Unsaturated Fatty Acids and Plasma Lipids

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DIETARY protein has been shown to depress the atherogenic effect of dietary cholesterol and fat in chickens.[1–3] The serum cholesterol, serum lipid and β-lipoprotein levels of birds fed a low protein diet were found to be higher than those fed a high protein diet. Furthermore, it was noted that the energy value of a diet as calculated from the total caloric intake of carbohydrate, fat and protein, had a relationship to serum cholesterol levels. The energy-to-protein (E/P) ratio of a food item for convenience can be calculated by dividing the total available calories in 100 gm. of the food item or diet by its protein content.

An analysis of variance[3] showed that at low or moderate levels of corn oil, statistically significant lower serum and carcass cholesterol levels were noted on a high protein diet as compared to a low protein diet (Table I). When the energy supplied by dietary corn oil was increased from 1.3 to 57.1 per cent, the serum cholesterol value decreased from 200 to 136 mg. per 100 ml. at a low protein level (E/P ratio 22.6) and from 166 to 129 mg. per 100 ml. at a high protein level (E/P ratio 11.5). Thus, dietary corn oil decreased serum cholesterol levels. However, the level of dietary protein influenced the cholesterol-depressing effect of corn oil in the chick, as lower serum cholesterol levels were noted in birds kept on the high protein diet as compared with those on the low protein diet. It was interesting to note that almost twice as much total carcass fat was found at an E/P ratio of 22.6 than at an E/P ratio of 11.5 or approximately 9 and 5 per cent, respectively. Variations in dietary fat or dietary protein did not alter the percentage of total carcass fat significantly as long as the E/P ratio remained constant.

The influence of the E/P ratio on serum and carcass cholesterol levels was independent of the type of dietary fat (Table II). A "hard" fat such as beef tallow gave results similar to a "soft" fat such as corn oil. That is, higher serum cholesterol levels were noted at the lower dietary protein levels whether the diet contained a "hard" or a "soft" fat. A larger percentage of carcass fat and higher carcass and serum cholesterol values were obtained at E/P ratios between 20 to 30 than at E/P ratios of 5 to 10 with either beef tallow or corn oil (Table III). However, dietary fat did have an effect over and above the E/P ratio; this effect was most pronounced at the high E/P ratios.

At comparable E/P ratios, lower serum cholesterol levels were noted in chicks fed a high fat diet as compared to a low fat diet. For example, birds fed 25 per cent beef tallow had lower serum cholesterol levels than those fed 0.2 per cent beef tallow, and those fed 20 per cent corn oil had lower levels than those fed 1 per cent corn oil. Although not strictly comparable because of differences in protein source and fat level, birds receiving 20 per cent corn oil also had lower carcass and serum cholesterol levels than those receiving 25 per cent beef tallow. On the other hand, when flooding of tissue with fat did not occur, i.e., at low fat levels, a high protein level tended to cancel out differences between the effect of "soft" versus

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TABLE I

Effect of Variations in Dietary Protein and Fat on Growth, Protein and Fat Composition and Carcass and Serum Cholesterol Levels of Chicks*

Dietary (per cent)		Energy from Fat (calories) (%)	Average Weight of Birds (gm.)	Carcass Composition (per cent)		Cholesterol Level (mg. per 100 ml.)	
Corn Oil	Protein			Fat	Protein	Carcass	Serum
E/P Ratio 22.6							
0.6	15.1	1.3	358	9.6	17.7	112	200
8.3	16.8	19.9	382	9.1	18.4	98	180
18.1	18.8	38.5	399	8.3	18.1	95	176
30.9	21.2	57.1	371	9.7	17.8	99	136
E/P Ratio 11.5							
0.6	30.0	1.3	405	4.1	18.8	87	166
8.4	33.4	19.6	449	4.7	19.0	80	140
18.2	37.7	37.9	440	5.7	18.6	76	136
31.1	42.3	56.3	434	5.6	18.7	75	129

* In this series 240 one day old male chicks were kept on the same basal diet for seven days and then divided into eight treatments of three replicates of ten birds each. They were kept in standard chick batteries in a randomized block design and fed the eight experimental rations *ad libitum* for a three-week period. Cholesterol values of six individual chicks from each treatment (two from each replicate) were used for an analysis of variance, the figures reported in this table represent an average of six cholesterol values in each treatment.

