The Pathogenesis of Neurogenic Hypercholesterolemia: V. Relationship to Hepatic Catabolism of Cholesterol[1] (37711)

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Following specific bilateral injury to its ventromedial nuclei, fornices, and medial portions of the lateral hypothalamic areas, the rat promptly exhibits a chronic hypercholesterolemia (1). This hypercholesterolemia was found to be independent of any dysfunction of the pituitary, thyroid, adrenal, or testicles (2). It was observed, however, to be associated with relatively slow removal of cholesterol from the blood stream by the liver (3).

Because of this latter finding and also because there is considerable evidence (4, 5) to suggest that changes in the rate of formation of bile acids also influence the serum level of cholesterol, we decided to investigate the capacity of the liver of the rat with the above hypothalamic injury to form and secrete bile acids. The results of this investigation indicate that this type of hypothalamic lesion diminishes the rate of hepatic synthesis and secretion of bile acids.

Methods. The rate of disappearance from plasma and conversion into bile acids of ^{3}H-labeled lipoprotein cholesterol. Fifteen of twenty-eight young-adult male rats (Long-Evans strain, weighing between 250 and 325 g) were subjected to two hypothalamic electrolytic lesions (2 mA for 10 sec) on each side (1.4 and 1.8 mm posterior to bregma, respectively, 0.75 mm lateral to the midline, and 9.5 mm beneath the surface of the brain). The remaining 13 rats serving as controls were subjected to the same operation, but no current was delivered. All animals were fed 30 ml/day of a high-cholesterol (273 mg/100 ml) liquid diet mixture consisting of evaporated milk (200 ml), glucose (80 g), Kaopectate Suspension (30 ml), vegetable oil (50 ml), NaCl (4 g), whole egg powder (36 g), Poly-Vi-Sol suspension (0.3 ml), and H_2O (189 ml).

The rats were bled before and also 7 and 14 days after operation, and the serum was analyzed for its cholesterol concentration (6). In view of the fact that in earlier studies (1, 2) hypercholesterolemia occurred in our rats only when the ventromedial nuclei, fornices, and medial portions of the lateral hypothalamic areas exhibited severe injury, satisfactory hypothalamic lesions were considered present in all rats whose serum cholesterol levels had increased at least 100% over their preoperative levels. On this basis, 11 of the 15 operated rats were selected as bearing satisfactory hypothalamic lesions. These 11 rats and the 13 control rats were then anesthetized with ether, and their bile ducts cannulated as previously described (7). Immediately after each rat was cannulated, it was injected intravenously with 2 ml of serum containing lipoprotein cholesterol-1α,2α-^{3}H (each dose contained 1.04×10^6 dpm of ^{3}H-cholesterol). Bile was then collected from all rats for 6 hr, after which time they were killed, their livers perfused with normal saline, and removed. Blood samples also were obtained immediately before, and again at the end of the bile collection. Cholesterol was determined quantitatively in blood, liver, and bile samples, and the specific radioactivity

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TABLE I. Rate of Disappearance and Conversion into Bile Acids of Intravenously Injected Labeled Cholesterol in Hypothalamic-Injured Rats.

	Hypothalamus-injured rats	Control rats
Number of rats	11	13
Average weight (g)	266*	301
Range	(236–277)	(286–300)
SEM[a]	± 4.3	± 3.4
Serum		
Cholesterol		
mg/100 ml serum	125*	78
Range	(100–153)	(55–109)
SEM	± 6.0	± 4.4
dpm × 10^4/mg cholesterol		
Immediately after injection	6.3	9.6
Range	(5.3–7.8)	(7.1–11.4)
SEM	± 0.4	± 0.5
6 hr after injection	2.5	2.3
Range	(2.0–3.1)	(2.1–2.6)
SEM	± 0.2	± 0.2
dpm × 10^4/ml		
Immediately after injection	7.9	7.5
Range	(5.5–10.4)	(4.5–10.5)
SEM	± 0.4	± 0.5
6 hr after injection	3.1*	1.8
Range	(1.8–4.4)	(1.2–2.7)
SEM	± 0.3	± 0.2
Liver (6 hr after serum injection)		
Weight (g)	11.1*	14.1
Range	(9.5–14.6)	(11.5–17.4)
SEM	± 0.5	± 0.5
mg cholesterol/total liver	277	310
Range	(179–379)	(119–382)
SEM	± 19.3	± 27.4
dpm × 10^3/mg cholesterol	0.9	1.2
Range	(0.3–1.6)	(0.6–1.9)
SEM	± 0.2	± 0.1
dpm × 10^5 total liver cholesterol	2.5***	3.9
Range	(0.9–4.6)	(2.3–7.5)
SEM	± 0.4	± 0.5
% of dose in liver	26.3***	40.1
Range	(8.9–44.4)	(25.3–72.4)
SEM	± 3.9	± 4.3
Bile (6 hr)		
Volume (ml)	3.74***	4.76
Range	(1.8–4.8)	(4.0–5.1)
SEM	± 0.50	± 0.1
Bile acids		
mg/ml	16.5	16.4
Range	(10.1–23.6)	(12.3–19.5)
SEM	± 1.5	± 0.8

