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Influence of dietary vitamin E and fat on depletion of a cholesterol depot in the liver of chicks

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With 1 figure and 3 tables

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When ingested in large quantities, both vitamin A and cholesterol are deposited in the liver as esters (1, 2). On subsequent withdrawal from the diet the deposits in the liver decline. In the case of vitamin A it has been shown that continuous ingestion of vitamin E during the depletion period markedly retards the depletion (3, 4), whereas a possible retarding effect of vitamin E on the depletion of cholesterol does not seem to have been thoroughly investigated.

The present study deals with the influence of vitamin E on depletion of a cholesterol depot in the liver of chicks. The influence of vitamin E was studied both with fat-free diets and with diets containing 10 % lard, a fat which at this level usually does not give rise to encephalomalacia when the diet is vitamin E deficient. All the diets contained the trace amount of selenite which is necessary for prevention of exudative diathesis in the absence of vitamin E.

Experimental

Sixty chicks were received day-old from a local dealer. They were divided equally into 6 groups and housed in battery brooders with electrical heating and wire screen bottom. Diets and tap water were available ad libitum.

During the first 15 days all the chicks received a vitamin E free starter ration containing 5 % lard but no added cholesterol, as indicated in table 1.

At the end of the 15-days period, the 10 chicks in group 1 were killed, their livers were taken out, weighed, wrapped in aluminum foil and stored at minus 20° C for determination of free, esterified and total cholesterol some weeks later.

The remaining 50 chicks received, for a period of 14 days, the vitamin E deficient diet containing 10 % palm kernel oil and 1 % cholesterol, indicated in table 1. (Fat is necessary for optimal absorption of cholesterol, and palm kernel oil was chosen because it has a very low content of polyunsaturated fatty acids.) Thereupon, the 10 chicks in group 2 were killed, their livers taken out and treated as described for group 1.

Table 1. Composition of diets.

	Starter ration	Cholesterol containing diet	Diets without added cholesterol used in the depletion period			
	All groups g	Groups 2–6 g	Group 3 g	Group 4 g	Group 5 g	Group 6 g
Casein, crude[1])	200.0	300.0	300.0	300.0	300.0	300.0
Gelatin	30.0	30.0	30.0	30.0	30.0	30.0
Salt mixture[2])	51.7	51.7	51.7	51.7	51.7	51.7
Vitamin mixture[3])	1.0	1.0	1.0	1.0	1.0	1.0
Cholin chloride	2.0	2.0	2.0	2.0	2.0	2.0
Dry yeast[4])	200.0	–	–	–	–	–
Corn starch	465,3	–	–	–	–	–
Sucrose	–	505.3	615.3	615.3	515.3	515.3
Palmkernel oil[5])	–	100.0	–	–	–	–
Lard[6])	50.0	–	–	–	100.0	100.0
Cholesterol	–	10.0	–	–	–	–
	1000.0 g	1000.0 g	1000.0 g	1000.0 g	1000.0 g	1000.0 g
Vitamin K substitute[7])	10.0 mg	10.0 mg	10.0 mg	10.0 mg	10.0 mg	10.0 mg
Selenium dioxide	1.4 mg	1.4 mg	1.4 mg	1.4 mg	1.4 mg	1.4 mg
All-rac.-α-tocopheryl acetate[8])	–	–	100.0 mg	–	100.0 mg	–

[1]) "Dairinex", from Dansk Mejeri Industri & Export Kompagni, Stege (Denmark).

[2]) The salt mixture indicated by *Dam & Søndergaard*, 1953 (5).

[3]) The vitamin mixture indicated by *Dam & Søndergaard*, 1953 (5). – Vitamins A and D_3 were given in the form of an aqueous colloidal solution; 0,1 ml by syringe to each chick twice a week, corresponding to 250 i.u. A, and 20 i.u. D_3 per chick per day. The solution was prepared as indicated by *Dam* et al., 1957 (6).

[4]) *Fleischmann* Yeast 50 B, from Standard Brands, Inc., New York (USA).

[5]) From A/S Dansk Sojakage Fabrik, Copenhagen (Denmark).

[6]) From Dansk Andels Svineslagteri, Hillerød (Denmark).

[7]) Dicalcium salt of 2-methyl-1,4-naphthoquinol-diphosphate, "Synkavit" Roche.

[8]) "Ephynal acetate", Roche.