Least significant difference of serum cholesterol = 26.3 and 28.0 mg. per 100 ml. and 5 and 1 per cent levels, respectively.

Least significant difference of carcass cholesterol = 13.3 mg. per 100 ml. at 5 per cent level.

"hard" fat on serum and carcass cholesterol levels. Chicks on 1.0 per cent corn oil and 0.2 per cent beef tallow had serum cholesterol values of 194 and 233 mg. per 100 ml. at an E/P ratio of 17 to 18, and 145 and 144 mg. per 100 ml. at an E/P ratio of 7 to 8, respectively.

The percentage of total carcass fat did not seem to be influenced by the type of dietary fat. In every case the percentage of total carcass fat seemed to be directly dependent on the E/P ratio and independent of the level or type of dietary fat. These results also seemed to indicate that the capacity to store cholesterol was significantly higher when the body tissues contained a larger percentage of fat.

In another series of experiments on "hard" and "soft" fat,[4] one day old chicks were kept for four weeks on diets which contained 10 per cent lard or corn oil and from 50 to 6 per cent protein (Table IV). Statistically higher serum cholesterol values were noted in birds which had received lard than those on corn oil and 25 or

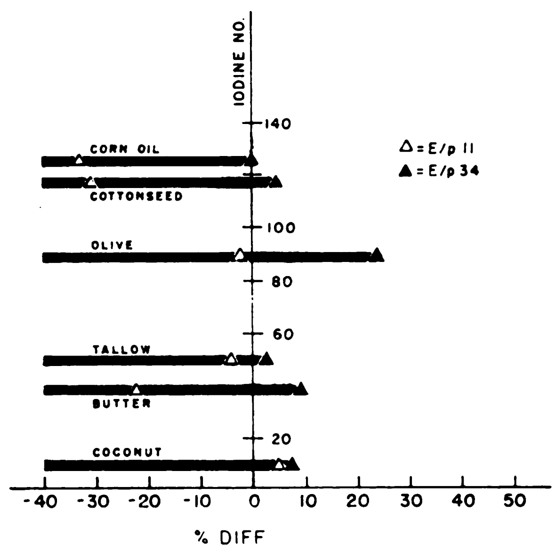

FIG. 1. The relationship of the iodine value of various fats to the serum cholesterol level in chicks fed low (E/P 34) and high (E/P 11) protein diets with deviations in mg. per 100 ml. from chicks fed corn oil at an E/P ratio of 34.

TABLE II

Effect of Energy/Protein Ratio on Percentage of Carcass Fat and Carcass and Serum Cholesterol Levels*

Dietary Protein (%)	E/P Ratio	Carcass Fat (%)	Cholesterol (mg. per 100 ml.)	
			Carcass	Serum
0.2 Per Cent Beef Tallow				
15	23	13.1	98	237
20	17	10.5	84	233
35	10	6.5	65	200
45	8	4.7	59	144
25 Per Cent Beef Tallow				
15	30	15.7	123	208
20	22	13.5	125	200
35	13	8.9	111	186
45	10	6.9	110	160

* In this series 600 one day old male chicks were divided into twenty treatments with four levels of fat (0.2, 10, 20 and 25 per cent) and five levels of protein (15, 20, 25, 35 and 45 per cent). The twenty experimental diets were fed ***ad libitum*** for a four-week period. The figures reported in this table represent an average of six cholesterol values for eight treatments, that is, only the two extreme levels of fat at each of the four protein levels. The linear regression coefficient for serum cholesterol on the basis of all twenty treatments = −2.0 mg. per cent when 1 per cent carbohydrate was replaced by 1 per cent protein.

