Hypocholesterolemic Effect Induced in Rats by Oil-Sardine (*Sardinella longiceps*) Fish and Sardine Oils Having Different Degrees of Unsaturation

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ABSTRACT AND SUMMARY

Oil-sardine (*Sardinella longiceps*) fish and its oil were found to have pronounced hypocholesterolemic effect in cholesterol bile salt stressed rats. Effect of fish was more than that observed with its oil. "Stearin" free or solvent winterized sardine oil with more unsaturation showed better effect than sardine oil as such. A significant correlation was observed between iodine value, ratio of polyunsaturated to saturated fatty acids and (2S-P) value of dietary fats, and logarithm of therminal serum total cholesterol (TC). Similarly a correlation between square root of iodine value and terminal serum TC was also observed. However, cholesterol lowering effect was not predictable on the basis of linoleic, arachidonic eicosapentaenoic, and docosahexaenoic acid contents.

INTRODUCTION

During the last two decades, the efficacy of polyunsaturated marine oils in lowering serum cholesterol level in experimental animals and man has been established and has been reviewed by Peifer (1). Hypocholesterolemic effect of fish in the diet of rats and human beings has been reported by Kinsell (2), Miller et al. (3), Harlow and Morton (4), and Peifer et al. (5).

In our earlier communication (6), the hypocholesterolemic effect of oil-sardine (*Sardinella longiceps*) fish and its oil in cholesterol bile salt stressed rats had been reported. In this experiment, diets contained 15% fat supplying about 30% of total calories and in test lipid groups sardine oil either as such or as fish supplied 10% fat. One of the objectives of the present investigation is to find the effect of sardine fish and its oil at a lower level of dietary fat (10%) and with a lower proportion (50%) of replacement by test lipids.

It has been suggested that the cholesterol-depressant activities of marine oils and their fatty acid fractions are closely related to their high total unsaturation which results from their contents of longer chain linolenate homologues (1). Khan et al. (7) have reported that a concentrate of cod liver oil with an iodine value (IV) of 375 was more effective than a concentrate with IV 315 in alleviating the hypocholesterolemia in the chicken. Against the above background, the present communication also reports the effect of two different fractions of sardine oil (having different degrees of higher unsaturation) incorporated in the diet at two different levels.

EXPERIMENTAL PROCEDURES

Adult male rats of Wistar strain were fed a stock diet (Group A, Table I) containing cholesterol and bile salt (sodium tauroglycocholate) for a period of 5 wk (pretest period) to ensure severe hypercholesterolemia. These were then divided into seven groups of seven rats each by completely randomized design and were fed test diets (Table I) for a period of 51-59 days. In Groups B, C, D, and F, test lipids either as such or as sardine fish replaced 50% of the hydrogenated vegetable (HVO) and refined groundnut oil taken together. In groups E and G, replacement was 75% and 25%, respectively. Pretest and test diets were essentially similar to those described by Peifer (1). Stock diets without fish and test lipids were prepared fresh at weekly intervals. Requisite quantity of sardine fish from a canned pack or test lipids was mixed with corresponding stock diet daily and given at 20 g (dry weight)/rat/day.

TABLE I

Composition of Test Diets

	Group A, %	Group B, %	Group C, %	Group D, %	Group E, %	Group F, %	Group G, %
Casein	18.0	18.0	18.0	18.0	18.0	14.1	18.0
Sucrose	64.0	64.0	64.0	64.0	64.0	64.0	64.0
Hydrogenated vegetable oil[a]	9.9	4.9	4.9	4.9	2.4	4.9	7.4
Cellulose	4.0	4.0	4.0	4.0	4.0	2.7	4.0
Salt mixture (30)	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin mixture (31)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitaminized oil[b]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Bile salt[c]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sardine oil	-	5.0	-	-	-	-	-
Stearin free sardine oil	-	-	5.0	-	7.5	-	-
Solvent winterized sardine oil	-	-	-	5.0	-	-	2.5
Oil-sardine fish[d] (moisture free basis)	-	-	-	-	-	10.2	-

[a]Vanaspati (Indian margarine).

