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Relative Distribution of Cholesterol in Plasma and Liver Compartments of Chicks Fed Different Fatty Acids. (29660)

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Numerous studies have been concerned with the influence of dietary lipids on serum cholesterol levels in man and other animals (1-7). Most of these studies have indicated that the hypocholesterolemic effect of certain dietary fats is related to their unsaturated fatty acid content. However, other reports have shown that unsaturation *per se* cannot account for the hypocholesterolemic effect of dietary lipids. Hegsted *et al*(8) have shown serum cholesterol levels to be positively correlated to the intake of unessential polyunsaturated and monounsaturated fatty acids in cholesterol-fed rats. Also, recent reports have shown medium chain triglycerides (a liquid fat composed of C_8 and C_{10} triglycerides having an iodine number of 0.2) to be hypocholesterolemic(9-10).

In a previous report(11), it was shown that dietary fats can influence the distribution of cholesterol between serum and liver. This effect of dietary fat was attributed to its fatty acid composition. The data presented here support this conclusion in chicks fed free fatty acids.

Materials and methods. Male Hy-line White Leghorn chicks were fed a commercial diet for 10 days. Ten chicks were then assigned to each of various experimental groups on the basis of body weight in such a manner as to have similar initial mean weights (105-108 g). 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 3-week experimental period.

The basal diet employed had the following composition in g/100 g of diet: Assay protein C-1,* 29.4; glycine, 0.4; DL-methionine, 0.3; salt mix,† 5.31; vitamin mix,† 4.0; glucose to 100. Fatty acids and cholesterol were added at a level of 10% and 2% of the diet, respectively, as indicated in the Tables. All additions to the basal diet were made at the expense of glucose. The purity of the fatty acids used was determined by gas chromatography. The stearic acid used was essentially pure, the oleic and lineoleic acids were 68 and 77% pure, respectively.

At termination of the experimental period, the chicks were bled by cardiac puncture, then sacrificed by cervical fracture. The livers were excised, blotted to remove excess blood, weighed, frozen and stored at —20°C until analyzed. Plasma cholesterol and lipid phosphorus were determined as previously described(13). Liver fat and cholesterol were determined as described earlier(11).

* Archer-Daniels Corp., Midland, Mich.
† See Leveille *et al.*(12).

TABLE I. Weight Gain, Plasma and Liver Lipids of Chicks Fed Different Fatty Acids With or Without Cholesterol.

Dietary supplement*	Body wt gain, g	Plasma		Liver†	
		Cholesterol, mg/100 ml	Lipid phosphorus, mg/100 ml	Fat, %	Cholesterol, mg/g
No added cholesterol:					
None	216 ± 21‡	140 ± 22	14.7 ± 2.0	4.4 ± .6	3.3 ± .5
Stearic acid	129 ± 24	117 ± 14	12.0 ± 1.4	4.8 ± .7	3.6 ± .3
Oleic acid	210 ± 20	116 ± 14	12.4 ± 1.3	4.4 ± .8	3.2 ± .5
Linoleic acid	198 ± 19	136 ± 11	11.3 ± 1.2	3.9 ± .4	3.0 ± .3
2% added cholesterol:					
None	179 ± 21	206 ± 67	11.9 ± 1.5	5.5 ±1.5	8.8 ±3.9
Stearic acid	167 ± 20	138 ± 35	10.9 ± 1.7	4.7 ± .8	4.8 ±2.2
Oleic acid	205 ± 33	901 ±150	19.5 ± 2.1	12.4 ±1.4	54.6 ±8.8
Linoleic acid	182 ± 22	1320 ±386	21.4 ± 4.9	8.6 ±1.1	34.4 ±5.4

* All fatty acids were fed at a level of 10% of the diet.
† Liver values are expressed on a wet weight basis.
‡ Mean for 10 chicks ± standard deviation.

The data were evaluated statistically by means of the "t" test.

