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Dietary Cholesterol and Serum Cholesterol-Esterifying Activity in Rabbits* (33629)

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Sperry (1) was the first to demonstrate that blood serum contains a cholesterol-esterifying enzyme. It is a liver produced (2) acyltransferase and the β-position of lecithin is the source of the required acyl groups (3, 4).

The activity in plasma of this enzyme, phosphatide: chloesterol fatty acid transferase, is increased in essential fatty acid (5), pantothenic acid and choline deficient rats (6) and decreased in inositol deficient rats (6). In these vitamin deficiency states, the increased activity of the enzyme is accompanied in male rats by increased concentrations of cholesterol esters in the tissues (6).

These observations suggest that the cholesterol-esterifying enzyme in plasma may serve an important role in the process of cholesterol ester deposition in the tissues. We have sought additional evidence for this concept by determining the change of activity of the enzyme in the serum of cholesterol-fed rabbits. Increased enzyme activity was observed accompanied by increased concentrations of cholesterol esters in some of the tissues.

Methods. Twelve rabbits, equally divided as to sex, with body weights in the range 2.5–3.0 kg were obtained from a commercial supplier. Two of the animals were used for the determination of the normal tissue cholesterol concentrations. Blood samples were obtained from an ear vein of each of the remaining animals at the start of the experiment and 5 days later. During this time the animals were fed a pelleted rabbit chow. [2] Sera from these blood samples were analyzed to obtain control levels of cholesterol and cholesterol-esterifying activity.

After the second blood samples were obtained, the rabbits were given the pelleted chow onto which cholesterol had been deposited from an ether solution to give a cholesterol concentration of 0.7 g/100 g of diet. The animals were continued on this diet for 6 weeks and blood samples were obtained, as above, at weekly intervals and the sera were analyzed for cholesterol and cholesterol-esterifying activity. At the end of the cholesterol feeding period, the animals were anesthetized, killed by exsanguination and samples of tissues were removed and frozen on dry ice. They were subsequently analyzed for their contents of cholesterol.

The activity of the cholesterol-esterifying enzyme in 0.50 ml of each serum sample was determined as previously described (6) using as substrate 2.0 ml of normal, heated rabbit serum containing cholesterol-1α-^{3}H.[3] Enzyme activities in the fresh experimental sera were expressed as dpm converted to cholesterol

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TABLE I. Cholesterol Concentrations in Serum and Tissues of Cholesterol-Fed Rabbits.

Weeks on cholesterol diet	Cholesterol concentrations[a]			Ratio free : ester
	Total	Ester	Free	
		Serum		
0	22.0 ± 2.2 (10)[b]	14.4 ± 1.6 (10)	7.0 ± 1.7 (10)	0.58 ± 0.15 (10)
1	118.4 ± 13.8 (9)	53.1 ± 4.3 (9)	65.2 ± 12.6 (9)	1.25 ± 0.26 (9)
2	241.3 ± 34.8 (10)	85.5 ± 9.2 (10)	155.8 ± 27.4 (10)	1.80 ± 0.27 (10)
3	242.4 ± 32.1 (10)	81.6 ± 7.5 (10)	160.9 ± 27.2 (10)	2.01 ± 0.33 (10)
4	297.2 ± 26.0 (10)	84.8 ± 8.5 (10)	212.4 ± 21.7 (10)	2.68 ± 0.30 (10)
5	280.5 ± 16.4 (8)	68.8 ± 4.8 (8)	211.4 ± 14.7 (8)	3.16 ± 0.27 (8)
6	325.4 ± 31.5 (9)	104.1 ± 4.1 (9)	221.3 ± 28.4 (9)	2.09 ± 0.22 (9)
		Liver		
0	4.0 ± 0.01 (2)	2.4 ± 0.10 (2)	1.6 ± 0.08 (2)	0.70 ± 0.06 (2)
6	6.9 ± 0.5 (10)	3.8 ± 0.17(10)	3.2 ± 0.44(10)	0.82 ± 0.08 (10)
		Kidney		
0	1.3 ± 0.3 (2)	0.2 ± 0.02 (2)	1.2 ± 0.2 (2)	6.49 ± 0.36 (2)
6	0.9 ± 0.04(10)[c]	0.3 ± 0.02(10)[c]	0.6 ± 0.03(10)[c]	2.30 ± 0.20 (10)[c]
		Heart		
0	0.7 ± 0.1 (2)	0.4 ± 0.02 (2)	0.4 ± 0.1 (2)	0.94 ± 0.27 (2)
6	1.1 ± 0.1 (10)[c]	0.5 ± 0.04(10)[c]	0.6 ± 0.04(10)	1.04 ± 0.08 (10)
		Adrenal		
0	13.3 ± 1.8 (2)	4.1 ± 0.5 (2)	9.8 ± 1.9 (2)	2.38 ± 0.19 (2)
6	11.9 ± 0.9 (10)	3.6 ± 0.5 (10)	8.4 ± 0.7 (10)	2.78 ± 0.45 (10)
		Skeletal muscle		
0	0.9 ± 0.1 (2)	0.3 ± 0.1 (2)	0.6 ± 0.03 (2)	2.21 ± 0.43 (2)
6	0.9 ± 0.1 (9)	0.4 ± 0.03 (9)	0.5 ± 0.04 (9)	1.57 ± 0.13 (9)