[b]Refined groundnut oil containing vitamins A (2000 IU), D (1000 IU), and E (100 IU) per g oil.

[c]Sodium tauroglycocholate.

[d]Equivalent to 25.5 g as it is basis, providing 3.9 g protein, 5 g lipids, and 1.3 g ash.

TABLE II

Fatty Acid Composition of Different Lipids Used (% by wt)

Fatty acid	Hydrogenated vegetable oil	Sardine oil (12)	Stearin free sardine oil (12)	Solvent winterized sardine oil (12)	Total lipids of sardine muscle (13)
Saturated					
12:0-15:0	--	10.7	9.3	9.4	8.3
16:0	21.6	22.9	17.9	13.4	26.9
17:0, 19:0 & 20:0	--	1.4	1.4	1.2	1.0
18:0	10.9	6.1	5.1	3.9	3.8
Monoenoic					
14:1, 15:1	--	2.4	2.2	1.2	--
16:1	--	11.2	14.8	13.9	6.7
17:1	--	0.3	0.6	--	--
18:1	64.5	7.0	9.2	6.7	15.4
19:1-24:1	--	0.2	0.2	--	6.1
Polyenoic					
16:2	--	2.0	2.1	2.4	--
16:3	--	1.6	2.3	0.8	--
16:4	--	0.8	0.9	1.3	--
18:2	3.0	2.3	5.1	4.4	4.3
18:3	--	1.0	2.0	2.7	0.8
18:4	--	4.0	3.5	5.2	1.7
20:2	--	0.3	0.9	0.2	--
20:3	--	--	--	--	0.8
20:4	--	1.5	2.4	1.7	0.7
20:5	--	13.2	11.2	18.2	10.6
22:2	--	0.1	--	--	--
22:4	--	--	--	--	1.2
22:5	--	--	--	--	0.8
22:6	--	11.2	9.4	14.1	8.8

TABLE III

Characteristics of Lipids Used

	Hydrogenated vegetable oil	Refined groundnut oil	Sardine oil	Stearin free sardine oil	Solvent winterized sardine oil	Lipid of sardine muscle
Iodine value	62.5	92.1	166.8	177.9	216.7	--
Cholesterol content, mg %	--	--	370	182	342	120
Free fatty acid, % as oleic acid	0.1	0.1	0.2	0.2	0.5	0.1
Saturated acids (%)	32.5	18-19[a]	41.1	33.7	27.9	40.0
Monoenoic acids (%)	64.5	53-63[a]	21.1	27.0	21.8	28.2
Polyenoic acids (%)	3.0	19-26[a]	38.0	39.8	51.0	29.7

[a]Reference (17).

Test lipids with different degrees of unsaturation and used in the present investigation were: (a) sardine oil (SO) prepared from fresh whole oil-sardine fish by cooking and pressing at the pilot plant of CFTRI Fish Technology Experiment Station, Mangalore, and added to diet of group B at 5% level: (b) "stearin" free sardine oil (SFSO) prepared in the laboratory by filtering off "stearin" that separated out during storage for a few months at ambient condition (25-28 C) and added to diets of rats of groups C and E at 5% and 7.5%, respectively; and (c) solvent (acetone) winterized sardine oil (SWSO) prepared in the laboratory by winterization at −28 C and by removal of solvent in a rotary flash evaporator under vacuum. This oil was added to the diets of rats of groups D and G at 5% and 2.5% levels respectively. To ensure an appropriate balance between polyenoic fatty acids of fish oils and antioxidants (8), sardine oils (SO, SFSO, SWSO) were mixed with alpha-tocopherol acetate (SO and SFSO, 75 mg/100 g and SWSO, 100 mg/100 g). The oils were packed and stored as described in our earlier communication (6).

