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Alimentary production of gallstones in hamsters

27. Influence of supplementation of the gallstone producing diet with squalene, cholesterol, certain other sterols, fish oil fatty acid ethyl esters, and modification of the basal diet on gallstone production and levels of cholesterol in serum and liver

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With 13 tables

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It has been found previously (1) that addition of cholesterol at the level of 1 % to a gallstone producing diet for young hamsters markedly reduced formation of cholesterol gallstones in hamsters of both sexes and greatly increased formation of amorphous pigmented gallstones in the female hamsters.

In the present study we have further examined the effects of dietary cholesterol on gallstone production in hamsters and compared these effects with those of squalene, cholestanol, 7-dehydrocholesterol, ergosterol, commercial preparations of lanosterol and soybean sterols, and the known protective effects of fish oil fatty acid ethyl esters (2) and substitution of rice starch for glucose in the basal diet (3, 4). In most cases, the influence of the various dietary manipulations on the levels of cholesterol in serum and liver was also examined.

Materials and methods

The following preparations of sterols, squalene, nonabsorbable antioxidant, and fats were used:

Colesterol, U.S.P., XVII, E. Merck, Darmstadt.

Lanosterol, Calbiochem.

β-Sitosterol, crystalline, practical grade from soybeans, Sigma, in the following referred to as "Soy bean sterols".

7-Dehydrocholesterol, Sigma.

Cholestanol, Sigma.

Squalene, grade 1, 98–100 %, Sigma.

Ionox 330 2,4,6-tri-(3′,5′-ditert.-butyl-4′hydroxybenzyl) mesitylene, gift from Shell Nederland Chemie, Rotterdam through Dansk Shell A/S, Copenhagen.

Palmkernel oil, gifts from Dansk Sojakagefabrik A/S, Copenhagen, and Århus Oliefabrik A/S, Århus.

Distilled menhaden oil fatty acid ethyl esters, gift from U.S. Fish and Wildlife Service, Bureau of Commercial Fisheries, Seattle, Wash. (U.S.A.).

Ergosterol, gift from F. Hoffmann-La Roche A.G., Basel.

Butter fat was prepared and stored as described in our paper (5).

The sterols were tested for purity by thin layer chromatography on Silica gel G (Merck, Darmstadt). Amounts of sterol 100 to 200 μg, solvent system: petroleum ether / diethyl ether / glacial acetic acid 70/30/1 (v/v/v). Visualization by charring with 50 % H_2SO_4.

Cholesterol gave only one spot. The other sterols gave each one spot followed by a tail of unidentified impurities. In the case of cholestanol the tail was very faint.

Squalene was also tested by thin layer chromatography on Silica gel G. Amount 70 μg, solvent system and visualization as above.

Squalene gave one spot accompanied by a few faint spots of unidentified impurities. The iodine value determined by the method of *Rosenmund* and *Kuhnhenn* (6) was 385.

Table 1. Fatty acid composition of the two principal fats used[1])

Fatty acid[2])	Palmkernel oil[3]) "a"	Palmkernel oil[3]) "b"	Menhaden oil fatty acid ethyl esters
8 : 0	0.5	0.1	
10 : 2	2.4	1.8	
12 : 0	58.4	58.5	trace
14 : 0	17.0	18.6	6.4
14 : 1			0.2
15 : 0 (or 14 : 2)			0.5
16 ald			0.2
16 : 0	7.9	8.8	16.9
16 : 1 ω 7			12.9
16 : 2 (or 17 : 0)			1.0
?			1.9
?			1.7
18 : 0	1.9	2.7	2.8
18 : 1 ω 9	10.0	9.0	11.9
18 : 2 ω 6	1.9	0.5	1.2
?			0.4
18 : 3 ω 3			0.3
?			0.5
?			1.2
?			0.1
20 : 1			4.9
20 : 2			0.4
?			0.3
20 : 4 ω 6			1.5
22 : 1			1.7
20 : 5 ω 3			20.0
24 : 1 (or 21 : 5)			0.9
22 : 5 ω 3			0.6
?			1.8
22 : 6 ω 3			7.6

[1]) Per cent of the methyl esters of the individual fatty acids in the methyl esters of the total fatty acids from each fat.

[2]) Indicated by number of carbon atoms and double bonds.

[3]) Two batches of palmkernel oil were used in the experiments.
"a" in G 148, G 155, G 156 and G 157.
"b" in G 158, G 159, G 161 and G 163.

The fatty acid patterns of palmkernel oil and distilled ethyl esters of fatty acids of menhaden body oil were determined as described in our paper (7) and are shown in table 1.

Measurements of the absorption in ultraviolet at 282 nm of the preparations of 7-dehydrocholesterol and ergosterol in absolute ethanol solution and comparison with the data for the absorption in references 20 and 9 showed these preparations to be approximately 95% pure.

The methods for determination of cholesterol were:

For liver cholesterol: the method described in our paper (7) utilizing the *Liebermann-Burchard* reaction in the form indicated in our paper (8).

For serum cholesterol: the method described by *Watson* (10), slightly modified, in which the color reaction is carried out on the serum without prior extraction, and the reagent mixture contains 2,5-dimethylbenzene-sulfonic acid. The blood samples, 2 ml, were taken by heart puncture under ether anesthesia in a syringe rinsed with 0.9% NaCl, left at room temperature for ca. 1 hour and centrifuged.

Table 2. Composition of the stock diet "Mouse pills 66" used in the breeding colony from which the hamsters were obtained

15% ground wheat
18% ground oats
8% ground barley
10% ground yellow corn
23% wheat bran
3% alfalfa meal
1.5% dried yeast
5% skim milk powder
2.5% meat-and-bone meal
8% fish meal
3% crushed sunflower seed
1% vitamin mixture[1])
2% mineral mixture[2])
100.0%

[1]) One g of the vitamin mixture contains:
1100 i. u. vitamin A
330 i. u. vitamin D_3
0.612 mg thiamine
0.816 mg riboflavin
2.625 mg pantothenic acid
0.408 mg pyridoxine hydrochloride
0.00255 mg cyanocobalamin
0.0153 mg biotin
0.153 mg folic acid
40.000 mg choline
8.000 mg alpha-tocopherol acetate
mixed with a blend of 98.75% finely ground wheat bran and 1.25% ethoxyquine to make a total of 1 g.

[2]) The mineral mixture consists of
48.425% calcium carbonate
35.000% sodium chloride
1.250% ferrous sulfate, dried (30% Fe)
0.150% cupric oxide (80% Cu)
0.150% manganese oxide (72% Mn)
0.025% potassium iodide (76.5% I)
15.000% magnesium oxide

If the absorbancies at 625 nm obtained with the color reagents and cholesterol are set at 100, then the absorbancies obtained with the other sterols will be:

For the method used with liver:		For the method used with serum:	
cholestanol	0	cholestanol	0
lanosterol	11	lanosterol	26
soybean sterols	66	soybean sterols	66
7-dehydrocholesterol	165	7-dehydrocholesterol	150
ergosterol	117	ergosterol	107

Thus, if the amount of sterol in a sample is calculated using the absorbancy valid for cholesterol, then the amount of cholesterol will be correctly determined if the cholesterol is accompanied by cholestanol, but the total amount of sterols will be underestimated. If the cholesterol in the sample is accompanied by lanosterol or soybean sterols, the total amount of sterols will also be underestimated. When the cholesterol in the sample is accompanied by 7-dehydrocholesterol or ergosterol, the total amount of sterol will be overestimated.

The hamsters

For the time being we have no breeding colony for hamsters in our laboratory, but are obtaining hamsters from the breeding center of the State Serum Institute.

According to information from the Serum Institute, the diet used in their breeding colony for hamsters is "Mouse Pills 66" of the composition indicated in table 2. As drinking liquid is used a mixture of 1 part of whole milk, heated in closed containers at 90° C for 15 minutes, and 1 part of tap water. The total content of fat in the pills 3.95%, and the fatty acid pattern of the fat, shown in table 3, were determined in our laboratory by methods previously used.

During the first 6 to 7 days after arrival of the hamsters in our laboratory they were given the commercial stock diet "Rostock Mixture" described in our paper (11) and tap water. Thereafter they received the experimental diets (and tap water) for a period of 42 to 44 days.

During the experiments the hamsters were housed and treated as described previously (1).

Table 3. Fatty acid pattern of the fat in the stock diet "Mouse pills 66" specified in table 2

Fatty acid[1])	As percent of total fatty acids	As percent of diet[2])
12 : 0	0.2	0.01
14 : 0	1.8	0.07
16 : 0	16.0	0.61
16 : 1 ω 7	2.3	0.09
18 : 0	3.2	0.12
18 : 1 ω 9	22.1	0.84
18 : 2 ω 6	36.6	1.39
18 : 3 ω 3	4.6	0.17
20 : 1	3.5	0.13
22 : 1	3.8	0.14
20 : 5 ω 3	2.5	0.09
22 : 6 ω 3	3.4	0.13

[1]) Indicated by number of carbon atoms and double bonds, and distance from the terminal methyl-group to nearest double bond.

[2]) Assuming total fatty acids equal to 95% of total fat.

