from salmon, mullet, and ocean perch were somewhat less effective than whole fish supplements in reducing the liver lipids of these hypercholesteremic rats. Rats receiving whole mullet had the lowest concentration of lipids in their livers, although this supplement was one of the least effective depressants for the blood lipids (Table IV). Both the fish and their oils promoted a decrease in the TC/TP ratios found in liver lipids due to the lower content of cholesterol found in liver tissues. The phospholipids were relatively constant components of the livers for all groups, i.e., 2.87 g and 2.89 g per 100 g tissue in the 25% casein control group and in those rats receiving menhaden.

TABLE IV

The Effects of Fish Supplements on Food Intakes and Plasma Lipids of Rats

Suppl.[a] group	Rats per group	Avg. food intakes		Plasma lipids[b]		
		Total	Suppl. lipids in diets	Total cholesterol (TC)	Total phospholipids (TP)	TC/TP
		g/rat/day	g/rat/day	mg/100 ml	mg/100 ml	
Menhaden						
Whole	7	15.4	.760	250±33	193±17	1.3
Lipids	7	13.7	.670	223±30	185±13	1.2
NL-solids	7	13.7	0.0	510±62	228±20	2.2
Silver salmon						
Whole	6	14.5	.770	193±7	179±14	1.1
Lipids	5	15.2	.805	162±14	148±8	1.1
NL-solids	7	16.9	0.0	614±74	262±15	2.3
Mullet						
Whole	5	16.3	.620	259±36	189±9	1.4
Lipids	6	16.5	.626	263±22	204±11	1.3
NL-solids	7	16.5	0.0	563±69	245±13	2.4
Ocean perch						
Whole	7	14.0	.350	198±18	198±10	1.3
Lipids	8	14.3	.358	190±11	176±14	1.1
NL-solids	8	14.8	0.0	552±86	246±18	2.2
Controls[c]						
18% casein	8	15.4	0.0	578±57	242±14	2.4
25% casein	6	15.3	0.0	507±46	262±11	2.2

[a] See footnotes to Table III for description of terms.
[b] Mean values ± the standard error of the mean.
[c] All rats were preconditioned with the 18% casein; one group was continued on this diet.

TABLE V

Terminal Body Weights and Liver Lipids of Rats Supplemented With Fish

Suppl. group	Terminal body weights	Liver lipids[a]			
		Total	Total cholesterol (TC)		Total phospholipids (TP)	TC/TP
	g	g/100 g tissue	g/100 g tissue	g/100 g lipid	g/100 g lipid	
Menhaden						
Whole	433	21.6	8.5	39.1± 0.9	12.5±0.6	3.1
Lipids	410	21.9	8.2	37.2± 2.3	13.7±1.5	2.7
NL-solids	417	24.4	10.9	44.8± 1.0	10.9±0.6	4.1
Silver salmon						
Whole	399	20.1	8.1	40.2± 1.8	14.1±1.5	2.8
Lipids	433	24.1	10.9	41.9± 1.1	12.2±1.2	3.4
NL-solids	436	26.7	11.7	43.9±11.7	9.5±0.6	4.6
Mullet						
Whole	463	18.9	7.0	37.2± 1.3	15.2±1.3	2.5
Lipids	412	23.4	9.2	39.3± 2.2	11.9±0.6	3.3
NL-solids	400	24.3	10.3	41.9±10.3	10.6±0.3	4.1
Ocean perch						
Whole	402	23.0	8.8	38.3± 1.6	13.7±1.5	2.8
Lipids	426	23.4	9.9	42.4± 1.1	11.8±1.1	3.6
NL-solids	381	23.4	10.3	42.8± 0.7	11.6±1.0	3.7
Controls						
18% casein	416	28.3	12.6	44.5± 1.4	9.3±0.5	4.8
25% casein	415	25.6	11.3	44.1± 1.1	10.6±0.9	4.2

[a] Mean values ± the standard errors of the means.