Oil-sardine fish in the form of canned packs (6) was used. Fish thus prepared had moisture, 60.1%; protein (N x 6.25), 15.2%; total lipids, 19.6%; and ash, 5.1%. Total lipids extracted by chloroform-methanol and re-extracted with chloroform (9) were fractionated by column chromatography (10) and found to be composed of 3.6% of phospholipids, 0.5% of free fatty acids, and 93.3% triglycerides. It also contained 2.5% of nonsaponfiable matter determined by the official and tentative methods of AOCS (11). 25.5 g of fish containing 5 g lipids, 3.9 g protein, and 1.3 g ash, was mixed with 89.8 g of stock diet (group F). Casein content of the diet was reduced in view of contribution of protein by fish. To account for 1.3 g of mineral (ash) content derived from fish, cellulose content was reduced accordingly. To meet the additional requirement of antioxidant on account of lipids from fish, HVO of group F was mixed with alpha-tocopherol acetate (95 mg/100 g).

Fatty acid compositions of SO, SFSO, and SWSO used, and total lipids of oil-sardine fish have been reported by Venkateswara Rao and Gedam (12) and Gopakumar and Nair (13). HVO of the brand used was analyzed for its fatty acid composition by GLC in the Varian Aerograph series 1400 S, fitted with flame ionization detector, and 8 ft x 1/8 in. stainless steel column packed with 15% diethylene glycol succinate on Chromosorb W, 60-80 mesh. Injection temperature was 210 C, detector temperature 230 C, and the column was run isothermally at 185 C. Airflow was 300 ml/min, the chart speed 20 in./hr. The peaks were measured by triangulation. Fatty acid composition and characteristics of different lipids are given in Tables II and III.

At the end of test period on the afternoon previous to the day of sacrifice, diet was withdrawn. Rats were

TABLE IV

Food Intake and Increase in Body Weight during Test Period

Group	Diet	Average body weight (g) Initial (pretest period)	Initial (test period)	Terminal (test period)	Increase (test period) mean ± SE	Food intake g/rat/day mean ± SE
A	Control	149	242	307	65 ± 10.4^{xy}[a]	12.7 ± 0.51^{x}
B	5% Sardine oil	154	249	296	47 ± 10.4^{y}	12.2 ± 0.51^{xy}
C	5% Stearin free sardine oil	155	245	311	65 ± 10.4^{xy}	11.9 ± 0.51^{xy}
D	5% Solvent winterized sardine oil[b]	158	247	308	61 ± 12.3^{xy}	12.3 ± 0.60^{xy}
E	7.5% Stearin free sardine oil	155	248	322	74 ± 10.4^{xy}	12.1 ± 0.51^{xy}
F	5% Lipids from sardine fish	151	255	350	96 ± 10.4^{x}	14.6 ± 0.51^{z}
G	2.5% Solvent winterized sardine oil[a]	148	214	294	80 ± 11.3^{xy}	10.9 ± 0.55^{y}
	Degree of freedom				39	39

[a]The means carrying the same superscript are not significant different.

[b]Two rats of group D and one rat of group G died during the course of experiment; hence for these two groups averages are based on five observations and six observations, respectively. (SE = standard error.)

anesthetized and blood collected from heart. Liver and heart were weighed and a portion from each organ was kept separately for the estimation of moisture, fat (petroleum ether extract), and cholesterol. Remaining portions along with aorta were kept in 10% formalin for subsequent histopathological examination. Moisture content was determined by drying at 50-60 under vacuum; fat content by extraction of the dry solids with petroleum ether (40-60 C) in a soxhlet apparatus. Total cholesterol (14), ester cholesterol (15), and phospholipids (16) were determined in aliquot portions of serum. Difference in total cholesterol and ester cholesterol was taken as free cholesterol.

Weighed quantity of heart and liver was homogenized with acetone-ethanol (1:1) and centrifuged. The residue was extracted twice. The combined extracts were made up to a volume. Total cholesterol (heart and liver) and ester cholesterol (liver only) were estimated in aliquot portions of the extract by the methods referred to above.

Results were subjected to appropriate statistical tests of significance like Duncan's new multiple range and multiple F test (18) and extension of multiple range test to group means with unequal number of replications at 5% level (19).

DISCUSSION

Growth Response (Table IV)

During the pretest period all the animals were given the same diet, but group G showed lower body weight than the other groups. No reason for this could be ascribed. Increase in weight during test period varied from 47 g to 96 g. Animals of group F given fish showed significantly greater increase in weight than that observed in group B given SO. Significantly greater increase in weight observed in group F may be partly explained by its significantly higher food intake. Differences in increase in weights between other groups were not statistically significant.

