Studies Concerning the Pathogenesis of Neurogenic Hypercholesterolemia I. Independence of Hypercholesterolemia and Biliary Excretion[1] (35873)

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Previously we reported (1) the production of a chronic hypercholesterolemic state in the rat by bilateral electrolytic injury of the hypothalamus, involving the ventral medial nucleus, the fornices and the medial portion of the lateral hypothalamic nucleus. The hypercholesterolemia induced was found to be greatly intensified by the inclusion of excess cholesterol in the animal's diet.

Following the discovery of this type of neurogenic hypercholesterolemia, we have been attempting to discern the intermediary mechanism(s) by which this brain lesion elevates the blood cholesterol. In this connection, the studies of Gutstein and his associates (2–4) were of interest to us because they were able to induce an *acute, transitory* elevation of the blood cholesterol of the rat fed fat, by stimulating an area in the lateral hypothalamic nucleus. They attributed the observed rise in blood cholesterol to biliary obstruction caused by constriction of the sphincter of Oddi resulting from the hypothalamic stimulation.

Although our procedure is quite different from that of Gutstein *et al.* (2–4), we thought it necessary to study our experimental rats to determine whether their *chronic* hypercholesterolemia was in any way due to obstruction to the free passage of bile. Our studies showed quite conclusively that biliary obstruction did not play a part in the genesis of this type of neurogenic hypercholesterolemia.

Methods. To determine whether interference in biliary flow was responsible for the hypercholesterolemia following induction of our specific hypothalamic injury, we performed the following series of studies.

In the first study, the hypothalamic lesion was induced as previously described (1) in 10 of 20 young adult male rats (Long Evans strain, weighing an av of 300 g). The animals were group pair fed a low cholesterol diet containing 43 mg of digitonin-precipitable Liebermann-Burchard positive sterols of vegetable origin/100 g of diet. The plasma cholesterol concentration was determined weekly for 3 weeks according to the method of Martinek (5) and then the animals were killed. The respective diameters of the common bile ducts of the rats with the hypothalamic lesion were inspected and compared with those of the control animals. Finally, liver sections were obtained, fixed, embedded in Epon plastic, sectioned (1 μ thickness) and stained with toluidine blue. These sections then were studied microscopically to determine the state of the bile canaliculi.

In the second study, the hypothalamic lesion was induced in 6 of 13 rats. All rats subsequently were group pair fed the above described low cholesterol diet. Four weeks later, the common bile duct of all rats was cannulated; and the bile was collected for 24 hr in the 5 surviving lesioned and the 7 control animals. The volume of bile and its cholesterol content were measured.

In the third study, 15 of 33 rats were operated and 2 small incisions were made in their common bile duct approximately 3 mm apart. One end of a small segment of polyethylene tubing (o.d. = 0.038 in.; i.d. = 0.023 in.) was introduced into the distal incision and passed along the lumen of the bile duct through the sphincter of Oddi to enter and lie freely in the duodenum. The other

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end of the tubing was introduced into the proximal incision and passed up the common bile duct toward the liver.

Sutures then were placed about those portions of the common bile duct enclosing the polyethylene tubing. This preparation thus allowed the free passage of bile into the duodenum unhindered by any possible constriction of the sphincter of Oddi or of the bile duct itself. Following this operation, all rats were group pair fed a diet containing whole egg, milk and cottonseed oil (cholesterol concentration: 273 mg/100 ml) for 4 weeks. The rats were bled every 2 weeks for determination of their plasma cholesterol concentration. At the end of the fourth week, 7 of the 13 surviving rats bearing the tubing were given the hypothalamic lesion. A similar hypothalamic lesion was induced in 8 of the 18 previously unoperated rats. Blood samples were obtained 1 and 2 weeks after the hypothalamic lesion had been produced. The first sample was analyzed only for its plasma cholesterol concentration; the second sample was analyzed also for bilirubin concentration. After the second blood sample had been obtained, all rats received bromsulfalein (5 mg/100 g of body wt) by iv injection. Blood samples were obtained 5 min after this injection and analyzed for their bromsulfalein content according to the method of Henry *et al.* (6).

In the fourth study, 15 of 28 rats were operated and a single ligature was placed about the common bile duct. At autopsy all ligated ducts were seen to be extremely dilated proximal to this ligation. Nine of these 15 duct-ligated rats also received the specific hypothalamic lesion. A similar hypothalamic lesion was induced in 5 of the 13 rats which were not subjected to bile duct ligation. All rats were group pair fed 30 ml/day of a liquid cholesterol-free diet consisting of egg white powder, sucrose, vegetable oil, NaCl, and H_2O. Blood samples were obtained 3, 5, and 7 days after operation and analyzed for cholesterol.

Since in every experiment each group of rats was restricted in its diet to the quantity of food freely consumed by those eating least, all rats consumed their entire rations each day and weight gains of experimental and control groups were comparable in every case.

Results. Both the biliary system and the hepatic parenchymal cells of the 10 rats killed 3 weeks after induction of the hypothalamic lesion appeared identical to their tissue counterparts in the control rats; this despite the chronic hypercholesterolemia (av plasma cholesterol during the 3 weeks, 116 mg/100) of rats bearing hypothalamic lesions compared to the normocholesterolemia (av plasma cholesterol, 56 mg/100 ml) observed in the control rats. Thus the common bile ducts of the operated rats appeared, on gross inspection, to be undilated. Similarly, on microscopic examination, no evidence of dilation of bile canaliculi or dystrophy of hepatic parenchymal cells could be discerned.

Similarly the average bile flow (16.5 ml/24 hr) in rats, 4 weeks after hypothalamic injury, was essentially the same as that (15.9 ml/24 hr) observed in the control rats. However the average cholesterol concentration of the bile (12.0 mg/100 ml) of the experimental rats was significantly less ($p<0.05$) than that (16.3 mg/100 ml) found in the bile of the control rats.