TABLE I (continued).

	Hypothalamus-injured rats	Control rats
Bile acids		
mg/total bile	58.3**	77.9
Range	(32.4–73.2)	(65.2–97.9)
SEM	± 5.3	± 4.
dpm × 10^3/mg bile acid	0.5	0.6
Range	(0.3–0.6)	(0.4–0.9)
SEM	± 0.05	± 0.1
dpm × 10^4 bile acids in total bile	2.8*	4.9
Range	(1.7–3.8)	(3.3–6.4)
SEM	± 0.3	± 0.4
Cholesterol		
mg/100 ml bile	34.6***	28
Range	(23–46)	(17–35)
SEM	± 2.4	± 1.5
mg/ total bile	1.3	1.3
Range	(0.8–2.3)	(0.9–1.7)
SEM	± 0.1	± 0.1
dpm × 10^3/mg cholesterol	5.2	5.9
Range	(2.2–9.0)	(3.9–11.2)
SEM	± 0.7	± 0.6
dpm × 10^4 cholesterol in total bile	0.5	0.6
Range	(0.1–0.9)	(0.1–0.9)
SEM	± 0.1	± 0.1

[a] SEM = standard error of mean.
* Value significantly different ($p < 0.001$) from corresponding value for control rats.
** Value significantly different ($p < 0.01$) from corresponding value for control rats.
*** Value significantly different ($p < 0.05$) from corresponding value for control rats.

of digitonin-precipitable sterols also was determined according to previously described methods (6). Bile acids in the bile samples were determined quantitatively by the method of Talalay (8) as modified by Admirand and Small (9). Radioactivity in bile acids was determined after bile salts were separated from cholesterol according to the method of Folch *et al.* (10).

The labeled lipoprotein cholesterol preparation was made by injecting intraperitoneally into each of 5 normal rats 0.5 mCi of cholesterol-1α,2α-^{3}H dissolved in 1,2-propanediol and bleeding them 48 hr later. Employing thin layer chromatography analysis according to the methods of Folch *et al.* (10), Chedid *et al.* (11) and Parker and Peterson (12), approximately 25% of the radioactivity in the pooled sera was found in the free cholesterol and 75% in the cholesterol esters.

Biliary excretion of newly synthesized bile acids and cholesterol. Eighteen of thirty-six young rats were subjected to hypothalamic injury as described above, and the remaining 18 rats served as control. All these rats were fed the above-described high-cholesterol diet (30.0 ml/day). One week later, the serum cholesterol concentration of all rats was determined. Employing the advent of hypercholesterolemia as the criterion for properly placed bilateral hypothalamic lesions, 15 of the original 18 experimental rats were selected for this phase of the study.

The 15 hypothalamus-injured and the 18 control rats were anesthetized with ether, and their bile ducts were cannulated. Bile secreted during the first hour was discarded, and the bile secreted during the next 5 hr was collected. The first hour's secretion was discarded in order to eliminate a large part

TABLE II. The Extent of Incorporation of Acetate-^{3}H into Biliary Bile Salts and Cholesterol in Hypothalamus-Injured Rats.