The experimental diets

The fat-free basal diet used in the present study was the diet containing 20.0 % casein, 74.3 % glucose, 5.0 % salt mixture, 0.5 % vitamin mixture and 0.2 % choline chloride as specified in our paper (12).

In order to facilitate intestinal absorption of cholesterol and other sterols, most of the diets used contained a certain amount of fat which was incorporated into the basal diet at the expense of glucose.

Sterols, squalene etc. were added to the diets containing the fat.

For protection of easily oxidizable supplements such as squalene, 7-dehydrocholesterol, ergosterol, fish oil fatty acid ethyl esters etc. 0.5 % of the non-absorbable antioxidant Ionox 330, in the following referred to as Ionox, was added to the diets in the experimental series in which these components occurred. This substance was also added to the diets in several of the other experimental series.

Plan of the different experimental series

The first three experimental series G 148, G 153, G 155 served to examine: 1. the effect of incorporating fat into the diets (10 % palmkernel oil, G 148; 8 % butter fat, G 153; 7 % palmkernel oil, G 155); 2. the influence of adding excessive amount (3 %) of cholesterol and squalene (G 148); 3. the influence of adding graded lower amounts of cholesterol (G 153, G 155); 4. the influence of adding Ionox, 0.05 % to the diets (G 153).

Since fairly high incidences of cholesterol gallstones were observed with the diet containing 7 % palmkernel oil, and Ionox had little influence on formation of gallstones, the following experimental series were carried out with diets containing 7 % palmkernel oil and 0.05 % Ionox.

In Exp. Series G 156 the effects of commercial soybean sterols are compared with the effects of cholesterol, in both cases at the dietary levels of 0.5 % and 1 %.

Exp. Series G 157 shows the effects of distilled menhaden oil fatty acid ethyl esters, cholesterol, commercial lanosterol, 7-dehydrocholesterol and squalene all at the 1 % dietary level.

Exp. Series G 158 shows the effects of squalene and distilled menhaden oil fatty acid ethyl esters, both at the 0.5 % and 1 % dietary level compared with the effect of substituting rice starch for glucose in the basal diet.

Exp. Series G 159 and G 160 show the effects of cholesterol and of squalene at the 1 % dietary level using a larger number of hamsters than in the foregoing series.

Exp. Series G 161 shows the effects of cholestanol at the levels of 0.125 % and 0.5 % compared with the effects of cholesterol at the same dietary levels and with the effects of ergosterol at the 0.5 % dietary level.

Exp. Series G 163 shows the effects of cholestanol at the dietary levels of 0.5 % and 1 %.

Results and discussion

The results are shown in tables 4 to 13 and 4 A to 13 A[1]).

1. Influence of the diets on production of gallstones

From Exp. Series G 148, G 153 and G 155 (tables 4, 5 and 6) it is seen that incorporation of the fats has reduced the incidence of cholesterol con-

[1]) Abbreviations used in the tables: m = males, f = females, C = cholesterol gallstones, M = mixed gallstones (i.e. gallstones containing both cholesterol and amorphous material), A = amorphous gallstones, 0 = no gallstones, hg = hectogram (100 g), dl = deciliter (100 ml).

taining gallstones, i.e. cholesterol gallstones (C-gallstones) and "mixed gallstones" (M-gallstones). The reduction – significant with 95 % probability[2]) for the males in G 148 and G 153 – was more marked among the male than among the female hamsters. The effect of incorporating the fats is partly due to the fats as such [it has previously been shown that fats rich in polyunsaturated fatty acids are particularly effective in preventing formation of cholesterol containing gallstones (3, 5)], and partly due to the concurrent moderate reduction of the amount of glucose in the diets.

Both cholesterol (G 148, G 153 and G 155) and squalene (G 148) markedly inhibited formation of cholesterol containing gallstones, and the inhibition was more marked in the males than in the females. As far as squalene is concerned, the difference in degree of inhibition in the two sexes seen in G 148 is not significant, but it is confirmed by the results from G 158, G 159 and G 160 (tables 9, 10 and 11).

Cholesterol, but not squalene, markedly stimulated production of amorphous pigmented gallstones in the female hamsters when added at the level of 0.5 % or more. This effect of cholesterol was also seen in our first study on the influence of dietary cholesterol (at the level of 1 %) on gallstone production (1) which was carried out with hamsters from our own breeding colony. But the more efficient inhibition of production of cholesterol containing gallstones in the males than in the females now observed was not seen in the earlier experiment (1). The reason for this must be the different origin and feeding of the hamsters before start of the experiments in the two studies.

If addition of 0.05 % Ionox to the diets has had any influence on gallstone production, this effect may have been a slight stimulation of the production of cholesterol containig gallstones (G 153).

Exp. Series G 156 (table 7) shows that commercial soybean sterols were less efficient than cholesterol as inhibitors of production of cholesterol containing gallstones and as stimulators of production of amorphous gallstones, both at the 0.5 % and the 1 % dietary level.

From Exp. Series G 157 (table 8) it is seen that at the 1 % dietary level, cholesterol, squalene and distilled menhaden oil fatty acid ethyl esters were almost equally (and almost fully) effective in protecting against formation of cholesterol containing gallstones, but only cholesterol markedly stimulated production of amorphous gallstones. 7-dehydrocholesterol was less efficient than cholesterol as inhibitor of production of cholesterol containing gallstones, and commercial lanosterol, apparently, intensified production of cholesterol containing gallstones. Taking males and females as one group, the difference in incidence of cholesterol containing gallstones obtained with 7-dehydrocholesterol and commercial lanosterol is significant with 95 % significance. Neither 7-dehydrocholesterol nor commercial lanosterol stimulated production of amorphous gallstones. Whether the effects of the lanosterol preparation are due to lanosterol itself or to impurities in the preparation can only be decided when a sufficient amount of the pure sterol has become available.

[2]) Assessed as indicated in reference (13).

Table 4. Exp. Series G 148. Summary of occurrence and non-occurrence of gallstones

Group no.	Diet characteristics	Number of animals in group	Animals having C gallstones only		Animals having M gallstones only		Animals having C and M gallstones		Animals having C and A gallstones		Animals having A gallstones only		Animals having no gallstones	
			no.	%	no.	%	no.	%	no.	%	no.	%	no.	%
1362	Fat-free,	11 m	7	63,7	0	0	0	0	0	0	0	0	4	36.3
	0.05% Ionox	12 f	8	66.7	1	8.3	2	16.7	0	0	0	0	1	8.3
1363	10% palmkernel oil,	11 m	2	18.2	0	0	0	0	0	0	0	0	9	81.8
	0.05% Ionox	12 f	8	66.7	0	0	0	0	0	0	0	0	4	33.3
1364	10% palmkernel oil,	12 m	0	0	0	0	0	0	0	0	0	0	12	100
	0.05% Ionox, 3% cholesterol	11 f	1	9.1	0	0	0	0	1	9.1	7	63.6	2	18.2
1365	10% palmkernel oil,	12 m	0	0	0	0	0	0	0	0	1	8.3	11	91.7
	0.05% Ionox, 3% squalene	11 f	1	9.1	0	0	0	0	0	0	1	9.1	9	81.8

Age of the animals at start of the experiment 32–42 days.
Duration of the feeding period 42–43 days.

Table 4.A Exp. Series G 148. Mean values of body weight, liver weight, and cholesterol in liver, with st. d.[1])

Group no.	Diet characteristics	No. of animals in group	Body weight at start	Body weight after 6 weeks	Weight gain in 6 weeks	Weight of liver	Cholesterol in liver: total in whole liver	Cholesterol in liver: total	Cholesterol in liver: ester	Cholesterol in liver: free	Cholesterol in liver: ester % of total
			g	g	g	g	mg	mg/hg	mg/hg	mg/hg	
1362	Fat-free 0.05% Ionox	11 m	53.3 ±2.2	77.5 ±2.6	24.2 ±3.4	3.50 ±0.12					
		12 f	50.8 ±1.9	75.8 ±2.7	25.0 ±3.3	3.25 ±0.22					
1363	10% palmkernel oil, 0.05% Ionox	11 m	53.3 ±2.0	84.1 ±2.7	30.8 ±3.4	4.42[10] ±0.26	10.0[10] ±0.5	229.8[10] ±6.4	41.3[10] ±1.9	188.6[10] ±6.1	18.0[10] ±0.6
		12 f	50.8 ±1.9	85.4 ±2.8	34.7 ±3.4	4.40[11] ±0.21	11.1[11] ±0.5	253.0[11] ±6.3	57.4[11] ±3.4	195.6[11] ±5.3	22.5[11] ±0.7
1364	10% palmkernel oil, 0.05% Ionox, 3% cholesterol	12 m	53.3 ±2.3	84.0 ±2.6	30.8 ±3.5	5.05 ±0.28	141.1	2793.8 ±138.5	2527.5 ±138.3	273.1 ±9.9	90.35 ±0.46
		11 f	50.7 ±1.8	80.2 ±2.8	29.5 ±3.3	5.13 ±0.29	227.2	4427.9 ±137.5	4124.0 ±136.9	303.9 ±12.9	93.60 ±0.4
1365	10% palmkernel oil, 0.05% Ionox, 3% squalene	12 m	53.3	77.9	24.6	3.85[11] ±0.15	12.4[11] ±1.3	317.4[11] ±24.0	92.1[11] ±24.0	225.4[11] ±5.8	25.4[11] ±4.6
		11 f	50.7 ±2.9	80.8 ±2.9	30.0 ±4.1	4.49 ±0.3	11.8 ±0.7	263.6 ±6.6	55.7 ±4.4	207.9 ±4.9	21.0 ±1.3

[1]) In cases where liver weight and cholesterol in liver were not determined in all the animals in a group, the number of animals on which the mean values of these data are based is indicated by superscripts.