During the subsequent 14 days – the depletion period – the remaining 40 chicks were given diets not containing added cholesterol. (The lard contained 105 mg cholesterol/100 g, determined after saponification.) Group 3 received the fat-free diet with addition of 0.01 % all-rac.-α-tocopheryl acetate, group 4 received the fat-free diet without addition of vitamin E, group 5 received the diet containing 10 % lard and 0.01 % all-rac.-α-tocopheryl acetate, whereas group 6 received the diet containing 10 % lard without addition of vitamin E. The compositions of the respective diets are shown in table 1.

The fatty acid patterns of the fats used appear from table 2.

At the end of the 14-days depletion period the chicks in all the four groups 3–6 were killed and their livers taken out for determination of cholesterol as in the foregoing two groups.

Table 2. Fatty acid composition of the dietary fats[1]). (Individual fatty acid methyl esters as area per cent of total fatty acid methyl esters).

Fatty acid[2])	Palmkernel oil	Lard
8:0	10.1	
10:0	6.9	
12:0	46.3	0.1
14:0	17.7	1.4
16:0	6.5	28.2
16:1 ω7		1.8
17:0 (16:2)		0.2
18:0	1.6	19.5
18:1 ω9	10.5	40.7
18:2 ω6	0.3	7.2
18:3 ω3		0.4
20:1		0.5

[1]) Determined by GLC of the methyl esters on a Beckman gas-chromatograph, model GC 4, using a diethylene glycol succinate phase coated on acid-washed Chromosorb W (AW). Methylation of the fatty acids was carried out by the method of *Stoffel, Chu* and *Ahrens* (1959) (7).

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

The methods used for determination of cholesterol were as follows:

To about 3 g of liver tissue were added 10 g of anhydrous sodium sulfate and 60 ml chloroform, whereupon the mixture was homogenized for 5 minutes in an electrically driven homogenizer (from Measuring and Scientific Equipment Ltd., London, England).

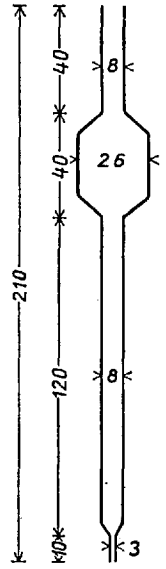

Fig. 1. Chromatographic tube. Scale in mm.

Table 3. Cholesterol in liver, and other data

Group No.	Diet characteristics			Number of chicks in group at end of feeding	Body weight at end of feeding	Weight of liver	$CHCl_3$ extract of liver
	During first 15 days	During following 14 days	During last 14 days				
					g	g	%
1	Vit. E-free starter ration			10	147.7 ±6.6	4.53 ±0.14	4.94 ±0.21
2	As above	Vit. E-free, 1% cholesterol, 10% palmkernel oil		10	267.5 ±17.9	8.33 ±0.54	7.53 ±0.24
3	As above	As above	Fat-free, 0.01% all-rac.-α-tocopheryl acetate	9	508.6 ±51.0	16.40 ±1.62	5.45 ±0.89
4	As above	As above	Fat-free, Vitamin E-free	10	495.9 ±20.4	14.87 ±1.19	5.51 ±0.87
5	As above	As above	10% lard, 0.01 all-rac.-α-tocopheryl acetate	10	515.3 ±32.9	12.76 ±0.83	3.88 ±0.09
6	As above	As above	10% lard, Vitamin E-free	9	514.6 ±42.8	15.21 ±1.27	3.89 ±0.11

The homogenate was filtered through a fritted glass filter funnel and washed with chloroform. The filtrate was evaporated to dryness *in vacuo* on a water bath of 40° C and filled up to 25 ml with petroleum ether (redistilled, b. p. 60–70° C) in a measuring flask. The esterified and free cholesterol contained in an aliquot part (usually 0.5 or 1 ml) of this solution were separated by column chromatography using the chromatography tube shown in fig. 1 [originally devised by Bro-Rasmussen and Hjarde (8)].

Silica gel G (Merck, Darmstadt) was activated by heating at 110° C for 24 hours. A plug of glass wool (Fiberglass, Pyrex Brand Wool, Filtering Fiber, Corning Glass Works, Corning N. Y., USA) was placed above the constricted outlet of the tube. One g of the activated silica gel was suspended in 10 ml redistilled petroleum ether and poured into the tube. The tube was connected with a nitrogen flask, and the column settled by nitrogen pressure while the tube was tapped lightly with a glass rod.

