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Abolition of Milieu-Induced Hyperlipemia in the Rat by Electrolytic Lesion in the Anterior Hypothalamus* (33860)

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The postprandial lipemia of the rat fed fat was observed in our previous studies to be exaggerated when this animal was exposed to white noise before and after ingestion of oil (1) or was placed in the central area of the general laboratory after oil ingestion (2). We designated this phenomenon as milieu-induced hyperlipemia and from the nature of the excitant we concluded that the initial stimulus for this elevation of the postprandial plasma triglyceride (PTG) is received and acted upon by the central nervous system (CNS) of the animal. However, the exact locus in the CNS where this stimulus initiates a lipid elevating mechanism and the final pathways and tissues or organs participating in this mechanism remains unknown.

In our last study (2), the removal of the adrenals and hypophysis did not abolish the milieu-induced postprandial hyperlipemia of the rat fed fat. In view of these findings, we decided to study what effect hypothalamic lesions might exert upon milieu-induced hyperlipemia. The results herein described, indicate that electrolytic lesions involving the anterior hypothalamic nuclei abolish milieu-induced hyperlipemia.

Methods. A. Induction of lesions in anterior and midhypoyhalamic areas. Young (12–14 weeks) male rats (Long-Evans strain) were employed in this study. Under pentobarbital anesthesia, two separate electrolytic lesions on each side were produced in the midhypothalamic or tuberal area, by means of the Kopf stereotaxic instrument. The first bilateral lesion was produced by an electrode placed 1.4 mm caudal to Bregma, 0.7 mm lateral to the midline, and 9.5 mm beneath the dorsal surface of the brain. The second bilateral lesion was induced with the electrode placed in the above same lateral and vertical coordinates but 1.7 mm caudal to Bregma. A direct current (2 mA for 10 sec) was used for the electrolytic injury. Control animals were treated exactly the same except the electrode was not activated.

A single lesion was produced on each side in the anterior or rostral hypothalamic area of rats of similar age, sex, and strain by an electrode inserted only 0.2 mm caudal to Bregma and with the same lateral and vertical coordinates described above. After operation, the rats were housed in groups of five and fed Simonsen laboratory chow.

At autopsy the brain of each rat was removed, fixed, embedded in paraffin, and serial sections (every 200 μ) were obtained. These were stained with Luxol fast blue and cresyl violet and then examined.

B. Pre- and postprandial chemical studies. After 2 weeks of convalescence, groups of experimental and control rats were starved for 15 hr and then brought into the central

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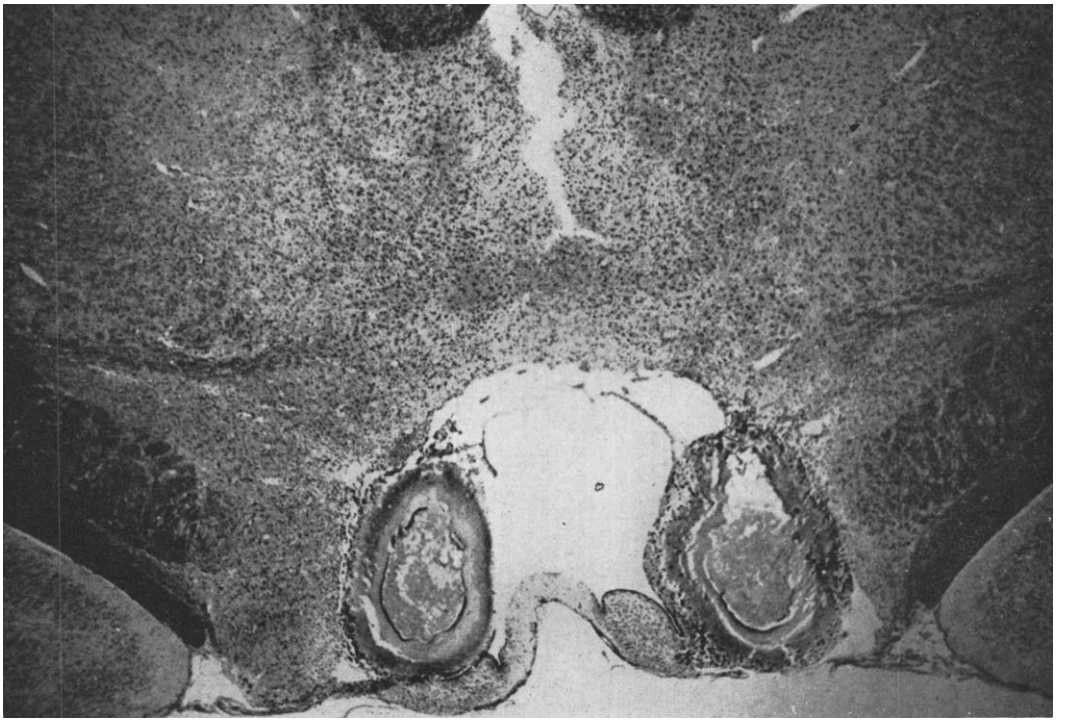