Table 5. Exp. Series G 153. Summary of occurrence and non-occurrence of gallstones

Group no.	Diet characteristics	Number of animals in group	Animals having C gallstones only		Animals having C and M gallstones		Animals having C and A gallstones		Animals having C and M and A gallstones		Animals having A gallstones only		Animals having no gallstones	
			no.	%	no.	%	no.	%	no.	%	no.	%	no.	%
1379	Fat-free	10 m	7	70	0	0	0	0	0	0	0	0	3	30
		10 f	7	70	1	10	0	0	0	0	1	10	1	10
1380	Fat-free, 0.05% Ionox	10 m	10	100	0	0	0	0	0	0	0	0	0	0
		10 f	8	80	0	0	0	0	0	0	0	0	2	20
1381	8% butter fat	10 m	0	0	0	0	0	0	0	0	3	30	7	70
		10 f	5	50	0	0	0	0	0	0	0	0	5	50
1382	8% butter fat, 0.05% Ionox	10 m	1	10	0	0	2	20	0	0	0	0	7	70
		10 f	8	80	0	0	1	10	0	0	0	0	1	10
1383	8% butter fat, 1% cholesterol	10 m	0	0	0	0	0	0	0	0	0	0	10	100
		10 f	0	0	1	10	1	10	0	0	5	50	3	30
1384	8% butter fat, 1% cholesterol, 0.05% Ionox	10 m	0	0	0	0	0	0	0	0	0	0	10	100
		10 f	0	0	0	0	2	20	1	10	5	50	2	20

Age of the animals at start of the experiment 36–45 days.
Duration of the feeding period 42–44 days.

Tab. 5A. Exp. Series G 153. Mean values of body weight, liver weight, and cholesterol in liver and serum, with st. d.[1])

Group no.	Diet characteristics	No. of animals in group	Body weight at start	Body weight after 6 weeks	Weight gain in 6 weeks	Weight of liver	Cholesterol in liver: total in whole liver	Cholesterol in liver: total	Cholesterol in liver: ester	Cholesterol in liver: free	Cholesterol in liver: ester % of total	Serum cholesterol
			g	g	g	g	mg	mg/hg	mg/hg	mg/hg		mg/dl
1379	Fat-free	10 m	59.1 ±2.5	80.5 ±3.1	21.4 ±3.9	3.95 ±0.22						145.1 ±6.1
		10 f	58.5 ±2.0	80.3 ±2.2	21.8 ±2.9	4.36 ±0.25						181.7 ±11.4
1380	Fat-free, 0.05% Ionox	10 m	58.9 ±2.8	79.4 ±1.6	20.5 ±3.2	3.87[7] ±0.17	9.0[7] ±0.6	233.7[7] ±10.0	43.7[7] ±5.2	190.0[7] ±8.9	18.4[7] ±1.5	144.9 ±5.6
		10 f	58.3 ±2.6	80.8 ±2.5	22.5 ±3.6	3.84 ±0.21	10.1 ±0.6	267.4 ±12.6	58.3 ±4.9	209.1 ±11.6	21.8 ±1.4	189.3 ±7.1
1381	8% butter fat	10 m	59.0 ±2.4	83.4 ±2.2	24.4 ±3.2	3.82 ±0.14						179.6 ±9.3
		10 f	58.4 ±2.4	80.8 ±3.4	22.4 ±4.1	4.14 ±0.23						206.8 ±8.1
1382	8% butter fat, 0.05% Ionox	10 m	59.0 ±3.1	88.5 ±2.4	29.5 ±4.0	4.59 ±0.20	9.5 ±0.4	208.4 ±5.8	27.0 ±1.8	181.4 ±5.5	13.0 ±0.9	189.4 ±4.7
		10 f	58.6 ±2.6	89.2 ±3.9	30.6 ±4.7	4.08 ±0.20	10.8 ±0.5	265.7 ±8.8	49.2 ±3.8	216.6 ±8.0	18.5 ±1.0	220.0 ±7.9
1383	8% butter fat, 1% cholesterol	10 m	59.1 ±2.6	89.6 ±3.1	30.5 ±4.4	5.67 ±0.20						306.0 ±11.9
		10 f	58.5 ±2.4	88.0 ±2.2	29.5 ±3.3	5.25 ±0.22						
1384	8% butter fat 0.05% Ionox 1% cholesterol	10 m	59.0 ±2.4	86.0 ±2.2	27.0 ±3.3	5.33 ±0.20	160.4 ±14.4	2990.6 ±183.0	2744.9 ±182.8	245.9 ±9.5	91.6 ±0.3	292.6 ±7.3
		10 f	58.4 ±2.2	88.6 ±3.2	30.2 ±3.8	5.53 ±0.26	300.1 ±34.8	5327.6 ±500.8	5030.6 ±500.6	296.9 ±16.1	94.0 ±0.6	360.0 ±13.4

[1]) In cases where liver weight and cholesterol in liver were not determined in all the animals in a group, the number of animals on which the mean values of these data are based is indicated by superscripts.

Table 6. Exp. Series G 155. Summary of occurrence and non-occurrence of gallstones

Group no.	Diet characteristics	Number of animals in group	Animals having C gallstones only		Animals having C and A gallstones		Animals having C and M and A gallstones		Animals having A gallstones only		Animals having no gallstones	
			no.	%	no.	%	no.	%	no.	%	no.	%
1389	Fat-free	10 m	9	90	0	0	0	0	0	0	1	10
		10 f	8	80	0	0	0	0	0	0	2	20
1390	7% palmkernel oil	10 m	5	50	0	0	0	0	0	0	5	50
		10 f	8	80	0	0	0	0	0	0	2	20
1391	7% palmkernel oil,	10 m	0	0	0	0	0	0	0	0	10	100
	0.125% cholesterol	10 f	7	70	0	0	0	0	0	0	3	30
1392	7% palmkernel oil,	10 m	0	0	0	0	0	0	0	0	10	100
	0.25% cholesterol	10 f	3	30	1	10	0	0	0	0	6	60
1393	7% palmkernel oil,	10 m	0	0	0	0	0	0	0	0	10	100
	0.50% cholesterol	10 f	0	0	0	0	1	10	5	50	4	40

Age of the animals at start of the experiment 33–41 days.
Duration of the feeding period 42–44 days.

Table 6A. Exp. Series G 155. Mean values of body weight, liver weight, and cholesterol in liver and serum, with st. d.[1])

Group no.	Diet characteristics	No. of animals in group	Body weight at start	Body weight after 6 weeks	Weight gain in 6 weeks	Weight of liver	Cholesterol in liver total in whole liver	Cholesterol in liver total	Cholesterol in liver ester	Cholesterol in liver free	Cholesterol in liver ester % of total	Serum cholesterol
			g	g	g	g	mg	mg/hg	mg/hg	mg/hg		mg/dl
1389	Fat-free	10 m	48.3	69.8	21.5	3.29[9]	8.5[9]	256.2[9]	45.0[9]	211.3[9]	17.3[9]	125.3
			±0.6	±1.3	±1.4	±0.11	±0.4	±5.7	±4.7	±2.2	±1.5	±5.7
		10 f	44.6	68.4	23.8	3.44[8]	9.2[8]	270.5[8]	58.9[8]	211.6[8]	21.7[8]	171.6
			±1.2	±2.9	±3.3	±0.17	±0.4	±8.2	±2.9	±5.9	±0.6	±8.1
1390	7% palmkernel oil	10 m	48.1	82.0	33.9	3.91	10.5	271.5	46.5	225.0	17.0	229.7
			±1.7	±3.1	±3.5	±0.17	±0.9	±20.5	±4.8	±19.9	±0.9	±8.2
		10 f	44.6	82.5	37.9	4.00	11.3	289.1	49.7	239.4	17.5	234.4
			±0.9	±2.2	±2.5	±0.30	±1.0	±26.7	±4.0	±26.6	±0.9	±13.6
1391	7% palmkernel oil, 0.125% cholesterol	10 m	48.2	82.3	34.1	4.17[9]	11.8[9]	282.6[9]	83.2[9]	199.4[9]	27.0[9]	232.8
			±1.8	±3.2	±3.7	±0.27	±1.2	±19.9	±19.6	±3.8	±3.9	±4.5
		10 f	44.5	76.2	31.7	3.42	9.6	280.8	54.5	225.3	19.3	258.7
			±1.0	±2.8	±3.0	±0.23	±0.7	±7.0	±3.7	±6.0	±1.1	±9.9
1392	7% palmkernel oil, 0.25% cholesterol	10 m	48.0	76.4	28.4	4.17	26.2	613.2	398.9	214.4	61.4	272.9
			±1.6	±2.8	±3.2	±0.17	±3.2	±60.8	±60.5	±5.4	±4.5	±9.5
		10 f	44.6	74.9	30.3	3.44[9]	10.5[9]	310.1[9]	68.6[9]	241.6[9]	22.1[9]	240.5
			±1.7	±2.9	±3.4	±0.33	±0.8	±7.6	±3.8	±6.6	±1.0	±13.9
1393	7% palmkernel oil, 0.5% cholesterol	10 m	48.3	81.5	33.2	4.35	91.1	2030.8	1790.2	240.6	91.1	274.2
			±1.5	±2.0	±2.5	±0.21	±12.5	±229.6	±229.4	±8.4	±12.5	±11.8
		10 f	44.6	71.1	26.5	3.82	87.5	2222.4	1971.9	250.5	87.5	321.4
			±1.9	±2.8	±3.4	±0.23	±13.9	±262.8	±262.4	±14.7	±13.9	±14.7

[1]) In cases where liver weight and cholesterol in liver were not determined in all the animals in a group, the number of animals on which the mean values of these data are based is indicated by superscripts.