Oils, especially fish oils, which contain highly unsaturated fatty acids are unstable and undergo oxidative and other changes during autoxidation and heating. It has been reported by Noboru Matsuo (20) that whereas autoxidized fish oils show poor (rather adverse) growth response discernible in a few weeks, fish oil as such exhibited no such adverse effect, rather good growth response was observed with fish oil fed at 20% level. From our present study, it appears that not only fish oil as such but also its fractions with higher degrees of unsaturation were apparently not autoxidized during storage and handling to show any growth depressant effect.

During the course of investigation, two rats of group D and one rat of group G died. Histopathology of different organs did not reveal any abnormality.

Terminal Serum Cholesterol and Phospholipids (Table V)

Serum cholesterol conforms to a log-normal distribution (21). In the present study, terminal serum cholesterol was found to vary widely from animal to animal. Statistical analysis was, therefore, carried out on the transformed variates (using logarithmic transformation).

SO, SFSO, SWSO and fish lowered terminal total cholesterol (TC), ester cholesterol (EC) and free cholesterol (FC) in comparison with the control group given HVO. Terminal TC and EC of all the test-lipid groups (B, C, D, E, F, and G) were significantly lower than the corresponding figure of the control group (A) given HVO. FC was significantly higher in groups A and B than the corresponding figure of groups C, D, E, and F. Serum phospholipids (PL) of different groups varied from 143-170 mg %; the difference between any two treatment means was not statistically significant. As expected, TC-PL ratio for all the groups (B, C, D, E, F, and G) given test lipids was significantly lower than that observed with the control group (A) given HVO.

Esterification of cholesterol enables it to be transported and metabolized. The lower the ratio of FC-EC in any treatment, the more beneficial is considered to be the effect. From this criteria, the effect of SWSO (group D, 5%) and SFSO (group E, 7.5%) were significant in comparison with HVO (group A) and SO (group B).

Sardine Fish and Its Oil

The group B (SO) and F (fish) had the same amount of test lipids in the diet. Yet fish was significantly more effective than its oil. The better effect observed with fish may be partly due to more intake of food by the group (14.6 g/rat/day for group F given fish as against 12.2 g for the group B given SO). Secondly, lipids of fish are not identical to oil pressed out. Sardine oil is composed of 98% triglycerides with unsaponifiable matter not more than 2% (22) whereas total lipids of fish contain 93.3% of triglycerides, 3.6% of phospholipids, 2.5% of unsaponifiable matter, and 0.5% of free fatty acids. Fatty acid composition of (a) total lipids extracted by chloroform and methanol and (b) oil extracted by cooking and pressing indicates that both contain equal amounts of saturated fatty acids (about 40%), but oil contains more polyenoic acids (about 40%) than that present in lipids of muscle (about 30%). From this consideration, oil should be more potent than fish itself but the reverse was observed from the results. Thus, a part of the difference in effect observed with fish and its pressed-out oil may be due to some unknown factors present in fish. In our earlier investigation (6), it was observed that at the 10% level, test lipids either as oil or as fish had no