FIG. 1. Photomicrograph of midhypothalamic area of rat brain (Luxol fast blue and cresyl violet stain ×40). The symmetrically-induced coagulative lesions have destroyed the ventromedial, dorsomedial, and paraventricular nuclei. Note the dilatation of the third ventricle.

area of the general laboratory where they were given 3.0 ml of cottonseed oil by oral intubation. They remained in the central area for 6 hr after intubation. Blood samples were obtained before and 6 hr after ingestion of oil. The fasting sample was analyzed for triglyceride (TG) according to the method of Van Handel and Zilversmit (3) and for glucose according to the method of Marks (4). The 6-hr sample was analyzed only for TG. Additional series of experimental and control rats were treated in identical fashion except that they were housed in a soundproof room for 72 hr before, and for 6 hr after, the ingestion of the oil.

C. Measurement of intestinal absorption and tissue distribution of fatty acids of dietary origin in rats with an anterior hypothalamic lesion. Six rats which later were found to have suffered rostral lesions and six control rats, after a preliminary starvation of 15 hr, were given 3.0 ml of cottonseed oil containing approximately 500,000 cpm of tripalmitin-^{14}C. Six hr later, the animals were bled and killed. The entire gastrointestinal tract including its contents, the entire liver and an epididymal fat pad were obtained from each animal. These tissues and aliquot of the terminal blood sample were anlayzed separately for their content of fatty acids of dietary origin, employing the labeled tripalmitin as an indicator as previously described (5).

D. Measurement of rate of disappearance of intravenously administered hypertriglyceridemic serum in (i) rats with a midhypothalamic lesion, and (ii) rats with an anterior hypothalamic lesion. Five rats which later were found to have suffered a severe tuberal lesion (*i.e.*, destruction of the ventromedial and dorsomedial nuclei) and five rats which were found to have suffered a severe rostral lesion (*i.e.*, bilateral destruction of the medial portion of the anterior hypothalamic nucleus) and 10 control rats, were given intravenously 2.0 ml of rat hyper-

