tain a variety of carbonyl and carboxyl compounds, indicating a random oxygen attack of the ester molecule. The ester was analyzed for carboxyl, ester, carbonyl, and hydroxyl groups and for unsaturation. Increased chain length showed increased susceptibility to oxidation. From the analytical values and the decomposition products a possible mechanism of thermal oxidation through a free radical system has been proposed. Unsaturation; hydroperoxide formation; instantaneous decomposition of the hydroperoxide to form various hydroxy, carbonyl, and carboxyl compounds; decomposition of these compounds to release carbon dioxide, carbon monoxide, and water; and polymerization through either carbon-carbon linkages or ester linkages have been suggested as a possible mechanism of thermal oxidation of fatty acid esters.

REFERENCES

1. Holman, R. T., "Progress in the Chemistry of Fats and Other Lipids," 2, 51, Academic Press Inc., New York (1954).
2. Powers, P. O., Ind. Eng. Chem., *41*, 304 (1949).
3. Bateman, L., Quart. Revs., Chem. Soc. (London), *8*, 147 (1954).
4. Farmer, E. H., and Sutton, D. A., J. Chem. Soc., 122 (1943).
5. Atherton, D., and Hilditch, T. P., J. Chem. Soc., 204 (1943).
6. Ross, John, Gebhart, A. I., and Gerecht, J. F., J. Amer. Chem. Soc., *71*, 282 (1949).
7. Swift, C. E., Dollear, F. G., and O'Connor, R. T., Oil and Soap, *23*, 355 (1946).
8. Skellon, J. H., J. Chem. Soc., 2020 (1950).
9. Zuidema, H. H., Chem. Rev., *38*, 197 (1946).
10. Kirk, R. E., and Othmer, D. F., "Encyclopedia of Chemical Technology," 1st Supplement, 401, The Interscience Encyclopedia Inc., New York (1957).
11. Shoruigin, P. P., and Kreshkov, A. P., J. Gen. Chem. (U.S.S.R.), *3*, 825 (1933); C. A. *28*, 6106 (1934).
12. Johnson, O. C., and Kummerow, F. A., J. Am. Oil Chem. Soc., *34*, 407 (1957).
13. Ellis, Rex, Gaddis, A. M., and Currie, G. T., Anal. Chem., *30*, 475 (1958).
14. Inouye, Y., and Noda, M., J. Agr. Chem. (Japan), *24*, 291 (1951).
15. "Official and Tentative Methods of the American Oil Chemists' Society," editor, V. C. Mehlenbacher, Chicago, Ill. (1946).
16. Smith, W. T. Jr., and Shriner, R. L., "The Examination of New Organic Compounds," 113, J. Wiley and Sons Inc., New York (1956).
17. Bryant, W. M. D., and Smith, D. M., J. Am. Chem. Soc., *57*, 57 (1935).
18. Fritsch, C. W., and Deatherage, F. E., J. Am. Oil Chem. Soc., *33*, 109 (1956).
19. Deatherage, F. E., and Mattill, H. A., Ind. Eng. Chem., *31*, 1425 (1939).
20. Sunderland, E., J. Oil Colour Chem. Assoc., *28*, 137 (1945).
21. Nobori, H., J. Soc. Chem. Ind. (Japan), *45*, Suppl. Binding, 453 (1942).
22. Perkins, E. G., Ph.D. thesis, Department of Food Technology, University of Illinois, Urbana, Ill. (1958).