TABLE V

Terminal Serum Total, Free and Ester Cholesterol and Phospholipids

Group	Diet	Total cholesterol (TC)		Free cholesterol (FC)		Ester cholesterol (EC)		Phospholipids (PL)	TC/PL	FC/EC
		mg/100 ml mean ± SE	Transformed variate mean ± 0.04	mg/100 ml mean	Transformed variate mean[a]	mg/100 ml mean ± SE	Transformed variate mean[b]	mg/100 ml mean ± SE	mean[c]	mean[d]
A	Control	321 ± 14	2.51^{w}	164	2.21^{w}	157 ± 14	2.19^{w}	170 ± 14	1.96^{w}	1.11^{w}
B	5% Sardine oil	167 ± 15	2.21^{x}	88	1.93^{wx}	79 ± 8	1.88^{x}	167 ± 14	1.00^{x}	1.14^{w}
C	5% Stearin free sardine oil	110 ± 9	2.03^{yz}	38	1.45^{yz}	70 ± 6	1.84^{x}	148 ± 14	0.75^{xy}	0.69^{wxy}
D	5% Solvent winterized sardine oil[e]	90 ± 11	1.94^{y}	23	1.30^{z}	68 ± 8	1.82^{x}	143 ± 17	0.63^{y}	0.34^{y}
E	7.5% Stearin free sardine oil	109 ± 11	2.02^{yz}	34	1.47^{yz}	75 ± 6	1.86^{x}	161 ± 14	0.70^{y}	0.46^{xy}
F	5% Lipids from sardine fish	117 ± 10	2.06^{yz}	47	1.59^{yz}	70 ± 9	1.83^{x}	170 ± 14	0.73^{xy}	0.78^{wxy}
G	2.5% Solvent winterized sardine oil[e]	139 ± 11	2.14^{xz}	63	1.74^{xy}	76 ± 6	1.88^{x}	148 ± 17	0.96^{xy}	0.87^{wx}
	Degree of freedom	39		38		38		38	38	37

[a]Means carrying the same superscripts (such as w,x,y,z) down a column are not significantly different. SE for D, ± 0.11; A, B, C, E, F, G, ± 0.10.
[b]SE for D, ± 0.05; A, B, C, E, F, G, ± 0.04.
[c]SE for D,G, ± 0.10; A, B, C, E, F, ± 0.09.
[d]SE for C,D, ± 0.17; G, ± 0.16, A, B, E, F, ± 0.14.
[e]Two rats of group D and one rat of group G died during the course of investigation.

significant difference in effect. On the other hand, in the present investigation where test lipids were given at the 5% level, fish was significantly more effective than the extracted oil. Thus, it is logical to conclude that at the 10% level (6), the effect of oil was so pronounced that possible effect of other factor(s) failed to manifest its additional effect which became clear only when test lipids were fed at a lower level of 5% as in the present investigation.

Peifer et al. (5) working on the lipid depressant activities of whole fish (menhaden, silver salmon, mullet, and ocean perch) and their component oils reported that hypocholesterolemic effect of the whole fish was duplicated by feeding proportionate amounts of oils found in these fish supplements. In the case of salmon, oil appeared to have a somewhat greater depressant activity than the fish itself. In the present investigation, sardin fish appeared to be more effective than its oil.

Quality of Dietary Fat and Hypocholesterolemic Effect

Various authors have proposed from time to time different quantitative relationships between the quality of dietary fat and its hypocholesterolemic effect. Ratio of polyunsaturated to saturated fatty acid contents by Jollife (23) and root of iodine value by Gunning et al. (24) have received attention. Keys et al. (25) have observed that 2S-P is a good index of dietary fat vis-a-vis its hypocholesterolemic effect where S and P are percentages of total diet calories furnished by saturated and polyunsaturated fatty acids. According to Hegsted (26) the effect observed with oils is due to combined contents of EFA (linoleic and arachidonic acids) and saturated fatty acids.

Iodine value (Wijs), cholesterol, saturated, linoleic, arachidonic, and other polyunsaturated fatty acid contents, ratio of polyunsaturated to saturated fatty acids, and (2S-P) values of dietary lipids used are given in Table VI. Dietary cholesterol was more or less the same in all the diets, and its effect is logically expected to be identical in all the groups.

Figures 1 and 2 give the correlation observed between terminal serum TC on the one hand and iodine value, square root of iodine value, ratio of polyunsaturated to saturated acid content and (2S-P) value of dietary lipids on the other hand. In these figures, group F given fish was not included. Square root of iodine value and P^1/S^1 gave highly significant correlation while with iodine value and (2S-P), it was significant.

Kinsell (27) and Grande (28) have rightly criticized the concept of polyunsaturated to saturated fatty acid ratio to predict serum cholesterol change as the ratio does not take into account the quantitative aspects of dietary fatty acids ingested. From the present investigation we may conclude that when total caloriese derived from dietary fat is about 20%, the ratio is a good index for the prediction of possible effect on serum TC.

