The Origins of Plasma Cholesterol

SANFORD OSCAR BYERS, PH.D.

THERE is a small positive correlation between the plasma cholesterol concentration and the development of clinical disease ascribed to atherosclerosis. This is true, however, only on a class or statistical basis. It is of no predictive value for individuals, whether "normal"[1] or diabetic.[2] Nevertheless the correlation justifies our studies concerning the origin and fate of plasma cholesterol.

Cholesterol enters the plasma either by absorption or by synthesis and release from the tissues.

EFFECT OF ABSORPTION

All exogenous cholesterol enters the blood by way of the thoracic lymph.[3,4] When cholesterol C-14 was given to a human by mouth, all radioactivity appeared in the lymph, none in the portal vein blood. Half of the lymph cholesterol C-14 was esterified, although all was given in the free form.[5] The newly absorbed cholesterol is not evenly distributed in the lymph but is preferentially concentrated in the chylomicrons (Fig. 1).[5,6,7]

The higher specific activity of the chylomicrons shows that exogenous cholesterol is probably built into them during passage from the intestinal mucosa to the lymph. The presence of these chylomicrons makes it possible to study the immediate fate of exogenous cholesterol separately from endogenous cholesterol.

After entering the blood, the chylomicrons leave again in a matter of minutes.[8] The major portion of the cholesterol enters the liver.[9,10] Once it has entered the liver, exogenous cholesterol is metabolized in the same way as endogenous cholesterol.[11] Absorbed cholesterol adds quantitatively to plasma cholesterol only during the persistence of alimentary lipemia. Its quantitative effect is slight and transient.[12]

EFFECT OF RELEASE FROM TISSUE (SYNTHESIS AND STORED EXOGENOUS CHOLESTEROL)

Almost all tissues synthesize cholesterol.[13] The newly synthesized cholesterol quickly appears in plasma.[10] The removal of various organs, however, does not alter plasma cholesterol responses, excepting only removal of the liver.[14,15,16] This holds true for hypercholesterolemia responses, such as bile duct ligation,[14] response to Triton® injection,[17] response to plasma albumin loss,[18] and also for restoration of normal plasma cholesterol values after acute reduction. If C-14 labeled acetate is given to a dog, C-14 labeled cholesterol is synthesized in almost all tissues, yet C-14 cholesterol appears in the plasma only if the liver is functioning.[15] The same observation has been made in heart-lung-liver preparations, using heart-lung preparations as controls.[16] Such data have justified the statement that the

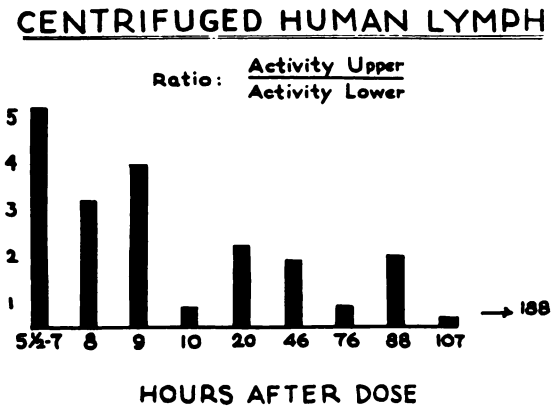

Fig. 1. Radioactivity from cholesterol-4-C^{14} absorbed into human intestinal lymph after oral administration. The upper, chylomicronous layer, shows preferential incorporation of radioactivity.

From the Harold Brunn Institute, Mount Zion Hospital, San Francisco.

Presented at the *Sixth Annual Symposium on Chemistry, Biochemistry and Metabolism of Lipids.* Institute for Metabolic Research, February 24–28, 1958, Oakland, California.

liver is the chief contributor to plasma cholesterol.

However, the liver is not the only possible contributor, for under special circumstances a substantial rise in plasma cholesterol can be brought about in the hepatectomized rat. One such condition is massive continuous infusion of phospholipid.[19] This action is shown by a variety of phospholipids, (except perhaps duck egg yolk lecithin purified through the cadmium salt which appears not to be active). This effect of phospholipid apparently mediates the hypercholesterolemia consequent upon biliary obstruction.[20] The hypercholesterolemia consequent on biliary obstruction can be duplicated in the normal animal by phospholipid injection to simulate the phospholipid rise which follows biliary obstruction. Since such phospholipid injection does not raise blood bile acids, the phospholipid rise seems a more immediate cause of hypercholesterolemia than does bile acid.