0.5 or 1 ml of the petroleum ether solution from the measuring flask was transferred to the column, rinsed with 0.5 ml petroleum ether, and thereafter with 1 ml diethyl ether: petroleum ether (40 : 60, v : v). During these procedures, the nitrogen pressure was regulated to provide a flow rate of one drop for every 3 seconds. The receiver was changed and the cholesterol ester eluted with 10 ml diethyl ether: petroleum ether (10 : 90, v : v), whereafter the free cholesterol was eluted with 10 ml undiluted diethyl ether. During the elution

for chicks fed as described in the text.

Esterified cholesterol		Free cholesterol		Total cholesterol (sum of esterified and free)		Total cholesterol determined directly		Esterified cholesterol
mg in 100 g liver	mg in whole liver	mg in 100 g liver	mg in whole liver	mg in 100 g liver	mg in whole liver	mg in 100 g liver	mg in whole liver	as % of total cholesterol
79.5 ±7.6	3.6 ±0.4	270.2 ±9.8	12.1 ±0.5	349.7 ±12.4	15.7 ±0.6	352.9 ±13.7	16.0 ±0.9	22.5 ± 1.6
2001.7 ±180.1	169.8 ±21.7	561.3 ±39.2	47.6 ±4.7	2563.0 ±184.7	217.4 ±22.2	2569.5 ±197.4	218.2 ±24.6	77.7 ± 1.5
173.9 ±44.7	26.6 ±4.9	248.5 ±8.6	40.4 ±4.0	422.4 ±45.6	67.0 ±6.3	419.0 ±42.7	66.8 ±7.1	37.6 ± 2.8
137.8 ±17.3	21.1 ±3.6	265.2 ±3.9	39.7 ±3.5	403.0 ±17.7	60.8 ±5.0	400.2 ±19.3	60.4 ±6.4	33.2 ± 2.4
56.0 ±8.3	7.3 ±0.8	247.6 ±9.8	31.6 ±2.3	303.6 ±12.8	38.9 ±2.4	308.8 ±8.6	39.6 ±3.0	18.4 ± 0.9
73.9 ±9.1	11.3 ±1.6	253.9 ±9.1	38.4 ±3.1	327.8 ±12.9	49.7 ±3.5	333.1 ±13.8	50.6 ±4.4	22.2 ± 1.8

the nitrogen pressure was regulated to provide a flow rate of one drop per second.

In order to check the effectiveness of separation and elution, one ml eluate was collected separately after each elution, and tested for presence of *Liebermann-Burchard* reactive material.

The two fractions containing the esterified and the free cholesterol, respectively, were evaporated to dryness under nitrogen, dissolved in chloroform, whereafter their contents of cholesterol were determined by the *Liebermann-Burchard* reaction as described in reference (9).

As a check on the determination of total cholesterol calculated as the sum of esterified and free cholesterol, a direct determination of total cholesterol was made by evaporating one ml of the petroleum ether solution from the measuring flask to dryness and dissolving the residue in chloroform, whereafter the content of total cholesterol was determined directly by the *Liebermann-Burchard* reaction as described (9).

Results

The results are presented in table 3 as mean values for each group with standard deviations.

The figures for cholesterol (total, esterified and free) in *the whole liver* are of particular interest, since these figures are less influenced by alterations in the weight of the liver than are the figures referring to 100 g liver. It should be noticed however, that, generally, the latter figures change in the same direction from group to group as do the figures for whole liver, the only exception being the unimportant differences with respect to free cholesterol in groups 3 and 4.

It is seen that during the period of cholesterol feeding, the total amount of cholesterol in the liver increased from 15.7 mg to 217.4 mg, which is about 14 times the initial amount. The free cholesterol increased from 12.1 mg to 47.6 mg, i. e., about 4 times, but the esterified cholesterol increased from 3.6 mg to 169.8 mg, i. e., about 47 times the initial amount. Before the cholesterol feeding, the esterified cholesterol was the minor part (22.5 %) of the total liver cholesterol, but after the cholesterol feeding it was the major part (77.7 %).