[Received December 8, 1958]

The Relationship of Corn Oil and Animal Fats to Serum Cholesterol Values at Various Dietary Protein Levels [1, 2]

M. G. KOKATNUR and F. A. KUMMEROW, Department of Food Technology, University of Illinois, Urbana, Illinois

THE NEED for a high fat, high cholesterol diet for the development of hypercholesteremia in a reasonable period of time has been recognized in many previous studies (1). In fact, these studies have been interpreted to indicate that a low fat, low cholesterol diet may serve as the best means of preventing hypercholesteremia (2, 3). An over-emphasis on dietary fat may have prevented consideration of other dietary components such as protein (4, 5). It would seem that a high fat diet would automatically lower protein intake as total food consumption would decrease. Furthermore, in experimental studies on the dietary factors which influence serum cholesterol levels, added fats and oils such as butter, margarine, and corn oil rather than whole milk, eggs, and meat have served as dietary sources of fat. The added fats upset dietary fat to protein ratios more than food items which contain both fat and protein. It would seem therefore that comparisons in diet in which only the fat is varied may yield misleading results or may be difficult to interpret.

In previous studies (6) we have attempted to correlate the fat and protein and the total metabolizable energy to the protein or E/P ratio of the diet with changes in carcass and serum cholesterol levels. In this presentation we have used previous studies as a means of introducing new data on the relationship of corn oil and animal fats to serum cholesterol levels.

[1] Presented at the 32nd Annual Fall Meeting, American Oil Chemists' Society, October 21, 1958, Chicago, Ill. The work was supported by research grant No. H-1819 from the National Institute of Health, U. S. Public Health Service, Department of Health, Education and Welfare.
[2] Portion of a thesis to be presented by M. G. Kokatnur as partial fulfillment of the requirements for the degree of Doctor of Philosophy in Food Technology.

Methods

In most of our studies, groups of chicks (New Hampshire Columbian Cross) were kept in three replicates of 10 birds each, on a nutritionally complete basal diet (Table I). The various experimental diets listed in Tables II, IV, and VI were modifications of the basal diet shown in Table I. The fat and protein levels of these diets were adjusted at the expense of Cerelose. The basal diet furnished all of the known amino acid, vitamin, and mineral requirements of the chick. The birds were weighed at weekly intervals, and the experiments were terminated after three to four weeks. Five ml. of blood from the two median weight chicks in each replicate were obtained *via* heart puncture, and in some treatments the weighed individual carcasses were digested with concentrated hydrochloric acid (1 ml./g. tissue) at 50°C. for 24

TABLE I
Composition of the Basal Diet

Ingredients	Percentage
Cerelose	57.11
Dracket assay protein	35.30
DL-Methionine	0.75
Glycine	0.30
Glista salts [a]	5.34
Choline chloride	0.20
Corn oil	1.00
Vitamins	+
Total	100.00

[a] (11) 0.88% NaCl, 0.002% $ZnCl_2$ 0.002% $CuSO_4 \cdot 5H_2O$, 0.0009% H_3BO_3, 0.0001% $CoSO_4 \cdot 7H_2O$, 0.004% KI, 0.14% Fe Citrate, 0.25% $MgSO_4 \cdot 7H_2O$, 0.90% K_2HPO_4, 0.065% $MnSO_4 \cdot H_2O$, 0.30% $CaCO_3$, 2.80% $Ca_3(PO_4)_2$.

hrs. The digest was cooled to room temperature, and the fat was removed by three separate extractions with Skellysolve F. The serum and carcass cholesterol values were determined by the Schoenheimer and Sperry method (7). All data were subjected to a statistical analysis (8).

Results and Discussion

The results indicated that the serum cholesterol levels were significantly lower at the higher protein levels (Table II). At a low-protein level a methionine-deficient diet significantly elevated serum cholesterol levels. A diet low in choline also significantly increased serum cholesterol levels at the 15% protein level but not in those fed higher levels of protein. A low choline, low methionine diet significantly increased serum cholesterol levels, but this elevating effect was again not obtained at the higher protein levels (9). The fact that the low-protein, methionine-deficient diet approximately doubled the serum cholesterol levels in our chicks, when compared with those on the low-protein choline deficient diet, agreed with the observation of Mann *et al.* (10). They found that, in cebus monkeys, hypercholesterolemia and consequent atherosclerosis were dependent upon a deficiency of sulfur amino acids but not of labile methyl groups. However the beneficial effect of high protein diets in preventing a cholesterol type of fatty liver cannot be explained wholly by the presence of methionine or by the lipotropic action of protein as a precursor of choline. The presence of adequate amounts of amino acids may therefore be necessary to provide for normal lipide and cholesterol metabolism.