Table 7. Exp. Series G 156. Summary of occurrence and non-occurrence of gallstones

Group no.	Diet characteristics	Number of animals in group	Animals having C gallstones only		Animals having M gallstones only		Animals having C and A gallstones		Animals having A gallstones only		Animals having no gallstones	
			no.	%	no.	%	no.	%	no.	%	no.	%
1394	7% palmkernel oil, 0.05% Ionox	10 m	4	40	0	0	0	0	0	0	6	60
		10 f	4	40	0	0	2	20	0	0	4	40
1395	7% palmkernel oil, 0.05% Ionox, 0.5% cholesterol	10 m	0	0	0	0	0	0	3	30	7	70
		10 f	0	0	0	0	0	0	9	90	1	10
1396	7% palmkernel oil, 0.05% Ionox, 1.0% cholesterol	10 m	0	0	0	0	0	0	3	30	7	70
		10 f	0	0	0	0	0	0	8	80	2	20
1397	7% palmkernel oil, 0.05% Ionox, 0.5% soybean sterols	10 m	2	20	0	0	0	0	0	0	8	80
		9 f	1	11.1	1	11.1	0	0	3	33.3	4	44.4
1398	7% palmkernel oil, 0.05% Ionox, 1.0% soybean sterols	10 m	0	0	0	0	0	0	0	0	10	100
		10 f	2	20	0	0	1	10	2	20	5	50

Age of the animals at start of the experiment 38–48 days.
Duration of the feeding period 42–44 days.

Table 7A. Exp. Series G 156. Mean values of body weight, liver weight and serum cholesterol, with st. d.

Group no.	Diet characteristics	No. of animals in group	Body weight at start g	Body weight after 6 weeks g	Weight gain in 6 weeks g	Weight of liver g	Serum cholesterol mg/dl
1394	7% palmkernel oil, 0.05% Ionox	10 m 10 f	65.2 ± 2.2 64.6 ± 2.9	87.6 ± 3.3 91.0 ± 2.7	22.4 ± 4.0 26.4 ± 3.9	3.95 ± 0.18 4.29 ± 0.30	202.6 ± 9.8 240.4 ± 6.9
1395	7% palmkernel oil, 0.05% Ionox, 0.5% cholesterol	10 m 10 f	65.2 ± 2.9 64.6 ± 2.5	87.3 ± 3.9 86.1 ± 3.4	22.1 ± 4.8 21.5 ± 4.2	4.47 ± 0.33 4.98 ± 0.17	262.7 ± 9.2 331.6 ± 17.7
1396	7% palmkernel oil, 0.05% Ionox, 1% cholesterol	10 m 10 f	65.2 ± 3.0 64.6 ± 2.0	89.4 ± 3.7 89.0 ± 1.9	24.2 ± 4.7 24.4 ± 2.7	5.24 ± 0.35 5.57 ± 0.26	325.2 ± 11.8 352.2 ± 19.4
1397	7% palmkernel oil, 0.05% Ionox, 0.5% soybean sterols	10 m 9 f	65.1 ± 2.3 64.9 ± 2.5	87.3 ± 3.6 88.4 ± 4.0	22.2 ± 4.2 23.6 ± 4.8	3.92 ± 0.23 4.04 ± 0.42	210.7 ± 12.4 207.3 ± 9.5
1398	7% palmkernel oil, 0.05% Ionox, 1 % soybean sterols	10 m 10 f	65.2 ± 2.9 65.6 ± 3.6	86.8 ± 3.5 91.6 ± 3.2	21.6 ± 4.5 26.0 ± 4.8	3.78 ± 0.21 4.09 ± 0.26	217.3 ± 6.4 208.8 ± 8.5

Table 8. Exp. Series G 157. Summary of occurrence and non-occurrence of gallstones

Group no.	Diet characteristics	Number of animals in group	Animals having C gallstones only		Animals having C and M gallstones		Animals having C and A gallstones		Animals having A gallstones only		Animals having no gallstones	
			no.	%	no.	%	no.	%	no.	%	no.	%
1399	7% palmkernel oil, 0.05% Ionox	10 m	5	50	0	0	0	0	1	10	4	40
		10 f	5	50	3	30	1	10	0	0	1	10
1400	7% palmkernel oil, 0.05% Ionox, 1% menhaden oil	10 m	0	0	0	0	0	0	2	20	8	80
		10 f	0	0	0	0	0	0	0	0	10	100
1401	7% palmkernel oil, 0.05% Ionox, 1% cholesterol	9 m	0	0	0	0	0	0	1	11.1	8	88.9
		10 f	0	0	0	0	1	10	7	70	2	20
1402	7% palmkernel oil, 0.05% Ionox, 1% commercial lanosterol	10 m	8	80	0	0	0	0	0	0	2	20
		10 f	9	90	0	0	0	0	0	0	1	10
1403	7% palmkernel oil, 0.05% Ionox, 1% 7-dehydrocholesterol	10 m	1	10	0	0	0	0	0	0	9	90
		10 f	3	30	0	0	0	0	0	0	7	70
1404	7% palmkernel oil, 0.05% Ionox, 1% squalene	10 m	0	0	0	0	0	0	0	0	10	100
		10 f	0	0	0	0	0	0	1	10	9	90

Age of the animals at start of the experiment 37–48 days.
Duration of the feeding period 42–44 days.

Table 8A. Exp. Series G 157. Mean values of body weight, liver weight, and cholesterol in liver and serum, with st. d.[1])

Group no.	Diet characteristics	No. of animals in group	Body weight at start	Body weight after 6 weeks	Weight gain in 6 weeks	Weight of liver	Cholesterol in liver: total in whole liver	Cholesterol in liver: total	Cholesterol in liver: ester	Cholesterol in liver: free	Cholesterol in liver: ester % of total	Serum cholesterol
			g	g	g	g	mg	mg/hg	mg/hg	mg/hg		mg/dl
1399	7% palmkernel oil, 0.05% Ionox	10 m	61.5 ±4.0	84.3 ±2.5	22.8 ±4.7	3.58 ±0.16	9.4 ±0.5	262.6 ±9.3	39.3 ±3.0	223.3 ±9.0	14.9 ±0.8	226.0 ±8.8
		10 f	59.3 ±1.4	83.5 ±1.7	24.2 ±2.2	4.00 ±0.14	10.3 ±0.3	260.6 ±8.4	41.8 ±2.4	218.7 ±8.1	16.1 ±0.7	242.6 ±5.3
1400	7% palmkernel oil, 0.05% Ionox, 1% Mh. f. a. ethyl esters[2])	10 m	61.5 ±2.8	87.1 ±2.9	25.6 ±4.0	3.79 ±0.13	8.4 ±0.3	220.6 ±6.8	30.5 ±2.5	190.2 ±6.3	13.7 ±1.0	159.7 ±5.5
		10 f	58.8 ±1.9	90.5 ±3.3	31.7 ±3.8	4.20 ±0.22	8.9 ±0.5	213.8 ±10.4	28.8 ±2.9	184.9 ±10.0	13.0 ±1.3	159.1 ±8.3
1401	7% palmkernel oil, 0.05% Ionox, 1% cholesterol	9 m	62.2 ±3.0	90.0 ±3.7	27.8 ±4.8	5.00 ±0.25	132.5 ±16.3	2605.8 ±231.1	2343.6 ±233.1	262.1 ±9.3	90.4 ±1.3	304.7 ±12.4
		10 f	59.3 ±1.5	82.3 ±2.5	23.0 ±2.9	5.23[9] ±0.24	207.7[9] ±14.3	3960.9[9] ±185.9	3683.0[9] ±181.2	277.9[9] ±26.4	92.9[9] ±0.7	367.5 ±10.9
1402	7% palmkernel oil, 0.05% Ionox, 1% commercial lanosterol	10 m	61.5 ±3.0	87.3 ±1.9	22.2 ±3.6	3.90[9] ±0.09	9.6[9] ±0.5	244.9[9] ±8.3	46.2[9] ±4.0	198.7[9] ±8.8	19.0[9] ±1.8	207.2 ±8.2
		10 f	59.5 ±2.3	83.9 ±2.8	24.4 ±3.6	4.57 ±0.25	10.8 ±0.7	229.9 ±8.4	40.9 ±3.6	189.0 ±8.6	17.9 ±1.7	255.2 ±6.2
1403	7% palmkernel oil, 0.05% Ionox, 1% 7-dehydrocholesterol	10 m	61.6 ±1.4	83.0 ±2.6	21.4 ±3.0	3.61 ±0.17	12.0 ±1.5	334.5 ±37.2	70.9 ±8.8	263.7 ±32.2	21.5 ±2.0	203.7 ±9.2
		10 f	59.3 ±1.9	79.0 ±2.2	19.7 ±2.9	3.98 ±0.20	12.9 ±1.7	327.5 ±42.1	60.6 ±8.5	267.0 ±35.1	18.4 ±1.4	210.6 ±4.6
1404	7% palmkernel oil, 0.05% Ionox, 1% squalene	10 m	61.6 ±2.7	87.6 ±1.9	26.0 ±3.3	4.11[8] ±0.17	11.8[8] ±1.0	285.8[8] ±16.4	68.6[8] ±15.2	217.2[8] ±6.0	22.6[8] ±3.1	216.5 ±8.1
		10 f	59.3 ±1.5	84.2 ±4.1	24.9 ±4.3	4.34[9] ±0.25	10.5[9] ±0.6	242.7[9] ±4.1	39.6[9] ±3.3	203.1[9] ±5.2	16.4[9] ±1.4	251.8 ±8.5

