OBSERVATIONS CONCERNING THE PRODUCTION AND EXCRETION OF CHOLESTEROL IN MAMMALS

V. The Relation of Biliary Retention of Cholesterol, Distention of the Biliary Tract, the Shunting of Bile to the Vena Cava, and the Removal of the Gastro-Intestinal Tract to the Hypercholesteremia Consequent on Biliary Obstruction*

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Although previous studies of this series (1, 2) have demonstrated that the liver is the source of plasma cholesterol, the mechanism regulating the plasma cholesterol level is still unknown. The present report describes the results of some studies concerning the mechanism underlying the accumulation of excess cholesterol in plasma following obstruction of the bile duct. These results indicate that the high concentration of cholesterol in plasma is not consequent on any alteration in the biliary or intestinal excretion of cholesterol, nor is it related to distention of the bile duct itself.

EXPERIMENTAL

Retention of Bile Cholesterol

Since bile contains cholesterol, retention of such cholesterol after biliary obstruction has been thought by some to be the cause of the excess cholesterol found in plasma after such obstruction. This seemed unlikely, because the amount of cholesterol excreted in the bile per day (3) is only about one-third of the amount accumulated in the plasma after 1 day of biliary obstruction; nevertheless, the possibility was explored further.

Ten male Long-Evans rats, averaging 328 gm., were submitted to biliary cannulation (3). The cannula was obstructed for 24 hours, then released, and bile collected during the following 24 hours. Plasma samples were obtained before cannulization, after 24 hours of obstruction, and 24 hours after release of the obstruction. The plasma samples and bile were analyzed for total cholesterol by published methods (3, 4).

As Table I demonstrates, the plasma cholesterol increased and then returned to normal following biliary obstruction and its release, respectively. Most im-

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important, moreover, in the first 24 hour period following release (during which time the plasma cholesterol returned to normal), the biliary concentration and daily output of cholesterol were 8.3 mg. per 100 cc. and 1.27 mg. per 24 hours, respectively,—values less than those found in the bile of normal rats (12.7 mg. per 100 cc. and 1.83 mg. per 24 hours (3)). This observed fall in plasma cholesterol without any concurrent increase in biliary excretion of cholesterol after release of biliary obstruction is a new indication that whatever the mechanism biliary obstruction sets going for the production of hypercholesteremia, it bears no direct relationship to either the retention or excretion of biliary cholesterol.

TABLE I

The Cholesterol Content of Plasma and Bile after Release of Biliary Obstruction

Rat	Weight	Plasma cholesterol			Bile volume	Bile cholesterol content after release of obstruction	
		Before obstruction	24 hrs. after obstruction	24 hrs. after release of obstruction			
	gm.	*mg./100 cc.*	*mg./ 100 cc.*	*mg./100 cc.*	*ml.*	*mg./100 cc.*	*mg./24 hrs.*
43	333	53	104	40	14.2	12.1	1.72
54	282	51	122	73	4.8	11.6	0.56
25	362	—	145	70	7.2	9.5	0.68
26	360	—	152	—	21.7	8.2	1.78
27	310	82	180	—	16.9	7.4	1.25
28	312	58	150	68	24.0	9.7	2.33
29	350	—	152	—	26.5	9.7	2.57
41	315	68	114	—	14.8	4.7	0.70
42	313	61	95	46	10.3	5.2	0.54
43	344	57	141	85	12.7	4.7	0.61
Average		61	136	64	15.3	8.3	1.27

Distention of the Biliary Tract

Conceivably, simple distention of the biliary tract after ligation of the common bile duct could bring about, in some way as yet unknown, the hypercholesteremia observed.

Effect of Prior Hepatic Denervation.—

In order to investigate the possibility that obstruction of the biliary duct might give rise to reflex nervous stimuli which in turn would lead to the observed excess plasma cholesterol, nine rats were anesthetized with ether and then complete denervation of the liver was accomplished just prior to ligation of the common bile duct by excision of the nerve trunks accompanying the hepatic artery.