There is a need for a reasonably simple, workable index which can be applied to dietary prescription for individuals with significantly raised plasma cholesterol level. Square root of iodine value appears to be the best index in this respect.

Essential fatty acids (linoleic and arachidonic) either as such or combined with saturated fatty acids had no correlation with terminal TC. In other words, with sardine oil serum cholesterol lowering effect is not due to its essential fatty acid contents. This confirms the result reported by Ahrens et al. (29) and Kingsbury et al, (30) that plasma cholesterol could be lowered by essential (corn oils), and nonessential (marine oils) fatty acids. The results corroborate that hypocholesterolemic activities of the marine oils or their fatty acid fractions are closely related to their contents of longer chain linolenate homologues (1). At the same time as reported earlier by Peifer et al. and also in the present investigation, hypocholesterolemic effect appeared

TABLE VI

Characteristics of Dietary Lipids of Different Groups[a]

	Group A	Group B	Group C	Group D	Group E	Group F	Group G
Iodine value (Wijs)	62.8	114.8	120.4	139.4	149.1	--	101.3
Total cholesterol (mg %)	500	519	509	517	514	528	508
Fatty acids (g/100 g diet)							
Saturated (S^1)	3.25	3.68	3.31	3.02	3.34	3.63	3.14
Polyunsaturated (P^1)	0.30	2.05	2.14	2.70	3.06	1.64	1.50
Linoleic (L)	0.30	0.27	0.41	0.37	0.46	0.37	0.34
Arachidonic (A)	--	0.08	0.12	0.09	0.18	0.04	0.04
Eicosapentaenoic	--	0.66	0.56	0.91	0.84	0.53	0.46
Docosahexaenoic	--	0.56	0.47	0.71	0.71	0.44	0.35
P^1/S^1	0.09	0.56	0.65	0.90	0.92	0.45	0.48
2S-P[b]	13.0	11.6	9.8	7.3	7.9	12.3	10.4
$(L+A)S^1 \times 100$	97.5	126.4	175.4	137.4	213.7	147.0	120.2

[a]Calories of diet – 426/100 go fo which 16% is from protein, 22% is from lipids, and 62% is from carbohydrate.

[b]S and P – % of calories in the diet contributed by saturated and polyunsaturated fatty acids respectively.

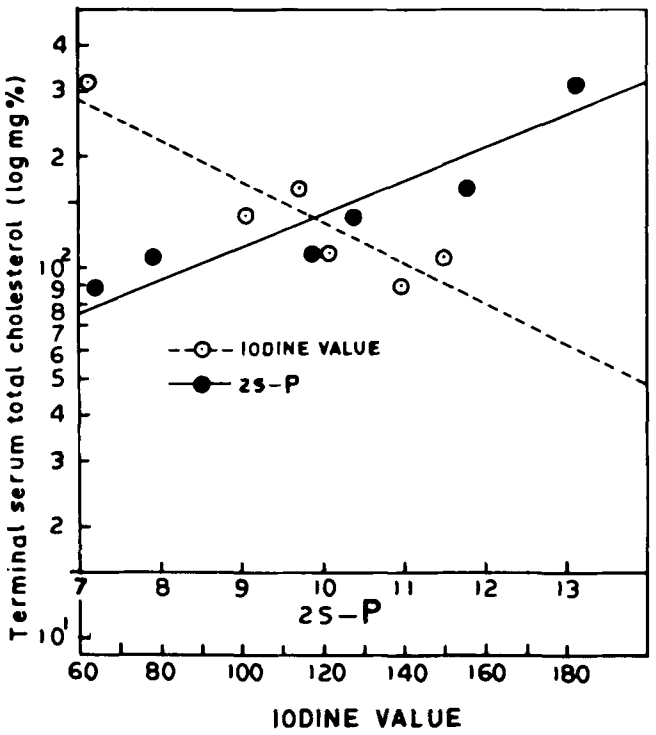

FIG. 1. Logarithm of terminal serum cholesterol (TC) plotted against iodine value (IV) and (2S-P) value of dietary fats. (S and P are percentages of total calorie in the diet contributed by saturated and polyenoic fatty acids respectively.) Regression of log TC on IV: Y = 2.8272 – 0.0059 x*. Regression of log TC on 2S-P: Y = 0.0808x + 1.3405* (* = significant).