[1]) In cases where liver weight and cholesterol in liver were not determined in all the animals in a group, the number of animals on which the mean values of these data are based is indicated by superscripts.

[2]) Mh. f. a. ethyl esters = Distilled Menhaden oil fatty acid ethyl esters.

Table 9. Exp. Series G 158. Summary of occurrence and non-occurrence of gallstones

Group no.	Diet characteristics	Number of animals in group	Animals having C gallstones only		Animals having C and M gallstones		Animals having C and A gallstones		Animals having A gallstones only		Animals having no gallstones	
			no.	%	no.	%	no.	%	no.	%	no.	%
1405	7% palmkernel oil, 0.05% Ionox	10 m	3	30	0	0	0	0	0	0	7	70
		9 f	6	66.7	0	0	0	0	0	0	3	33.3
1406	7% palmkernel oil, 0.05% Ionox, 1% menhaden oil	10 m	0	0	0	0	0	0	0	0	10	100
		10 f	0	0	0	0	0	0	0	0	10	100
1407	7% palmkernel oil, 0.05% Ionox, 0.5% menhaden oil	10 m	0	0	0	0	0	0	0	0	10	100
		10 f	4	40	0	0	0	0	1	10	5	50
1408	7% palmkernel oil, 0.05% Ionox, 1% squalene	10 m	0	0	0	0	0	0	0	0	10	100
		10 f	0	0	0	0	0	0	0	0	10	100
1409	7% palmkernel oil, 0.05% Ionox, 0.5% squalene	10 m	0	0	0	0	0	0	0	0	10	100
		10 f	0	0	1	10	1	10	2	20	6	60
1410	7% palmkernel oil, 0.05% Ionox, rice starch instead of glucose	8 m	0	0	0	0	0	0	0	0	8	100
		10 f	0	0	0	0	0	0	1	10	9	90

Age of the animals at start of the experiment 36–46 days.
Duration of the feeding period 42–44 days.

Table 9A. Exp. Series G 158. Mean values of body weight, liver weight, and cholesterol in liver and serum, with st. d.[1])

Group no.	Diet characteristics	No. of animals in group	Body weight at start	Body weight after 6 weeks	Weight gain in 6 weeks	Weight of liver	Cholesterol in liver: total in whole liver	Cholesterol in liver: total	Cholesterol in liver: ester	Cholesterol in liver: free	Cholesterol in liver: ester % of total	Serum cholesterol
			g	g	g	g	mg	mg/hg	mg/hg	mg/hg		mg/dl
1405	7% palmkernel oil, 0.05% Ionox	10 m	62.8 ±2.1	84.4 ±2.8	21.6 ±3.5	4.01 ±0.25	8.4 ±0.6	215.9 ±7.0	30.3 ±1.5	185.7 ±6.8	14.0 ±0.6	209.6 ±9.2
		9 f	60.9 ±2.3	86.4 ±2.9	25.5 ±3.7	4.23 ±0.30	9.6 ±0.6	228.7 ±7.4	39.4 ±2.8	190.2 ±6.8	16.8 ±1.0	222.9 ±16.8
1406	7% palmkernel oil, 0.05% Ionox, 1% Mh. f. a. ethyl esters[2])	10 m	62.8 ±2.6	78.0 ±3.7	15.2 ±4.5	3.40 ±0.16	7.7 ±0.4	228.6 ±7.8	24.8 ±2.4	203.8 ±7.4	10.8 ±0.9	120.5 ±6.4
		10 f	60.8 ±1.4	90.7 ±3.5	29.9 ±3.8	4.65 ±0.27	9.6 ±0.6	207.6 ±7.8	26.9 ±1.8	181.7 ±7.6	12.8 ±0.5	148.3 ±14.6
1407	7% palmkernel oil, 0.05% Ionox, 0.5% Mh. f. a. ethyl esters[2])	10 m	62.9 ±2.6	94.3 ±3.4	31.4 ±4.3	4.12[9] ±0.27	9.3[9] ±0.5	227.7[9] ±6.2	30.3[9] ±2.2	197.4[9] ±5.8	13.2[9] ±0.7	186.9 ±10.3
		10 f	60.8 ±1.2	83.5 ±3.4	22.7 ±3.6	4.03 ±0.23	9.0 ±0.5	226.9 ±2.9	30.0 ±2.9	196.9 ±8.6	13.2 ±0.7	178.2 ±3.0
1408	7% palmkernel oil, 0.05% Ionox, 1% squalene	10 m	62.8 ±1.8	90.0 ±3.0	27.2 ±3.5	4.37 ±0.27	11.0 ±1.0	251.5 ±14.4	56.0 ±11.3	195.2 ±2.8	21.3 ±3.2	224.9 ±9.1
		10 f	60.8 ±2.7	88.1 ±3.5	27.3 ±3.4	4.35 ±0.27	10.5 ±0.6	245.4 ±10.3	49.6 ±4.8	195.1 ±9.1	20.2 ±1.8	225.3 ±11.7
1409	7% palmkernel oil, 0.05% Ionox, 0.5% squalene	10 m	62.8 ±2.1	83.5 ±2.7	20.7 ±3.4	3.97[9] ±0.17	10.6[9] ±1.1	263.6[9] ±15.9	59.8[9] ±13.0	203.8[9] ±9.2	21.2[9] ±3.2	204.0 ±12.3
		10 f	60.9 ±2.3	80.4 ±1.8	19.5 ±2.9	4.06[9] ±0.15	9.0[9] ±0.5	220.1[9] ±5.8	36.4[9] ±4.2	183.7[9] ±4.1	16.4[9] ±1.7	207.5 ±8.6
1410	7% palmkernel oil, 0.05% Ionox, rice starch instead of glucose	8 m	63.4 ±2.0	86.8 ±4.0	23.4 ±4.4	3.82[7] ±0.32	8.6[7] ±0.6	229.1[7] ±13.1	38.7[7] ±2.5	190.4[7] ±12.8	17.2[7] ±1.1	165.1 ±10.9
		10 f	60.9 ±2.5	77.6 ±3.1	16.7 ±4.0	3.32[9] ±0.30	7.5[9] ±0.4	234.0[9] ±11.6	35.6[9] ±4.2	198.4[9] ±10.5	15.1[9] ±3.8	163.8 ±8.4

[1]) In cases where liver weight and cholesterol in liver were not determined in all the animals in a group, the number of animals on which the mean values of these data are based is indicated by superscripts.

[2]) Mh. f. a. ethyl esters = Distilled Menhaden oil fatty acid ethyl esters.

Table 10. Exp. Series G 159. Summary of occurrence and non-occurrence of gallstones

Group no.	Diet characteristics	Number of animals in group	Animals having C gallstones only		Animals having A gallstones only		Animals having no gallstones	
			no.	%	no.	%	no.	%
1411	7% palmkernel oil, 0.05% Ionox	18 m	3	16.7	0	0	15	83.3
		20 f	12	60	0	0	8	40
1412	7% palmkernel oil, 0.05% Ionox 1.0% cholesterol	20 m	0	0	3	15	17	85
		20 f	0	0	7	35	13	65
1413	7% palmkernel oil, 0.05% Ionox, 1.0% squalene	20 m	0	0	0	0	20	100
		20 f	1	5	2	10	17	85

Age of the animals at start of the experiment 37–47 days.
Duration of the feeding period 42–44 days.

Table 10A. Exp. Series G 159. Mean values of body weight and liver weight with st. d.