As Table I reports, the insertion of the polyethylene tubing into the bile duct, to forestall any possible biliary obstruction in the lesioned rats by possible constriction of the sphincter of Oddi did not prevent the occurrence of the hypercholesterolemia usually observed after induction of the hypothalamic lesion. Table I also illustrates that there was no excess retention of bilirubin in the plasma of the rats with lesions (whether or not provided with the polyethylene "shunt" prior to induction of the hypothalamic lesion) nor was there any retardation in the hepatic uptake of bromsulfalein.

Figure 1 shows clearly that the hypothalamic injury evokes its own specific hypercholesterolemic response even in rats subjected to total biliary obstruction. Thus the average plasma cholesterol concentration of these bile duct-ligated rats bearing hypothalamic lesions (3 days after both surgical procedures), was 228 mg/100 ml, a value significantly ($p<.01$) greater than that (163 mg/100 ml)

TABLE I. The Plasma Cholesterol, Bilirubin, and Bromsulfalein Excretion in Normal and Bile Duct Cannulated Rats After Hypothalamic Injury.

			Weeks after duct cannulation				Weeks after hypothalamic lesion					
	Initial av		2		4		1		2			
No. of rats	Wt (g)	Plasma cholesterol (mg/100 ml)	Av wt	Av plasma choles.	Av wt	Av plasma choles.	Av wt	Av plasma choles.	Av wt	Av plasma choles.	Av plasma bilirubin (mg/100 ml)	Bromsulfalein (% remaining after 5 min)
A. Rats subjected to duct cannulation and later hypothalamic injury												
7	300	71	335	111	364	95	383	186[a]	399	214[a]	0.23	22.6
Range		(68–74)		(67–164)		(75–110)		(119–226)		(146–314)	(0.2–0.4)	(20–24)
SE		±2.4		±12.3		±4.6		±14.1		±24.6	±0.01	±0.53
B. Control rats subjected to duct cannulation only												
6	305	70	328	117	356	101	371	96	377	101	0.20	22.5
Range		(68–74)		(86–140)		(87–115)		(79–115)		(60–121)	(0.1–0.25)	(21–24)
SE		±3.1		±13.7		±4.6		±6.5		±9.4	±0.05	±0.43
C. Control rats subjected to hypothalamic injury only												
8	307	68	360	108	390	96	406	172[b]	429	176	0.20	23.5
Range		(62–74)		(90–128)		(86–114)		(138–221)		(114–224)	(0.1–0.25)	(118–27)
SE		±2.9		±4.4		±3.4		±9.4		±15.2	±0.02	±1.25
D. Control intact rats												
10	304	67	354	101	384	95	397	104	407	103	0.20	22.0
Range		(67–72)		(62–140)		(68–121)		(74–125)		(72–133)	(0.1–0.25)	(20–24)
SE		±3.1		±7.9		±6.8		±6.5		±5.7	±0.04	±0.49

[a] Significantly greater ($p < .001$) than corresponding values shown in Sect. B.

[b] Significantly greater ($p < .001$) than corresponding values shown in Sect. D.

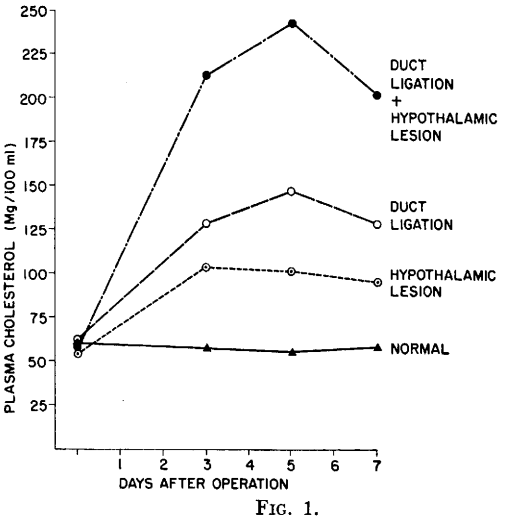

FIG. 1.

found in rats subjected to bile duct ligation alone. As Fig. 1 further illustrates, this difference persisted during the total 7-day postoperative period.

Discussion. The present results indicate decisively that the chronic hypercholesterolemia we consistently observed after making a bilateral hypothalamic injury involving the ventral medial nuclei, the fornices, and the medial portion of the lateral hypothalamus (1) cannot be due to any interference in the flow of bile coming after the induction of the brain lesion. The final experiment in which induction of the hypothalamic lesion was observed to effect an additional rise in the plasma cholesterol of rats also subjected to total biliary obstruction, clearly points to some additional mechanism (following the brain lesion) other than inhibition of bile flow, which is responsible for the pathogenesis of the hypercholesterolemia observed.

These results, however, do not negate the validity of the conclusions reached by Gutstein and his associates (3, 4) concerning the role of biliary obstruction in the pathogenesis of the transient hypercholesterolemia they observed in their experimental rats. Undoubtedly their preparation is one that is entirely different from ours. Thus they unilaterally and acutely stimulated an area in the lateral hypothalamus whereas we bilaterally induced a chronic lesion in the specific hypothalamic areas described above. Secondly, they observed only a modest, transient rise in both the plasma cholesterol and triglyceride; whereas our experimental animals exhibited a marked, chronic rise of plasma cholesterol but not of triglyceride (1).

Summary. The possible role of the biliary system in the pathogenesis of hypercholesterolemia observed in rats following bilateral hypothalamic injury involving the ventral medial nuclei, the fornices, and the medial portions of the lateral hypothalamus was investigated. The hypercholesterolemia was found not to be mediated by any abnormality in the flow of bile.

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