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Role of Hyperlipemia in the Genesis of Hypercholesteremia.* (22076)

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It has long been known that an hypercholesteremia, either occurring clinically or induced experimentally, is usually accompanied by a rise in the concentration of plasma phospholipid and sometimes also of neutral fat. This frequently observed association of hypercholesteremia with hyperlipemia has not been investigated in respect to the possible *sequential* relationship of these multiple lipid rises. Instead it has been more or less assumed, in lieu of direct evidence, that the lipid rises observed in hypercholesteremia are merely associative phenomena. Recently, however, Allbrink, Man and Peters(1), noting the physical association of plasma cholesterol with neutral fat in chylomicra, have posed the question of whether an excess of plasma neutral fat might not dissolve additional cholesterol thus preventing its inclusion in soluble lipoprotein, leading thereby in some manner to a state of hypercholesteremia.

In view of this last possibility, it was decided to induce a sustained state of hyperlipemia in the rat by continuous parenteral injection of 2 types of lipid mixture (containing only neutral fat and lecithin) and then observe the possible changes in plasma cholesterol resulting therefrom. The results obtained in this study, as herein reported, indicate that a state of hypercholesteremia quickly follows the induction of a prolonged hyperlipemia.

Materials and methods. Male rats (Long Evans strain) 12-16 weeks of age were used in all of the experiments. (a) Production of sustained hyperlipemia was accomplished in rats by rapid intravenous administration of 3 ml of either of 2 different lipid emulsions (B† and Z‡) followed by *continuous* intravenous administration of the same emulsion at 0.9 to 1.2 ml/hour for 12 hours. This continuous intravenous administration to an unanesthetized rat was made possible by its enclosure in the Bollman type of cage(2). Prior to the experiment these rats had been placed for 7-15 days on a diet comprised of Purina Chow enriched with cholic acid (2%) and olive oil (2%). Each rat also received an additional 3 ml of olive oil by stomach tube at 72, 48 and 24 hours prior to the start of experiment. All food was withheld from rats for 24 hours immediately prior to and during the experi-

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† B lipid emulsion (kindly furnished by Don Baxter) is composed as follows: Sesame Oil, U.S.P. 10; Dextrose, U.S.P. 4.5; Purified Soya Bean Lecithin 0.65; Water, q.s. 100 ml. Lipid is present as finely divided particles of 1 μ or less in diameter.

‡ Z lipid emulsion (kindly furnished by D. B. Zilversmit of University of Tenn.) is composed as follows: Coconut Oil 10; Purified Soya Bean Lecithin 1.0; Dextrose 5.0; Water, q.s. 100 ml. Lipid particles are approximately the same diameter as in B emulsion.

ment. This pre-feeding regimen was adopted after preliminary studies had demonstrated that the usual rapid decline in hyperlipemia after a single injection of a fat emulsion could be slowed if the animal's fat absorption previously had been enhanced. Plasma samples obtained before, immediately after the initial injection of 3 ml of fat emulsion, and 12 hours after beginning of continuous intravenous injection, were analyzed for cholesterol(3) and phospholipid(4). The total lipid of the samples was analyzed by the method of Bragdon(5). Control animals were fed same regimen but received glucose solution (5%) intravenously in lieu of fat emulsion. (b) *Partial hepatectomy.* Three animals were prefed and later administered lipid emulsion as described above except that just prior to lipid administration, the rats were anesthetized with ether and a portion (approximately 2.7 g) of liver removed. (c) *Production of hyperlipemia by injection of protamine.* Freshly prepared protamine sulfate solution (salmine)§ solution (2 mg in 3 ml normal saline solution) was injected into a series of rats given 3 ml of olive oil just prior to injection and into a control series fasted for 24 hours. Both series were given additional intravenous injections of 2 mg of protamine at 3, 6 and 9 hours following initial injection. For additional control purposes, a third series of rats were given 3 ml of olive oil and injections of dextrose solution (5%) comparable in volume to the volumes injected in the first 2 series. Plasma samples obtained before and 12 hours after the initial injection were analyzed for cholesterol and total lipid. (d) *In vitro extraction of cholesterol from hypercholesteremic serum by lipid suspension.* Pooled hypercholesteremic but non-turbid serum (cholesterol concentration; 128 mg/100 ml) was obtained from bile ligated, cholate fed rats(6). A lipid suspension was obtained as follows: The 24-hour intestinal lymph of 5 rats fed 3 ml of olive oil was collected and submitted to ultracentrifugation (r.c.f. = 13,000-27,000) for 30 minutes. The resulting top creamy layer was removed and suspended in 5 ml of H_2O. This

