Influence of Dietary Factors on Plasma Lipid Relationships in the Growing Chick. (26923)

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The relationship of serum phospholipid to cholesterol has received considerable attention. Data have been presented(1) demonstrating an increasing cholesterol-phospholipid (C/P) ratio in patients suffering from conditions known to be predisposing to atherosclerosis. The cholesterol-fed chicken(2,3) or rabbit(4,5) and the cholesterol-fed, thiouracil-treated dog(6) all have been shown to have higher C/P ratios than comparable controls.

The level of glyceride in serum has received little attention. Some evidence has been presented indicating that serum glyceride level may be related to atherogenesis(7).

This report presents data on the plasma lipid components of chicks fed differing levels of protein and cholesterol; the data presented illustrate differences in plasma lipid relationships in normal and cholesterol-fed chicks.

Methods. Male Hy-line chicks were used in these studies. The animals were housed in electrically heated cages with raised wire floors, were fed a commercial diet for one week, then distributed to the various treatment groups on the basis of body weight. The dietary variables introduced are indicated in the table of results and the diets were in all other respects adequate.

Body weights and food consumption were determined weekly. Food and water were supplied *ad libitum*. At the end of the experimental period, blood was drawn by cardiac puncture, heparin being used as an anticoagulant.

The collected plasma was extracted with 2:1 chloroform-methanol. The extract was evaporated to dryness under a stream of nitrogen and redissolved in hexane. The lipids were then separated by silicic acid chromatography(8). The eluted fractions were quantitated by the following methods: glycerides by the method of Van Handel and Zilversmit(9) as modified by Leveille *et al.*(10), cholesterol by the method of Searcy and Bergquist(11) and phospholipids by the method of Fiske and Subbarow(12).

Excreta were collected and homogenized; an aliquot of the freeze-dried homogenate was used for bile acid determination by the method of Mosbach *et al.*(13).

Results and discussion. The influence of dietary protein and cholesterol on weight gain, serum lipids and bile acid excretion is presented in Table I. The data demonstrate an increase in all plasma lipids at the lower protein level fed. The observed increase in plasma cholesterol is in accord with the results of previous studies(14,15,16). The increased bile acid excretion noted for the chicks fed the high protein diet is in agreement with the findings of Nishida *et al.*(17), who have shown that chicks fed a high protein diet excrete labeled cholesterol in the bile at a faster rate than do chicks fed a low level of dietary protein. The increase in plasma lipids due to cholesterol feeding is evident from the data in Table I.

The relationship between plasma lipids was studied with the data presented in Table I and similar data obtained in other studies. The correlation coefficient for total plasma cholesterol and phospholipids, in chicks fed a cholesterol-free diet, was found to be highly significant ($r = 0.90$, $P < 0.01$) (Fig. 1). Ester and free cholesterol levels were also significantly correlated to the plasma phospholipid level ($r = 0.90$ and 0.77, respectively). If the C/P ratio is calculated from any point on the regression line shown in Fig. 1, the ratio remains constant, indicating that cholesterol and phospholipid levels increase at the same rate in the chick fed an essentially cholesterol-free diet. If the data for cholesterol-fed chicks are similarly treated, a significant correlation ($r = 0.62$, $P<0.02$) between plasma cholesterol and phospholipid levels results. However, in contrast to the data for chicks fed cholesterol-free diets, calculation of C/P ratios from the regression line ($y =$

TABLE I. Influence of Dietary Protein and Cholesterol on Weight Gain, Plasma Lipids and Bile Acid Excretion in the Growing Chick.

Dietary* protein level, %	Dietary cholesterol, %	Body wt gain, g†	Plasma lipids: Cholesterol, mg/100 ml Ester	Free	Total	Glycerides, mM/l	Lipid phosphorus, mg/100 ml	Bile acid excretion, g/kg body wt/day‡
10	—	89 ± 22§	107 ± 18§	24 ± 7§	131 ± 23§	1.48 ± .63§	9.46 ± 2.49§	3.06
10	.30	84 ± 34	378 ± 86	146 ± 61	523 ± 144	3.59 ± 1.06	12.60 ± 1.63	4.71
20	—	228 ± 18	74 ± 18	27 ± 8	102 ± 20	.41 ± .19	6.41 ± 1.94	4.45
20	2.00	245 ± 21	340 ± 61	169 ± 44	509 ± 104	.95 ± .38	11.30 ± 2.63	5.49

* Sesame oil meal supplemented with 0.90% L-lysine HCl served as the protein source.
† Wt gain from 7th to 28th day; animals fed a commercial diet for days 1-6.
‡ Sum of cholic and deoxycholic acid for one 24-hr collection.
§ Mean of 8 animals ± stand. dev.