Group no.	Diet characteristics	No. of animals in group	Body weight at start g	Body weight after 6 weeks g	Weight gain in 6 weeks g	Weight of liver g
1411	7% palmkernel oil, 0.05% Ionox	18 m	59.4 ±2.2	91.3 ±3.7	31.9 ±4.3	4.49 ±0.20
		20 f	59.5 ±1.3	89.4 ±2.3	29.9 ±2.6	4.55 ±0.20
1412	7% palmkernel oil, 0.05% Ionox, 1% cholesterol	20 m	60.0 ±1.7	87.6 ±3.1	27.6 ±3.5	4.63 ±0.22
		20 f	59.6 ±1.6	87.2 ±2.2	27.6 ±2.7	4.87 ±0.17
1413	7% palmkernel oil, 0.05% Ionox, 1% squalene	20 m	60.1 ±2.3	87.6 ±3.1	27.5 ±3.9	3.98 ±0.77
		20 f	59.5 ±1.3	91.7 ±2.2	32.2 ±2.5	4.55 ±0.17

Exp. Series G 158 (tables 9, 9A) shows that at the 1% dietary level, squalene and distilled menhaden oil fatty acid ethyl esters afforded full protection against formation of cholesterol containing gallstones, and that the same effect could be obtained by substituting rice starch for glucose in the diet. At the 0.5% dietary level, squalene and distilled menhaden oil faffy acid ethyl esters gave only full protection against formation of cholesterol containing gallstones in the males. In the females,

where the protection was only partial, squalene may have been slightly more efficient than distilled menhaden oil fatty acid ethyl esters.

Ergosterol at the 0.5 % dietary level (Exp. Series G 161, tables 12, 12 A), was less effective than cholesterol in inhibiting formation of cholesterol containing gallstones, and did not stimulate production of amorphous gallstones. Under the polarizing microscope, small needle-shaped birefringent crystals could be seen in the bladder bile from 5 of the 10 male hamsters in the ergosterol group listed as not having cholesterol containing gallstones. The nature of these crystals was not identified.

Cholestanol (Exp. Series G 161 and G 163, tables 12, 12 A and 13, 13 A) at dietary levels of 0.125, 0.5 and 1 % gave no protection against production of cholesterol containing gallstones, but rather seems to have intensified production of such gallstones. It must be noted, however, that the cholesterol containing gallstones occurring in the cholestanol fed group contained a certain amount of another sterol besides cholesterol. The sterols of these gallstones could be separated by thin layer chromatography on Silica gel (moving phase chloroform/diethylester/glacial acetic acid, 97:2.3:0.5 (v:v:v) and migrated as cholesterol and cholestanol resp.

2. Influence of the diets on serum cholesterol

The levels of serum cholesterol in the various groups of the experimental series (except G 148, G 159 and G 160) are recorded in the tables marked A. Some of the tables marked A also contain data for liver cholesterol.

In most cases there is a tendency for the mean value of serum cholesterol to be higher for the females than for the males in the same group, and in most cases where cholesterol containing gallstones occur the incidence of such gallstones is higher among the females than among the males. Otherwise the relationship between serum cholesterol and occurrence of cholesterol containing gallstones is very variable.

Incorporation of the fats (8 % butter fat or 7 % palmkernel oil) which decreased the incidence of cholesterol containig gallstones in the male hamsters caused increase of serum cholesterol in both sexes (table 5 A and 6 A).

Addition of cholesterol at the levels of 0.5 % or 1 % which markedly decreased the incidence of cholesterol containing gallstones in hamsters of both sexes caused very marked increase of serum cholesterol (tables 5 A, 6 A, 7 A, 8 A).

Addition of squalene 0.5 % or 1 % which gave approximately the same degree of protection against cholesterol containing gallstones as that which could be obtained with dietary cholesterol at the same levels did not markedly raise serum cholesterol (tables 8 A, 9 A).

With soybean sterols (table 7 A), commercial lanosterol (table 8 A) 7-dehydrocholesterol (table 8 A), and ergosterol (table 12 A), no attempt was made to determine individual sterols in the serum. Therefore, the figures for "serum cholesterol" in these groups are not strictly comparable with the figures for serum cholesterol in the other groups in the same series, and do not warrant definite conclusions.

With distilled menhaden oil fatty acid ethyl esters, 1 % (tables 8 A, 9 A), and 0.5 % (table 9 A), protection against production of cholesterol

containing gallstones was clearly accompanied by lowering of serum cholesterol, and the same was true in the group receiving the diet with rice starch instead of glucose (table 9 A).

It is not likely that the low level of serum cholesterol *per se* is the cause of the protection against production of cholesterol containig gallstones seen in these groups, since protection against this type of gallstones can also be obtained when the level of serum cholesterol is high such as is the case in the cholesterol fed groups.

Decreased biosynthesis of cholesterol in the liver is the most likely cause of the protection against cholesterol containing gallstones obtained by addition of distilled menhaden oil fatty acid ethyl esters, by replacement of dietary glucose by starch, or by addition of squalene or cholesterol to the diet.

This assumption is supported by the following observations:

In hamsters, decrease of biosynthesis of cholesterol has been found when starch is introduced into the diet instead of glucose [*in vivo* with whole animals (14)], *in vitro* with liver slices (15), and when a polyunsaturated fatty acid in the form of ethyl linoleate is added to the high glucose diet [*in vitro* with liver slices (15)]. The inhibition was found to occur before mevalonate (15). Dietary cholesterol which increases serum cholesterol, and dietary squalene which has little influence on serum cholesterol have been shown to inhibit biosynthesis of cholesterol in rats [*in vitro* with liver slices (16)]. The inhibition takes place at a stage before mevalonate [shown for dietary cholesterol (17)] viz. at the reduction of 3-hydroxy, 3-methyl-glutarate to mevalonate [shown for dietary cholesterol (18)]. Finally, in many mammals biliary cholesterol is assumed to depend largely on the hepatic synthesis of cholesterol (19).

The decrease of serum cholesterol coinciding with the protection against cholesterol containing gallstones seen in the groups receiving distilled menhaden oil fatty acid ethyl esters or the diet in which glucose is replaced by starch, apparently, is also a consequence of the inhibition of biosynthesis of cholesterol. Assuming that squalene inhibits the biosynthesis of cholesterol in hamsters at the stage before mevalonate without interfering with its own conversion to cholesterol it is understandable that serum cholesterol may remain largely unaltered or be slightly increased if a sufficiently large amount of squalene is taken up from the intestine.

Cholestanol 0.5% and 1% (tables 12A, 13A) which seems to have increased production of cholesterol containing gallstones, lowered the level of serum cholesterol. The mechanisms by which these effects of cholestanol are brought about remain to be examined.

3. Influence of the diets on the content of cholesterol in the liver

In the cases where the livers were assayed for cholesterol, substantial changes of the mean values of total cholesterol and of percentage of esterified cholesterol in total cholesterol were only found in the groups receiving cholesterol in the diet.

Incorporation of the fats, 8% butter fat or 7% palmkernel oil, both of which caused significant increases of serum cholesterol, did not produce significant increases of liver cholesterol (tables 5A, 6A).

Table 11. Exp. Series G 160. Summary of occurrence and non-occurrence of gallstones

Group no.	Diet characteristics	Number of animals in group	Animals having C gallstones only		Animals having C and M gallstones		Animals having A gallstones only		Animals having no gallstones	
			no.	%	no.	%	no.	%	no.	%
1414	7% palmkernel oil, 0.05% Ionox	20 m	8	40	0	0	0	0	12	60
		20 f	13	65	1	5	0	0	6	30
1415	7% palmkernel oil, 0.05% Ionox, 1% cholesterol	20 m	0	0	0	0	4	20	16	80
		20 f	0	0	0	0	11	55	9	45
1416	7% palmkernel oil, 0.05% Ionox, 1% squalene	20 m	0	0	0	0	0	0	20	100
		20 f	1	5	0	0	0	0	19	95

Age of the animals at start of the experiment 36–46 days.
Duration of the feeding period 42–44 days.

Table 11A. Exp. Series G 160. Mean values of body weight and liver weight with st. d.

Group no.	Diet characteristics	No. of animals in group	Body weight at start g	Body weight after 6 weeks g	Weight gain in 6 weeks g	Weight of liver g
1414	7% palmkernel oil, 0.05% Ionox	20 m	56.6 ± 1.6	86.9 ± 2.3	30.3 ± 2.8	4.30 ± 1.80
		20 f	59.1 ± 1.6	92.3 ± 2.2	33.2 ± 2.7	4.40 ± 0.20
1415	7% palmkernel oil, 0.05% Ionox, 1% cholesterol	20 m	56.6 ± 1.5	85.8 ± 2.3	29.2 ± 2.7	4.94 ± 0.80
		20 f	59.2 ± 1.4	93.0 ± 2.1	33.9 ± 2.5	5.61 ± 0.80
1416	7% palmkernel oil, 0.05% Ionox, 1% squalene	20 m	56.6 ± 1.6	88.1 ± 2.3	32.5 ± 2.8	4.20 ± 0.20
		20 f	59.2 ± 1.4	94.6 ± 2.3	32.5 ± 2.7	4.90 ± 0.20

Table 12. Exp. Series G 161. Summary of occurrence and non-occurrence of gallstones

Group no.	Diet characteristics	Number of animals in group	Animals having C gallstones only		Animals having C and M gallstones		Animals having C and A gallstones		Animals having M gallstones only		Animals having A gallstones only		Animals having no gallstones	
			no.	%	no.	%	no.	%	no.	%	no.	%	no.	%
1417	7% palmkernel oil, 0.05% Ionox	10 m	3	30	0	0	0	0	0	0	0	0	7	70
		10 f	7	70	0	0	0	0	0	0	1	10	2	20
1418	7% palmkernel oil, 0.05% Ionox, 0.125% cholestanol	10 m	9	90	0	0	0	0	0	0	0	0	1	10
		10 f	7	70	0	0	1	10	1	10	0	0	1	10
1419	7% palmkernel oil, 0.05% Ionox, 0.5% cholestanol	10 m	6	60	0	0	1	10	0	0	0	0	3	30
		10 f	8	80	1	10	0	0	0	0	0	0	1	10
1420	7% palmkernel oil, 0.05% Ionox, 0.125% cholesterol	10 m	1	10	0	0	0	0	0	0	0	0	9	90
		10 f	5	50	0	0	1	10	0	0	0	0	4	40
1421	7% palmkernel oil, 0.05% Ionox, 0.5% cholesterol	10 m	1	10	0	0	0	0	0	0	1	10	8	80
		10 f	0	0	0	0	0	0	0	0	8	80	2	20
1422	7% palmkernel oil, 0.05% Ionox, 0.5 % ergosterol	10 m	0	0	0	0	0	0	0	0	0	0	10[1])	100[1])
		10 f	5	50	0	0	0	0	0	0	1	10	4	40

Age of the animals at start of the experiment 36–46 days.