§ Eli Lilly and Co. control No. 8367-571094 DH.

TABLE I. Total Lipid and Cholesterol Response of Intact and Partially Hepatectomized Rat to Continuous Injection of Lipid Emulsion.

Rats				Blood plasma								
				Total lipid (mg/100 ml)			Phospholipid (mg/100 ml)			Plasma cholesterol (mg/100 ml)		
No.	Given	Avg wt (g)	Total amt inj. (ml)	A	B	C	A	B	C	A	B	C
10	Control rats, dextrose sol.	301 (282–314)	12.5 (11.8–13.3)	286 (147–286)	—	219 (185–263)	101 (90–124) ± 2.3	—	125 (107–142) ± 3.5	62 (52–76) ± 2.24	—	80 (69–96) ± 2.44
13	B fat	299 (235–368)	15.2 (13.8–16.4)	189 (131–292) ± 13.2	—	681 (500–958) ± 53.0	97 (81–114) ± 4.8	185 (93–310) ± 38	196 (120–271) ± 15.4	53 (35–78) ± 3.4	60 (48–80) ± 2.4	124 (80–195) ± 11.2
5	Z fat	318 (304–328)	15.2 (14.8–15.4)	177 (138–296) —	1888	864 (604–1140) —	121 (100–176) —	198 (184–238) —	251 (181–327) —	58 (48–70) —	62 (51–67) —	128 (88–180) —
3	Partial hepatectomized rats, B fat	310 (302–318)	14.6 (14.0–15.2)	190 (181–200) —	—	562 (523–597) —	—	—	—	50 (47–51) —	54 (45–64) —	69 (67–73) —

A = Before inj. B = Immediately after initial inj. C = 12 hr after inj.
Values in parentheses = Range; ± values = Stand. error of the mean.

TABLE II. Total Lipid and Cholesterol Response of Intact Rat to Intermittent Injections of Protamine.

Rats			Blood plasma			
			Total lipid (mg/100 ml)		Plasma cholesterol (mg/100 ml)	
No.	Given	Avg wt (g)	A	B	A	B
11	Protamine	311 (292–340)	138 (121–152) ± 5.9	238 (175–324) ± 21.4	48 (33–65) ± 3.6	60 (38–77) ± 3.4
9	Protamine & olive oil	325 (300–344)	204 (151–273) ± 20.3	563 (263–1390) ± 66.0	56 (39–78) ± 4.0	119 (96–174) ± 7.9
9	Olive oil	310 (302–318)	218 (148–356) ± 22.4	287 (210–394) ± 54	50 (47–51) ± 3.8	69 (67–73) ± 7.4

A = Before inj. B = 12 hr after inj.
Values in parentheses = Range; ± values = Stand. error of mean.

latter suspension was considered to consist almost entirely of a mixture of chylomicronous lipids, relatively free of protein(7) and had a cholesterol concentration of 148 mg and a total lipid concentration of 4440 mg/100 ml. Two ml of the lipid suspension were mixed with 2 ml of hypercholesteremic serum, shaken at room temperature for 6 hours and then the mixture ultracentrifuged. The resulting top creamy layer (approximately 0.9 ml) was removed, suspended in 5 ml of water and analyzed for cholesterol. The lower layer also was similarly analyzed. For control purposes, the identical procedure was repeated substituting a solution of H_2O for the hypercholesteremic serum.