Discussion

For GLC analyses of complex mixtures of esters, such as those prepared from tissue lipids and marine oils, an instrument equipped with an Argon β-ionization detector was used. However, the response of such detectors is nonlinear for some long chain polyunsaturated esters (14), whereas our experience has shown that mixtures of saturated esters (12:0 to 22:0) are readily assayed within a probable error of ±5% using a thermal conductivity detector. For these reasons we used the hydrogenated samples for the chain length analyses of the marine oil esters and unhydrogenated samples for determining the relative amounts (percentages) of saturated and unsaturated components within each chain length. Corrections for the nonlinear response of the β-ionization detector have also been determined for those pure polyunsaturated esters that are available. Only one correction factor, 1.25 for the area of linolenate com-

ponent, was used in deriving the analytical data reported here; corrections were not required for oleate and linoleate under the outlined experimental conditions (see Analytical Methods). Studies with model mixtures of pure esters suggest that calculations based on these procedures are more accurate than those determined by relating the area of each component to the total area obtained from all components in a GLC-chromatogram.

The studies reported here revealed that the lipid depressant activities exhibited by four marine oils could be duplicated by ingestion of the whole fish products which contain these oils. In hypercholesterolemic rats, both the fish and fish oils promoted a more favorable balance between the cholesterol and phospholipid components found in blood and liver (i.e., a lower TC/TP). Observations of Mead and Gauze (17) suggest that a low TC/TP value is probably one of the best indexes of a normal distribution of lipids in the blood vascular tissues.

Although Wood and his coworkers (18) have reported that unsaponifiables from certain fish liver oils are effective cholesterol depressants in chickens, it would seem unlikely that the small amounts of marine sterols found in these supplements (Table I and Fig. 1) were responsible for the lipid depressant activities of the four species of fish. The diets contained between 5 and 20 times more cholesterol than the marine sterols; the bile acid components of the experimental diets would also be expected to mask the effects of the marine sterols found in the fish supplements. Furthermore, our other studies (3,4) and those of De Groot and Reed (7) have shown that the unsaponifiables from the whole body oils of tuna and menhaden, and the liver oil from cod, have little influence on the circulating lipids of hypercholesterolemic rats.

Results from these studies demonstrate that it is possible to bring about a significant reduction in blood and tissue lipids of hypercholesterolemic rats by very small dietary changes. The oil supplements from ocean perch represented only 5.5% of the total caloric contents of the diets (¼ of the dietary fat); yet this small alteration in the chemical composition of the diet was enough to promote nearly a 50% reduction in the combined cholesterol and phospholipid components of the blood (Table I). It is not apparent, however, why doubling the intake of a similar oil (menhaden) failed to promote a greater reduction in blood lipids. The lipid depressant activities of the marine oils may be a reflection of a combination of factors including their total unsaturation (1,2), the type of unsaturation found in their fatty acid components, and the different proportions of specific fatty acids found in the marine oils.

The apparent differences in the effects of the whole fish and their component oils on liver lipids may be related to the lipotropic activities of the fish proteins. Other investigators (8) have observed that dietary protein concentrations, and the amino acid components of proteins, can influence both liver and blood lipids under similar experimental conditions.

Acknowledgment

The author thanks John Champlin for his technical assistance in some of the GLC analyses made for this study.

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[Received February 1, 1962]

Hexane and Ethanol as Peanut Oil Solvents

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Abstract

Absolute ethanol is a better solvent for extracting peanut grits than 95% ethanol, with hexane intermediate in its action. More nonlipids solids are extracted by 95% ethanol than absolute ethanol as compared with none by hexane. Ethanol-extracted oils are slightly higher in color and free fatty acids than hexane-extracted oils.

PREVIOUS WORK in this laboratory has shown that peanut flakes disintegrate upon contact with a solvent, producing powder difficult to handle in a continuous extractor. Extraction rates were therefore determined on two sizes of peanut grits using three solvents: hexane, 95% ethanol, and absolute ethanol. While ethanol, as pointed out by Rao and Arnold (3), is not practical for general extraction use in this country, it has advantages for use in countries such as India where many peanuts are grown. Since preliminary examination of early experimental results indicated that the ethanols were apparently extracting more non-glyceride material than hexane, determinations were made of the amounts of solid non-glyceride material and upon the purity of the oil extracted.

Two sizes of grits were used: through 14-mesh and retained on 20-mesh, and through 20-mesh and retained on 30-mesh. The extractions were carried out in glassware rate extraction apparatus similar to that used in previous studies in this laboratory (1) except somewhat larger. The extraction chamber was 12 inch high by 2 inch diam. Both extraction chamber and solvent were heated to the desired temperature