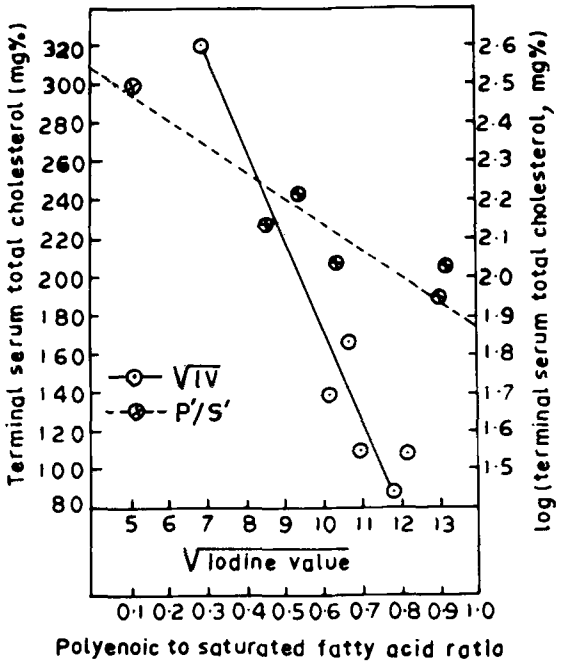

FIG. 2. Terminal serum cholesterol (TC) plotted against P^1/S^1 value and square root of iodine value (IV) of dietary fats. (P^1/S^1- Polyenoic to saturated fatty acid ratio of diet.) Regression of log TC on P^1/S^1: Y = 2.5026 – 0.5961x**. Regression of TC on square root of IV: Y = 705.08 – 51.75 x** (** = highly significant).

to have no relationship with eicosapentaenoic and docosahexaenoic acids either singly or taken together, and also total polyunsaturated fatty acids.

SFSO and SO had the same contents (about 40%) of polyunsaturated fatty acids, the removal of "stearin" only decreased the proportion of saturated fatty acid content with a corresponding increase in monounsaturated acids. This change greatly improved the efficacy of the product (SFSO), in comparison with its mother oil. It seems that with SFSO, its contribution to the extent of 5% in the diet was sufficient to exhibit maximum effect, further increase in amount was without any additional advantage.

SWSO had 50% polyunsaturated fatty acids, saturated acids contributing 28%. This change made it more effective than SFSO not to speak of SO. Results indicate that for maximum possible effect, about 3.5% SWSO in the diet would have been sufficient (5% was more than sufficient and 2.5% was insufficient to exhibit full effect).

Liver and Heart (Tables VII and VIII)

None of the test lipids including those from fish as such had any significant effect on the weight (total or calculated as per 100 g body weight basis), moisture, fat, cholesterol (total and ester) contents of liver. Similarly, weight, moisture, and total cholesterol contents of heart were unaffected by any test lipids.

Results of Peifer et al. (5) indicated that certain fractions of menhaden oil could effectively mobilize cholesterol out of livers of hypercholesterolemic rats. We did not find any evidence of the same.

Histopathological Study

Liver showed normal lobular architecture in all the groups. There was fatty change in liver cells in the centrolobular and midzone of lobula. Fatty change was minimal to severe in groups A to F. In group G, it was nominal. In other words, none of the treatments brought any additional change. Aortas were examined for possible atherosclerosis which was not observed in any group in-

