PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 442–445 (1974)

Neurogenic Hypercholesterolemia: Influence Upon Lipoproteins[1] (37827)

SANFORD O. BYERS, MEYER FRIEDMAN, AND ROBERTA NEUMAN

Harold Brunn Institute, Mt. Zion Hospital and Medical Center, Box 7921, San Francisco, California 94120

We have reported (1, 2) the experimental induction of neurogenic hypercholesterolemia by bilateral injury of the ventromedial nuclei, the fornices, and the medial portions of the lateral hypothalamic areas in the rat. In light of the extensive current interest in lipoprotein electrophoretic patterns and the distribution of cholesterol among ultracentrifugally separated lipoprotein classes, it seemed appropriate to examine whether this type of neurogenic hypercholesterolemia was accompained by alteration in lipoprotein. Accordingly, the present study compares the electrophoretic separation of lipoproteins from the plasma of normal rats with that of rats bearing the above-described hypothalamic injury; it also sets forth the distribution of cholesterol among centrifugally separated lipoproteins.

Methods. Production of hypothalamic lesions. Hypothalamic lesions were placed in male Long–Evans rats 10–12 weeks old and weighing approximately 300 g. Each rat received two electrolytic lesions (2 mA for 10 sec) on each side (1.4 and 1.8 mm, respectively, posterior to the bregma, 0.75 mm lateral to the midline, and 9.5 mm beneath the surface of the brain). The site of the brain lesions was not checked at autopsy since previous work (1, 2) has shown correct placement of the lesions to be indicated by a rise in plasma cholesterol. Control rats were sham-operated. All rats were fed 30 ml/day of a previously described (2) liquid diet containing about 270 mg of cholesterol/100 ml ("high-cholesterol diet").

Serum lipoproteins were separated into three electrophoretic fractions (beta, prebeta, and alpha lipoproteins) on agarose[2] by the method of Elevitch and Austin (3). The fractions were stained with fat red 7B dye and the intensity of stain measured *in situ* by direct reading, using a photometric densitometer. The sum of areas under the total densimetric curve was considered to represent 100% lipoprotein; individual areas representing the separated lipoprotein fractions were then assigned percentage values as fractions of 100%.

The sera from animals bearing hypothalamic lesions (experimental group) were selected for combination into nine pools; sera from the control group were similarly selected for combination into nine pools. Sera of similar cholesterol concentration were pooled together so that the concentration of cholesterol in a pool was closely similar to the various concentrations of the respective sera making up that pool. Each pool was separated into four fractions by ultracentrifugal flotation essentially as described by Hatch and Lees (4).

Each serum pool was centrifuged at a temperature of 8–10° for 30 min at 25000*g* using a prechilled Beckman Spinco SW39 swinging bucket rotor and 0.5 × 2 in. tubes. After this centrifugation, approximately the upper 2 mm of fluid (4 drops) was removed from the uppermost surface of the serum in each tube with a dropper pipet. This layer contained any slight milky

[1] This work was supported by Grant HL00119 of the National Heart and Lung Institute and by the Parke–Davis Fund.

[2] These determinations were performed in the Clinical Laboratory at Mt. Zion Hospital and Medical Center.

turbidity and is designated "chylomicrons."

The subnatants of 2 tubes (from which the chylomicron layer had been removed) were pooled and 5.7 ml used to fill a fresh centrifuge tube which was centrifuged for 16 hr at 116,000*g*. After centrifugation, the tube was sliced and the top 1 cm of fluid removed; this portion is designated as very low density lipoprotein (VLDL). The subnatant below the VLDL, including all gelatinous material, was mixed with ½ its volume of a salt solution of density 1.182. The salt solution was 0.195 *m* in NaCl, 2.44 *m* in NaBr, and contained 0.1 g EDTA/liter. This mixture was centrifuged for 20 hr at 116,000*g* and the top 1 cm then removed. This is the density = 1.062 top and contains the low density lipoproteins (LDL). The subnatant below the LDL was mixed to homogenize all gelatinous material. This fraction contains the high-density lipoprotein (HDL).

