Effects of Medium- and Long-Chain Saturated Triglycerides on Blood and Liver Cholesterol of Chickens and Rats.* (29225)

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The availability of synthetic, edible fats with a simplified fatty acid composition marked an advance in ultimate clarification of the complex effects which natural fats exert on blood lipids. Although several workers (1,2) have suggested that degree of fatty acid unsaturation was closely related to the hypocholesterolemic effects of certain oils, others (3,4,5) have observed either a serum-cholesterol-lowering effect or no adverse effect in human subjects following ingestion of a medium-chain, saturated triglyceride.

In studies on rats it had also been noted that the animals fed saturated triglycerides of C_6-C_{10} chain length (MCT) had significantly lower serum cholesterol values than those given saturated triglycerides of C_{12} to C_{18} chain length (LCT). Low serum cholesterol values were associated with low liver cholesterol levels and vice versa(6,7). In the present investigations, the plasma and liver cholesterol response of the chicken to feeding of MCT and LCT was compared with that of the rat.

Experimental. MCT and LCT were prepared from coconut oil and other palm kernel oils by fractionation of the split fatty acids and reconstitution into triglycerides of the desired fractions after hydrogenation. MCT represented about 15% of the original coconut oil and was a clear, thin, odorless liquid containing carbon chains C_6 to C_{10}. LCT represented about 60% of the original oil and was a solid fat containing carbon chains C_{12} to C_{18}.

Groups of 30 male, cross-bred chickens were placed on the experimental diets at one day of age for a period of 4 weeks. In one experiment MCT or LCT was provided at 8% and in the other at 12% of the total diet. The basal diet used was a conventional corn-soybean chick starting ration supplying approximately 2% ether extractable material. At 2 weeks and again at 4 weeks of age blood was drawn by heart puncture for plasma cholesterol determination using the extraction procedure of Searcy and Bergquist(8) followed by the colorimetric method of Zlatkis *et al*(9). At the end of the second experiment 10 birds from each treatment group were sacrificed with chloroform; their livers were quickly removed, blotted dry, weighed, and subsequently homogenized with chloroform-methanol (2:1). Lipids were then cold-extracted by continuous shaking, followed by filtration, and cholesterol determined by the Zlatkis method.

Matching groups of 8 weanling rats of the Columbia-Sherman strain were placed on highly purified, complete diets. The latter contained 20% fat and 30% casein and were consumed until death. Details of the animals' selection and the diet composition have been previously reported(6). The rats were anesthetized with chloroform, blood was withdrawn from the heart, and serum cholesterol determined according to Schoenheimer and Sperry(10).

Results. Table I shows the results obtained for growing chickens. Plasma cholesterol was significantly higher ($P<0.01$) for the animals fed 8 or 12% MCT than for those given either level of LCT. The differences were greater at 2 weeks than at 4 weeks. The 4-week comparison of the control group receiving no fat with those receiving MCT or LCT showed that MCT increased the plasma cholesterol level beyond the normal level, but that LCT did not effect a reduction below normal. At the 12% level of triglyceride supplementation there was a marked growth retardation, particularly with MCT. Except for the reduced food intake and growth rate, however, the birds appeared

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TABLE I. Effect of Medium- and Long-Chain Saturated Triglycerides on Growth Rate and Blood and Liver Cholesterol in the Chicken.

Fat supplement*	Body wt (g) 2 wk	Body wt (g) 4 wk	Plasma cholesterol (mg %) 2 wk	Plasma cholesterol (mg %) 4 wk	Liver cholesterol (mg % wet wt) 4 wk
MCT, 8%	125 ± 2†	303 ± 10	156 ± 7	139 ± 4‡	
LCT, 8%	127 ± 3	314 ± 10	118 ± 4	128 ± 3‡	
None	162 ± 4	390 ± 9	151 ± 7	142 ± 5	468 ± 5
MCT, 12%	126 ± 4	296 ± 8	178 ± 9	162 ± 6	448 ± 15
LCT, 12%	150 ± 4	348 ± 13	125 ± 8	141 ± 3	481 ± 10

* MCT—medium-chain, saturated triglyceride; LCT—long-chain, saturated triglyceride.
† Mean body weights ± S.E. for group of 30 male chickens per treatment.
‡ Mean values ± S.E. for 19 birds per treatment; for all other plasma and liver cholesterol determinations 10 birds per treatment were used.

healthy. Liver cholesterol values show the opposite picture from that observed for plasma. They were lowest for the MCT-fed birds and significantly higher ($P<0.01$) for the LCT-fed birds.

Table II gives serum cholesterol values for rats receiving either MCT or LCT. In contrast with the chicken studies, MCT-fed rats had significantly lower ($P<0.01$) serum cholesterol levels than LCT-fed animals. With the younger rats a slightly reduced growth rate was associated with MCT feeding, as it had been for the chickens.

Discussion. The results emphasize the caution that must be exercised in generalizing experimental results obtained with one animal species to another. Thus, the blood cholesterol results previously observed in the rat (7) and reconfirmed by the present study, were just the opposite of those observed with growing chickens. During the early weeks of life, fat constitutes a relatively more important source of calories for the chicken than for the rat since after hatching an appreciable amount of yolk material is still available for absorption. The large percentage of fat from unabsorbed yolk may be responsible also for the differences in blood cholesterol values noted between the 2- and 4-week sampling periods.