TABLE VII

Terminal Weights and Moisture Contents of Liver and Heart

Group	Diet	Liver			Heart		
		Total wt (g) Mean ± SE[a]	Wt (g)/100 g body wt Mean ± SE	Moisture % Mean ± SE	Total wt (g) Mean ± SE	Wt (g)/100 g body wt Mean ± SE	Moisture % Mean ± SE
A	Control	13.7 ± 0.9	4.5 ± 0.2	58.4 ± 2.1	1.5 ± 0.1	0.49 ± 0.03	75.5 ± 0.4
B	5% Sardine oil	13.5 ± 0.9	4.5 ± 0.2	59.7 ± 2.1	1.3 ± 0.1	0.45 ± 0.03	75.6 ± 0.4
C	5% Stearin free sardine oil	14.0 ± 0.9	4.5 ± 0.2	57.6 ± 2.1	1.4 ± 0.1	0.44 ± 0.03	75.8 ± 0.4
D	5% Solvent winterized sardine oil[b]	14.4 ± 1.0	4.7 ± 0.3	56.6 ± 2.5	1.7 ± 0.1	0.54 ± 0.03	74.5 ± 0.5
E	7.5% Stearin free sardine oil	14.0 ± 0.9	4.4 ± 0.2	60.1 ± 2.1	1.4 ± 0.1	0.42 ± 0.03	76.6 ± 0.4
F	5% Lipids from fish	16.0 ± 0.9	4.6 ± 0.2	58.5 ± 2.1	1.6 ± 0.1	0.47 ± 0.03	75.7 ± 0.4
G	2.5% Solvent winterized sardine oil[b]	12.5 ± 0.9	4.3 ± 0.3	60.3 ± 2.2	1.2 ± 0.1	0.42 ± 0.03	76.2 ± 0.5
	Degree of freedom	39	39	39	39	39	39

[a]SE = standard error.

[b]Two rats of group D and one rat of group G died during the course of investigation.

TABLE VIII

Fat and Cholesterol Content of Liver and Heart

Group	Diet	Fat (liver) mg/100 g fresh tissue Mean ± SE	Total cholesterol (liver) mg/g fresh tissue Mean ± SE	Ester cholesterol (liver) mg/g fresh tissue Mean ± SE	Total cholesterol (heart) mg/g fresh tissue Mean ± SE
A	Control	17.3 ± 2.1	38.3 ± 4.1	33.1 ± 4.8	1.6 ± 0.3
B	5% Sardine oil	15.7 ± 2.1	35.1 ± 4.1	28.2 ± 4.4	1.7 ± 0.3
C	5% Stearin free sardine oil	19.5 ± 2.1	38.4 ± 4.1	32.4 ± 4.4	1.5 ± 0.3
D	5% Solvent winterized sardine oil	17.6 ± 2.5	37.8 ± 4.9	34.0 ± 5.2	2.1 ± 0.3
E	7.5% Stearin free sardine oil	16.4 ± 2.1	31.6 ± 4.1	27.3 ± 4.4	1.0 ± 0.3
F	5% Lipids from sardine fish	18.6 ± 2.1	41.8 ± 4.1	34.7 ± 4.4	1.0 ± 0.3
G	2.5% Solvent winterized sardine oil	16.0 ± 2.3	31.0 ± 4.5	26.7 ± 4.8	0.9 ± 0.3
	Degree of freedom	39[a]	39[a]	38[a,b]	35[a,c]

[a]Two rats of group D and one rat of group G died during the course of investigation.

[b]Ester cholesterol (liver) could not be estimated for one rat of group A.

[c]Total cholesterol (heart) could not be estimated for one rat each of groups D, E, F, and G.

cluding the control one. Sections of aorta were normal in all the groups.

The present investigation clearly indicates the efficiency of sardine fish and its oil even when these contributed 5% lipids in the diet. The same effect could be observed at a lower level of incorporation in the diet when mother oil was processed to increase its total unsaturation. However, cholesterol lowering effect was not predictable on the basis of linoleic, arachidonic, eicosapentaenoic, and docosahexaenoic acid contents. No adverse effect of treatments either on growth or on the histopathology of liver and aorta was observed. The investigation gives an additional support to already accumulated evidence to the importance of fish for dietary prescription of individuals with significantly raised plasmic cholesterol.

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ERRATUM

In "Determination of Sultones in Anionic Surfactants," by W.D. MacMillan and H.V. Wright [*JAOCS* 54:163 (1977)] under Experimental Procedures—Batchwise Ion-Exchange (page 163, column 2), the quantity of methylene chloride should be 1500 ml rather than 300 ml.