The concentration of cholesterol in the original sera was determined using ferric chloride–glacial acetic acid according to the directions of Martinek (5). Cholesterol in centrifugally separated lipoprotein-containing serum fractions was extracted into chloroform by the method of Folch (6). After evaporation of chloroform at 50°, the residual cholesterol was determined by the Liebermann–Burchard reaction as used by A. T. Ness (7). Triglyceride was determined by the method of Soloni (8).

Serum for electrophoretic separation was obtained by bleeding rats from the tail while they were lightly anesthetized with ether (1 week after placing lesions) and later by exsanguination from the abdominal aorta while fully anesthetized (2 weeks after placing lesions). Animals whose sera were to be separated by centrifugation were bled by exsanguination at the end of 2 weeks.

Results. Electrophoretic separation. The data are set forth in Table I. In operated rats, cholesterol concentration was significantly higher ($P < 0.0001$) and post-operative body weight significantly lower ($P < 0.0001$) than similar values in control rats. The remaining measurements, i.e., triglyceride concentration and distribution

TABLE I. Distribution of Dye Among Electrophoretically Separated Lipoproteins from Rats with Hypothalamic Lesions.

		1 week post operation						2 weeks post operation					
					% of dye Lipoprotein areas						% of dye Lipoprotein areas		
No. of rats	Pre-operation weight (g)	Weight (g)	Serum cholesterol (mg/100 ml)	Serum triglyceride (mg/100 ml)	Beta	Prebeta	Alpha	Weight (g)	Serum cholesterol (mg/100 ml)	Serum triglyceride (mg/100 ml)	Beta	Prebeta	Alpha
					Rats with hypothalamic injury								
11	330 ± 4.8[a]	301* ± 4.9	119* ± 5.9	34 ± 7.4	50 ± 2.2	16 ± 1.6	34 ± 1.7	316* ± 4.0	137* ± 8.0	22 ± 5.7	46 ± 2.7	17 ± 1.6	33 ± 2.0
					Control rats								
10	326 ± 4.9	336 ± 8.6	60 ± 3.1	22 ± 3.1	47 ± 2.0	19 ± 1.2	33 ± 1.9	351 ± 4.7	67 ± 4.4	17 ± 3.2	46 ± 1.1	18 ± 1.7	35 ± 2.2

[a] Values are means ± SEM.

* Value significantly ($P < 0.001$) different from corresponding control value.

TABLE II. The Distribution of Cholesterol Among Centrifugally Separated Serum Lipoproteins.

Rats bearing hypothalamic lesions										
Cholesterol										Average ± SEM
mg/100 ml pooled serum	169	160	148	135	128	126	109	91	85	128 ± 10*
% in chylomicrons	5.9	10.1	6.6	6.1	10.8	4.5	3.4	4.0	2.1	5.9 ± 1
% in VLDL	62.0	49.1	58.3	52.0	47.7	44.5	49.9	33.7	49.9	49.7 ± 2.8*
% in LDL	17.3	17.6	16.2	17.2	18.0	13.4	19.3	35.3	22.3	19.6 ± 2.2
% in HDL	14.8	23.2	18.9	24.6	23.3	37.7	27.4	26.9	25.7	24.7 ± 2.2*
Control rats										
Cholesterol										Average ± SEM
mg/100 ml pooled serum	88	71	69	67	63	59	54	51	46	63 ± 4.5
% in chylomicrons	7.0	8.0	5.7	5.0	4.1	5.4	8.5	3.1	3.4	5.6 ± 0.7
% in VLDL	44.9	31.7	33.2	29.2	32.8	32.2	30.3	20.4	28.6	31.5 ± 2.2
% in LDL	19.5	22.6	17.3	18.2	23.8	24.1	21.8	36.4	20.9	23.1 ± 2.2
% in HDL	28.6	37.2	43.8	47.6	39.8	38.3	39.7	40.1	46.9	40.2 ± 2.0

* Significantly different from corresponding value of control group ($P < 0.001$).

of fat red 7B dye among beta, prebeta, and alpha lipoproteins, were essentially the same in the two groups of rats.