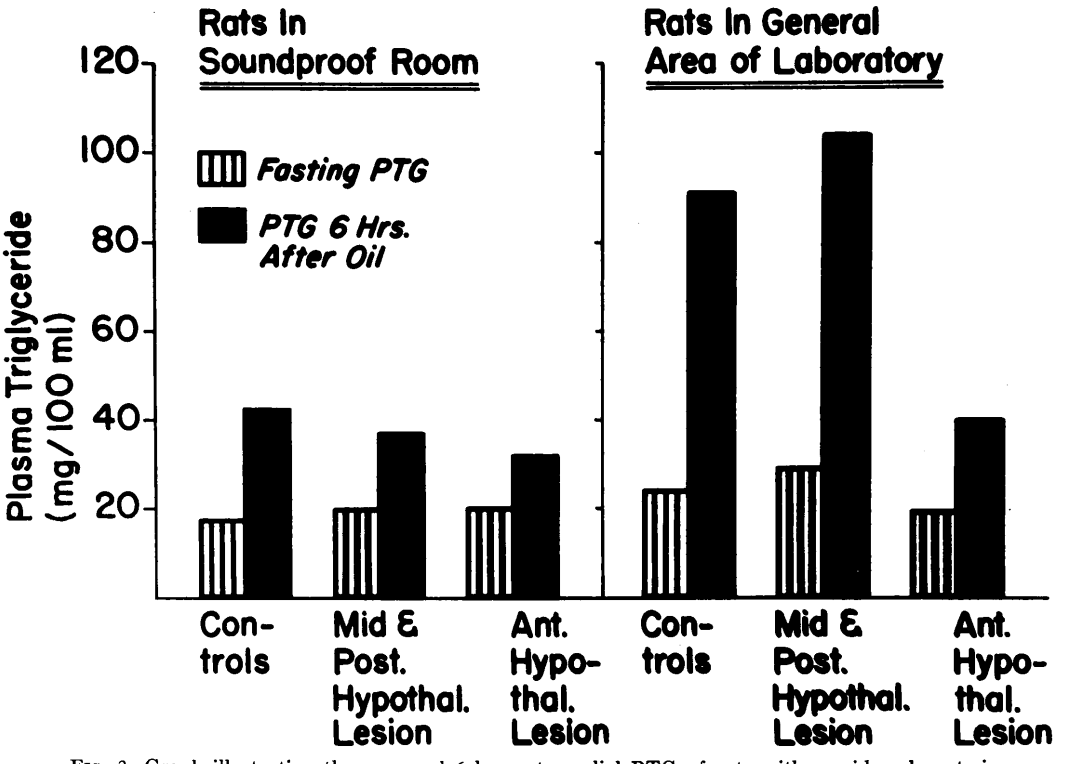

FIG. 2. Graph illustrating the pre- and 6-hr postprandial PTG of rats with a mid and posterior hypothalamic lesion and rats with an anterior hypothalamic lesion.

lipemic serum (av TG conc = 2250 mg/100 ml). This serum was obtained from rats that 24 hr previously had received 200 mg of Triton WR-1339. Blood samples were obtained immediately, 1, and 2 hr after injection of the serum in all animals. In addition, another blood sample was obtained from the rats with an anterior or rostral hypothalamic lesion and the control rats 6 hr after injection of the hyperlipemic serum.

Results. A. Pre- and postprandial PTG of rats bearing midhypothalamic lesions. Twenty of 30 operated rats which exhibited severe tuberal lesions (see Fig. 1) survived. The ventromedial, dorsomedial, paraventricular, and sometimes the anterior part of the posterior hypothalamic nuclei were destroyed in these animals. The typical aggressive behavior usually produced by this lesion was seen in all the rats. For the first few days following the operation, these rats ate somewhat less than the 30 control rats, but after the first week, the hypophagia disappeared and at the end of 10 days the average weight of the experimental rats was approximately the same as that of the controls.

As Fig. 2 illustrates, these lesions did not alter either the fasting or postprandial PTG either in the soundproof room or in the general laboratory. Thus the fasting PTG (20 mg/100 ml) of the 10 experimental rats housed in the soundproof room was essentially the same as that (17 mg/100 ml) of the 21 similarly housed control rats. Likewise (see Fig. 2) the average postprandial PTG (37 mg/100 ml) of the experimental rats was about the same as that (41 mg/100 ml) of the controls.

A similar equivalence of fasting and postprandial PTG values was observed in the experimental and control rats when they were housed in the central area of the general laboratory. Thus the average serum PTG of the experimental rats rose to 104 mg/100 ml 6 hr after oil ingestion and the average serum PTG of the control rats rose to 91 mg/100