The average plasma total cholesterol content of these rats after 24 hours of obstruction was 143 mg. per 100 cc., while the corresponding figure in eight control rats subjected to ligation alone was 151 mg. per 100 cc. These results indicate that the hypercholesteremia after ligation occurs independently of nervous stimulation of the liver.

Cholesterol Content of Liver before and after Biliary Obstruction.—

Distention of the bile duct might possibly cause hypercholesteremia by back pressure of bile perfusing or compressing the liver so as to release tissue cholesterol. However, analyses of the cholesterol content of liver lobes removed surgically from ten rats, before and 24 hours after obstruction, indicated that no significant loss of cholesterol from hepatic tissue took place. The average cholesterol content of the liver was 8.73 mg. per gm. (dry weight) before and 8.26 mg. per gm. after biliary obstruction.

The Effect of Retention of Bile without Biliary Obstruction or Distention.—

Although the preceding observations made it clear that biliary tract distention *per se* did not produce the hypercholesteremia observed after ligation of the biliary duct by either reflex nervous stimulation, or by compression of hepatic tissue with subsequent release of stored cholesterol, it was still possible that the distention interfered in some other manner with the mechanisms underlying the hepatic synthesis and discharge of cholesterol into the plasma.

In order to investigate this latter possibility, rats were subjected to an operation which produced a state in which bile was retained without an accompanying significant distention of the biliary tract.

The operation consisted of the following procedures:—The rats were anesthetized with ether, the abdomen opened, and the end of a curved polyethylene catheter (diameter: 0.023 inch) was introduced into the inferior vena cava *via* a right lumbar vein. The catheter was kept patent by intermittent injection of normal saline solution during a few minutes until it was joined by means of a steel tube to a polyethylene catheter of the same diameter, previously introduced into the biliary duct. As soon as the two catheters were joined, bile could be seen to enter the inferior vena cava. This would be expected in view of the fact that the maximal "secretory" pressure of bile in the rat is 23 cm. of this fluid (3). The condition of the rats 24 hours after operation was good. Their average blood pressure was 122 mm. of Hg and their average packed cell volume, 42 per cent indicating that no significant hemolysis had occurred. Although rats subjected to this type of operation have, in our experience, survived for many days, the majority of the present series were examined at the end of 24 hours in order to be certain that the anastomosis was functioning and that no distention of the bile duct had occurred. Twenty-three successful instances were obtained. Twelve control rats were subjected to bile duct ligation alone.

During this form of biliary retention, hypercholesteremia again occurred. The degree, however, was somewhat less than that found in the twelve control rats subjected to bile duct ligation (see Table II). The significance of this dif-

ference is uncertain. Perhaps the hypercholesteremia after biliary obstruction is due chiefly, if not entirely, to the retention of a substance or substances which would normally be excreted in the bile.

The Possible Role of Intestinal Activity in the Production of Hypercholesteremia after Biliary Obstruction

Although the experiments just described have demonstrated that the retention of bile cholesterol is not responsible for the hypercholesteremia observed after biliary obstruction, the possibility remains that perhaps a normal intestinal excretion or destruction of cholesterol is interfered with in some way under such circumstances. If this did happen, one would expect that removal of the intestines alone would produce hypercholesteremia.

TABLE II

Hypercholesteremia after Biliary Obstruction or Anastomosis of Bile Duct and Inferior Vena Cava

Type of operation	No. of rats	Average weight	Cholesterol	
			Before operation	24 hrs. after operation
		gm.	*mg./100 cc.*	*mg./100 cc.*
Biliary obstruction	12	314	46 (33–67)*	132 (95–190)
Anastomosis of bile duct and inferior vena cava	23	261	49 (28–72)	97 (63–144)

* Figures in parentheses give range of individual values.

To investigate this possibility, animals were studied from which the gastro-intestinal system had been removed.

This was accomplished as follows: The rat was anesthetized with ether and the bile duct was cannulated, directing the flow of bile outside the body. Then the terminal end of the colon and the abdominal portion of the esophagus were ligated and severed. The inferior mesenteric artery and all branches of the superior mesenteric artery except the hepatic artery were ligated and the mesenteric attachments of the stomach, intestines, and spleen were ligated and cut. In this manner, a rat was obtained whose liver was functioning to some extent but whose entire gastro-intestinal tract had been removed.