[a] Concentrations of cholesterol in serum are given as mg/100 ml; in the remaining tissues concentration is mg/g.

[b] Mean ± SE of mean; number of animals contributing a sample for analysis is given in parentheses.

[c] Significantly different from control value, $p \leq 0.02$, Fisher's t test; all serum values were significantly different from control values.

esters per 0.50 ml of serum during the incubation time of 2.0 hr. Cholesterol concentrations in the sera and in the tissues were determined as previously described (6) by the ferric chloride method of Rosenthal and Jud (7).

Results. The cholesterol contents of the sera, livers, kidneys, and other tissues are given in Table I. Cholesterol-esterifying activities of the serum, obtained at weekly intervals, are given in Table II. Since the concentration of free cholesterol in the serum increased markedly as a result of the cholesterol feeding, the observed cholesterol-esterifying activity was multiplied by a factor to correct for the dilution of the labeled free cholesterol in the substrate by the free cholesterol in the 0.50 ml of serum assayed. The cholesterol concentrations and serum enzyme activities were the same for the two sexes.

Discussion. The cholesterol-esterifying activity of serum became elevated as a result of the feeding of cholesterol to rabbits on an otherwise normal diet. The increased enzyme activity, probably reflecting increased enzyme concentrations (5), was noted 1 week after the start of cholesterol feeding and correlated well ($r = 0.96$) with the increased concentration of free cholesterol in the serum during the first 5 weeks of cholesterol feeding. The degree of correlation of enzyme ac-

TABLE II. Cholesterol Esterifying Activity in Serum of Cholesterol-Fed Rabbits.

Weeks on cholesterol diet	Observed activity[a]	Isotope dilution factor[b]	Corrected activity
0	2838 ± 215 (10)[c]	1.00	2838 ± 215 (10)
0	3281 ± 277 (10)	1.00	3281 ± 277 (10)
1	2328 ± 169 (9)	2.66	6192 ± 169 (9)
2	1472 ± 131 (10)	5.25	7728 ± 131 (10)
3	1507 ± 252 (10)	5.40	8138 ± 252 (10)
4	1409 ± 181 (10)	6.87	9680 ± 181 (10)
5	1533 ± 174 (9)	6.84	10,486 ± 174 (9)
6	1253 ± 317 (9)	7.13	8934 ± 317 (9)

[a] Activity = dpm incorporated into cholesterol esters/0.50 ml of serum/2.0 hr.