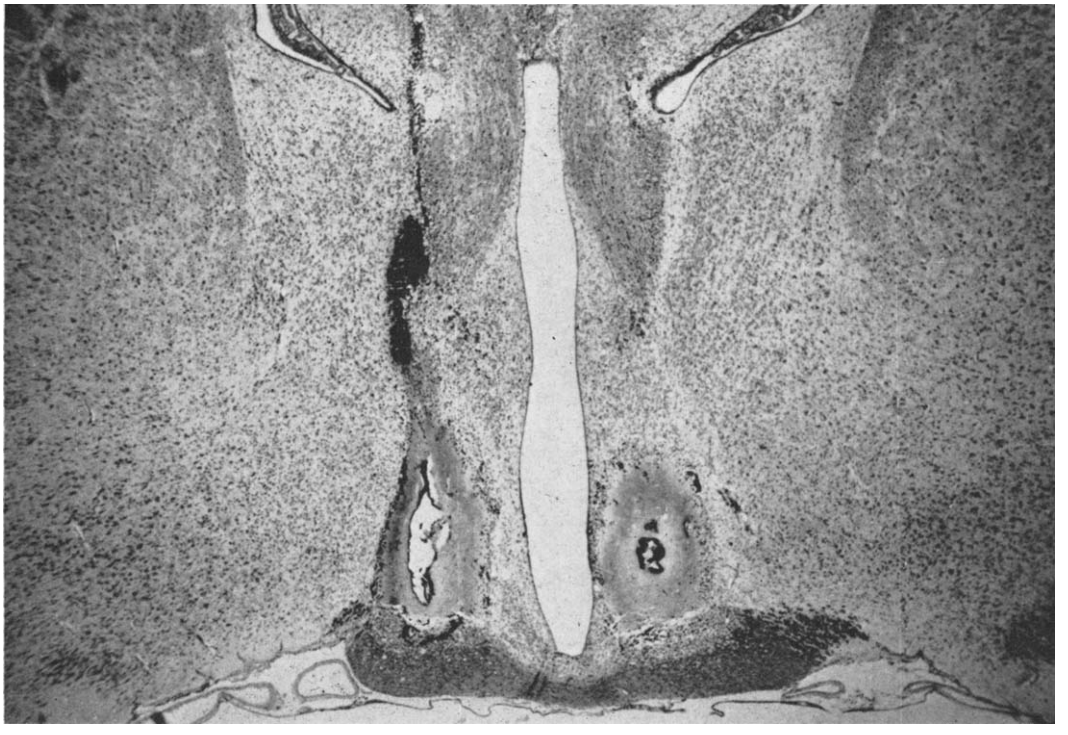

FIG. 3. Photomicrograph of anterior hypothalamic area of rat brain (Luxol fast blue and cresyl violet stain ×40). The symmetrically placed lesions have destroyed the medial portion of the anterior hypothalamic nuclei. The optic chiasm underlies the lesion.

ml. The average fasting plasma glucose (139 mg/100 ml) of the experimental rats was essentially the same as that (145 mg/100 ml) of the controls.

B. Pre- and postprandial PTG of rats bearing anterior hypothalamic lesions. Twenty-seven of 35 lesioned rats which exhibited rostral lesions (see Fig. 3), destroying the entire medial portion of the anterior hypothalamic nucleus, survived. Occasionally, the posterior part of the preoptic nucleus also was found to be injured. However, the optic chiasm, and the supraoptic and paraventricular nuclei were not injured. Similar to the rats who had received the tuberal or midhypothalamic damage, these rats with rostral lesions also ate poorly the first few days after operation but within 2 weeks, their average weight approximated that of the controls. They did not display, however, the wildness and aggressiveness of the rats bearing tuberal lesions.

The average fasting PTG (20 mg/100 ml) of the seven experimental rats housed in the soundproof room was not significantly different (see Fig. 2) from that (17 mg/100 ml) of the control rats. Similarly the average 6-hr PTG (32 mg/100 ml) of the experimental rats housed in the soundproof room was not significantly different from that (41 mg/100 ml) of the control rats.

A very marked difference was observed, however, between the average postprandial PTG (40 mg/100 ml) of the 20 experimental rats housed in the central area of the general laboratory and that (91 mg/100 ml) of the control rats (see Fig. 2). Actually, the postprandial PTG of the lesioned rats, as Fig. 2 illustrates, was essentially the same, whether they were housed in the soundproof room or in the central area of the general laboratory. In short, rats with this type of anterior hypothalamic lesion did not appear to be susceptible to milieu-induced hyperlipemia. Their average fasting PTG, however, was approximately the same as that of the

TABLE I. Intestinal Absorption of TG in Rats with Anterior Hypothalamic Lesions.