Results. Table I shows the data obtained for body weight gain and for plasma and liver lipids. The chicks fed stearic acid without cholesterol did not grow as well as did those receiving the other fatty acid supplements or the fat-free diet ($P<0.001$). The plasma cholesterol level of chicks fed a cholesterol-free diet was slightly but significantly depressed by supplements of stearic or oleic acids, but not by linoleic acid, as compared to the group fed the fat-free diet. When fed cholesterol, chicks in the group receiving the stearic acid supplement demonstrated lower plasma cholesterol levels than any other group ($P<0.001$). Linoleic and oleic acids significantly elevated plasma cholesterol levels of cholesterol-fed chicks ($P<0.001$) with chicks fed linoleic acid having the higher plasma cholesterol levels ($P>0.001$). The plasma lipid phosphorus levels followed a trend similar to that observed for plasma cholesterol. The fatty acid supplements lowered lipid phosphorus levels in chicks fed a cholesterol-free diet ($P<0.01$). In cholesterol-fed chicks, oleic and linoleic acid supplements elevated plasma lipid phosphorus levels ($P<0.001$). However, there was no significant difference between the lipid phosphorus levels of chicks fed oleic or linoleic acids. When cholesterol-supplemented diets were fed, oleic and linoleic acids significantly increased both liver fat and liver cholesterol levels ($P<0.001$). Liver fat and liver cholesterol levels were significantly higher in chicks fed oleic in comparison to linoleic acids ($P<0.001$).

Because of the disparity between liver and plasma cholesterol levels of cholesterol-fed chicks receiving oleic and linoleic acid supplements, the distribution of cholesterol between liver and plasma compartments was studied. Plasma cholesterol was calculated using a value of 7% of body weight for plasma volume(14). These data are presented in Table II. The cholesterol in the plasma and liver compartments were generally lowered by the fatty acid supplements in chicks fed a cholesterol-free diet. There were some changes in distribution of the cholesterol between the two pools. Stearic acid supplementation increased the proportion found in the liver ($P<0.010$) and linoleic acid had the opposite effect ($P<0.025$). In the cholesterol-fed chicks, stearic acid decreased plasma and liver cholesterol ($P<0.05$) but did not alter the distribution of cholesterol as compared to the group fed the fat-free diet.

Oleic and linoleic acids markedly increased cholesterol levels in both the plasma and liver compartments of cholesterol-fed chicks ($P<0.001$). Linoleic acid-fed chicks had significantly greater amounts of cholesterol in plasma but less in liver than did oleic acid-fed

TABLE II. Distribution of Cholesterol in the Plasma-Liver Pool of Chicks Fed Different Fatty Acids With or Without Cholesterol.

Dietary supplement*	Liver cholesterol	Plasma cholesterol†	Total plasma-liver cholesterol	% of total cholesterol in liver
No added cholesterol:				
None	36.7 ± 6.2‡	31.9 ± 6.2	68.6 ± 9.4	53.5 ± 5.9
Stearic acid	31.9 ± 3.4	18.6 ± 2.3	50.8 ± 10.7	63.2 ± 2.6
Oleic acid	35.9 ± 8.2	25.6 ± 2.8	61.5 ± 9.9	57.9 ± 4.8
Linoleic acid	26.2 ± 5.2	29.2 ± 3.1	55.4 ± 6.8	47.0 ± 4.8
2% added cholesterol:				
None	91.9 ± 45.2	41.3 ±13.7	134.1 ± 57.0	67.5 ± 8.0
Stearic acid	52.6 ± 27.4	26.4 ± 7.4	79.0 ± 34.1	64.8 ± 5.9
Oleic acid	814.8 ±126.5	195.7 ±30.0	1010.6 ±118.2	80.3 ± 4.2
Linoleic acid	429.8 ± 63.3	262.5 ±69.4	692.3 ± 95.7	62.5 ± 7.4

* All fatty acids were fed at a level of 10% of the diet.
† Plasma cholesterol was calculated assuming a plasma volume of 7% of body wt.
‡ Mean for 10 animals ± standard deviation.

animals ($P<0.001$). Oleic acid-fed chicks had 46% more cholesterol in the plasma-liver pool than did those fed linoleic acid. This difference was highly significant ($P<0.001$). Thus, the distribution of cholesterol between the two compartments was significantly altered ($P<0.001$) by oleic acid feeding. As can be seen from Table II, 62.5% of the plasma-liver cholesterol was found in the liver compartment of linoleic acid-fed chicks as compared to 80.3% for chicks fed oleic acid.