Average body weight at 0.2 per cent tallow, 331 ± 64 gm.; at 25 per cent tallow 272 ± 45 gm.

TABLE III

Effect of Energy/Protein Ratio on Percentage of Carcass Fat and Carcass and Serum Cholesterol Levels*

Dietary Protein (%)	E/P Ratio	Carcass Fat (%)	Cholesterol (mg. per 100 ml.)	
			Carcass	Serum
1 Per Cent Corn Oil				
20	18	10.8	71	194
30	12	7.1	78	167
40	9	4.8	63	161
50	7	3.0	56	145
20 Per Cent Corn Oil				
20	23	13.9	79	159
30	15	8.6	77	119
40	12	6.2	40	114
50	9	5.0	44	105

* In this series 240 one day old female chicks were divided into eight treatments of three replicates of ten birds each and fed the experimental diets ***ad libitum*** for a three-week period. The figures reported in this table represent an average of six cholesterol values in each treatment. The linear regression coefficient = −2.2 mg. per 100 ml. when 1 per cent carbohydrate was replaced with 1 per cent protein.

Average body weight at 1 per cent corn oil, 239 ± 2.6 gm.; at 20 per cent corn oil, 241 ± 6.4 gm.

12.5 per cent protein. Thus, in comparison with lard, corn oil did have an effect over and above the E/P ratio at a particular protein level. However, this effect was most pronounced on a low protein diet of high E/P ratio; on a high protein diet of low E/P ratio, a numerical but not a statistical difference in serum cholesterol was noted between lard and corn oil.

The effect on serum cholesterol of various edible oils and fats at low and high dietary protein levels is represented in Figure 1. In this figure the serum cholesterol level of chicks fed corn oil and a low protein diet (E/P ratio, 34) has been used as a point of reference.[5–7] The results indicated that the substitution of olive oil, tallow, butterfat or coconut oil for corn oil increased serum cholesterol values in chicks. At a higher protein level (E/P ratio, 11) all of the fats except coconut oil gave lower serum cholesterol values than corn oil at an E/P ratio of 34. Thus, for chicks, the serum cholesterol value is dependent on the dietary protein level as well as the degree of unsaturation of the dietary fat.

Mattil[8] recently stated that the linoleic acid content of the visible fat in our diet has increased from 6 per cent in 1880 to 17 per cent in 1956 (Table V). In order to determine whether a shift in the consumption of a mixture of edible fats can shift serum cholesterol values in chicks, a mixture of fats was prepared from the per capita consumption of visible fats in 1921 as compared with 1956 (Table VI). These data and methods of procedure suffer from two defects: one, whether or not the per capita consumption of fat as listed[9] is accurate;

TABLE IV
The Effect of the E/P Ratio and the Type of Dietary Fat on Weight Gain and Serum Cholesterol Level in Chickens

Dietary Protein (%)	E/P Ratio	10 Per Cent Dietary Fat				
		Lard		Corn Oil		
		Average Weight Gain (%)	Serum Cholesterol (mg. per 100 ml.)	Average Weight Gain (per cent)	Serum Cholesterol (mg. per 100 ml.)	Difference (mg. per 100 ml.)
50.0	8	415	119	430	95	+24
25.0	16†	450	178	474	117	+61
12.5	32†	256	268	281	194	+74
9.0	45†	170	271	172	194	+77
6.0*	67†	47	222	63	206	+16

* Chicks fed a diet containing 6 per cent protein showed extremely poor growth. Four out of sixteen birds were lost during the experiment because of protein deficiency.
† Difference in serum cholesterol levels between lard and corn oil treatments significant at 5 per cent level.
Linear regression coefficient for serum cholesterol: Lard = +1.74, mg. per 100 ml. when E/P ratio increased by one; corn oil = +1.94, mg. per 100 ml. with E/P ratio increased by one.

and two, whether or not the composition of the fats themselves have changed. The latter may not be true for lard and butterfat, but the composition of the shortenings and margarine offered for sale in 1956 are not similar to those on sale in 1921.

