Lipid Changes in Plasma, Alpha Lipoproteins, Liver and Aorta of Chicks Fed Different Fats. (28022)

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The influence of dietary fat on blood cholesterol levels has received considerable attention. In man, there is some agreement that, other factors being equal, unsaturated fats tend to depress serum cholesterol while saturated lipids appear to be hypercholesterolemic(1,2,3). The results of certain studies employing rats or chicks as experimental animals suggest that the effect of dietary lipid on serum cholesterol in these species may be analogous to that observed in man(4,5,6,7). Other reports have demonstrated that for the rat and chick the level of certain fatty acids may be of greater significance than degree of unsaturation *per se* in altering serum cholesterol levels. Thus, Hegsted *et al.*(8) have shown that in the cholesterol-fed rat, serum cholesterol is negatively related to the dietary level of saturated and essential unsaturated (linoleic and arachidonic acids) fatty acids, and positively correlated to intake of nonessential polyunsaturated and monounsaturated fatty acids. In the growing cholesterol-fed chick, Adamson *et al.*(9) have demonstrated that unsaturated fats caused greater elevations in serum and liver cholesterol levels than saturated fats. Keys *et al.*(3) have shown that in man saturated fatty acids elevate serum cholesterol, while polyunsaturated acids have a depressing effect; oleic acid was reported to be without influence.

Obviously, some of the reports cited indicate the existence of certain discrepancies between man, rat and chick in reactivity of serum cholesterol to dietary fat. The data presented here demonstrate that the influence of different dietary fats on plasma cholesterol differs in chicks fed a diet containing or free of cholesterol. These results further illustrate the need of studying cholesterol changes in tissues other than plasma or serum.

Materials and methods. Male Hy-line White Leghorn chicks were fed a commercial diet for one week. The chicks were then distributed to the various experimental groups on a body weight basis in such a manner as to have similar initial mean weights (62-65 g). Ten chicks were assigned to each group. The chicks were reared in heated cages having raised wire floors. Food and water were supplied *ad libitum*. Food consumption and body weights were determined at weekly intervals throughout the 4-week experimental period.

The composition of the basal diet was as follows in g/100 g of diet: Soybean oil meal (47% protein), 42.55; vitamin mixture,* 0.40; choline Cl, 0.20; salt mixture,* 4.00; glucose to 100. An additional 5.96% soybean oil meal was added to the fat-supplemented diets at the expense of glucose to maintain a constant calorie:protein ratio. All diets supplied 15 calories of metabolizable energy per gram of protein. All additions to the basal diet were made at the expense of glucose. Fat level in the basal diet was low, all fat being supplied by the soybean oil meal (1.17% fat), amounting to 0.57 and 0.50% in the fat-supplemented and unsupplemented diets, respectively. The diets employed contained only marginal levels of sulfur amino acids.

The animals were bled by cardiac puncture at termination of the experimental period, using a heparinized syringe. The animals were then sacrificed and the liver and aorta excised and stored in the frozen state until analyzed.

Plasma lipoproteins were separated by a polyanion precipitation technic(11) and the α-lipoproteins remaining in the supernatant were analyzed. Plasma and α-lipoprotein cholesterol and lipid phosphorus were determined as previously described(10). The livers were analyzed individually while the aortas were pooled by experimental group for analysis. The weighed tissues were homoge-

* For composition, see Leveille *et al.*(10).

TABLE I. Influence of Dietary Fat and Cholesterol on Weight Gain; Plasma, Liver and Aorta Lipids of the Chick.

Fat*	Body wt gain (g)†	Plasma		Liver			Aorta		
		Cholesterol (mg/100 ml)	Lipid P (mg/100 ml)	Fat (%)	Cholesterol (mg/g)‡	Lipid P (mg/g)‡	Fat (%)	Cholesterol (mg/g)‡	Lipid P (mg/g)‡
No added cholesterol:									
None	247 ± 39§	176 ± 23	16.15 ± 1.82	5.94 ± .72	4.74 ± .30	1.07 ± .11	3.13	3.73	.30
Coconut oil	267 ± 25	190 ± 35	16.65 ± 1.56	5.51 ± .72	4.55 ± .35	1.06 ± .08	3.29	3.56	.32
Olive "	273 ± 44	220 ± 30	14.55 ± 1.79	4.65 ± .60	4.18 ± .56	.85 ± .10	2.98	3.80	.30
Corn "	257 ± 27	223 ± 39	15.10 ± 2.86	4.83 ± .79	4.26 ± .58	.96 ± .09	2.69	4.54	.35
1% cholesterol added:									
None	237 ± 29	401 ± 67	17.06 ± 1.85	7.53 ± 1.11	12.55 ± 2.11	1.38 ± .10	3.42	5.44	.31
Coconut oil	286 ± 37	1533 ± 248	25.92 ± 2.84	9.91 ± 1.75	29.39 ± 5.77	1.27 ± .17	4.14	5.92	.31
Olive "	278 ± 42	1121 ± 188	16.87 ± 2.53	13.42 ± 2.19	48.33 ± 4.77	1.05 ± .11	3.46	7.66	.36
Corn "	300 ± 29	1188 ± 186	18.36 ± 2.16	9.68 ± 1.14	31.94 ± 3.39	1.04 ± .10	3.15	6.85	.36

