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The Effect of Some Marine Oils and Squalene on the Plasma Cholesterol in Chicks

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INTRODUCTION

The effect of fish oils on the plasma or serum cholesterol levels of chicks was studied by MARCH and BIELY (1959) and by DAM et al. (1959). The Canadian group found addition of herring oil to a normal diet to lower the serum cholesterol level. DAM et al. (1959) investigated the effect of dietary cod liver oil and linseed oil in chicks fed normal and cholesterol-supplemented diets. With the normal diet the oils were without effect on the plasma cholesterol level, whereas with 1 % dietery cholesterol, linseed oil caused higher plasma cholesterol levels than did cod liver oil. WOOD and BIELLY (1960 a) in experiments with a diet containing 1 % added cholesterol, found herring oil not to lower the serum cholesterol level whereas liver oils from some teleost fishes lowered it as compared with the level observed on the diet without added fat. The active factor in the liver oils was located in the unsaponifiable matter (WOOD and BIELY 1960 b) and was found to be closely related to the vitamin A fraction (WOOD 1960 a). Vitamin A enriched corn oil simulated the effect observed with ling-cod liver oil, whereas corn oil itself was without any cholesterol lowering effect (WOOD and TOPLIFF 1961). The work of the Canadian group was summarized by WOOD (1960 b).

The present investigation was started to obtain information on whether some Norwegian fish oils would lower the cholesterol level in the plasma of chicks. We were interested only in the effect of the oils which could not be ascribed to their vitamin A content. Within each experiment all groups given extra fat in the diet were therefore given vitamin A palmitate up to the amount of vitamin A present in the supplementary oil with highest vitamin A content. Arachis oil was used as a reference oil in all experiments. The following fish oils were tested: Cod liver oil, herring oil, dogfish liver oil, basking shark liver oil. In addition the effects of soya-bean oil, margarine fat and purified squalene were investigated. Squalene was included as a consequence of information

given by Mr. B. Rivlin on the effect of a hydrocarbon derived from shark liver oil (personal communication). The oils were tested as additions to a normal basal diet with and without added cholesterol.

METHODS

Day-old male white leghorn chicks from a local hatchery were for one week given the basal diet reported in Table 1. They were then weighed and divided into groups in which each of the chicks were of about equal body-weight, within 10 g. From these groups an equal number of chicks were taken at random for each experimental group, usually they were taken from two groups. The experimental groups usually consisted of five chicks. They were given the experimental diets for two weeks after which they were weighed and killed. Blood was collected in heparinized centrifuge tubes and cholesterol in the plasma was determined by the method of KLUNGSØYR, HAUKENES & CLOSS (1958). Cholesterol was mixed into the basal diet from a solution in ethyl ether, which was then allowed to evaporate during the mixing. The oils and fats used were mixed into the daily portions of the diet which were offered to each group of chicks.

The experimental design is evident from the data given in the tables. The results were subject to analysis of variance.

Table 1. Percentage composition of the basal diet.

Maize meal	55,8
Wheat bran	27.4
Casein.....	11.8
Dried brewers yeast	2.0
$Ca_3 (PO_4)_2$	2.0
NaCl	1.0
	100.0

To 10 kg portions of this diet were added 2 g $MnSO_4$. 4 aq and 0.05 g riboflavine. To all the supplementary fats were added 0.2 mg DL-α-tocopherol per g of oil.

RESULTS

Eleven experiments were carried out. The results are summarized in Tables 2—7.

Expts 1—3/60 (Table 2). Premilinary tests were made with arachis oil, cod liver oil, herring oil and dogfish liver oil at the 10 % level, basal diet without added fat served as a control. In all experiments the oils were tested with the diet containing 1 % added cholesterol, in Expt. 3/60 also

Table 2. Plasma cholesterol levels in chicks fed arachis oil, cod liver oil, herring oil and dogfish liver oil at a level of 10 % in diets without added cholesterol or with 1% added cholesterol.