During the subsequent depletion period of 14 days, the total amount of liver cholesterol declined in all groups, and the esterified cholesterol again became the minor part of the total cholesterol. The esterified cholesterol declined to twenty odd mg in the groups receiving the fat-free diets, and to about 10 mg in the groups receiving the diets with 10 % lard, whereas the free cholesterol only declined to about 40 mg in the groups receiving the fat-free diets, and to thirty odd mg in the groups receiving the diets with 10 % lard. As a consequence thereof, the changes of total cholesterol, while largely following the changes of esterified cholesterol, were slightly less marked than the latter.

Considering first the fraction in whole liver which exhibited the greatest decline: *the esterified cholesterol,* it can be seen that the difference in decline resulting from adding vitamin E to the fat-free diet (group 3 versus group 4) was nonsignificant ($0.4 > p > 0.3$), whereas the difference in decline resulting from adding vitamin E to the diet containing 10 % lard (group 5 vs. group 6) was moderately significant ($0.05 > p > 0.02$) and consisted in acceleration of the depletion. The difference in decline resulting from adding lard to the diets also consisted in acceleration of the depletion; with the vitamin E deficient diet, this effect of lard (grop 6 vs. group 4) was moderately significant ($0.05 > p > 0.02$), and with the diet containing vitamin E (group 5 vs. group 3) it was highly significant ($0.01 > p > 0.001$). (The fact that in groups 5 and 6, the lard supplied approximately 220–294 mg cholesterol to each chick during the depletion period further emphasizes the accelerating effect of lard on cholesterol depletion.)

Regarding *the free cholesterol,* there were no significant differences between any of the groups 3, 4, 5, and 6, the difference exhibiting the greatest significance ($0.1 > p > 0.05$) being that between groups 5 and 6 (vitamin E vs. no vitamin E in the diet with lard).

Discussion

It is evident from the results that the depletion of a depot of cholesterol in the liver of chicks is not influenced by dietary vitamin E in the same way as is the depletion of a depot of vitamin A.

When the diet in the depletion period was fat-free, no significant stabilizing effect of vitamin E on the cholesterol depot could be found, and when the diet contained 10 % lard, addition of vitamin E was rather found to accelerate the removal of cholesterol from the depot. In the latter case, vitamin E might be thought to influence the metabolism of linoleic acid, which in turn will influence the metabolism of the stored cholesterol ester. However, a clarification of these relationships will require further experimentation.

The acceleration of the removal of cholesterol from the liver of chicks resulting from addition of lard to the diet during the depletion period, especially when the diet contained vitamin E, reminds of the results of previous experiments in which chicks were reared on cholesterol-free diets with or without 10 % peanut oil (10), or on cholesterol-free diets with and without 10 % cod liver oil or 10 % linseed oil (11). In all these cases the content of total cholesterol in the liver was lower when the diet contained fat than when the diet was fat-free. (In these older experiments, the results were expressed as mg total cholesterol per 100 g liver, but the weight of the livers showed only small variations from group to group within each experiment.) In an experiment (12) in which 10 % hydrogenated peanut oil was used, the content of cholesterol in the liver was not lower when the diet contained fat than when the diet was fat-free. The effect of fats on liver cholesterol observed in the experiments (10) and (11) could, therefore, be related to the content of polyenoic fatty acids in the fats.

Summary

Six groups of day-old chicks, 10 in each group, were given a vitamin E deficient starter ration containing 5 % lard for a period of 15 days.

At the end of this period, the chicks in group 1 were killed. The mean content of cholesterol in their livers was 15.7 mg (3.6 mg esterified + 12.1 mg unesterified cholesterol), liver weight 4.53 g.

The remaining 5 groups received, for a period of 14 days, a vitamin E deficient diet containing 1 % cholesterol and 10 % palm kernel oil.

Then the chicks in group 2 were killed. The mean content of cholesterol in their livers was 217.4 mg (169.8 mg esterified + 47.6 mg unesterified cholesterol), liver weight 8.33 g.

Thereafter, the remaining 4 groups received, for a period of 14 days, diets without added cholesterol as follows:

Group 3: a fat-free diet + 0.01 % all-rac.-α-tocopheryl acetate.

Group 4: a fat-free diet without vitamin E.

Group 5: a diet with 10 % lard + 0.01 % all-rac.-α-tocopheryl acetate.

Group 6: a diet with 10 % lard without added vitamin E.