Another material whose continuous massive injection will raise blood cholesterol is triglyceride fat. The action of triglycerides is not mediated by phospholipid, although phospholipid also increases after triglyceride injection.[21] The triglyceride must be given continuously, or it escapes from the plasma, even in the hepatectomized rat.[22] Triglyceride fat accumulation apparently mediates the hypercholesterolemia consequent upon Triton injection,[22–25]

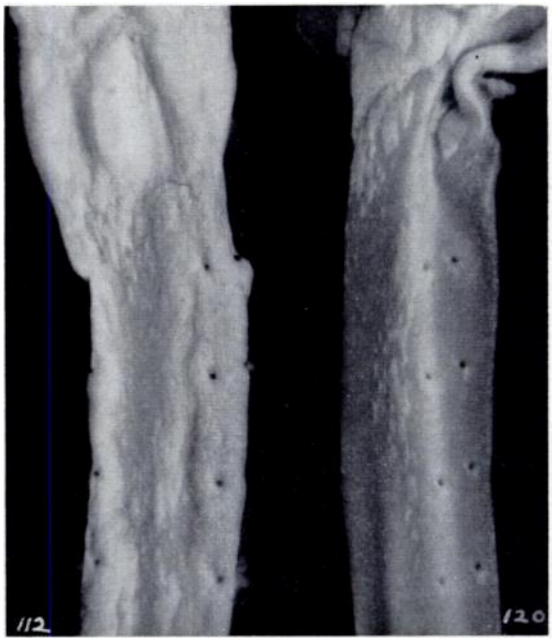

Fig. 2. Appearance of aorta from an untreated atherosclerotic rabbit (#112, at left side) contrasted with that from a matched control rabbit treated with repeated infusions of phospholipid.

and also the hypercholesterolemia of nephrosis.[26] The action of phospholipid or triglyceride is to impede the exit of cholesterol from plasma to the liver, while not affecting its entrance into the plasma.[20–25]

Where does the cholesterol, which appears in the plasma after these agents, come from?

TABLE I

(A) Effect of Phosphatide Infusion on Aortic and Coronary Atherosclerosis

Number of rabbits	Average plasma cholesterol during cholesterol feeding		Average plasma cholesterol at beginning of experiment		Number of infusions	Gross aortic atherosclerosis (0–5)			Microscopic examination of left coronary artery (% occlusion)		
	Phosphatide infused	Controls	Phosphatide infused	Controls		Phosphatide infused	Controls	Difference (%)	Phosphatide infused	Controls	Difference (%)
7	981	972	131	138	8	2.7	4.9	82	35	58	81

(B) Effect of Phosphatide Infusion on Aortic Cholesterol and Lipid Contents of Atherosclerotic Rabbits

Number of rabbits	Aortic cholesterol (g/100 g)			Aortic total lipid (g/100 g)		
	Phosphatide infused	Controls	Difference (%)	Phosphatide infused	Controls	Difference (%)
7	15.2 (±1.8)	23.0 (±1.7) +<0.001	51	47.2 (±4.7)	64.6 (±5.1) +<0.01	37

Quantitative analyses have not been sufficiently accurate to assign its proper contribution to each tissue. One such tissue is the liver. But comparable plasma concentrations can be obtained in the absence of the liver. We believe the source to be a general contribution from many tissues. The adrenal is one where a quantitative deficit may be demonstrated.[23,27] In other tissues biologic and analytic variations have so far prevented a clear demonstration.

One other tissue which can be shown to lose cholesterol to plasma is the atherosclerotic aorta.[28] This has been shown by analysis following repeated injection of phospholipid into atherosclerotic rabbits. The effect of infusions repeated over three weeks is shown in Table I. Evaluation of aortal atherosclerosis at the end of this period is presented in Figure 2.

REFERENCES

1. GOFMAN, J. W. *et al.*, and the Committee on Lipoproteins and Atherosclerosis of the National Advisory Heart Council: Evaluation of serum lipoprotein and cholesterol as predictors of clinical complications of atherosclerosis. *Circulation* 14: 691, 1956.
2. LOWRY, A. D. and BARACH, J. H.: Value of lipoprotein and cholesterol determinations in diabetic patients who developed cardiovascular complications. *Circulation* 17: 14, 1958.
3. BIGGS, M., FRIEDMAN, M., and BYERS, S. O.: Intestinal lymphatic transport of absorbed cholesterol. *Proc. Soc. Exper. Biol. & Med.* 78: 641, 1951.
4. CHAIKOFF, I. L., BLOOM, B., SIPERSTEIN, M. D., KIYASU, J. Y., REINHARDT, W. O., DAUBEN, W. G., and EASTHAM, J. F.: Lymphatic transport of absorbed cholesterol C^{14}. *J. Biol. Chem.* 194: 407, 1952.

5a. BYERS, S. O., KELLY, K., FRIEDMAN, M., and BIERMAN, H.: The route of absorption of cholesterol in the human being. *Clin. Res. Proc.* 4: 215, 1956.

b. HELLMAN, L., FRAZELL, E. L., and ROSENFELD, R. S.: Direct measurement of cholesterol absorption via thoracic duct in man. *J. Clin. Investigation* 37: 901, 1958.

6a. ALBRINK, M. J., GLENN, W. W. L., PETERS, J. P., and MAN, E. B.: The transport of lipids and chyle. *J. Clin. Investigation* 34: 1467, 1955.

b. BYERS, S. O. and FRIEDMAN, M.: Role of chylomicra in transport of cholesterol and lipid. *Am. J. Physiol.* 179: 79, 1954.

