The Cause of Persisting Hypercholesteremia in Rabbit after Cessation of Cholesterol Feeding.* (24017)

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Our comparative study of cholesterol metabolism of the rabbit and rat undertaken(1), suggested that hypercholesteremia developed more readily in the rabbit than in the rat, when both species ingested excess cholesterol because of several observed metabolic differences. First, the rabbit was observed to absorb a larger fraction of its ingested cholesterol than the rat. Secondly, the liver of the rabbit appeared far less capable of removing excess cholesterol from blood than the liver of the rat. This difference in rate of hepatic withdrawal of cholesterol from plasma did not appear, however, to be due to any peculiarity in rabbit plasma but apparently to some comparative defect in the liver itself. For various reasons(1,2,3), we believed that this defect resided in the hepatic reticulo-endothelial system. Another peculiarity of the rabbit fed excess cholesterol is persistent hypercholesteremia long after cessation of cholesterol feeding. The reason for this lag in return of plasma cholesterol to a normal level has not been determined despite its possible importance, as Zilversmit(4) has recently pointed out. It was therefore thought advisable to investigate this phenomenon. The results of our study suggest that the lag is due to prolonged and slow discharge into plasma of the cholesterol previously accumulated in tissues during the cholesterol feeding period.

Methods. A. *Injection of rabbit hypercholesteremic serum.* To determine and compare rate of disappearance of injected rabbit hypercholesteremic serum with that of rat hypercholesteremic serum, 8 normal, young male rabbits (avg wt: 1211 g) were given 10 ml of pooled rabbit hypercholesteremic serum (avg cholesterol: 802 mg/100 ml) obtained from rabbits made hypercholesteremic by ingestion of excess dietary cholesterol. This serum was turbid and on microscopic examination abounded in chylomicra. An additional 10 rabbits (avg wt: 1226 g) were given 10 ml of pooled rat hypercholesteremic serum (avg cholesterol: 750 mg/100 ml) obtained from rats made hypercholesteremic by experimental elevation of plasma bile acid(5). Rat serum was translucent and completely devoid of chylomicra. A sample of blood was obtained from each rabbit of both series before, immediately, 12 and 24 hours after injection. These samples were analyzed for total cholesterol(6).

B. *Acute reduction of plasma cholesterol of hypercholesteremic rabbits.* Fifteen male rabbits (avg wt: 1890 g) were given a diet containing 2% cholesterol and 3% cotton seed oil for 3 months, then they were bled and again given their usual ration of Purina rabbit chow. After 40 days upon the latter diet, these rabbits were anesthetized with ether, and an inlying plastic catheter was inserted into the right external jugular vein of 9 rabbits(7). One week later, each rabbit was given *via* catheter, approximately 20 mg of heparin and then several minutes later, 40 to 50 cc whole blood was removed by syringe and immediately replaced with 40 to 50 cc of whole blood from a normocholesteremic rabbit. This procedure was repeated 4 or 5 times, always employing a new normocholesteremic rabbit for each exchange, to decrease progressively the plasma cholesterol content of the hypercholesteremic rabbit. Blood samples were obtained from hypercholesteremic rabbits before and immediately, 24, 48, 72, and 120 hours after these blood exchanges, and their cholesterol concentration determined. Estimation of the hematocrit in several animals determined before and immediately after the exchange transfusion, revealed that no significant change had oc-

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TABLE I. Rate of Disappearance of Rabbit and Rat Hypercholesteremic Serum in the Rabbit.

No. of rabbits		Avg wt	Plasma cholesterol (mg/100 ml) Before inj.	Immed. after inj.	12 hr after inj.	24 hr after inj.
		A. Rabbits given rabbit hypercholesteremic serum				
8		1211	68	162	106	106
	Range	855–1815	44–97	143–201	68–180	73–155
	S.E. mean		3.5	5.1	4.9	3.7
		B. Rabbits given rat hypercholesteremic serum				
11		1226	59	149	115	107
	Range	840–1482	30–95	130–182	83–137	66–157
	S.E. mean		2.4	4.8	3.6	3.1

curred. The remaining 6 hypercholesteremic rabbits employed as controls, were bled at the same intervals, except for intervals immediately and 120 hours after exchange procedure. A similar exchange was done on 4 normocholesteremic rabbits, their removed blood (40-50 ml) being replaced with hypercholesteremic blood obtained from hypercholesteremic rabbits plasmapheresed above. Here again the *initially* withdrawn blood of at least 2 hypercholesteremic rabbits was given in the first 2 blood replacements effected in the normocholesteremic rabbit. The blood withdrawn from the normocholesteremic rabbits in each exchange was discarded. Blood samples were obtained at similar times as described above.