TABLE II (9)
Comparison of Total Serum Cholesterol Values (mg. %) on Various Diets

Prot. level (%)	Complete diet	Low methionine diet	Low choline diet	Low methionine low choline
15	261 ± 38[a]	706 ± 117	356 ± 44	583 ± 121
20	219 ± 20	....	....	477 ± 64
30	222 ± 15	222 ± 26	190 ± 13	212 ± 15
35	173 ± 9	....	....	195 ± 9

[a] Mean ± standard error of mean.

An adequate level of dietary protein prevented an abnormal increase in serum cholesterol when carbohydrate was replaced by fat (Table III). The incorporation of increasing amounts of beef tallow to the point where more than 50% of the caloric intake was supplied by fat did not increase the serum cholesterol level significantly, when compared with the serum cholesterol level of birds which had received no dietary fat. In fact, a statistical analysis of the data seemed to indicate that some dietary fat was desirable.

TABLE III*
Serum Cholesterol Levels in Chicks Fed Varying Amounts of Protein and Beef Tallow

	Serum cholesterol as mg. %			
Protein Tallow	0.2%	10%	20%	25%
45%	144	168	141	160
35	200	206	193	186
25	219	168	190	201
20	233	215	187	200
15	237	224	216	208

Average body weight at 0.2% tallow, 331 ± 64 g.; at 25% tallow, 272 ± 45 g.

* Linear regression analysis. Average linear regression coefficient for serum cholesterol at all protein levels = −1.1 mg. % when 1% carbohydrate was replaced by 1% tallow.

The addition of (0.1 or 0.3%) dietary cholesterol did not influence significantly the effect of dietary protein on either a low or a high level of dietary fat (Table IV). There were no differences in the average weight gains between the group receiving 1% or 20% corn oil. The presence of dietary cholesterol did not seem to influence the feed consumption and weight gain at each dietary protein level. In spite of a lower protein intake the serum cholesterol values were lower in chicks which had received 20% corn oil as compared with those on 1% corn oil. Dietary corn oil did seem to help lower serum cholesterol, especially at a high dietary protein level. At a 40% level of protein and 20% corn oil, four-week-old chicks had numerically lower serum cholesterol levels than those on only 1% corn oil.

An inverse linear relationship seemed to exist between the serum cholesterol level and the absolute intake of protein. The largest increase in serum cholesterol was noted in birds which had consumed the least amount of protein (5). No apparent relationship existed between serum cholesterol and differences in caloric (calculated metabolizable energy) intake or differences in the percentage of calories supplied by dietary fat when the protein intake was high or adequate.

These results did not indicate however whether dietary protein caused a mere shift of cholesterol from the serum to the carcass or actually aided in the catabolism of cholesterol. We therefore kept various groups of day-old chicks on diets of varying fat and protein levels and after four weeks determined the amount of carcass fat and cholesterol as well as the serum cholesterol level (Table V). The fat and protein were so adjusted as to result in an energy-to-protein ratio of 22.6 and 11.5; the latter diets thus contained approximately twice as much protein as the former at each level of fat. The E/P ratio of a a food item or diet can be calculated by dividing the total available calories in 100 g. of the food item or diet by its protein content.