Ten successful preparations of this type were obtained. For control purposes, twelve rats were subjected to the same operation, except that their bile ducts were not cannulated but instead were ligated.

The general condition of both types of eviscerated rats was surprisingly good for about 24 hours after operation. During this period they were lively, they urinated freely, and did not appear dehydrated. They usually had a blood pressure of 80 to 100 mm. of Hg. It is of interest that the average hematocrit

reading of ten eviscerated rats with ligated bile ducts fell from 42 to 39 per cent during the 24 hours. The hematocrit reading of twelve rats with cannulated ducts fell from 44 to 40 per cent during the same period.

It will be seen (Table III) that the rats deprived of intestines and stomach exhibited no significant change in plasma cholesterol whereas those animals subjected to the same procedures plus biliary obstruction exhibited a marked hypercholesteremia. Obviously intestinal activity *per se* had nothing to do with the hypercholesteremia occurring after biliary obstruction.

TABLE III

The Plasma Cholesterol after Viscerectomy with and without Free Bile Flow

Type of operation	No. of rats	Average weight	Plasma cholesterol (total)	
			Before operation	After operation
		gm.	*mg./100 cc.*	*mg./100 cc.*
Viscerectomy with free flow of bile	10	225	50 (40–64)*	51 (32–80)
Viscerectomy with biliary obstruction	12	249	51 (40–64)	97 (76–110)

* Figures in parentheses represent the range.

DISCUSSION

Previous observers (5, 6) have suggested that the hypercholesteremia occurring after biliary obstruction is not due to a retention of the cholesterol in the bile, and the present data make plain that this is the case, the bile cholesterol having no relation to the hypercholesteremia.

The present report also demonstrates that the distention of the biliary duct system resulting from obstruction does not exert a direct role in the genesis of the hypercholesteremia. Finally, the data indicate that retention of bile does not produce the observed hypercholesteremia by altering some intestinal action upon cholesterol.

One must conclude from this study that whenever bile is retained in plasma, either by ligation of the bile duct or anastomosis of the latter to the inferior vena cava, the process leads to a hypercholesteremia which is occasioned neither by the abnormal retention of cholesterol which otherwise would be excreted in bile, nor by a possible abnormal retention stemming from changed intestinal excretion of the substance. These results suggest the possibility that biliary obstruction may lead to the retention in plasma of one or more substances, other than cholesterol, which in turn effect a rise in plasma cholesterol by some process independent of gastro-intestinal activity.

The identity of physiological substances capable of stimulating hypercholesteremia is of great medical importance. The chief constituents of bile are the

bile acids. Therefore, we are investigating the ability of bile acids to produce hypercholesteremia in both the bile duct ligated rat and in the normal animal.

SUMMARY

Plasma cholesterol was observed to fall after release of biliary obstruction without any concurrent change in biliary excretion of cholesterol.

Excision of the nerve trunks accompanying the hepatic artery did not change the degree of hypercholesteremia following biliary obstruction.

No significant change in the cholesterol content of hepatic tissue occurred following biliary obstruction.

Animals with gastro-intestinal systems removed exhibited no significant change in plasma cholesterol, whereas those subjected to gastro-intestinal removal plus biliary obstruction exhibited a marked hypercholesteremia.

Anastomosis of the bile duct to the inferior vena cava resulted in a hypercholesteremia similar to that occurring after obstruction of the bile duct.

CONCLUSIONS

1. The increase in plasma cholesterol following biliary obstruction in the rat is not due to retention of the cholesterol normally excreted in the bile.
2. Possible changes in intestinal excretion or absorption after biliary obstruction were not responsible for the hypercholesteremia occurring after the latter phenomenon.
3. Retention of bile itself independently of obstruction or distention of the bile duct causes hypercholesteremia.
4. The investigation of bile acids as hypercholesteremic agents is suggested.

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