7. FREDRICKSON, D. S., MCCOLLESTER, D. L., HAVEL, R. J., and ONO, K.: Early steps in transport and metabolism of exogenous triglyceride and cholesterol; in *Chemistry of Lipides as Related to Atherosclerosis, A Symposium.* Thomas, Springfield, Ill., 1958.
8. NEVEU, T., BIOZZI, B., BENACERROF, B., STIFFEL, C., and HALPERN, B. N.: Role of reticulo-endothelial system in blood clearance of cholesterol. *Am. J. Physiol.* 187: 269, 1956.

9a. FRIEDMAN, M., BYERS, S. O., and GUNNING, B.: Fate of injected cholesterol in the animal body. *Am. J. Physiol.* 172: 309, 1953.

b. GOULD, R. G.: Lipid metabolism and atherosclerosis. *Am. J. Med.* 11: 209, 1951.

c. FRIEDMAN, M., BYERS, S. O., and SHIBATA, E.: Absorption and fate of ingested cholesterol. *J. Exper. Med.* 98: 107, 1953.

10. HELLMAN, L., ROSENFELD, R. S., and GALLEGHER, T. F.: Cholesterol synthesis from C^{14} acetate in man. *J. Clin. Investigation* 33: 142, 1954.
11. HELLMAN, L., ROSENFELD, R. S., EIDINOFF, M. L., FUKUSHIMA, D. K., and GALLEGHER, T. F.: Isotopic studies of plasma cholesterol of endogenous and exogenous origins. *J. Clin. Investigation* 34: 48, 1955.
12. FRIEDMAN, M., ROSENMAN, R. H., and BYERS, S. O.: Deranged cholesterol metabolism and its possible relationship to human atherosclerosis. A review. *J. Gerontology* 10: 60, 1955.
13. FRIEDMAN, M., BYERS, S. O., and ST. GEORGE, S.: Cholesterol metabolism. *Ann. Rev. Biochem.* 25: 613, 1956.

14a. FRIEDMAN, M., BYERS, S. O., and MICHAELIS, F.: Role of the liver in restoration of plasma cholesterol after experimentally induced hypercholesteremia. *Am. J. Physiol.* 164: 789, 1951.

b. BYERS, S. O., FRIEDMAN, M., and MICHAELIS, F.: The source of excess plasma cholesterol after ligation of the bile duct. *J. Biol. Chem.* 188: 637, 1951.

15. ECKLES, N. E., TAYLOR, C. B., CAMPBELL, D. J., and GOULD, G.: The origin of plasma cholesterol and the rates of equilibration of liver plasma and erythrocyte cholesterol. *J. Lab. & Clin. Med.* 46: 359, 1955.
16. TENNENT, D. M., ZANETTI, M. E., ATKINSON, D. I., KURON, G. W., and OPDYKE, D. F.: The endogenous origin of blood cholesterol. *J. Biol. Chem.* 228: 241, 1957.
17. FRIEDMAN, M. and BYERS, S. O.: The mechanism responsible for the hypercholesteremia induced by triton WR-1339. *J. Exper. Med.* 97: 117, 1953.
18. ROSENMAN, R. H., FRIEDMAN, M., and BYERS, S. O.: Plasma lipid interrelationships in experimental nephrosis. *J. Clin. Investigation* 36: 1558, 1957.
19. FRIEDMAN, M. and BYERS, S. O.: Role of hyperphospholipidemia and neutral fat increase in

plasma in the pathogenesis of hypercholesteremia. *Am. J. Physiol.* 186:13, 1956.

20. FRIEDMAN, M. and BYERS, S. O.: Interrelationship of plasma cholate and phospholipid concentrations and their resultant effect on plasma cholesterol in biliary obstruction. *Am. J. Physiol.* 188:337, 1957.
21. FRIEDMAN, M. and BYERS, S. O.: Hyperlipemia in the genesis of hypercholesteremia. *Proc. Soc. Exper. Biol. & Med.* 90:496, 1955.
22. FRIEDMAN, M. and BYERS, S. O.: Mechanism underlying hypercholesteremia induced by triton WR-1339. *Am. J. Physiol.* 190:439, 1957.
23. PATNODE, R. A.: Studies on the effect of triton (WR-1339) on guinea pig tissues. I. Lipide chemistry of lung, liver, spleen, adrenals and blood. *J. Exper. Med.* 107:33, 1958.
24. HUDGINS, P. C. and PATNODE, R. A.: Studies on the effect of triton on guinea pig tissues. II. Fragility of blood cells. *J. Exper. Med.* 107:43, 1958.
25. PATNODE, R. A. and HUDGINS, P. C.: Studies on the effect of triton (WR-1339) on guinea pig tissues. III. Tuberculin hypersensitivity and *in vitro* cytolysis of leucocytes by tuberculin. *J. Exper. Med.* 107:55, 1958.
26. ROSENMAN, R. H. and SMITH, M.: Relationship between concentrations of albumin and lipids in plasma of experimentally nephrotic rats. *Am. J. Physiol.* 191:40, 1957.
27. FRIEDMAN, M. and BYERS, S. O.: Unpublished observations.
28. FRIEDMAN, M., BYERS, S. O., and ROSENMAN, R. H.: Resolution of aortic atherosclerotic infiltration in the rabbit by phosphatide infusion. *Proc. Soc. Exper. Biol. & Med.* 95:586, 1957.