Fat supplement	Mg cholesterol per 100 ml (Mean ± standard error)					
	Expts. without added cholesterol			Expts. with 1% added cholesterol		
	1/60	2/60	3/60	1/60	2/60	3/60
Without added fat	(4)* 166 ± 1.5	(5) 177 ± 10.2	(5) 55 ± 2.7	(5) 575 ± 96.7	(5) 401 ± 62.4	(5) 352 ± 57.4
Arachis oil			(5) 156 ± 10.3	(5) 305 ± 51.0	(5) 385 ± 32.6	(5) 518 ± 17.5
Cod liver oil			(5) 135 ± 11.4	(3) 257 ± 87.4	(4) 349 ± 58.7	(5) 423 ± 72.1
Herring oil			(5) 129 ± 2.0	(5) 208 ± 81.3	(5) 623 ± 143.1	(5) 556 ± 67.9
Dogfish liver oil			(5) 107 ± 10.2	(4) 423 ± 84.9	(4) 707 ± 132.1	(5) 427 ± 115.0

* Number of chicks.

Table 3. Plasma cholesterol levels in chicks fed arachis oil, soya-bean oil, cod liver oil, herring oil and margarine fat at a level of 10% in diets without added cholesterol and with 1% added cholesterol.

Fat supplement	Mg cholesterol per 100 ml (Means over and differences between Expts. 2/61 and 3/61).			
	Without added cholesterol		1 % added cholesterol	
	Mean 2/61—3/61	Differences	Mean	Differences 2/61—3/61
Without added fat	158	—42	354	— 57
Arachis oil	155	—15	488	— 43
Soya-bean oil	165	+18	536	—186
Cod liver oil	120	+15	472	—377
Herring oil	147	—13	440	— 50
Margarine fat	139	+10	430	—108
Standard error (48d.f.)	± 9.8	± 13.9	± 51.8	± 73.3

with the diet without added cholesterol. Since some of the chicks died during the experiments the results are presented separately for each experiment.

Without added cholesterol dogfish liver oil produced the lowest plasma cholesterol level (Expt 3/60). This effect was significant when tested in an analysis of variance. The next lower level was obtained with herring oil, but in this case the effect was non-significant.

With added cholesterol cod liver oil produced low plasma cholesterol levels in Expts 1 and 2/60, but in Expt 3/60 the effect was not so clear.

Expts 2 and 3/61 (*Table 3*). Arachis oil, soya-bean oil, cod liver oil, herring oil and margarine fat were tested at the 10 % level in diets without and with 1 % added cholesterol.

Analysis of variance indicated significant treatment effect between the groups given the diet without added cholesterol, with added cholesterol the treatment effect was non-significant.

Without added cholesterol cod liver oil gave a significantly lower plasma cholesterol level than the other fats, between the latter the differences were non-significant.

The mean differences between experiments were non-significant with both types of diet.

Table 4. Plasma cholesterol levels in chicks fed arachis oil and cod liver oil at a level of 10% in diets with 0, 0.25, 0.5 and 1.0% added cholesterol.

Cholesterol level in the diet	Mg cholesterol per 100 ml		
	Mean over oils and expts.	Difference arachis oil — cod liver oil	Difference Expt 1/61 — Expt 4/60
0	158	+ 50	+18
0.25	185	+ 13	−23
0.5	262	+ 68	+41
1.0	462	+167	−39
Standard error (64 d.f.)	± 22.5	± 31.9	± 45.0

Expts 4/60 and 1/61 (*Table 4*). Arachis oil and cod liver oil were tested at the 10 % level with diets containing 0.25, 0.5 and 1.0 % cholesterol. The basal diet without added cholesterol served as a control.

Arachis oil gave higher plasma cholesterol levels than did cod liver oil with all diets. The effect was most pronounced at the 1 % cholesterol level.

When the results obtained were pooled in the analysis of variance the difference between oils was significant only at the 0.5 and 1 % cholesterol levels. However, when the results obtained on the diet without added cholesterol were treated separately, the cod liver oil gave significantly lower plasma levels than those obtained with arachis oil in the diet.

Expts 4 and 5/61 (*Table 5*). Arachis oil and cod liver oil were tested at the 2.5, 5 and 10 % levels with the diets without and with 1 % added cholesterol. The level of fat had no significant effect on the plasma cholesterol level. With both types of diet the mean cholesterol level in the plasma was higher in the chicks given arachis oil than in those given cod liver oil. This effect was significant with the diet with 1 % added cholesterol, but not with the diet without such addition. With the latter diet cod liver oil gave a lower plasma level than arachis oil in Expt 4/61, whereas in Expt 5/61 the levels were about equal.