Discussion. The data reported demonstrate the ability of dietary oleic acid to alter the distribution of cholesterol between plasma and liver. In a previous study(11), we reported a similar effect in chicks fed dietary fats containing a high percentage of oleic acid (olive oil) or linoleic acid (corn oil). Similar results have also been reported in cholesterol-fed male rats(15). The results obtained were attributed to the fatty acid content of the dietary lipids. In the present study, similar results were obtained in chicks fed free fatty acids, thereby supporting our original conclusions.

The disparity observed in cholesterol distribution between plasma and liver cholesterol for chicks fed oleic or linoleic acids illustrates the inadequacy of plasma cholesterol measurements in reflecting the influence of a dietary lipid on cholesterol deposition. Such an observation gains significance when considered in view of the reported hypocholesterolemic effect of olive oil (predominantly oleic acid) in man(1). It is quite possible that this oil may elevate tissue cholesterol levels while depressing serum levels. Consequently, changes in serum cholesterol must be evaluated with caution.

The fatty acids fed had relatively little effect on plasma and liver cholesterol when added to the cholesterol-free diets. This observation would indicate that the alterations observed in the cholesterol-fed animals are mediated through effects on cholesterol absorption and storage rather than by an influence on synthetic and catabolic pathways.

The enhancing effect of mono- and polyunsaturated fatty acids on cholesterol absorption previously reported(16,17) is corroborated by the data herein presented. If liver and plasma cholesterol content can be taken as a measure of cholesterol absorption, chicks fed oleic or linoleic acids show a greatly enhanced cholesterol absorption as compared to chicks fed a fat-free diet (Table II). Stearic acid on the other hand appeared to depress cholesterol absorption.

Summary. Growing male chicks were fed a fat-free diet or this diet supplemented with stearic, oleic or linoleic acid, with or without added cholesterol. In cholesterol-fed chicks, dietary oleic or linoleic acid increased plasma and liver cholesterol levels and plasma lipid phosphorus levels. Oleic acid-fed chicks had lower plasma and higher liver cholesterol levels than did linoleic acid-fed animals. The total liver-plasma cholesterol pool was higher in oleic than in linoleic acid-fed chicks, but

there was a significant difference in distribution of cholesterol between these two compartments. Total liver lipids paralleled liver cholesterol levels. The fatty acids fed had relatively little effect on the plasma and liver cholesterol levels of chicks receiving a cholesterol-free diet.

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Viabilities of Frozen Mammalian Cells Following Slow Thawing.* (29661)

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For many mammalian cell lines, a freezing rate of between 1 and 40°C per minute will permit good recovery of cells when they are thawed rapidly(1), but slow thawing has been reported to reduce viability(2,3). Fast thawing of 1-ml aliquots in 1.2 ml sealed ampuls is readily achieved by agitation in a 37°C water bath. This is also true for small pieces of skin. Larger masses of tissue, such as the dog kidney, cannot be thawed at a sufficiently rapid rate by immersion in a water bath. We previously reported(4) that more rapid thawing could be produced by the use of diathermy with no apparent damage to tissue culture cells and diathermy is now being explored(5) for thawing frozen kidney. The present study was undertaken to find conditions which would produce good recovery after slow thawing of frozen mammalian cells in tissue culture with the hope that the knowledge gained might be effectively applied to preservation of larger frozen organs that would be thawed slowly. Accordingly, means were sought to protect cells against damage during slow thawing. This investigation includes a comparison of the viability of frozen mammalian cells following rapid *vs* slow thawing in the presence of glycerol or dimethylsulfoxide and with the pH of the medium adjusted to different values

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