TABLE IV
Relationship of Dietary Protein, Fat, and Cholesterol to Serum Cholesterol Levels

Protein	Av. feed cons.	1% Dietary corn oil Dietary cholesterol			Av. feed cons.	20% Dietary corn oil Dietary cholesterol		
		0.0	0.1	0.3%		0.0	0.1	0.3%
%	g.	mg.%	mg.%	mg.%	g.	mg.%	mg.%	mg.%
20	364	170	173	190	294	179	175	182
30	346	167	166	165	283	147	139	160
40	318	140	162	150	272	117	118	109

These results indicated that the E/P ratio had a significant effect on serum cholesterol levels. At low or moderate levels of corn oil, statistically significant lower serum and carcass cholesterol levels were noted at an E/P ratio of 11.5 as compared to a ratio of 22.6. At a high level of corn oil, lower carcass but not lower serum cholesterol values were noted at an E/P ratio of 11.5. When (6) the energy supplied by dietary corn oil was increased from 1.3 to 57.1%, the serum cholesterol value decreased from 200 to 136 mg. % at an E/P ratio of 22.6 and from 166 to 129 mg. % at an E/P ratio of 11.5.

The influence of the E/P ratio on serum and carcass cholesterol levels was independent of the type of dietary fat. A "hard" fat such as beef tallow gave results similar to a "soft" fat, such as corn oil. How-

TABLE V (6)

Effect of Variations in Dietary Protein and Fat on Growth and Fat Content and Carcass and Serum Cholesterol Levels of Chicks

Dietary Corn Oil	Dietary Protein	Energy from fat (calories)	Average weight of birds	Carcass fat	Cholesterol level Carcass	Cholesterol level Serum
%	%	%	g.	%	mg.%	mg.%
E/P ratio 22.6						
0.6	15.1	1.3	358	9.6	112	200
8.3	16.8	19.9	382	9.1	98	180
18.1	18.8	38.5	399	8.3	95	176
30.9	21.2	57.1	371	9.7	99	136
E/P ratio 11.5						
0.6	30.0	1.3	405	4.1	87	166
8.4	33.4	19.6	449	4.7	80	140
18.2	37.7	37.9	440	5.7	76	136
31.1	42.3	56.3	434	5.6	75	129

Least significant difference of serum cholesterol = 26.3 mg. % at 5% level.
Least significant difference of carcass cholesterol = 13.3 mg. % at 5% level.
The basal diet for this experiment has been given in Table I in (9).

ever dietary fat did have an effect over and above the E/P ratio at a particular fat level as seen from results of an experiment in which either corn oil or lard was used at the same E/P ratio. This effect was most pronounced at the high E/P ratios (Table VI). At low E/P ratios only a numerical but not a statistical difference in serum cholesterol was noted between lard and corn oil. However as the E/P ratio increased, these differences became significant and lower serum cholesterol levels were noted in birds which had been fed corn oil. The data were subjected to regression analysis to study the trend of the cholesterol levels between lard and corn oil treatments (Figure 1).

We have previously shown that abnormal amounts of lipide are not found in the serum unless the protein level of the diet is inadequate (9). Lipoproteins therefore seem to be needed to mobilize fat and to help increase the lipide storage capacity of the tissue. As amino acids are dynamically involved in protein metabolism, the sporadic intake of protein may not insure an adequate and continual supply of lipide-carrying proteins. A good E/P ratio in breakfast, lunch, and dinner from day to day may be favorable for a high lipide-carrying capacity. Caloric intake and protein seem to have a complementary effect on general lipide metabolism. A beefsteak twice a week may add substantially to the average total protein intake but may not make up for a coffee and doughnut type of lunch the other days of the week.

TABLE VI*

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% Dietary fat, Lard: Average weight gain	Lard: Serum cholesterol	Corn oil: Average weight gain	Corn oil: Serum cholesterol
%		g.	mg.%	g.	mg.%
50.0	8	415	119	430	95
25.0	16[a]	450	178	474	117
12.5	32[a]	256	268	281	194
9.0	45[a]	170	271	172	194
6.0[b]	67[a]	47	222	63	206

[a] Difference in serum cholesterol levels between lard and corn oil treatments significant at 5% level.
[b] Chicks fed a diet containing 6% protein showed extremely poor growth. Four out of 16 birds were lost during the experiment because of protein deficiency.
* Linear regression analysis Fig. 1
Linear regression coefficient for serum cholesterol
Lard = +1.74, mg. % when E/P ratio increased by one.
Corn oil = +1.94, mg. % when E/P ratio increased by one.

