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Received Dec. 26, 1969. P.S.E.B.M., 1969, Vol. 131.

The Induction of Neurogenic Hypercholesteremia* (33971)

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A central nervous influence on plasma cholesterol concentration was suggested by our observations (1) on accountants and other individuals subjected to time–pressure stress, and by our study in collaboration with Dr. C. G. Gunn (2) in which we observed plasma cholesterol rises in rabbits receiving electrical stimulation in the diencephalon—a finding confirmed by Gutstein *et al.* (3). However, in our study (2), the area of the hypothalamus responsible for the observed plasma cholesterol rise was not determined because stimulation of various hypothalamic loci (*e.g.*, the anterior hypothalamic area, the supraoptic nuclear area, the ventral medial nucleus and the lateral hypothalamic area) all induced the observed rise in cholesterol. These results suggested either that many hypothalamic nuclei and specific areas were concerned with the plasma cholesterol level or that only one or more specific areas were being stimulated despite the anatomical location of the inserted electrode. In the present study relatively discrete electrolytic lesions were induced in the hypothalamus of the rat in the attempt to determine first whether such lesions could alter the plasma cholesterol of this species and secondly whether such areas could be more securely identified.

Methods. In the first study, hypothalamic lesions were placed in male Long-Evans rats weighing approximately 300 g each. Bilateral lesions were induced in the first group of 12 rats by insertion of electrodes 0.04–0.08 mm posterior to bregma, 0.075 lateral to midline (*L* coordinate) and 0.95 mm below the brain surface (*D* coordinate). Bilateral lesions were induced in the second group of 15 rats by insertion of electrodes at the same lateral and vertical coordinates but 0.12 to 0.16 mm posterior to bregma. A dc current of 2 mA was applied for 20 sec in all rats. Eighteen control rats were subjected to similar electrode insertions but no current was applied. The animals were fed a low cholesterol diet of vegetable origin (containing 43 mg of digitonin precipitable Liebermann-Burchard positive sterol per 100 g of diet). Two weeks after operation, all rats were bled and the samples were analyzed for plasma cholesterol concentration (4).

In the second study, 4 groups of 15 rats each were subjected to two electrode insertions on each side (0.14 and 0.18 mm posterior to bregma) but the lateral and vertical coordinates employed varied (see Table I) with each group in an attempt to damage specific nuclear areas or tracts. Two weeks after operation, these rats were bled and the samples were analyzed for cholesterol.

In the third study, 2 groups of 15 rats each received 2 electrolytic lesions (2 mA for 10 sec) on each side (0.14 and 0.18 mm, respectively posterior to bregma, 0.075 mm lateral to the midline and 0.95 mm beneath the surface of the brain). The first group was fed the low cholesterol diet and bled 3, 7, 14, 21, and 28 days for plasma cholesterol determinations. In addition, 14 days after operation, following a prior fast of 15 hr they were given 3 ml of cottonseed oil by stomach tube. Blood sample were obtained before and 6 hr after this feeding. The fasting samples were analyzed for cholesterol, triglyceride (5), and phospholipid (6), and the postpran-

* Aided by grants from the National Institutes of Health, National Heart Institute, HE-00119 and HE-03429.

TABLE I. The Plasma Cholesterol Response to Hypothalamic Injury.

Coordinates[a] employed		No. of rats	Av wt		Nuclei and tracts involved[b]		Av plasma cholesterol (mg/100 ml)
			Before operation	2 Weeks after operation			
D	*L*				Destroyed	Injured	
0.95	0.075 (Controls)[c]	10	298	333	—	—	69 ± 2.3 (51–89)
0.95	0.075	8	300	322	VMN, DMN, AN, F	LHA	128 ± 13.2 (84–206)
1.0	0.075	10	302	323	VMN, AN, F	LHA	119 ± 7.3 (95–152)
0.90	0.075	5	297	302	DMN, F	LHA	101 ± 2.6 (95–112)
0.95	0.05	11	304	323	VMN, DMN, AN	—	69 ± 2.6 (54–87)

[a] The *A–P* coordinates were 0.14 and 0.18 posterior to bregma in all rats.

[b] VMN = ventromedial nucleus; DMN = dorsomedial nucleus; AN = arcuate nucleus; F = fornices; LHA = lateral hypothalamic area.

[c] No current was applied.

dial sample for triglyceride. The second group of 18 rats received the same lesions but were fed a high cholesterol diet of whole egg, milk, and oil (cholesterol content: 273 mg/100 ml). These were bled weekly for plasma cholesterol determinations. Ten control rats subjected only to electrode probing were fed the low cholesterol and 17 control rats, the high cholesterol diet. These were bled at similar intervals. At 28 days, all animals were killed, their brains were obtained, and the site and extent of the lesions therein were studied.