Duration of the feeding period 42–44 days.

[1]) 5 out of the 10 male hamsters in the ergosterol group listed as not having gallstones had some small double-refringent needle-shaped crystals in the bladder bile.

Table 12A. Exp. Series G 161. Mean values of body weight, liver weight, and serum cholesterol, with st. d.

Group no.	Diet characteristics	No. of animals in group	Body weight at start g	Body weight after 6 weeks g	Weight gain in 6 weeks g	Weight of liver g	Serum cholesterol mg/dl
1417	7% palmkernel oil, 0.05% Ionox	10 m 10 f	60.6 ± 3.5 62.5 ± 1.4	89.3 ± 4.3 97.5 ± 2.7	28.7 ± 5.6 35.0 ± 3.1	4.45 ± 0.29 4.79 ± 0.20	227.7 ± 6.2 248.2 ± 8.2
1418	7% palmkernel oil, 0.05% Ionox, 0.125% cholestanol	10 m 10 f	60.8 ± 4.0 62.4 ± 1.7	87.9 ± 4.0 93.2 ± 2.8	27.1 ± 5.7 30.8 ± 3.3	4.04 ± 0.25 4.64 ± 0.29	199.5 ± 9.1 261.8 ± 7.2
1419	7% palmkernel oil, 0.05% Ionox, 0.5% cholestanol	10 m 10 f	60.8 ± 2.0 62.4 ± 2.6	88.5 ± 3.8 91.7 ± 2.0	27.7 ± 4.3 29.3 ± 4.2	4.03 ± 0.25 4.35 ± 0.20	188.1 ± 4.4 219.2 ± 9.3
1420	7% palmkernel oil, 0.05% Ionox, 0.125% cholesterol	10 m 10 f	60.9 ± 1.8 62.1 ± 2.0	88.7 ± 3.8 94.6 ± 3.8	27.8 ± 3.5 32.0 ± 4.3	4.59 ± 0.24 5.06 ± 0.32	227.0 ± 4.6 273.3 ± 11.0
1421	7% palmkernel oil, 0.05% Ionox, 0.5% cholesterol	10 m 10 f	60.8 ± 1.7 62.5 ± 1.8	84.1 ± 2.0 94.5 ± 3.1	23.3 ± 2.6 32.0 ± 3.5	4.67 ± 0.21 5.42 ± 0.24	279.9 ± 9.5 319.6 ± 11.8
1422	7% palmkernel oil, 0.05% Ionox, 0.5% ergosterol	10 m 10 f	60.8 ± 5.4 62.3 ± 2.2	86.5 ± 3.0 91.4 ± 3.8	25.7 ± 6.2 29.1 ± 4.4	4.25 ± 0.14 4.85 ± 0.27	188.6 ± 6.6 222.4 ± 11.0

Table 13. Exp. Series G 163. Summary of occurrence and non-occurrence of gallstones

Group no.	Diet characteristics	Number of animals in group	Animals having C gallstones only		Animals having A gallstones only		Animals having no gallstones	
			no.	%	no.	%	no.	%
1429	7% palmkernel oil, 0.05% Ionox	10 m	5	50	0	0	5	50
		10 f	9	90	0	0	1	10
1430	7% palmkernel oil, 0.05% Ionox, 0.5% cholestanol	10 m	8	80	0	0	2	20
		10 f	10	100	0	0	0	0
1431	7% palmkernel oil, 0.05% Ionox, 1% cholestanol	10 m	9	90	0	0	1	10
		10 f	9	90	0	0	1	10

Age of the animals at start of the experiment 38–47 days.
Duration of the feeding period 42 days.

Table 13A. Exp. Series G 163. Mean values of body weight, liver weight, and cholesterol in liver and serum, with st. d.

Group no.	Diet characteristics	No. of animals in group	Body weight at start	Body weight after 6 weeks	Weight gain in 6 weeks	Weight of liver	Cholesterol in liver: total in whole liver	Cholesterol in liver: total	Cholesterol in liver: ester	Cholesterol in liver: free	Cholesterol in liver: ester % of total	Serum cholesterol
			g	g	g	g	mg	mg/hg	mg/hg	mg/hg		mg/dl
1429	7% palmkernel oil, 0.5% Ionox	10 m	60.3 ±2.5	84.0 ±3.3	23.7 ±4.1	3.76 ±0.23	9.6 ±0.8	240.4 ±6.1	37.5 ±2.0	202.9 ±5.2	15.6 ±0.6	231.6 ±6.1
		10 f	52.6 ±0.6	77.0 ±1.7	24.4 ±1.8	3.81 ±1.8	9.9 ±0.6	260.2 ±10.1	48.4 ±4.8	211.8 ±9.1	18.4 ±1.0	263.5 ±8.1
1430	7% palmkernel oil, 0.5% Ionox, 0.5% cholestanol	10 m	60.3 ±2.5	80.5 ±3.4	20.2 ±4.2	3.68 ±0.19	8.2 ±0.5	222.0 ±5.9	25.0 ±1.8	197.0 ±5.6	11.3 ±0.8	188.7 ±7.7
		10 f	52.6 ±1.4	79.4 ±2.6	26.8 ±2.9	3.93 ±0.25	9.6 ±0.4	246.1 ±6.6	36.0 ±1.7	210.1 ±6.4	14.7 ±0.7	241.7 ±6.1
1431	7% palmkernel oil, 0.05% Ionox, 1% cholestanol	10 m	60.4 ±2.8	84.1 ±2.7	23.7 ±3.8	3.91 ±0.15	9.1 ±0.4	232.0 ±5.6	28.9 ±1.6	203.2 ±5.4	12.4 ±0.4	204.4 ±2.8
		10 f	52.5 ±1.7	73.6 ±2.0	21.1 ±2.6	3.70 ±0.15	8.9 ±0.4	241.6 ±4.2	31.8 ±1.2	209.8 ±4.0	13.2 ±4.2	229.2 ±4.4

From Exp. Series G 155 (table 6 A) it is seen that addition of 0.125 % cholesterol was not sufficient to produce significant increases of liver cholesterol. Increases began in the group receiving 0.25 % cholesterol, and very great increases occurred in both sexes in the group receiving 0.5 % cholesterol. Here, the mean values of total cholesterol were above 2000 mg per 100 g liver, and the percentage of esterified cholesterol in total cholesterol were about 90.

Since individual sterols were not determined in the groups receiving commercial lanosterol (table 8 A), 7-dehydrocholesterol (table 8 A) and ergosterol (table 12 A), the values for "liver cholesterol" in these groups are subject to reservations similar to those holding for "serum cholesterol".

In Exp. Series G 158 (table 9 A), squalene, 1 % and 0.5 %, caused moderate but significant increases of liver cholesterol in the male hamsters but not in the females. In Exp. Series G 157 (table 8 A), the increase of liver cholesterol obtained with 1 % squalene was not significant in the males, and in the females it was negative.

Distilled menhaden oil fatty acid ethyl esters 1 % (table 8 A) caused significant decreases in liver cholesterol in males an females in G 157, but this was not confirmed in G 158 (table 9 A).

Replacement of dietary glucose with rice starch (table 9 A) did not lead to significant changes of liver cholesterol.

Thus, protection against production of cholesterol containing gallstones has occurred both with greatly increased and with largely unaltered contents of cholesterol in the liver.

4. Other observations

Since in many of our earlier experiments which were carried out with hamsters from our own breeding colony, the weight of the testes varied greatly from time to time, the weight of the testes was also recorded in the present experiments. It was found to be between 1.0 and 1.6 g at the end of each experiment.

Summary

Dietary squalene was found to counteract formation of cholesterol containing gallstones in young hamsters.

Complete or almost complete protection against production of cholesterol containing gallstones could be obtained in hamsters of both sexes by addition of 1 % squalene, 1 % cholesterol or 1 % distilled menhaden oil fatty acid ethyl esters to the gallstones producing diet used, or by substitution of rice starch for glucose in this diet.