	Hypothalamus-injured rats	Control rats
Number of rats	15	18
Average weight (g)	288*	320
Range	(272-307)	(296–352)
SEM	± 2.7	± 3.4
Average plasma cholesterol (mg/100 ml)	148*	69
Range	(112–175)	(53–84)
SEM	± 6.9	± 2.1
Liver		
Weight (g)	11.6*	14.8
Range	(9.2–14.4)	(12.8–17.1)
SEM	± 0.4	± 0.3
mg cholesterol/total liver	304	335
Range	(200–387)	(154–582)
SEM	± 18.8	± 28.9
dpm/mg cholesterol	36.7	42.8
Range	(21–51)	(23–65)
SEM	± 2.5	± 3.1
dpm × 10^4 total liver cholesterol	1.04	1.21
Range	(0.7–1.4)	(0.9–1.8)
SEM	± 0.1	± 0.1
Bile (5 hr)		
Volume	3.5*	4.3
Range	(2.9–4.2)	(3.8–5.3)
SEM	± 0.1	± 0.1
Bile acids		
mg/ml	14.3*	27.0
Range	(5.9–32.6)	(18–50)
SEM	± 2.1	± 2.5
mg/total bile	49.5*	117.0
Range	(18–96)	(75–266)
SEM	± 6.8	± 13.5
dpm/mg bile acid	483	345
Range	(239–898)	(132–590)
SEM	± 65.	± 36.9
dpm × 10^4 bile acids in total bile	1.9*	3.5
Range	(0.9–2.5)	(1.9–5.2)
SEM	± 0.9	± 0.2
Cholesterol		
mg/100 ml	23.7**	29.2
Range	(124–43)	(22–38)
SEM	± 2.4	± 1.1
mg/total bile	0.9**	1.2
Range	(0.4–1.3)	(0.9–1.6)
SEM	± 0.1	± 0.1
dpm × 10^4/mg cholesterol	1.1	0.7
Range	(0.3–2.9)	(0.3–1.3)
SEM	± 0.2	± 0.1

TABLE II (continued).

	Hypothalamus-injured rats	Control rats
Cholesterol		
dpm $\times 10^4$ cholesterol in total bile	1.2	1.30
Range	(0.8–2.1)	(0.9–1.7)
SEM	$\pm$ 0.1	$\pm$ 0.1

* Value significantly different ($p < 0.001$) from corresponding value for control rats.
** Value significantly different ($p < 0.05$) from corresponding value for control rats.

of the animal body's pool of already formed bile acids. Immediately prior to the collection of the 5-hr bile sample, each rat was injected intravenously with 0.5 mCi Na acetate-^{3}H.[1]

At the end of the bile collection, the rats were bled, killed, their livers perfused free of blood with normal saline solution, and removed. Cholesterol was determined quantitatively in the blood serum, liver, and bile samples, and the specific radioactivity of digitonin-precipitable sterols in these samples also was determined. The concentration and radioactivity of the bile acids in the bile samples also was determined.

Results. The rate of disappearance from plasma and conversion into bile acids of endogenous cholesterol. As noted previously (13), ^{3}H-labeled lipoprotein cholesterol, after its injection, did not disappear as rapidly from the plasma of the hypothalamus-injured rats as it did from the plasma of the control rats. Thus, 6 hr after its injection, the average dpm of the cholesterol present in 1 ml of plasma of the 11 experimental rats was significantly greater (see Table I) than that observed in the 13 control rats. In accordance with this delay in egress from the plasma, the hepatic accumulation of labeled cholesterol was significantly less (see Table I) 6 hr after administration of the labeled cholesterol in the experimental rats than in the control rats. This difference in the rate of efflux of labeled cholesterol from the plasma of the experimental rat could have been due to some alteration in its hepatic function. It could also have been due to the greater initial dilution of the injected labeled lipoprotein cholesterol effected by the greater amount of unlabeled cholesterol present in the plasma of the experimental rat, leading to a relatively slower rate of disappearance of the labeled cholesterol. The initial dissimilarity, but final similarity, in the specific activities of the plasma cholesterol of the experimental and control rats (see Table I) is consonant with the latter explanation.

The rats with hypothalamic lesions excreted significantly less bile than the control rats during the 5-hr period of collection. As a consequence of this reduced biliary flow, the total quantity of bile acids excreted by the experimental rats during the 5-hr period was significantly reduced, although the concentration of bile acids in the bile of the experimental rats was not reduced (see Table I). Due to this same reduction in the total bile flow, the amount of ^{3}H-labeled bile acids excreted by the experimental rats (see Table I) was also considerably reduced.