Results. Eight of the 12 rats of the first study which had received the more anteriorly placed lesions survived the 2 week period. Their average plasma cholesterol (72 mg/100 ml; SE mean: ±3.7) was essentially the same as that (66 mg/100 ml; SE mean: ±2.2) of the controls. All 8 rats exhibited severe damage of the preoptic, anterior hypothalamic, suprachiasmatic and paraventricular nuclei. Ten of the 15 rats of the first study which had received the more posteriorly placed lesion survived the 2-week period. These experimental rats showed anorexia for a few days; despite the initial anorexia, however, by the end of 2 weeks they had ingested approximately as much food as the controls and their average weight (325 g) at this time was approximately the same as the (311 g) of the controls. Their average plasma cholesterol content (95 mg/100 ml; SE mean: ±8. however, was significantly greater ($p<0.001$) than that (62 mg/100 ml; SE mean: ±3.1) of the controls. All 10 rats exhibited severe bilateral destruction of the dorsomedial (DMN), arcuate (AN), and ventromedial (VMN) nuclei, fornices and some injury to the medial portion of the lateral hypothalamic area (LHA) (see Fig. 1A and B).

In the second study, three of the four operated groups of rats exhibited a significant elevation (see Table I) in their average plasma cholesterol. The fourth group whose lesion had been placed only 0.05 mm lateral to the midline, did not exhibit any change in plasma cholesterol. All four groups exhibited transitory postoperative anorexia and permanent sham rage. As Table I indicates, in the first group, severe destruction occurred in the VMN, DMN, AN, and fornices; the medial portion of the LHA was also damaged. Except for DMN destruction, similar lesions were observed in the second group. In the third group, the VMN and AN escaped dam-

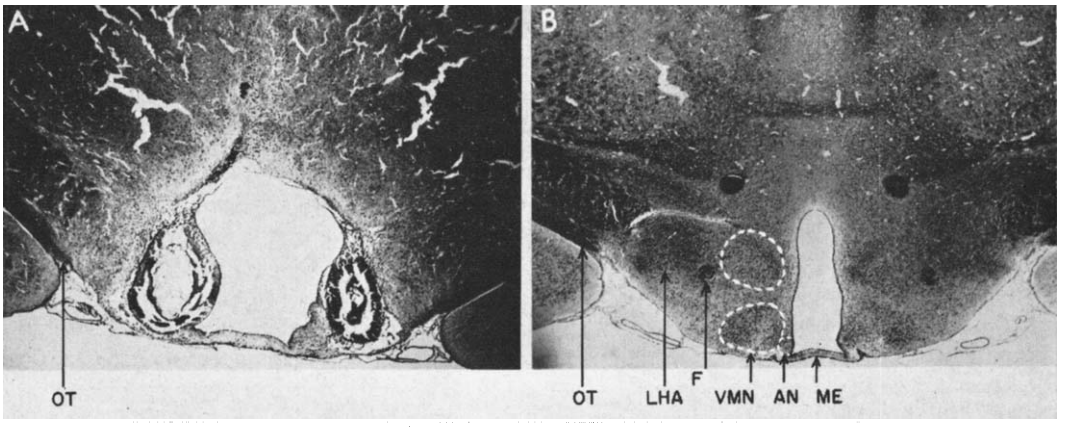

FIG. 1A and B. Microphotograph of (A) hypercholesteremia-inducing lesion in the midhypothalamic area of the rat, and (B) normal intact midhypothalamus (×20. Comparison of the two photographs illustrates that the bilateral lesion present in (A) has destroyed the ventromedial (VMN), the dorsomedial (DMN) and arcuate (AN) nuclei leaving the median eminence (ME) intact. Note in (A) also that the fornix (F) and a considerable portion of the lateral hypothalamic area (LHA) lying between the fornix and optic tract (OT) has been damaged. The third ventricle is enlarged in (A) due to the destruction of a considerable amount of periventricular tissue.

age. In the fourth group, only the VMN, DMN, and AN were destroyed, the fornices and LHA escaping injury.

