Cholesterol and Phospholipid Concentration in Hepatic Lymph and Bile During Phosphatide Induced Hypercholesteremia.* (23582)

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Hypercholesteremia induced by infusion of various phosphatides(1) does not occur unless sustained elevation of plasma phospholipid is maintained for at least several hours by continuous infusion of suitable phosphatide. This necessitates administration of a rather large amount of phosphatide (*e.g.*, 20 mg or more/hour in the rat) because of continual escape of administered phosphatide from the circulation. One of the organs responsible for this rapid withdrawal of phosphatide from plasma is the liver. This is believed to be so not only because this organ has been found(2) capable of rapidly withdrawing phospholipids from blood but also because we have observed(3) that the liverless animal invariably exhibited a far higher plasma phospholipid than the normal rat when both were infused with equivalent amounts of phosphatide.

It therefore seemed important to us to study the hepatic lymph and bile of animals receiving phosphatide in an effort to discover the possible mechanism(s) by which the liver removes excess phospholipid from the circulating blood.

Methods. A. *Effect of hepatectomy upon rate of disappearance of injected phosphatide.* A group of 14 male, adult rats (Long Evans Strain) was anesthetized and subjected to functional hepatectomy(3), immediately after which each received 90 mg of crude brain lecithin† in 3.0 ml of 5% dextrose-saline solution by immediate intravenous injection. They were bled before, immediately, and 3 hours after the injection. A group of 10 normal control rats were similarly injected with the same amount of lecithin and bled at the same intervals. The plasma samples were analyzed for phospholipid(4). B. *Effect of phosphatide infusion upon phospholipid content of hepatic and intestinal lymph.* Nine adult rats were anesthetized and after the hepatic lymph duct was cannulated and lymph began to be collected(5), each rat was injected intravenously with 3.0 ml of a 2% suspension of brain lecithin in 5% dextrose-normal-saline solution and then infused continuously at an approximate rate of 1.0 ml per hour with the same suspension of phosphatide. This infusion was continued as long as lymph continued to flow (4-12 hours). A series of 6 control rats was subjected to the identical procedures except a 5% dextrose-saline solution was substituted for the phosphatide emulsion. Plasma samples were obtained before and immediately after initial injection of the phosphatide and also at the end of the experiment. These samples together with the total lymph collections were analyzed for phospholipid and cholesterol(6). For comparative purposes, another series of 15 adult rats (starved for 36 hours prior to operation) was anesthetized, the intestinal lymph duct was cannulated(7) and the lymph collected for 10 hours. Then 8 of these 15 rats were injected and infused as described above for 10 hours with a 3% lecithin suspension in 5% dextrose-normal-saline solution. The remaining 7 rats for control purposes were infused with 5% dextrose-saline solution. Plasma samples were obtained be-

TABLE I. Rate of Disappearance of Injected Phosphatide in Liverless Rat.

No. of rats	Avg wt (g)	Plasma phospholipid (mg/100 ml) Before inj.	Immed. after inj.	3 hr after inj.
		A. Control normal rats		
10	272	110 ± 2.6*	414 ± 16.4	178 ± 7.8
		B. Hepatectomized rats		
14	274	108 ± 3.1	504 ± 18.2	421 ±12.8

* ± S.E. of mean in all cases.

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† "Lecithin (animal), 90% pure", purchased from Nutritional Biochemicals Corp., Cleveland, O.

TABLE II. Phospholipid and Cholesterol Content of Hepatic and Intestinal Lymph during Continuous Infusion of Phospholipid.