* All fats added at a level of 10% of diet.
† Body weight gain from 7th through 35th day of age.
‡ Wet weight.
§ Mean for 10 animals ± standard deviation.

nized with 25 volumes of methanol and made up to 75 volumes with chloroform. The homogenates were heated in a 50°C water bath for 20-30 minutes and were then filtered over anhydrous Na_2SO_4 and the residue washed with chloroform. The filtrates were evaporated to dryness under a stream of O_2-free nitrogen, redissolved in chloroform and refiltered over anhydrous Na_2SO_4 into tared flasks and the solvent evaporated under a stream of O_2-free nitrogen. After storing the dry samples in an evacuated desiccator overnight, total lipids were determined gravimetrically. The lipids were then dissolved in a known volume of chloroform, and cholesterol and lipid phosphorus were determined on appropriate aliquots by the colorimetric procedures employed for plasma.

Results. The influence of the various dietary treatments employed on weight gain and on plasma, liver and aortic lipids, is presented in Table I. Addition of fat to the basal diet appeared to improve weight gain in most instances, although this increase was statistically significant ($P<0.05$) only in the cholesterol-supplemented groups. Plasma cholesterol levels of the groups receiving the low-fat diet were lower than those for the fat-supplemented groups. There were no significant differences between plasma cholesterol levels of chicks fed the various oils without added cholesterol, although the groups fed olive or corn oil had slightly higher levels than did the coconut oil-fed group. Addition of cholesterol to the diets resulted in significant increases in plasma cholesterol levels in all cases ($P<0.001$). The group receiving coconut oil had plasma cholesterol levels significantly higher than the groups fed either olive or corn oil ($P<0.005$).

The oils fed resulted in slight alterations in amount of fat and cholesterol in the livers of animals not fed cholesterol. However, addition of cholesterol to the diet significantly elevated total lipids as well as the cholesterol and lipid phosphorus levels ($P<0.005$) (Table I). The fat- and cholesterol-supplemented groups demonstrated significantly higher hepatic fat and cholesterol levels than did animals fed the cholesterol-supplemented low-fat diet ($P<0.001$). In the fat-supple-

TABLE II. Influence of Dietary Fat and Cholesterol on Plasma Alpha-Lipoprotein Cholesterol and Lipid Phosphorus.

	Lipid bound to α-lipoprotein			
	Cholesterol		Lipid P	
Fat supplement*	mg/100 ml plasma	% of total	mg/100 ml plasma	% of total
No added cholesterol:				
None	106 ± 18†	61 ± 10	9.29 ± 1.18	58 ± 4
Coconut oil	125 ± 22	67 ± 14	10.38 ± 1.10	63 ± 7
Olive "	138 ± 36	62 ± 12	7.26 ± 1.48	50 ± 10
Corn "	125 ± 28	57 ± 11	7.74 ± 1.74	52 ± 12
1% added cholesterol:				
None	112 ± 22	28 ± 6	6.92 ± .92	41 ± 6
Coconut oil	121 ± 18	8 ± 2	5.50 ± .89	21 ± 6
Olive "	104 ± 20	10 ± 2	6.02 ± .94	39 ± 6
Corn "	101 ± 8	8 ± 1	4.30 ± .68	23 ± 3

* All fats fed at a level of 10% of diet.

† Mean for 10 animals ± standard deviation.

mented groups receiving cholesterol, olive oil produced significantly higher liver fat and cholesterol levels than did either coconut or corn oil ($P<0.001$).

In the cholesterol-fed chicks the aorta cholesterol level is much higher for the olive oil-fed chicks than for any other group, and the coconut oil-supplemented group shows the lowest value for the fat-supplemented groups.