The biliary excretion of cholesterol, however, did not appear to be affected in the hypothalamus-injured rats. Although the concentration of cholesterol was significantly greater in the individual bile volumes collected from the experimental rats, because of the comparatively reduced biliary flow, the average total biliary excretion of cholesterol during the 5-hr period of bile collection was no greater in the experimental rats than in the control rats. Table I also shows that the amount of ^{3}H-labeled bile cholesterol excreted by the experimental rats was approximately the same as that excreted by the control rats.

The extent of incorporation of acetate-^{3}H into bile acids. As was found in the preceding

[1] New England Nuclear Company, 100 mCi/m*M*.

study, Table II again illustrates that the hypothalamus-injured rat chronically secretes less total bile and certainly considerably less bile acids than the normal rat. Table II illustrates the fact that the experimental rat excreted considerably less newly synthesized (*i.e.*, ^{3}H-labeled) bile acids than the control rats during the 5-hr collection period. It is of interest, however, that no significant difference was observed between experimental and control animals in the rate of incorporation of acetate-^{3}H into hepatic cholesterol (see Table II).

Discussion. In a preceding study (13), we observed that hepatic as well as the intestinal rate of cholesterol synthesis did not appear to be altered in our rats with hypothalamic lesions. Hence, increased production of cholesterol probably was not responsible for the hypercholesterolemia observed in these rats. The present studies again revealed no significant change in their hepatic rate of synthesis of cholesterol (as measured by the rate of incorporation of ^{3}H-labeled acetate into cholesterol).

In addition, the present studies indicated that the liver of the hypothalamus-injured rat becomes smaller than that of the control rat. This liver chronically excretes less bile and bile salts, and it lags in its conversion of labeled cholesterol into labeled bile acids. Finally, the liver also appears to lag in its ability to incorporate acetate-^{3}H into newly synthesized bile acids. In other words, formation of bile acids is retarded regardless of whether such acids are made from preformed cholesterol coming to the liver from plasma or from cholesterol newly formed in the liver itself.

In view of the fact that there is considerable evidence at hand to indicate that hypercholesterolemia usually ensues when the usual conversion of cholesterol into bile acids is retarded, it seems most likely to us that one of the causes of the hypercholesterolemia occurring so rapidly and consistently in the rat after induction of our particular hypothalamic lesion may be a hepatic defect in the conversion of cholesterol into bile acids.

Summary. The rate of disappearance of ^{3}H-labeled cholesterol and its conversion into bile acids were measured in rats subjected to hypothalamic damage to their ventral medial nuclei, fornices, and the medial portions of their lateral hypothalamic areas. A significant reduction was found in both processes in the experimental rats. Also, the rate of incorporation of acetate-^{3}H into newly synthesized bile acids was found to be reduced in hypothalamus-injured rats. These findings suggest that the hypercholesterolemia regularly appearing after this type of hypothalamic injury stems from a relative lag in the catabolic conversion of cholesterol into bile acids.

1. Friedman, M., Byers, S. O., and Elek, S. R., Proc. Soc. Exp. Biol. Med. **131**, 759 (1969).

2. Friedman, M., Byers, S. O., and Elek, S. R., Amer. J. Physiol. **223**, 473 (1972).

3. Friedman, M., Byers, S. O., and Elek, S. R., Proc. Soc. Exp. Biol. Med. **142**, 359 (1973).

4. Byers, S. O., and Friedman, M., Proc. Soc. Exp. Biol. Med. **143**, 551 (1973).

5. Rosenman, R. H., Byers, S. O., and Friedman, M., J. Clin. Endocrinol. Metab. **12**, 1287 (1952).

6. Byers, S. O., and Friedman, M., Atherosclerosis **11**, 373 (1970).

7. Byers, S. O., and Friedman, M., Amer. J. Physiol. **168**, 297 (1952).

8. Talalay, P., *in* "Methods of Biochemical Analysis" (David Glick, ed.), Vol. 8, p. 119. Interscience Publishers, Inc., New York (1960).

9. Admirand, W. H., and Small, D. M., J. Clin. Invest. **47**, 1043 (1968).

10. Folch, J., Lees, M., and Sloane-Stanley, G. H., J. Biol. Chem. **226**, 497 (1957).

11. Chedid, A., Haux, P., and Natelson, S., Clin. Chem. **18**, 384 (1972).

12. Parker, F., and Peterson, N. F., J. Lipid Res. **6**, 455 (1965).

13. Byers, S. O., and Friedman, M., Amer. J. Physiol. accepted for publication (1973).

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