149.73 + 0.287x, where y = phospholipid level in mg/100 ml and x = total cholesterol in mg/100 ml) results in increasing ratios at the higher plasma cholesterol levels. These results demonstrate a significant difference in the relationship of these 2 lipid classes to each other in normal and cholesterol-fed chicks. It would appear from these data, that in the chick fed a cholesterol-free diet, the increase in plasma cholesterol is endogenous in nature and is accompanied by a corresponding increase in phospholipids. In the cholesterol-fed chick, the exogenous cholesterol is reflected in elevated plasma levels; and although plasma phospholipids increase in an apparent attempt to maintain a normal relationship, such is not attained and an abnormal ratio results. The significance of the C/P ratio in atherogenesis is at this time speculative; however, the possible importance of an abnormal ratio should be considered.

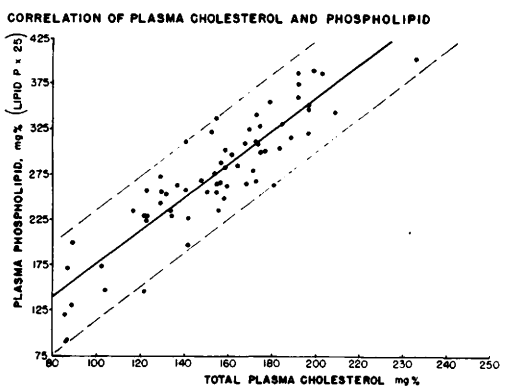

FIG. 1. Relationship of total plasma cholesterol and phospholipid levels in the growing chick. Upper and lower lines represent the 5% confidence limits calculated for the regression line (y = 1.86x − 9.26, where y = plasma phospholipids and x = total plasma cholesterol in mg/100 ml).

The chicks fed cholesterol had higher plasma glyceride levels (Table I) than did chicks fed a cholesterol-free diet; however, no correlation was found between the plasma glycerides and total cholesterol (r = 0.004) or phospholipids (r = −0.011) in the chick not receiving added cholesterol in its diet.

Summary. Chicks fed 20% dietary protein had lower plasma total cholesterol, phospholipid and glyceride levels than did chicks fed a 10% level of protein. The chicks fed the higher level of protein excreted 4.45 g of bile acids per kilo of body weight per day as compared to 3.06 g for the chicks fed the 10% level of protein. A highly significant positive correlation was observed between plasma total cholesterol and phospholipid levels in chicks fed cholesterol-free diets. The data illustrate that cholesterol and phospholipid increase at similar rates in animals not fed cholesterol, but that in the cholesterol-fed chick cholesterol increases at a faster rate, thereby resulting in an elevated cholesterol-phospholipid ratio.

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Swelling of Heart and Liver Mitochondria from Magnesium Deficient Rats and Its Reversal.* (26924)

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Heart mitochondria isolated from rats fed a magnesium deficient diet have a low P/O ratio(1). The uncoupling effect of thyroxine on oxidative phosphorylation is reversed by dietary magnesium(2). However, it is not clear whether the effect of thyroxine and magnesium is related to the biochemical or to the physical integrity of the mitochondrial system(3).

This paper presents a study of the swelling of mitochondria of the heart and liver of rats fed a magnesium deficient diet. Of particular interest is the observation that these mitochondria rapidly became swollen, although their magnesium content was not significantly decreased. ATP† reversed the swelling of mitochondria isolated from the heart and liver of both control and magnesium deficient rats. EDTA prevented the swelling of mitochondria isolated from liver(4,5), but did not prevent the swelling of heart mitochondria, which contained almost twice the amount of magnesium found in liver mitochondrial fraction.

Methods. Young albino male rats, weighing 45 to 50 g were fed diets containing the following constituents per 100 g of diet: casein (purified) 20; sucrose 30; wheat starch 34; salt-mixture of Jones Foster(6) with $CaCO_3$ and $MgSO_4$ removed, 3.0; vitamins A, D and E mixture 1.0; soybean oil 10.0; and $CaCO_3$ 2.0. The vitamins A, D and E mixture contained 5 g of halibut liver oil, 1 g of α-tocopherol acetate and 34 g of soybean oil. The amounts of the water-soluble vitamins used in the diet have been listed elsewhere(7). Magnesium was added to the control diet in the form of MgO to supply 48 mg of magnesium per 100 g of diet. The magnesium deficient diet contained less than 2 mg % of magnesium.

The rats were housed in individual cages, and fed *ad libitum*. The temperature was maintained at 20 ± 2°C. The rats were weighed twice weekly and observed daily for

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† Abbreviations: ATP, adenosine triphosphate; EDTA, ethylene-diaminetetraacetic acid; Tris, tris (hydroxymethyl) aminomethane; DPN, diphosphopyridine nucleotide.