No. of rats	Av wt (g)	Plasma triglyceride 6 hr after oil (mg/100 ml)	Total amount of TG absorbed in 6 hr (mg)	Absorbed dietary TGFA: In total plasma vol (mg)	Absorbed dietary TGFA: In liver (mg)	Absorbed dietary TGFA: In fat pad (mg/g of tissue)
			Rats with anterior hypothalamic lesions			
6	322	38	587	3.61	57	1.23
	Range	(23–58)	(550–660)	(1.8–6.3)	(22–81)	(0.74–1.94)
	SE mean	± 5.3	± 28	± 0.6	± 10.2	± 0.17
			Control rats			
6	373	132	881	14.0	104	1.65
	Range	(88–188)	(770–940)	(8.8–24.8)	(92–121)	(0.83–2.79)
	SE mean	± 15.4	± 32	± 2.4	± 4.1	± 0.29

control animals. Also their average fasting plasma glucose (128 mg/100 ml) was not significantly different from that (145 mg/100 ml) of the control rats.

C. Intestinal absorption and tissue distribution of dietary derived fatty acids. As Table I demonstrates, rats with anterior hypothalamic lesions absorbed significantly less ($p<0.001$) of the administered triglyceride than the controls. As might be expected from this lesser absorption (see Table I), the average total hepatic content (57 mg) of fatty acid of dietary origin in the rats bearing the rostral lesion also was significantly less ($p<0.001$) 6 hr after the administration of the oil than that (104 mg) observed in the control rats. Although a lesser amount of fatty acid of dietary origin also was found (see Table I) in the sample of adipose tissue of the experimental rats, than in a similar sample of the control animals, the difference was not statistically significant.

D. Disappearance of intravenously administered pooled hyperlipemic serum. The respective rates of disappearance of the TG contained in the hyperlipemic serum appeared to be approximately the same (see Table II) in the experimental rats (whether

TABLE II. Rate of Disappearance of Injected Hyperlipemic Serum in Rats with Hypothalamic Lesions.

No. of rats	Av wt (g)	Plasma triglyceride (mg/100 ml) after injection (hr): Immediately	1	2	6
		A. Rats with midhypothalamic lesions			
5	298	470	325	181	
	Range	(445–486)	(286–354)	(150–205)	
	SE mean	± 6.3	± 13.6	± 7.9	
		B. Rats with anterior hypothalamic lesions			
5	302	474	300	184	35
	Range	(442–494)	(241–342)	(120–248)	(23–57)
	SE mean	± 5.9	± 12.2	± 8.2	± 6.6
		C. Control rats			
10	294	490	323	187	50
	Range	(372–548)	(290–378)	(135–216)	(36–88)
	SE mean	± 3.2	± 8.1	± 4.9	± 8.6

the lesion was induced in the rostral or tuberal area of the hypothalamus) as in the control rats.

Discussion. In previous studies (2, 5) we reported the occurrence of milieu-induced hyperlipemia in oil-fed rats,—a phenomenon not able to be abolished either by adrenalectomy or hypophysectomy. Injury of the anterior hypothalamic area however, as was done in the present study, appeared to abolish this type of hyperlipemia in the oil-fed rat. Apparently diminution in the intestinal absorption of the administered fat was the mechanism by which the hypothalamic lesion abolished the milieu-induced hyperlipemia. The results obtained with the parenterally administered triglyceride further strengthened this view.

Various authors have entertained the possibility that the hypothalamus may influence the metabolism or disposition of lipid substances ever since Hetherington and Ranson (6) demonstrated that bilateral injury of the ventromedial nucleus might lead to obesity. However, clearcut evidence of such involvement has been lacking although it seems fairly well established (7, 8) that destruction of the ventromedial nucleus may lead to a reduction in the discharge of FFA from adipose tissue. Also Gutstein *et al.* (9) recently have reported that acute stimulation of the lateral hypothalamic nucleus in the fat fed rat may lead to a slight rise in its PTG level. However, the possible physiological analogue of this type of stimulation, namely the severe injury or destruction of the ventromedial nucleus as accomplished in the present study did not lead to any abnormal change in the PTG of the fed rat.

Summary. An electrolytic lesion placed in the anterior hypothalamus of rats fed 3 ml of cottonseed oil prevented the milieu-induced postprandial hyperlipemia usually observed in these animals. Thus the average 6-hr postprandial plasma triglycerides (PTG) of the lesioned rats was 40 mg/100 ml and that of the controls was 91 mg/100 ml. A similar lesion placed in the tuberal area of the hypothalamus was not similarly effective.

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