A simulated mixture of butterfat, lard, shortening and margarine bought as stock items in a supermarket and formulated as listed for the per capita consumption for 1921 and for 1956 was fed to chicks at a 10 per cent level in a low and high protein diet. The results indicated that on a low protein diet (E/P ratio of 34) both mixtures increased the serum cholesterol values when compared with corn oil (Fig. 2). However, on a high protein diet at an

TABLE V
Total Percentage of Linoleic and Saturated Fatty Acids Consumed in Visible Fats[8]

Year	Linoleic Acid (%)	Saturated Acids (%)
1880	6	49
1910	17	43
1920	13	45
1956	17	33

TABLE VI
Per Capita Consumption of Fats[9] (in pounds)

Fat	1921	1956
Butter	16.0	8.7
Lard	10.6	9.9
Margarine	2.0	8.2
Shortening	6.9	10.9
Edible oils	3.5	10.0

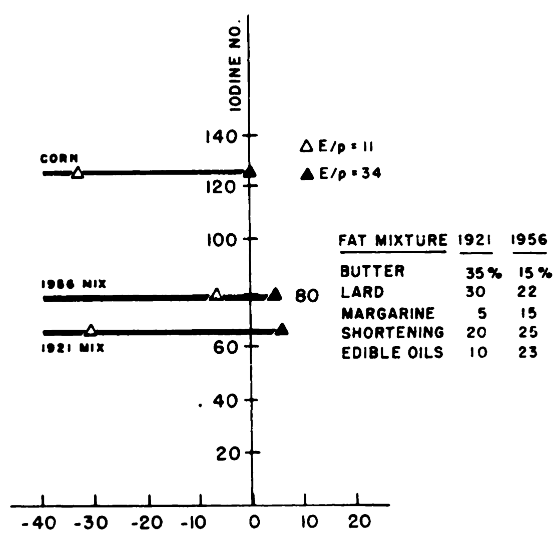

FIG. 2. The relationship of dietary protein level to the serum cholesterol level in mg. per 100 ml. in chicks fed two composite fat mixtures as compared with chicks fed corn oil.

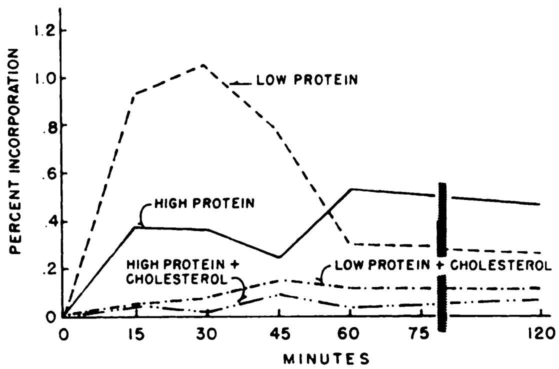

FIG. 3. The percentage of incorporation of acetate-1-C^{14} into liver cholesterol in chicks which had been kept on low and high protein diets.

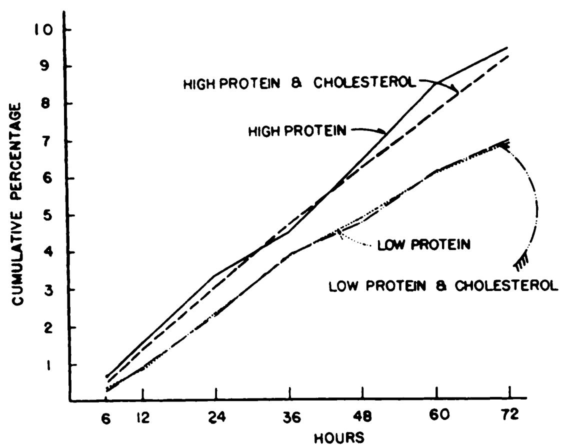

FIG. 4. The cumulative percentage excretion of cholesterol-4-C^{14} as bile acids obtained from chicks kept on low and high protein diets.

