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# Methionine Decholesterolization in Old Hens.*†

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Choline has been shown in our previous studies to have a decholesterolizing effect in old hens.[1] Methionine has been found to take part in a reversible reaction with choline in the animal body[2] and to substitute for choline[3] and be effective in lipid metabolism disturbances in the liver of mammals.[4,5] It therefore seemed logical to study methionine, just as we have choline and inositol, as a possible decholesterolizing agent in old hens with hypercholesterosis.

Forty-four 2-year-old white leghorn hens were individually caged and fed the standard Texas A & M laying mash. The initial blood lipid levels were determined by the methods of Bloor[6] for total cholesterol, Bloor and Knudson[7] for cholesterol esters, and King[8] for total and inorganic phosphorus.

Methionine, 0.5 g, was placed daily in the gullet of each of 24 of the old hens. One hen died of liver disease. After feeding the methionine for 18 to 29 days, the hens were bled a second time and the blood lipids were again determined, but, as shown in Table I, very little decholesterolization had occurred. The administration of methionine was continued, third bleedings were made after 36 to 51 days, and the bloods chemically analyzed as before.

When random samples of blood showed

TABLE I.
Effect of Methionine on Cholesterol in Blood and Tissue of Old Hens.

| | Blood cholesterol, mg/100 ml | | Lipid Phosphorus, mg/100 ml | Tissue cholesterol, mg/100 g | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Aorta | | Heart | | Liver | |
| | Total | Esters | | Total | Esters | Total | Esters | Total | Esters |
| Controls | | | | | | | | | |
| 20 old hens | 195 | 147 | 10.7 | 253 | 188 | 233 | 172 | 303 | 223 |
| S.D.* | ±18 | ±20 | ±2 | ±50 | ±47 | ±48 | ±36 | ±50 | ±38 |
| 23 old hens | | | | | | | | | |
| 1st bleeding | 228 | 171 | 11.2 | | | | | | |
| S.D. | ±33 | ±23 | ±2.3 | | | | | | |
| Methionine | | | | | | | | | |
| 2nd bleeding | 171 | 126 | 14.3 | | | | | | |
| 18-29 days S.D.* | ±29 | ±28 | ±2.4 | | | | | | |
| 3rd bleeding | 138 | 95 | 16.6 | 156 | 102 | 135 | 91 | 276 | 169 |
| 36-57 days S.D.* | ±25 | ±24 | ±4 | ±21 | ±21 | ±19 | ±13 | ±94 | ±63 |

* S.D.—Standard Deviation.

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that the cholesterol levels were significantly lowered, the hens were sacrificed and blood collected for lipid analysis. The aorta, about a quarter gram of heart muscle and a similar mass of liver were individually and accurately weighed, then macerated with sand and 1 to 3 ether alcohol solution. The extract of each organ was analyzed and the amounts of total cholesterol and cholesterol esters in 100 g of each tissue calculated. Twenty old hens fed as simultaneous controls were also sacrificed and the blood and tissues likewise treated. The results are set down as controls in Table I.

It is evident that the total blood cholesterol and cholesterol ester levels definitely decreased and the phospholipids rose after the old hens had been given 0.5 g methionine daily for about 3 weeks. After 5 to 7 weeks treatment, significantly lower levels of the blood cholesterol and cholesterol esters were established, and the aorta, heart, and liver showed significantly lower lipid levels than those of the control series. Methionine therefore, seems to act as a decholesterolizing agent in old hens.

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## Fibrinogenolytic Demonstration of Activation and Inhibition of Tryptase in Plasma Protein Fraction-I ("Antihemophilic Globulin").*

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Previous experience has shown the superiority of lysis of fibrinogen[1] over fibrinolysis[2] in the assay of tryptic enzymes. Recent revival of the idea[3] that the natural blood-clotting system involves the same protease (serum-tryptase) that is responsible for fibrinolysis[4] and other proteolytic phenomena is based upon experimental analogies with crystalline pancreatic trypsin. The ability of trypsin to restore or improve the clotting of hemophilic blood *in vitro*[5] and *in vivo*[6] suggests a plasma tryptase deficiency in hemophilia, for which not-altogether-conclusive evidence is afforded by data[7,8] on proteases demonstrable by the Delezenne and Pozerski (1903) chloroform-activation method, and by similar finding[9] of such proteases in certain "globulin" plasma protein fractions, which the Harvard investigators[10] have developed in application to the clinical treatment of hemophilia. The present observations employ a fibrinogenolytic reaction[1] to establish some fundamental considerations with respect to the development of "tryptase" activity in fibrinogen-rich human plasma fractions, as compared with similar materials of bovine plasma origin.

*Reagents.* 1. H.F.: human plasma Fraction I, courtesy of Drs. Cohn, Minot, Edsall, and colleagues (Harvard Medical School);[10-12]

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