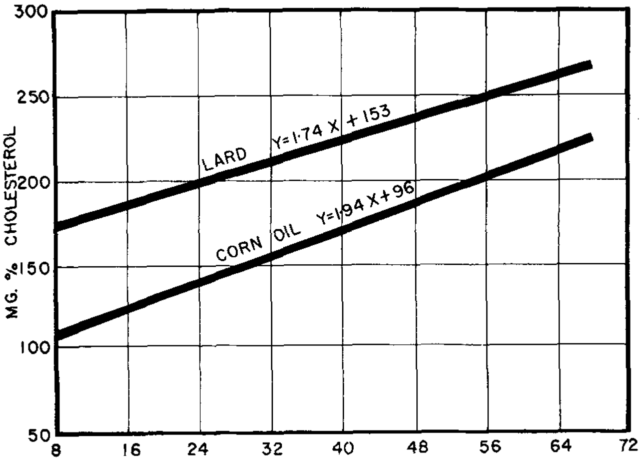

FIG. 1. Serum cholesterol vs. E/P ratio at 10% level of fat (* footnote Table IV).

It is conceivable that an excessive intake of protein is not desirable as the excess would be deaminated, and some of these resulting de-aminated amino acids could also be converted to acetyl coenzyme A and fat or to cholesterol. However, if one assumes that cholesterol and other serum lipides are connected with atherogenesis, then the relationship of the E/P ratio to the lipide-carrying capacity of the serum may be important to atherosclerosis.

Summary

A high level of dietary protein lowered serum cholesterol. Incorporation of increasing amounts of beef tallow did not increase the serum cholesterol levels significantly, and some dietary fat was found to be desirable. Addition of up to 0.3% of dietary cholesterol did not significantly increase the serum cholesterol levels on either low or high levels of dietary fat or protein. The energy protein (E/P) ratio of a diet had a significant effect on serum and carcass cholesterol levels. The type of fat had an effect on serum cholesterol levels, but with lowering of the E/P ratio this effect became negligible.

Acknowledgment

The authors wish to thank A. Ueno and T. Nishida for their valuable help and H. M. Scott for advice and use of facilities in the poultry department.

REFERENCES

1. Katz, L. N., and Stamler, Jeremiah, "Experimental Atherosclerosis," Charles C. Thomas Publishing Company, Springfield, Ill. (1953).
2. Keys, Ancel, J. Chron. Dis., *4*, 364 (1956).
3. Katz, L. N., Stamler, Jeremiah, and Pick, Ruth, Fed. Proc., *15*, 885 (1956).
4. Moyer, A. W., Kritchevsky, David, Logan, J. B., and Cox, H. P., Proc. Soc. Exper. Biol. and Med., *92*, 736 (1956).
5. Kokatnur, Mohan, Rand, N. T., Kummerow, F. A., and Scott, H. M., J. Nutrition, *64*, 177 (1958).
6. Kokatnur, Mohan, Rand, N. T., and Kummerow, F. A., Circulation Research, *6*, 424 (1958).
7. Schoenheimer, Rudolph, and Sperry, W. M., J. Biol. Chem., *106*, 745 (1934).
8. Duncan, D. E., Biometrics, *11*, 1 (1955).
9. Nishida, Toshiro, Takenaka, Fumio, and Kummerow, F. A., Circulation Research, *6*, 194 (1958).
10. Mann, G. V., Andrus, S. B., McNally, A., and Stare, F. J., J. Exper. Med., *98*, 195 (1953).
11. Glista, W. A., doctoral thesis, University of Illinois (1951).

[Received December 12, 1958]