E/P ratio of 11, the 1921 mixture gave results which were closer to corn oil than the 1956 mixture in spite of the higher iodine value of the latter. Thus, there may be other factors in addition to the dietary protein level and the degree of unsaturation in the dietary fat which influence serum cholesterol levels in the chick.

The manner in which dietary protein lowers serum cholesterol levels in chicks is not known. Preliminary results in our laboratory indicate that the anabolism as well as the catabolism of cholesterol is influenced by the dietary protein level. A higher initial incorporation of acetate-1-C^{14} into cholesterol occurred in the livers of birds which had been kept on the low protein diets as compared with those on the high protein diets (Fig. 3). The presence of dietary cholesterol stimulated the incorporation of more acetate-1-C^{14} into liver cholesterol in chicks which had been kept on the low protein diet as compared with those on the high protein diet. However, dietary cholesterol significantly depressed the rate of incorporation of acetate-1-C^{14} into cholesterol. Although dietary cholesterol depressed cholesterol biosynthesis, it stimulated the rate of incorporation of acetate-1-C^{14} into fatty acids. The ratio of incorporation of acetate-1-C^{14} into fatty acids was larger in the presence of dietary cholesterol than in the absence of cholesterol.

The results of studies of cholesterol catabolism indicated that more cholesterol-4-C^{14} was excreted by birds which had received the high protein diet as compared with those on the low protein diet during a seventy-two-hour test period (Fig. 4). The presence of dietary cholesterol did not seem to affect the rate of cholesterol-4-C^{14} excretion. Chicks in which the bile duct had been cannulated excreted only 8 per cent of the cholesterol-4-C^{14} as bile acids, while rats excreted 52 per cent of cholesterol-4-C^{14} as bile acids during the same period of time. Furthermore, the increased flow of bile due to the cannulation itself was a factor in cholesterol metabolism.

Cannulation of the bile duct caused a significant decrease in serum cholesterol and serum lipoprotein levels, while ligation of the bile duct caused a significant increase. In the latter case the lipoprotein pattern became more heterogeneous with a significant increase of the lipoprotein in the low S_f range. These results indicated a close relationship between bile flow and serum cholesterol and lipoprotein levels.

It is thus apparent that both the anabolism and catabolism of cholesterol are influenced by nutritional as well as physiologic factors. However, how these factors influence the development of atherosclerosis is still to be determined.

Dam and co-workers[10,11] noticed an increase in the cholesterol content of the aorta of the chick when fat and cholesterol had been included in the dietary regimen. This increase occurred whether the fat was unhydrogenated or hydrogenated. However, as Geer et al.[12] have pointed out, an analysis for cholesterol

TABLE VII
Lipid Content of Human Aorta

	Extractable Lipid (%)
Adventitia	9.5
Media	2.9
Intima with lesion	4.9
Intima without lesion	1.3

content of the entire aorta may not reflect differences in the cholesterol content of the involved and non-involved areas. We have noted differences in the total lipid content of the adventitia, media and intima layer of a human aorta (Table VII). Furthermore, the involved area contained more extractable lipid than the non-involved area.

CONCLUSIONS

It is evident dietary as well as physiologic factors influence the serum cholesterol level. Although the essential unsaturated fatty acids may be involved in cholesterol transport, it does not necessarily follow that an excess of dietary linoleic acid is desirable. The proportion of dietary carbohydrate, fat and protein over a protracted period of time may influence the degree of formation of plaques and atherosclerosis more dominantly than "soft" fats. It seems that the arterial wall *per se* should receive as much study as the serum cholesterol value.

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