Ultracentrifugal separation. Table II shows that the high serum cholesterol concentration of rats with lesions is carried chiefly in the VLDL ($P < 0.001$). In consequence, the percent of cholesterol associated with other lipoprotein classes is somewhat smaller than the corresponding value in control rat serum. This depression of percentage attains statistical significance ($P < 0.001$) in the HDL. It should be noted that the *weight* of cholesterol in each lipoprotein class increases when concentration of serum cholesterol increases, despite a decrease in percentage (in classes other than VLDL).

Discussion. Camejo (10) and Koga *et al.* (11) have characterized the serum lipoproteins of Sprague–Dawley rats, Windmueller and Levy (12) those of the Osborne–Mendel strain, whereas Mills and Taylaur (13) include Wistar rats in a survey of lipoproteins of 18 animals. The distribution and composition of lipoproteins vary among these three strains of rat and, it may be presumed, are also different in the Long–Evans rats we used. Additionally, our animals, both those bearing hypothalamic injuries and intact animals, ate a high-cholesterol diet. It is probably by reason of diet, rather than strain, that our intact animals show a high percentage of plasma cholesterol in the HDL: 40% (see Table II) as compared with 18.7% in the Sprague–Dawley strain (10). The additional cholesterol observed in sera from rats bearing hypothalamic lesions is evidently carried on the VLDL.

Lipoproteins separated by both electrophoretic and ultracentrifugal methods have been correlated (9) as follows: pre-β and VLDL; β and LDL; α and HDL. It is therefore anomalous that no change was noted in the distribution of dye among electrophoretic fractions from rats with lesions despite considerable change in cholesterol distribution among centrifugally separated fractions. The explanation may lie in preferential staining by the dye of the triglyceride moiety of lipoprotein. The triglyceride content of serum lipoprotein did not differ between normal and injured rats (see Table II) since the hypothalamic lesion does not alter serum triglyceride concentration.

Summary. Neurogenic hypercholesterolemia was induced in cholesterol-fed Long–Evans male rats by bilateral injury of the ventromedial nuclei, the fornices, and the medial portions of the lateral hypothalamic areas. Serum lipoproteins from these rats and from sham-operated controls were separated into three electrophoretic fractions (beta, prebeta, and alpha) and

into four fractions by ultracentrifugal flotation (chylomicrons, VLDL, LDL, and HDL). No differences were observed between operated and control groups in the distribution of fat red 7B dye among electrophoretic fractions. The excess cholesterol present in the serum of rats with neurogenic hypercholesterolemia was observed to be carried chiefly in the VLDL.

1. Friedman, M., Byers, S. O., and Elek, S. R., Proc. Soc. Exp. Biol. Med. **131**, 759–762 (1969).

2. Friedman, M., Byers, S. O., and Elek, S. R., Amer. J. Physiol. **223**, 473–479 (1972).

3. Elevitch, F. R., and Austin, D. G., "Lipoprotein Electrophoresis in Agarose" in Tech. Bull. Analytical Chemistry Inc., Palo Alto, Calif., 1970.

4. Hatch, F. T., and Lees, R. S., Advances Lipid Res. **6**, 1–68 (1968).

5. Martinek, R. G., Clin. Chem. **11**, 495–504 (1965).

6. Folch, J., Lees, M., and Sloane-Stanley, G. H., J. Biol. Chem. **226**, 497–509 (1957).

7. Ness, A. T., Pastewka, J. V., and Peacock, A. C., Clin. Chim. Acta. **10**, 229–237 (1964).

8. Soloni, F. G., Clin. Chem. **17**, 529–534 (1971).

9. Noble, R. P., Hatch, F. T., Mazrimas, J. A., Lindgren, F. T., Jensen, L. C., and Adamson, G. L., Lipids **4**, 55–59 (1969).

10. Camejo, G., Biochemistry **6**, 3228–3241 (1967).

11. Koga, S., Horowitz, D. L., and Scanu, A. M., J. Lipid Res. **10**, 577–588 (1969).

12. Windmueller, H. G., and Levy, R. I., J. Biol. Chem. **242**, 2246–2254 (1967).

13. Mills, G. L., and Taylaur, C. E., Comp. Biochem. Physiol. **40B**, 499–501 (1971).

Received Sept. 18, 1973. P.S.E.B.M., 1974, Vol. 145.