In the third study, the 10 surviving experimental rats fed the low cholesterol diet, exhibited a significant rise in their average plasma cholesterol content 72 hr after operation (see Fig. 2) which persisted during the entire experimental period. Their average

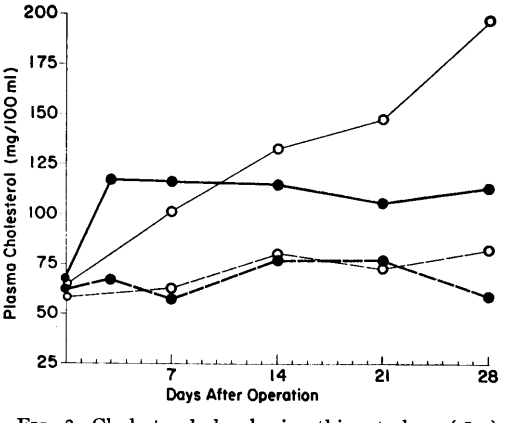

FIG. 2. Cholesterol levels in this study: (●–), experimental rats fed low cholesterol diet; (●- -), control rats fed low cholesterol diet; (○–), experimental rats fed high cholesterol diet; (○- -), control rats fed high cholesterol diet.

plasma phospholipid content (252 mg/100 ml) 2 weeks after operation was also significantly higher ($p<0.001$) than that (134 mg/100 ml of the controls. However their fasting plasma triglyceride (31 mg/100 ml) and 6-hr postprandial plasma triglyceride values (115 mg/100 ml) were essentially the same as those (30 and 99/100 ml, respectively) of the controls, The 12 surviving rats fed the high cholesterol diet (see Fig. 2) exhibited a significant rise in their average plasma cholesterol 7 days after operation, despite their initial anorexia, and in the succeeding weeks this rise continued and eventually reached a value of 199 mg/100 ml 4 weeks after operation. The intake of food by these experimental rats was approximately half of that taken by the controls during the first postoperative week, but subsequently they ingested approximately the same amount of food so that at the end of the experimental period the average weights of the experimental and control rats were essentially the same. All the brains of the two groups of experimental rats of the third study revealed severe destruction of the VMN, DMN, AN and fornices; also injury of the medial portion of the lateral hypothalamic area.

Discussion. In a previous study (7) electrolytic destruction of the anterior portion of the hypothalamic area in the rat was observed to abolish the milieu-induced postprandial lipemia which otherwise would usually occur. This phenomenon was not observed in the rat given a tuberal lesion. Destruction of nuclei in the anterior portion of the hypothalamus (*i.e.*, the preoptic, supraoptic, suprachiasmatic, anterior hypothalamic, and paraventricular nuclei) in the present study was not found to induce any change in the concentration of cholesterol in plasma. Moreover, *isolated* destruction of just the ventromedia, dorsomedia, or arcuate nuclei, failed to alter the cholesterol level. However, when a lesion destroyed the fornices and the medial portion of the lateral hypothalamis (at the same plane in which the ventromedial nucleus lies), together with the ventromedial or dorso-medial nucleus, a hypercholesteremia promptly ensued. Thus, although it is clear that damage to the fornices and lateral hypothalamic area must be present for hypercholesteremia to evolve, it is not similarly explicit whether either of the medial nuclei also must be damaged or destroyed at the same time. The results of the present experiments, then, improve upon those obtained in the earlier, stimulation studies (2) in the respect that they implicate at least two relatively specific areas (*i.e.*, the fornix and the medial portion of the lateral hypothalamic area) in the hypercholesteremic reaction; nevertheless they are similar to those earlier results in that they do not eliminate the possibility that either of the medial nuclei also may be necessary for the induction of hypercholesteremia.

The mechanism by which the above described hypothalamic lesion induced hypercholesteremia remains to be determined. It can be said at this time that while a diet rich in cholesterol intensified the hypercholesteremia, the latter also occurred in a diet practically devoid of cholesterol. Indeed, hypercholesteremia was observed (72 hr after operation) in most of the rats who had avoided the intake of any food during this period. Furthermore, there were no differences observed in the physical activities between the experimental and control animals. Therefore it seems probable at this time that at least one of the components responsible for the hypercholesteremia observed, is of endogenous provenance. But whatever the mechanism for this type of hypercholesteremia proves to be, the present results suggest that the central nervous system does bear a relationship of some kind that may be relevant to the maintenance and regulation of the plasma cholesterol and phospholipid concentration of the normal animal.

Summary. A chronic hypercholesteremia almost invariably results in the rat after placement of an electrolytic lesion which involves the fornix, medial part of the lateral hypothalamus and one of the two medial nuclei of the hypothalamus. This hypercholesteremia is not accompanied by any discernible changes in plasma triglyceride.

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Received Jan. 29, 1969. P.S.E.B.M., 1969, Vol. 131.