No. of rats	Avg wt (g)	Avg amt. phospholipid infused (mg)	Avg plasma phospholipid (mg/100 ml)		Avg plasma cholesterol (mg/100 ml)		Lymph			
			Before initial inj.	Immed. after initial inj.	Before initial inj.	End of infusion	Duration of collect. (hr)	Vol (ml)	Cholesterol (mg/100 ml)	Phospholipid (mg/100 ml)
					I. *Hepatic lymph: A. Rats infused with phosphatide*					
9	327	226 ± 11.0*	103 ± 3.2	206 ± 7.3	323 ±15.0	47 ± 2.1	117 ± 8.6	9.7 ± 1.1	2.6 ± .4	34 ± 2.4	123 ± 8.7
						B. Control rats infused with dextrose-saline				
6	320		107 ± 4.4	129 ± 3.8	51 ± 2.2	63 ± 3.6	9.8 ± 1.2	1.0 ± .2	30 ± 3.0	80 ± 7.8
					II. *Intestinal lymph: A. Rats infused with phosphatide*					
8	281	325 ± 12.0	106 ± 3.9	506 ± 7.4	1069 ± 8.2	59 ± 5.2	169 ± 8.2	10	9.9 ± .8	21 ± 1.7	88 ± 6.2
						B. Control rats infused with dextrose-saline				
7	285		104 ± 4.1		65 ± 6.2	58 ± 4.2	46 ± 3.8	10	6.3 ±1.1	26 ± 2.1	95 ± 7.4

* ± S.E. of mean in all cases.

fore and at the end of the experiment in all of the rats; in the experimental group, an additional sample was obtained immediately after the initial injection of phosphatide. The plasma samples together with the total lymph collections were analyzed usually for both phospholipid and cholesterol. C. *Effect of phosphatide infusion upon phospholipid content of hepatic bile.* A group of 10 adult rats was anesthetized, the bile duct cannulated, and bile collected for 24 hours. Five of these rats also received phosphatide as described above during the entire period of 24 hours. The remaining 5 rats received 5% dextrose-normal saline solution instead of phosphatide. Plasma samples obtained before, immediately, and 24 hours after the start of the infusion were analyzed for phospholipid and cholesterol. The total bile collection was analyzed for phospholipid.

Results. The rate of disappearance of a single injection of phosphatide was (See Table I) markedly retarded in the liverless rat. Whereas the normal animal exhibited an average decline of 57% in its plasma level of phosphatide, 3 hours after the injection, only a fall of 16% was observed in the hepatectomized rat after the same period of time.

Continuous infusion of phosphatide into rats subjected to hepatic lymph collection led to a marked elevation of plasma phosphatide (Table II) and also as a consequence of the latter elevation, the usually observed hypercholesteremia. The lymph of these rats collected during this period (Table II) as compared to the control animals exhibited little or no change in cholesterol content but a significantly increased phospholipid concentration. On the other hand, intestinal lymph obtained from a group of rats infused with phosphatide showed no higher cholesterol or phospholipid content (Table II) than the intestinal lymph of the control animals.

Similar to intestinal lymph, the bile of rats infused with phosphatide exhibited no greater content of phospholipid (Table III) than that of the control series infused with dextrose-normal saline solution.

Discussion. The results of the present experiments confirm the fact that the liver of

TABLE III. Phospholipid Content of Hepatic Bile during Continuous Infusion of Phosphatide. 5 rats/series.

Avg wt (g)	Avg amt phospholipid infused (mg)	Avg plasma phospholipid (mg/100 ml)			Avg plasma cholesterol (mg/100 ml)		Bile (24 hr)		
		Before initial inj.	Immed. after initial inj.	End of infusion†	Before initial inj.	End of infusion†	Vol (ml)	Avg phospholipid mg/100 ml	mg/24 hr
A. Rats infused with phosphatide									
304	830 ± 8.0*	107 ± 2.4	305 ± 10.4	422 ± 32.0	55 ± 2.1	137 ± 18.2	14.2 ± 1.7	8.0 ± .8	1.2 ± .1
B. Control rats infused with dextrose-saline									
306		109 ± 3.0	91 ± 2.2	112 ± 2.3	54 ± 1.2	62 ± 2.2	15.6 ± .4	8.5 ± .4	1.4 ± .1

* ± S.E. of mean in all cases.