At the end of this period, all the chicks in groups 3 to 6 were killed. The contents of cholesterol in their livers were as follows:

Group 3: 67.0 mg (26.6 mg esterified + 40.4 mg unesterified cholesterol), liver weight 16.40 g.

Group 4: 60.8 mg (21.1 mg esterified + 39.7 mg unesterified cholesterol), liver weight 14.87 g.

Group 5: 38.9 mg (7.3 mg esterified + 31.6 mg unesterified cholesterol), liver weight 12.76 g.

Group 6: 49.7 mg (11.3 mg esterified + 38.4 mg unesterified cholesterol), liver weight 15.21 g.

It is concluded that additional vitamin E to the fat-free diet had no significant influence on the decrease of the cholesterol depot in the liver during the depletion period, whereas addition of vitamin E to the diet containing 10% lard resulted in a somewhat intensified decrease of the cholesterol depot in the liver during this period.

Addition of lard to the diet intensified the decrease of the cholesterol depot in the liver during the depletion period, especially when the diet contained vitamin E.

Thus, the influence of vitamin E on the depletion of a cholesterol depot in the liver is different from the influence of vitamin E on the depletion of a depot of vitamin A.

Zusammenfassung

Sechs Gruppen von Küken, 10 in jeder Gruppe, erhielten von einem Alter von 1 Tag an während 15 Tagen eine „Starter Ration", welche 5% Schweineschmalz, aber kein Vitamin E enthielt. Dann wurden die Küken in Gruppe 1 getötet. Der durchschnittliche Gehalt an Cholesterin in der Leber war 15,7 mg (3,6 mg verestertes + 12,1 mg unverestertes Cholesterin). Gewicht der Leber 4,53 g.

Die übriggebliebenen 5 Gruppen erhielten während 14 Tagen eine Vitamin-E-freie Nahrung, welche 1% Cholesterin und 10% Palmkernöl enthielt. Die Küken in Gruppe 2 wurden danach getötet. Der durchschnittliche Gehalt an Cholesterin in der Leber war 217,4 mg (169,8 mg verestertes + 47,6 mg unverestertes Cholesterin). Gewicht der Leber 8,33 g.

Die restlichen 4 Gruppen wurden dann während 14 Tagen mit den folgenden Nahrungen gefüttert:

Gruppe 3: Fettfreie Nahrung + 0,01% all-rac.-α-Tocopherylacetat.

Gruppe 4: Fettfreie Nahrung ohne Vitamin E.

Gruppe 5: Nahrung mit 10% Schweineschmalz + 0,01% all-rac.-α-Tocopherylacetat.

Gruppe 6: Nahrung mit 10% Schweineschmalz ohne Vitamin E.

Nach Beendigung dieser Fütterungsperiode wurden sämtliche Küken in den Gruppen 3–6 getötet. Der durchschnittliche Gehalt an Cholesterin in der Leber und das Gewicht der Leber in den einzelnen Gruppen waren:

In *Gruppe 3:* 67,0 mg (26,6 mg verestertes + 40,4 mg unverestertes Cholesterin), Gewicht der Leber 16,40 g.

In *Gruppe 4:* 60,8 mg (21,1 mg verestertes + 39,7 mg unverestertes Cholesterin), Gewicht der Leber 14,87 g.

In *Gruppe 5:* 38,9 mg (7,3 mg verestertes + 31,6 mg unverestertes Cholesterin), Gewicht der Leber 12,76 g.

In *Gruppe 6:* 49,7 mg (11,3 mg verestertes + 38,4 mg unverestertes Cholesterin), Gewicht der Leber 15,21 g.

Es wird gefolgert, daß Zulage von Vitamin E zur fettfreien Nahrung keinen signiflkanten Einfluß auf die Abnahme des Cholesteringehaltes der Leber in der Karenzperiode gehabt hat, während Zulage von Vitamin E zu der 10% Schweineschmalz enthaltenden Nahrung die Abnahme des Cholesteringehalts der Leber etwas gefördert hat.

Zulage von Schweineschmalz beschleunigte die Abnahme des Cholesteringehalts der Leber, besonders wenn die Nahrung Vitamin E enthielt.

Der Einfluß von Vitamin E auf die Abnahme des Cholesteringehalts der Leber ist somit verschieden von dem Einfluß von Vitamin E auf die Abnahme des Gehalts an Vitamin A.

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