Results. As Table I indicates, a continuous state of hyperlipemia characterized by a rise in both total lipid and phospholipid content of rat's plasma, was obtained by continuous injection of either B or Z lipid emulsion. Induction of this hyperlipemia resulted in a marked rise in cholesterol content of plasma. Thus when hyperlipemia was induced by injection of B emulsion, the average plasma cholesterol content increased from 53 mg before to 124 mg/100 ml in 12 hours, a rise of 134%. Approximately similar results in respect to cholesterol were obtained following parenteral administration of Z emulsion (See Table I), although its injection led to a greater elevation of plasma lipid, possibly due to its greater phospholipid content. Continuous injection of dextrose solution into control animals did not elevate their total plasma lipid but a moderate rise in their plasma phospholipid (24%) and cholesterol (29%) occurred. These latter rises however cannot be ascribed to administration of dextrose solution because they invariably occur in fat fed rats following any type of experimental procedure (*e.g.*, routine bleeding for samples).

Induction of lipemia in partially hepatectomized rats did not lead to a significant rise in plasma cholesterol. This failure of cholesterol to rise in partial absence of the liver suggests that the excess cholesterol observed in plasma of above intact rats after induced lipemia came from a hepatic and not an intestinal source. Starvation of all intact animals 24 hours prior to experimental procedure also suggests a source other than the intestine of the excess cholesterol.

The results following injection of protamine also suggested that experimental induction of lipemia alone induces a cholesteremia. Thus (Table II) although *intermittent* injection of protamine into the fasted rat produced a possible moderate increase in total lipid, it did not induce marked hypercholesteremia. However when the same dosage of protamine was given to rats fed olive oil, a significant hyperlipemia occurred and a hypercholesteremia (Table II). These results are in agreement with those found by Szasz and Constantinides(8).

In vitro exposure of pooled hypercholesteremic serum to the suspension of intestinal lymph lipids resulted in a fall of cholesterol content of serum from 2.56 to 1.0 mg and a

corresponding rise in cholesterol content of chylomicron lipids from 2.4 to 3.4 mg. The cholesterol content of control chylomicron lipid mixture remained unchanged. In other words, the lipid mixture was capable of extracting over half of the cholesterol originally present in clear hypercholesteremic serum.

Discussion. Our findings indicate that hypercholesteremia resulted whenever a sufficient degree of sustained hyperlipemia was induced by the methods employed. Furthermore, it appears that this cholesterol rise will occur regardless of whether the hyperlipemia ensues, because too much lipid is presented by parenteral administration to the organism for its complete removal from blood, or because the organism is prevented by protamine injection from removing its usual amount of lipid from blood. Cholesterol thus retained also originates primarily in the liver. Apparently a portion of the cholesterol normally discharged into plasma and then later taken up again by the liver is "trapped" in the excess lipid circulating in plasma. Ability of a lipid suspension derived from intestinal lymph to extract cholesterol existing in soluble, ostensibly lipoprotein form(9) in hypercholesteremic serum further suggests the possibility of such "trapping." Apparently then lipid can dissolve cholesterol existing in plasma, as Allbrink, Man and Peters(1) have suggested, and this occurs even when cholesterol is present in soluble lipoprotein bondage.

While previously hypercholesteremia and hyperlipemia have been considered independent processes, the demonstration that a rise in plasma lipid quickly leads to hypercholesteremia suggests that it is necessary to consider the possibility that an excess rise of cholesterol in plasma (when attended by hyperlipemia) may be passively induced resultant of *primary* derangement in plasma lipids other than cholesterol. This induced hypercholesteremia could have been effected by accumulation in plasma of either neutral fat, phospholipid or both lipids present in the emulsions. Present studies are in progress to determine the magnitude of particular responsibility of each of these other lipid species.

Summary. Induction of hyperlipemia in rats by continuous intravenous injection of lipid mixtures containing only neutral fat and soya lecithin quickly led to hypercholesteremia. These results suggest that in hypercholesteremia associated with hyperlipemia, the primary derangement may lie not in the cholesterol but in some phase of the fat metabolism.

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