Contrary to dietary cholesterol, dietary squalene did not stimulate production of amorphous gallstones in the female hamsters.

The incidence of amorphous gallstones in the female hamsters was high already at the 0.5 % level of dietary cholesterol.

Commercial soybean sterols and 7-dehydrocholesterol fed at the 1 % dietary level, and ergosterol fed at the 0.5 % dietary level afforded some protection against production of cholesterol containing gallstones, but the degree of protection obtained with these sterols was less than that which could be obtained with cholesterol fed at the same dietary levels. In the group of hamsters fed ergosterol, small needle-shaped crystals could be observed in this bladder bile of several of the animals not having gallstones.

Commercial lanosterol fed at the 1% level, and cholestanol fed at levels of 0.125%, 0.5% and 1% furthered production of cholesterol containing gallstones. The cholesterol containing gallstones produced in hamsters fed cholestanol contained some cholestanol besides cholesterol.

With all the protective agents tested, complete protection against production of cholesterol containing gallstones was particularly easy to obtain in the male hamsters. With the unsupplemented basal diets which contained a moderate amount of fat (7% or 10% palmkernel oil or 8% butter fat), in order to facilitate intestinal absorption of sterols, the incidence of cholesterol containing gallstones also was lower among the male than among the female hamsters. This circumstance is ascribed to the origin and preexperimental feeding of the hamsters used in the present study.

Protection against production of cholesterol containing gallstones was accompanied: 1. by lowered serum cholesterol in the groups fed distilled menhaden oil fatty acid ethyl esters or the diet with rice starch instead of glucose, 2. by greatly increased serum cholesterol in the groups fed cholesterol, and 3. by largely unaltered serum cholesterol in the groups fed squalene.

Protection against production of cholesterol containing gallstones was accompanied: 1. by inconsistently lowered liver cholesterol in the groups fed distilled menhaden oil fatty acid ethyl esters, 2. by largely unaltered liver cholesterol in the group receiving the diet with rice starch instead of glucose, 3. by greatly increased liver cholesterol in the groups fed cholesterol, and 4. by inconsistently increased or unaltered liver cholesterol in the groups fed squalene.

The cause of the protection against production of cholesterol containing gallstones obtained by addition of squalene, cholesterol or distilled menhaden oil fatty acid ethyl esters to the gallstone producing diet or by substitution of glucose by rice starch in the diet is discussed. Inhibition of biosynthesis of cholesterol in the liver is held to be the most likely cause.

Zusammenfassung

Squalen, mit der Nahrung eingegeben, schützt junge Hamster vor Bildung von cholesterinhaltigen Gallensteinen.

Vollständige oder fast vollständige Schutzwirkung gegen Bildung von cholesterinhaltigen Gallensteinen konnte in Hamstern von beiden Geschlechtern erreicht werden durch Zugabe von 1% Squalen, 1% Cholesterin oder 1% destillierten Äthylestern von Menhaden-Öl-Fettsäuren zu der Nahrung, die Gallensteine hervorruft, oder durch Austausch der in der Nahrung enthaltenen Glucose durch Reisstärke.

Im Gegensatz zu Cholesterin, das mit der Nahrung eingegeben wird, bewirkte mit der Nahrung zugeführtes Squalen nicht erhöhte Bildung von amorphen Gallensteinen in den weiblichen Hamstern.

Schon bei einem Gehalt von 0,5% Cholesterin in der Nahrung war das Vorkommen von amorphen Gallensteinen in den weiblichen Hamstern beträchtlich.

Handelsübliche Sojabohnen-Sterine und 7-Dehydrocholesterin je in der Höhe von 1% der Nahrung und Ergosterin in der Höhe von 0,5% der Nahrung zeigten Schutzwirkung gegen Bildung von cholesterinhaltigen Gallensteinen. Die mit diesen Sterinen erreichte Schutzwirkung war aber geringer als jene, die man mit Cholesterin in denselben Höhen erreichen konnte.

Ein Handelsprodukt von Lanosterin in der Höhe von 1% der Nahrung und Cholestanol (Dihydrocholesterin) in den Höhen von 0,125%, 0,5% und 1% förderten die Bildung von cholesterinhaltigen Gallensteinen. Die cholesterinhaltigen Gallensteine der mit Cholestanol gefütterten Hamster enthielten außer dem Cholesterin auch etwas Cholestanol.

Mit sämtlichen untersuchten Agenzien, die eine Schutzwirkung zeigten, war es besonders leicht, volle Schutzwirkung gegen Bildung von cholesterinhaltigen Gallensteinen in den männlichen Hamstern zu erreichen. Mit den nichtsupplementierten Basalnahrungen, welche, um die Darmresorption der Sterine zu erleichtern, eine mäßige Menge von Fett (7% oder 10% Palmkernöl oder 8% Butterfett) enthielten, war die Inzidenz von cholesterinhaltigen Gallensteinen auch geringer unter den männlichen als unter den weiblichen Hamstern. Eine Erklärung hierfür ist wahrscheinlich in dem Ursprung und der Fütterung der Hamster vor dem Versuch zu finden.

Die Schutzwirkung gegen Bildung von cholesterinhaltigen Gallensteinen wurde begleitet von: 1. herabgesetztem Serum-Cholesterin in den Gruppen, die die Nahrung mit Zugabe von destillierten Äthylestern von Menhaden-Öl-Fettsäuren oder die Nahrung mit Reisstärke anstatt Glucose erhielten, 2. mit sehr erhöhtem Serum-Cholesterin in den mit Cholesterin gefütterten Gruppen und 3. mit im wesentlichen unverändertem Serum-Cholesterin in den mit Squalen gefütterten Gruppen.

Die Schutzwirkung gegen Bildung von cholesterinhaltigen Gallensteinen wurde begleitet von: 1. unregelmäßig herabgesetztem Leber-Cholesterin in den Gruppen, die die Nahrung mit Zugabe von destillierten Äthylestern von Menhaden-Öl-Fettsäuren erhielten, 2. mit im wesentlichen unverändertem Leber-Cholesterin in der Gruppe, die die Nahrung mit Reisstärke anstatt Glucose erhielt, 3. mit sehr erhöhtem Leber-Cholesterin in den mit Cholesterin gefütterten Gruppen und 4. mit unregelmäßig erhöhtem oder im wesentlichen unverändertem Leber-Cholesterin in den mit Squalen gefütterten Gruppen.

Die Ursache der Schutzwirkung gegen Bildung von cholesterinhaltigen Gallensteinen, die man durch Zugabe von Squalen, Cholesterin oder destillierten Äthylestern von Menhaden-Öl-Fettsäuren oder durch Austausch von Glucose durch Reisstärke in der Nahrung erreicht, wird erörtert. Hemmung der Biosynthese von Cholesterin in der Leber wird als die wahrscheinlichste Erklärung angenommen.

References

1. *Dam, H., I. Prange* and *E. Søndergaard,* Z. Ernährungswiss. **9,** 43 (1968). – 2. *Christensen, F., I. Prange* and *H. Dam,* Z. Ernährungswiss. **4,** 186 (1964). – 3. *Dam, H.* and *F. Christensen,* Z. Ernährungswiss. **2,** 36 (1961). – 4. *Prange, I., F. Christensen* and *H. Dam,* Z. Ernährungswiss. **3,** 59 (1962). – 5. *Dam, H., I. Prange* and *F. Christensen,* Z. Ernährungswiss. **6,** 97 (1965). – 6. *Rosenmund, K. W.* and *W. Kuhnhenn,* Z. Untersuchg. d. Nahrungsgenußmittel **46** 154 (1923). – 7. *Søndergaard, E., I. Prange* and *H. Dam,* Z. Ernährungswiss. **12,** 1 (1973). – 8. *Dam, H., I. Prange* and *E. Søndergaard,* Acta Pathol. Microbiol. Scand. **31,** 172 (1952). – 9. *Deuel, H. J.* jr., The Lipids, I., p. 748 (New York 1951). – 10. *Watson, D.,* Clin. Chem. Acta **5,** 637 (1960). – 11. *Prange, I., E. Søndergaard* and *H. Dam,* Z. Ernährungswiss. **12,** 92 (1973). – 12. *Dam, H., I. Prange* and *E. Søndergaard,* Z. Ernährungswiss. **11,** 80 (1972). – 13. *Koller, S.,* in *H. M. Rauen,* Biochemisches Taschenbuch, Bd. 2, S. 988 (Berlin 1964). – 14. *Jensen, B.* and *H. Dam,* Biochem. Biophys. Acta **125,** 367 (1966). – 15. *Muroya, H.,* Japan. Arch. Chirurgie **37,** 76 (1968). – 16. *Langdon, R. G.* and *K. Bloch,* J. Biol. Chem. **202,** 77 (1953). – 17. *Gould, R. G.* and *G. Popjak,* Biochem. J. **66,** 51 (1957). – 18. *Siperstein, M. D.* and *M. J. Guest,* J. Clin. Invest. **39,** 642 (1960). – 19. *Byers, S. O.* and *M. Friedman,* Amer. J. Physiol. **168,** 297 (1952). – 20. *Cook, R. P.,* Cholesterol, p. 92 (New York 1958).

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