TABLE II. Effect of Medium- and Long-Chain Saturated Triglycerides on Growth Rate and Serum Cholesterol of Rats.

Fat supplement*	Body wt (g)	Serum cholesterol (mg %)
MCT, 20%	248 ± 10†	73 ± 4
LCT, 20%	262 ± 13	112 ± 7
MCT, 20%	391 ± 11‡	68 ± 4
LCT, 20%	393 ± 12	96 ± 8

* MCT—medium-chain, saturated triglyceride; LCT—long-chain, saturated triglyceride.
† Mean body weights ± S.E. for groups of 8 male albino rats, 86 days old.
‡ Mean body weights ± S.E. for groups of 8 male albino rats, 156 days old.

The association of low liver cholesterol with high serum cholesterol in chickens as well as the opposite relationship in rats fed either a low-fat diet(11) or one containing corn oil(12) shows how difficult it is to predict organ cholesterol concentration from serum levels.

The present findings with saturated triglycerides challenge the concept that only polyunsaturated fatty acids possess hypocholesterolemic activity. Elsewhere(13) we have reported evidence that avian atherogenesis is retarded by the sterol and not by the unsaturated fatty acid fraction of certain natural fats.

Summary. Studies were carried out with chickens and rats to relate the ingestion of medium- and long-chain saturated triglycerides to the blood and liver cholesterol level. It was found that MCT significantly elevated the plasma cholesterol level of chickens compared with LCT. The reverse observation was made in rats where MCT was effective in lowering serum cholesterol. On the other hand, MCT-fed chickens and rats had a significantly lower liver cholesterol level than LCT-fed individuals. The significance of these findings with reference to the relationship between dietary fat and blood lipid concentration is discussed.

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1. Ahrens, E. H., Jr., Hirsch, J., Insull, W., Jr., Tsaltas, T. T., Blomstrand, R., Paterson, M. L., *Lancet,* 1957, v1, 943.

2. Keys, A., Anderson, J. T., Grande, F., *ibid.,* 1957, v2, 959.

3. Kaunitz, H., Slanetz, C. A., Johnson, R. E., Babayan, V. K., *Fed. Proc.,* 1959, v18, 485.

4. Hashim, S. A., Arteaga, A., Van Itallie, T. B., *Lancet,* 1960, v1, 1105.

5. Beveridge, J. M. R., Connell, W. F., Haust, H. L., Mayer, G. A., *Can. J. Biochem. Physiol.,* 1959, v37, 575.

6. Kaunitz, H., Slanetz, C. A., Johnson, R. E., *J. Am. Oil Chem. Soc.,* 1959, v36, 322.

7. Kaunitz, H., *Metabolism,* 1962, v11, 1187.

8. Searcy, R. L., Bergquist, L. M., *Clin. Chim. Acta,* 1960, v5, 192.

9. Zlatkis, F., Zak, B., Boyle, A. J., *J. Lab. Clin. Med.,* 1953, v41, 486.

10. Schoenheimer, R., Sperry, W. M., *J. Biol. Chem.,* 1934, v34, 581.

11. Alfin-Slater, R. B., Aftergood, L., Wells, A. F., Deuel, H. G., Jr., *Arch. Biochem. and Biophys.,* 1954, v52, 180.

12. Avigan, J., Steinberg, D., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 814.

13. Fisher, H., Weiss, H. S., Griminger, P., *ibid.,* 1963, v113, 415.

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Enhancement of Vascular Permeability by Mixtures of C'1 Esterase and Normal Serum.* (29226)

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Recent investigations have demonstrated that purified human C'1 esterase, an enzyme derived from the first component (C'1) of complement, has the ability to increase vascular permeability in guinea pig skin(1). By means of inhibitors, inactivation by heat and pH, and use of subcomponents of C'1, it was shown that the permeability activity was referable to C'1 esterase rather than an extraneous factor in the preparation. Histopathological studies, including electron microscopy, and the suppressive effect of an anti-histaminic drug were consistent with the hypothesis that enhanced vascular permeability might be mediated by release of a histamine-like substance.

The present study was undertaken to test the possibility that C'1 esterase functioned as a permeability factor by interacting with other humoral substances to form an agent capable of releasing a pharmacologic effector of inflammation. Experiments were performed in which concentrations of C'1 esterase, subthreshold for direct action in guinea pig skin, were added to guinea pig serum *in vitro* and the mixtures then tested for permeability activity *in vivo*. The data to be presented, in fact, provide evidence for the participation of serum constituents in the mechanism of action of C'1 esterase. Preliminary experiments further suggest the possible identity of these serum constituents with the complement system.

Materials and methods. Vascular permeability was tested in guinea pig skin according to Miles and Wilhelm(2), as further described by Ratnoff and Lepow(1). *Phosphate buffered saline* (PBS), solution A of Dulbecco(3), was used as diluent in all permeability assays. *Purified human C'1 esterase* was prepared and assayed by the methods of Haines and Lepow(4). The preparations employed

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