Expt 6/61 (*Table 6*). Mixed methyl esters prepared from cod liver oil and fractions of these obtained by molecular distillation were tested at levels corresponding to 10 % of the undistilled methyl ester mixture in the diet containing 1 % added cholesterol. Arachis oil and cod liver oil at the 10 % level and the basal diet without added fat served as controls. The three latter diets were also tested without added cholesterol.

Table 5. Plasma cholesterol levels in chicks fed arachis oil and cod liver oil at levels of 0, 2.5, 5.0 and 10.0 % in diets without added cholesterol and with 1 % added cholesterol.

Fat levels	Mg cholesterol per 100 ml									
	Without added cholesterol					1 % added cholesterol				
	Mean over oils	Difference Arachic oil –cod liver oil	SE (48d.f.)	Difference Expt 4/61 – Expt 5/61	SE (48d.f)	Mean over oils	Arachis oil –cod liver oil	SE (48d.f)	Expt 4/61 –Expt 5/61	SE (48d.f)
0	177.1			−25.0		411.2			− 65.2	
2.5	158.9	+11.4		− 3.2		423.5	+ 60.1		+ 38.9	
5.0	153.2	+ 2.1	±10.46	− 5.9	±10.46	516.9	+161.4	±85.16	−111.2	±85.16
10	153.8	+19.6		+ 5.0		411.7	+152.5		− 12.1	
Mean over fat level										
Expts 4/61 5/61	155.3	+11.0	±6.04			450.7	+124.7	±49.17		
Expt 5/61	156.0	−29.9	± 8.54			436.6	+ 96.0	± 69.54		
Expt 4/61	154.6	− 7.8				464.7	+153.3			

Table 6. Plasma cholesterol levels in chicks fed arachis oil, cod liver oil and cod liver oil methyl esters* in diets without added cholesterol and with 1% added cholesterol.

Fat supplement	Mg cholesterol per 100 ml			Data on methyl esters				
	Without added cholesterol	1% added cholesterol		% of total	F.f.a %	Iodine number %	Sap. number	Vit. A IU/g
No fat added	144	353						
Arachis oil	175	545						
Cod liver oil (C.l.o.)	146	298						
Standard error (12 d.f.)	±26.4	±68.7						
C.l.o. methyl esters		296			0.25	167.2	184.2	530
Fraction I		283	±59.2	10.8	0.05	78.4	207.4	130
II		280	(40 d.f.)	10.8	0.10	106.9	198.2	140
III		315		17.0	0.20	134.2	191.8	450
IV		404		25.2	0.30	177.0	184.3	150
V		326		15.8	0.45	253.8	171.6	1100
VI		287		9.8	0.85	225.0	159.5	5800
Standard error (28 d.f.)		±54.7						

* Arachis oil, cod liver oil and cod liver oil methyl esters were fed at a level of 10 %, while the methyl ester fractions were given in amounts such as to correspond to 10 % addition of the original methyl ester mixture.

The cod liver oil methyl esters were kindly supplied by A/S J.C. Martens & Co., Bergen, to which the authors are indebted.

No significant differences were observed between the fractions of the methyl esters, nor between these and the controls. Between the three control groups used on each type of diet the differences were also insignificant. However, cod liver oil gave lower mean plasma cholesterol levels than arachis oil with both types of diet. All groups grew well, except the group given the undistilled methyl ester mixture which grew at a lower rate than the remaining groups. This did not influence the cholesterol level.

Expt 7/61 (*Table 7*). Arachis oil, basking shark liver oil and squalene were tested at the 10, 5 and 2 % levels respectively with diets without and with 1 % added cholesterol. Eight chicks were used on each treatment, subdivided into replicates of four chicks.

Without added cholesterol arachis oil gave a significantly higher plasma cholesterol level than the two other oils, between the latter the difference was non-significant.

With added cholesterol basking shark liver oil and squalene gave lower plasma cholesterol than arachis oil. This effect was, however, non-significant.

Table 7. Plasma cholesterol levels in chicks fed arachis oil, basking shark liver oil and squalene in dies without added cholesterol or with 1% added cholesterol.

