Cholesterol-Esterifying Activity in Serum of the Cholesterol-fed Rabbit[1] (35752)

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Mechanisms for the accumulation of cholesterol esters during genesis of atheromata have not yet been adequately established. There have, however, been suggestions that hypercholesterolemia may be accompanied by increased cholesterol-esterifying activity—presumably lecithin:cholesterol acyltransferase (LCAT) activity—in plasma (1) or in arterial tissue (2). Such observations have prompted the hypothesis that altered LCAT activity may be involved in abnormal deposition of cholesterol ester in tissues.

Existing literature provides conflicting information on the relationship between hypercholesterolemia and LCAT activity. Monger and Nestel (3) reported a positive correlation between unesterified cholesterol concentration and cholesterol esterifying enzyme activity in human plasma. Wells and Rongone (1) have reported that cholesterol-feeding induces increased LCAT activity in serum of normal rabbits. On the other hand, Shapiro *et al.* (4) reported decreased LCAT activity in hypercholesterolemic serum of several species. All of these studies employed assay systems in which substrate concentrations were variable from assay to assay. We have therefore restudied the question, using an assay system in which concentrations of both substrates were constant at the start of incubation. Results are described below.

Methods. The general design was as follows. Sera of cholesterol-fed and control rabbits were freed of lipoprotein by ultracentrifugation, and LCAT activity of the lipoprotein-free infranate was assayed. Lecithin was added at constant concentration in the form of mixed lipoproteins by employing a single plasma sample throughout the experiment.

New Zealand male rabbits, averaging 1800 g, were fed Purina Chow with 1% cholesterol and 10% olive oil for 1, 2, 3, 4, 5 or 11 weeks. Control animals received Purina Chow alone. Serum cholesterol was determined periodically by the procedure of Abell *et al.* (5). Each rabbit was anesthesized with sodium pentobarbital. Thoracic cavity was opened and blood was withdrawn from the heart. Serum was adjusted to a density of 1.21 with KBr and centrifuged at 107,000*g* in a Spinco Model L ultracentrifuge for 24 hr. Tube was cut at a preset height with a tube slicer. Lipoprotein, contained in the upper portion of the tube, was discarded. Infranatant (approx 4 ml) was dialyzed against a large volume (4 liter) of 0.9% saline overnight and assayed for LCAT activity by the procedure of Glomset and Wright (6).

Dialysis of normal and hypercholesterolemic infranate produced an increase in volume, the extent of which was monitored by determination of protein concentration (7) before and after dialysis. Ratio of predialysis protein concentration to postdialysis concentration was 1.21 ± 0.06 (mean ± standard error) in 4 hypercholesterolemic (11 week) samples, and 1.23 ± 0.05 in 4 normocholesterolemic samples. Dilution of the enzyme was therefore considered constant, on the average. Results were corrected for dilution by estimating that 0.82 ml of predialysis serum yielded 1.00 ml of postdialysis serum.

Prior to use in the assay system, human plasma and a 5% solution of bovine albumin

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TABLE I. Terminal Plasma Cholesterol Concentrations.[a]

Time on diet (weeks)	Plasma cholesterol (mg/dl)	
	Cholesterol-fed rabbits	Control rabbits
1	994 ± 546	60 ± 17
2	1246 ± 448	81 ± 28
3	633 ± 430	53 ± 10
4	769 ± 219	58 ± 15
5	798 ± 504	50 ± 13
11	1749 ± 442	34 ± 6

[a] Values are mean ± standard deviation. Each group consisted of four animals.

were heated individually at 60° for 0.5 hr to destroy LCAT enzyme activity. A large volume of pooled human plasma was treated in this way and stored at —20° in order to have enough for the length of the study. A suspension of cholesterol-4-^{14}C in albumin solution was prepared by the procedure of Porte and Havel (8) the day before each group of assays. One ml of the suspension of cholesterol-4-^{14}C in albumin was added to 8 ml of the previously heated substrate plasma. One ml of the dialyzed serum to be assayed was added, and the mixture was shaken for 3 hr at 37°. For a control, one ml of 0.9% saline was used in place of the enzyme solution. After incubation, serum lipid was isolated by the procedure of Folch *et al.* (9). The lipid was fractionated by thin-layer chromatography using silica gel G as a support. The plate was developed using 5% ethyl ether in petroleum ether and was sprayed with 0.02% dichlorofluorescein in ethanol. Cholesterol ester area, viewed under UV light, was scraped into a counting vial and suspended in a thixotropic gel consisting of 43 g of Hyamine 10X, 26 g of Thixcin R (Baker Chemical Company), 3.45 *g* of 2,5-diphenyloxazole and 86 mg of 1,4-bis-2(5-phenyloxazolyl)-benzene (10).

Radioactivity measurements were made in a Packard liquid scintillation spectrometer. Counting rate of the saline-containing vial was subtracted from the counting rate of each unknown.

Results. The cholesterol-fed animals became severely hyperlipidemic, as indicated by the terminal serum cholesterol values shown in Table I. Atheromata developed regularly, with histological changes discernible in the aorta by the end of the of the first week and gross atheromata by the end of the fourth week.

Results of incubation, expressed as total radioactivity of the resulting cholesterol ester, are shown in Table II. At all intervals studied, LCAT activity in serum of cholesterol-fed animals was indistinguishable from that of control animals.

Discussion. Raz *et al.* (11) have demonstrated that the rate of cholesterol esterification by LCAT is strikingly dependent on substrate concentration. Using human material, cholesterol esterification increased progressively as total (mixed) lipoprotein concentration was increased up to a free cholesterol

TABLE II. Esterification of Cholesterol-4-^{14}C in Assay System.

Diet	Total radioactivity in cholesterol ester[a]; weeks from start of experiment					
	1	2	3	4	5	11
Normal	4622	4880	5421	4770	5139	5217
	5562	5851	5350	6141	5295	4982
	5084	6768	6462	5201	5060	5781
	4512	5734	6032	4199	4763	4371
Mean ± SE	4945 ± 208	5808 ± 334	5816 ± 229	5078 ± 355	5064 ± 97	5088 ± 253
Cholesterol containing	4653	7575	5781	5225	5170	4888
	5452	6321	6188	5381	4167	5938
	4872	5961	6110	4802	5460	4418
	4253	5562	6580	5679	5123	5726
Mean ± SE	4808 ± 217[b]	6355 ± 504[b]	6165 ± 142[b]	5272 ± 158[b]	4980 ± 243[b]	5243 ± 309[b]

[a] C.p.m. incorporated in 3 hr by enzyme derived from 0.82 ml of serum.

[b] Mean value not significantly different from corresponding control value.

concentration of 0.7 m*M*; further increase in substrate concentration resulted in a decline in esterification rate. Control of substrate concentration is therefore essential to an unbiased assay. Validity of the method employed here—involving ultracentrifugal removal of lipoprotein from the enzyme-containing sample—is based on the fact that LCAT activity of plasma is associated with proteins of density >1.21 (11).

We conclude from this experiment that cholesterol feeding has no influence upon plasma LCAT activity in the rabbit. Presumably, cholesterol-induced atherogenesis in this species is not dependent upon augmented LCAT activity in plasma.

Summary. Serum LCAT activity was studied in hypercholesterolemic rabbits, employing an assay mixture in which initial substrate concentrations were fixed. Activity of the enzyme was indistinguishable from that in normal control serum.

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