Results. As Table I illustrates, excess cholesterol contained in serum of the hypercholesteremic rabbit appeared to disappear as rapidly after its injection into the normal rabbit as did the rat hypercholesteremic serum. However as previously pointed out(1), the plasma of either series was not completely cleared of excess cholesterol even at 24 hour period.

Experimental reduction of cholesterol in plasma of hypercholesteremic rabbit was observed (Fig. 1) to be followed within the next few days by a gradual return to much higher plasma cholesterol values, despite the fact that the animal had been and continued to be upon an almost completely cholesterol free diet.

On the other hand, the 4 initially normocholesteremic rabbits made acutely hypercholesteremic by exchange procedure quickly returned to the same level observed in rabbits that had received rat hypercholesteremic serum.

The 6 control hypercholesteremic rabbits continued to lose some of their cholesterol during the experimental period as would be expected.

Discussion. In previous study of cholesterol metabolism of the rabbit(1) it was observed that the cholesterol present in serum of hypercholesteremic rabbit disappeared after its injection into the rat just as rapidly as the cholesterol of rat hypercholesteremic serum. Similarly in the present experiments, the rabbit also exhibited no greater delay in ridding itself of cholesterol present in rabbit hypercholesteremic serum than that in rat hypercholesteremic serum. Thus the profound difference in lipoprotein spectrum of these 2 types of sera(8,9) did not appear to influence

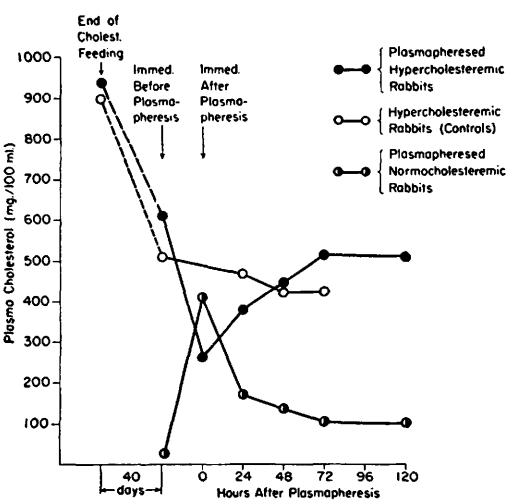

FIG. 1. Effects of acute changes in plasma cholesterol both in hypercholesteremic and normocholesteremic rabbits.

ability of either species to rid themselves of excess cholesterol contained therein. The conclusion therefore appears inescapable that delay in return to normal of cholesterol of the rabbit previously fed excess cholesterol cannot be due to some intrinsic lipoprotein phenomenon in rabbit hypercholesteremic serum which then allows such serum to retain excess cholesterol therein.

The return of plasma cholesterol of hypercholesteremic rabbits to a markedly higher level after its acute reduction by exchange transfusion, of course indicated that such rabbits are receiving cholesterol in their plasma from some tissue(s) either containing an excess of this sterol or else synthesizing and discharging it, at a rate in excess of its rate of hepatic removal and destruction(1). Since the rate of synthesis of cholesterol is not elevated in the cholesterol fed, hypercholesteremic rabbit(1,10), and since an excess of cholesterol remains in various tissues, particularly the liver and aorta(11,12) of rabbits previously fed cholesterol, it would appear that cholesterol rebound after exchange transfusion was due to escape of such tissue excess cholesterol into plasma. Such an escape might continue until some new balance or equilibrium had been established between cholesterol present in tissue and that in plasma perfusing such tissue. It was of interest that normocholesteremic rabbits when made hypercholesteremic by exchange transfusion with hypercholesteremic serum quickly returned almost to their normocholesteremic status, again demonstrating that this type of hypercholesteremic plasma itself possessed no more intrinsic cholesterol, retaining property than that of rat hypercholesteremic serum.

Summary. 1. Normal rabbits were able to rid their plasma of excess cholesterol just as rapidly after such cholesterol was administered in the form of rabbit hypercholesteremic serum, as when administered in the form of rat hypercholesteremic serum. 2. Hypercholesteremic rabbits whose plasma level of cholesterol was reduced by exchange transfusions exhibited a return to higher levels in succeeding days. 3. Normocholesteremic rabbits whose level of cholesterol was elevated by exchange transfusions quickly reverted to their previous normocholesteremic status. 4. These facts suggest that the chronic discharge of previously stored tissue cholesterol is responsible for the long persistent hypercholesteremia in the rabbit after cessation of cholesterol feeding.

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