† 24 hr.

the normal rat is capable of rapidly removing large quantities of an injected phosphatide from the circulating plasma. However, it is not at all clear how phosphatide is withdrawn or what becomes of it when it is withdrawn.

Certainly the small gain in the phosphatide content of hepatic lymph suggests that little excess phosphatide accumulates in the hepatic tissue fluids despite its high concentration in the plasma and it continual escape therefrom. The failure of bile also to exhibit any increase in its phospholipid content during the massive infusion of this lipid strongly suggests that the ability of the liver to remove excess phosphatide from plasma is not due to its hepatobiliary excretory capacity. This last finding conforms with our previous observations(8) that the phospholipid rise in plasma after biliary obstruction is due primarily to a preceding rise of plasma bile acids.

It is of interest that despite the presence of hypercholesteremia in the phosphatide infused rats, no increase in the cholesterol content of either hepatic or intestinal lymph was observed. Earlier studies(9) have revealed that excess cholesterol in hepatic lymph occurs only when there is an excess in plasma of cholesterol carried in a form which is relatively freely diffusible (*e.g.*, in the rat injected with the hypercholesteremic serum obtained from a rat with biliary obstruction). Excess cholesterol does not occur in hepatic lymph when the excess plasma cholesterol is carried in a form in which it is seemingly "trapped" or inextricably bound to lipid such as occurs in the developing stages of the triglyceride induced hypercholesteremia observed in the nephrotic state(10) and after the injection of Triton(9). These latter data therefore suggest that the excess cholesterol appearing in plasma after continuous infusion of phosphatide is present in some relatively indiffusible state. Some of the excess cholesterol however does escape from the blood because the liver of the *starved* rat invariably exhibits an increase in cholesterol content after a 12 hour period of massive phosphatide infusion(3).

Summary. Rate of disappearance of intravenously injected phosphatide from plasma

was markedly retarded in the liverless rat. When hypercholesteremia was provoked in normal rats by constant infusion of phosphatide, the elevated blood cholesterol concentration was not reflected in any rise of cholesterol level in hepatic or intestinal lymph, or in bile. The elevated phospholipid level was not reflected in bile or intestinal lymph, but did cause a moderate rise in the phospholipid level of hepatic lymph.

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Effects of Dietary Fat upon Plasma Polyunsaturated Acids.* (23583)

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It has been amply demonstrated that plasma cholesterol concentration can be altered by changes in type of dietary fat(1-4). Dietary cholesterol and physiological stresses which induce hypercholesterolemia accelerate development of essential fatty acid (EFA) deficiency(5,6). It is commonly known that cholesteryl esters and phospholipids of plasma contain highly unsaturated acids. It has been postulated that polyunsaturated acids are required for normal transport of cholesterol and other lipids(7).

To test this hypothesis, a study of the effects of alterations in dietary fat upon polyunsaturated acids of plasma was desirable. The fatty acid composition of human plasma lipids was therefore determined in individuals in whom the effect of dietary fat upon plasma cholesterol was being studied. The difficulty of the analysis for polyunsaturated acids limited the number of individuals to be studied. The results presented here are from 4 individuals from Exp. I and II, and 8 individuals from Exp. III, IV, V, and VI of an investigation published earlier(8). In addition, an experiment was performed on one case of familial hypercholesterolemia in whom the effects of dietary ethyl linoleate were observed.

Exp. A. Four normal volunteers were fed experimental diets prepared under supervision of dietitian in the University Hospital in Lund. They consumed sufficient amounts of the diets to maintain their body weights constant. Blood was drawn periodically for analysis of total plasma cholesterol(9) and of plasma polyunsaturated acids(10). Two subjects were changed from their unrestricted diet to one in which 40% of calories was provided by corn oil, but in which no other fat was added. This regime was maintained for 4 weeks, then the diet was replaced for another 4 weeks by one in which butterfat provided 40% of the calories. The other 2

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