	Mg cholesterol per 100 ml			
	Without added cholesterol		1% added cholesterol	
	Mean	Difference Replicate 1 −replicate 2	Mean	Difference Replicate 1 −replicate 2
10 % Arachis oil	194	−34	501	−113
5 % Basking shark liver oil	133	+ 4	312	+ 84
2 % Squalene	167	−15	389	+111
Standard error (18 d.f.)	± 11.5	± 23.0	± 54.0	± 108.0

DISCUSSION

The cholesterol level varied between 140 and 180 mg per 100 ml plasma in the rats fed the diet without added fat. This is of the same order of magnitude as reported by the Canadian and Danish groups (MARCH and BIELY, 1959; WOOD and BIELY, 1960 a, 1960 b; WOOD, 1960 a; DAM et al., 1959). As these authors reported experiments with, as well as with-

out added cholesterol, it was found of interest to apply both techniques in the present study. The results are, however, discussed separately for the two types of feeding.

In experiments with added cholesterol cod liver oil in most instances gave lowered blood cholesterol compared with the groups given arachis oil. In some experiments, however, this effect could not be observed (Expts 3/60 in Table 2, 3/61 in Table 3, and Expt 5/61 at the 2.5 % fat level in Table 5). When the cholesterol level for the groups "cod liver oil" and "no fat added" were compared, the picture was somewhat similar. Usually the cod liver oil groups showed lowered values, exception were Expts 3/60 in Table 2 and 3/61 in Table 3. It should, however, be pointed out that both compared with arachis oil and no fat added, the effect varied greatly. Basking shark liver oil and squalene, the main component of the unsaponifiable matter of this oil, gave a lowering of cholesterol.

The possibility that the effect of cod liver oil could be derived from certain fractions of the oil was considered (Expt. 6/61 in Table 6). Six methyl ester fractions with iodine numbers ranging from 78 to 254 were tested. They showed generally equal effects on the cholesterol level as did cod liver oil and the original methyl ester mixture. In Expts 2 and 3/61 (Table 3) soya-bean oil and margarine fat were included. Compared with arachis oil, soya-bean oil showed no cholesterol-lowering effect, whereas margarine fat gave a slight effect.

It would be expected that if an oil has a specific effect on the cholesterol level in chicks, this should also be observed in chicks fed no added cholesterol in the diet. This was tried in a parallel series of experiments. Generally cod liver oil was found to give lower plasma cholesterol levels than the groups given arachis oil or with no fat added. This effect was observed also for all the marine oils tested: herring oil, dogfish liver oil and basking shark liver oil. In the present study dogfish liver oil showed the greatest effect. Maragrine fat also lowered the cholesterol under these conditions, whereas soya-bean oil showed no effect.

In all experiments the oils were adjusted with regard to the vitamin A content of the diet. The relative effects of the different oils thus cannot be explained as derived from this vitamin. The groups with no added fat were, however, for practical reasons, not supplemented with vitamin A. Wood and Topliff (1961) found effect of vitamin A. It should be pointed out that they fed oils with very high vitamin A-contents, thus their dogfish liver oils contained 7—8000 and their ling cod liver oils 19 000 to 42 000 I.U. vitamin A per g. The maximum vitamin A content for any oil tested in the present study was 1 800 I.U. per g.

Generally it may be concluded that the marine oils showed a cholesterol lowering effect in chicks, but not consistently. The effect of basking shark liver oil and squalene is of special interest, but the results are too few to allow speculations with regard to this point.

SUMMARY

The effect of some fish oils on the plasma-cholesterol level of chicks were studied in eleven experiments. Soya-bean oil, margarine fat and squalene were also included in the study. Arachis oil was used as a reference in all experiments.

In experiments with added cholesterol, cod liver oil usually showed lowered values compared with arachis oil and no fat added, but the effect varied greatly. Thus no final conclusions could be drawn. Soya-bean oil did not show any effect compared with arachis oil, whereas basking shark liver oil, dogfish liver oil and squalene lowered the cholesterol level. Methyl ester fractions of cod liver oil with iodine numbers ranging from 78 to 254 were without effect.

In experiments without added cholesterol, cod liver oil in most cases gave significantly lower cholesterol levels than did arachis oil. This effect was also observed for herring oil, dogfish liver oil, basking shark liver oil and squalene.

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