In Table II data are presented on the cholesterol and lipid phosphorus bound to the plasma α-lipoproteins. There was a slight decrease in the absolute levels of both when cholesterol was fed. However, there was a highly significant decrease in percentage of total plasma cholesterol found in the α-lipoprotein fraction when cholesterol was fed. Similar but less dramatic changes occurred in percentage of lipid phosphorus bound to the α-lipoproteins.

Discussion. Maximal weight gains were not obtained, probably because of the low sulfur amino acid content of the diets. Although a low level of dietary sulfur amino acids elevates serum cholesterol in the chick (10), the relative effect of other factors is still evident(12).

The results obtained here demonstrate the enhancing effect of dietary fat on cholesterol absorption. It has been shown previously that the fatty acid fraction of the ingested fat is responsible for increased absorption (13). It also appears that unsaturated fatty acids and particularly oleic acid are the most effective in improving cholesterol absorption in the rat(14). This finding correlates with the observation of Hegsted *et al.*(8) that oleic acid has a hypercholesterolemic effect in the cholesterol-fed rat.

Our results indicate that olive oil, and therefore probably oleic acid (olive oil contains approximately 65% oleic acid), enhances cholesterol absorption to a gerater extent than essential polyunsaturated (corn oil – 42% linoleic) or saturated (coconut – 90% saturated) fatty acids in the chick, as has been reported for the rat(8). This is based on the assumption that total circulating and hepatic cholesterol is a reflection of cholesterol absorption. Employing a value of 7% of body weight for plasma volume of the chick(15), total hepatic and circulating cholesterol was calculated to be 766, 893 and 706 mg for the coconut, olive and corn oil-fed groups, respectively. These calculations indicate that the chicks fed olive oil absorbed 127 and 157 mg more cholesterol (or 17 and 22%) than did chicks fed coconut or corn oil, respectively. It is also significant that, although the olive oil-fed chicks had a greater hepatic-circulating cholesterol level than did animals fed coconut or corn oil, this was not reflected by the plasma cholesterol level, since 70% of the cholesterol was found in the liver as compared to 51 and 58% for the coconut and corn oil-fed chicks, respectively. Similar results have been reported for rats fed coconut or corn oil(16). Therefore, particularly since the aorta cholesterol level as was noted for liver cholesterol is not reflected by

plasma levels in the olive oil-fed chick, the importance of examining lipid levels in tissues other than plasma is evident. In the present study, the animals fed corn oil had slightly higher liver cholesterol levels than did chicks fed coconut oil; however, the difference was not statistically significant. In human subjects, corn oil has been found to depress, and hydrogenated coconut oil to elevate both serum and liver cholesterol levels (17). However, the fact that the hypocholesterolemic effect of corn oil in humans is not due to a shift of cholesterol from the circulation to the liver does not preclude the possibility that such may occur with other fats such as olive oil, reputed to be hypocholesterolemic in man(1).

The changes noted for lipoprotein cholesterol corroborate an earlier report from this laboratory(11) and are in accord with similar changes reported for the rat(18). The decrease in percentage of total plasma cholesterol bound to the α-lipoprotein would necessitate an increase in the β-fraction. Since the absolute amount of cholesterol found in the α-fraction remains essentially constant, the plasma cholesterol increase caused by cholesterol feeding in the chick must occur exclusively in the β-fraction.

Summary. Growing chicks were fed either a low-fat diet or this diet supplemented with coconut, olive or corn oil, with and without added cholesterol. In the absence of dietary cholesterol, the fats fed produced higher plasma cholesterol levels than did the low-fat control diet; no significant differences between fats were observed.

In the cholesterol-fed chick, coconut oil induced a significantly higher plasma cholesterol level than did either olive or corn oil. With cholesterol feeding, all of the fat-supplemented groups had higher plasma, liver and aorta cholesterol levels than did the group fed the low-fat control diet. In spite of lower plasma cholesterol levels, the olive oil, cholesterol-fed chicks had higher liver cholesterol levels than chicks fed coconut oil and cholesterol. The lipid phosphorus levels, in general, paralleled cholesterol.

The percentage of plasma cholesterol and lipid phosphorus bound to the plasma α-lipoproteins decreased with increasing plasma cholesterol levels. The absolute amount of cholesterol bound to the α-lipoproteins remained essentially unchanged, demonstrating that the increase in cholesterol occurs exclusively in the β-lipoprotein fraction.

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