[b] The amount of cholesterol-1α-^{3}H was the same in each incubation vessel while the total free cholesterol was composed of 0.14 mg from the 2 ml of heated, normal rabbit plasma substrate plus the free cholesterol in the 0.50-ml sample of serum being assayed. Thus the total free cholesterol in the incubation vessels was 0.175, 0.466, 0.919 mg, etc., when sera from rabbits fed cholesterol for 0, 1, 2, etc., weeks, respectively, were assayed. Therefore the specific activity of cholesterol in the first vessel was diluted by a factor of 2.66 in the second vessel and by a factor of 5.25 in the third vessel, etc. Observed enzyme activities were multiplied by these dilution factors to obtain corrected enzyme activities which would be directly comparable.

[c] Mean ± SE of mean; number of animals contributing a sample for analysis is given in parentheses. All activities obtained are significantly different from control values, $p \leq 0.02$, Fisher's t test.

tivity and concentration of cholesterol esters in the serum was less but still high (r = 0.86).

A highly significant positive correlation has been demonstrated in humans (8) between the concentration of free cholesterol in plasma and the rate of cholesterol esterification in plasma. However, cholesterol feeding in rats has been reported (9) to decrease the cholesterol-esterifying activity of sera. The significance of this reported decrease is uncertain because the diet employed contained bile salts in addition to cholesterol; bile salts have been shown (10, 11) to be inhibitors of the cholesterol-esterifying enzyme in plasma. In addition, since it was also demonstrated that cholesterol feeding leads to a decrease in the synthesis of arachidonic acid from linoleic acid, an increase in the cholesterol-esterifying activity of plasma might well have been expected (5).

Shapiro *et al.* (12) reported that, in chickens with hypercholesteremia produced by feeding for 1 month a semisynthetic diet supplemented with 2% cholesterol and 5% corn oil, the serum cholesterol-esterifying activity was reduced to about 13% of the control value. The results obtained after feeding a 2% cholesterol diet to rabbits for 2 months were equivocal. In our experiments, the cholesterol-esterifying activity of serum was determined in rabbits at weekly intervals after initiating the feeding of a 0.7% cholesterol diet. Though this regimen was continued for only 6 weeks, the results indicate that the serum cholesterol-esterifying activity increased to a maximum value in 4–5 weeks and then began to decline. The decline may be due to the general toxicity of exogenous cholesterol in these animals (13).

In agreement with previous results in male rats (6), it was also observed in the present study that in increased level of cholesterol-esterifying activity in serum was associated with increased concentrations of cholesterol esters in certain of the tissues examined. Again, a role in cholesterol ester deposition in the tissues is suggested for the cholesterol esterifying enzyme in plasma. However, no sex difference was noted in the present study in which the cholesterol was supplied exogenously in contrast to the previous work in which the cholesterol was supplied endogenously. It may be presumed therefore

that exogenous cholesterol supplies suitable substrate for cholesterol esterification in both male and female animals where endogenous cholesterol does so only in the male animal.

The phosphatide: cholesterol fatty acid transferase, responsible for cholesterol esterification in plasma, is apparently produced in the liver (2) but is not identical to the enzyme which has been measured in hepatocytic organelles (3, 14). Since cholesterol feeding does produce an increase in the level of activity of the plasma enzyme and since exogenously derived cholesterol is engulfed by the Kupffer cells of the liver, the possibility suggests itself that such cells, when stimulated, might be the source of the plasma enzyme. This possibility appears unlikely, however, since endogenously derived cholesterol does not stimulate the Kupffer cells (15, 16) though such cholesterol may be associated with increased levels of activity of the plasma enzyme (6). In addition, we have stimulated the reticuloendothelial system by injecting diethylstilbesterol subcutaneously, 1.0 mg/day for 6 days, into male rats (17) and have found the level of activity of the plasma cholesterol-esterifying enzyme to be unchanged or even decreased.

Summary. The feeding of a normal diet containing 0.7% cholesterol to rabbits results in increased levels of activity of the plasma cholesterol-esterifying enzyme, lecithin: cholesterol fatty acid transferase. The increased level of enzyme activity occurs within one week after the beginning of cholesterol feeding. Associated with the increased levels of enzyme activity are increased concentrations of cholesterol esters in liver, kidney and heart tissue but not in adrenal or skeletal muscle tissue. No sex differences were observed.

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