TABLE I. Radium Retention in Rachitic and Vit. D-Treated Rats.

	Group	No. of rats	Bone ash, %	Mean radium retention, μg
I.	Normal control	9		3.98±.128* (3.61-4.77)
		5†	60.78 (60.38-61.35)	
II.	Untreated rachitic	14		2.90±.141* (1.97-3.65)
		5†	52.93 (45.99-57.40)	
III.	Vit. D-treated rachitic	13		4.36±.063* (4.02-4.76)

* Stand. error.
† Killed at same time that radium was inj. into other animals of group.

the bone ash determinations on animals from the rachitic and control groups, sacrificed at time of injection of radium into the rest of the animals of the groups. The rachitic animals showed significantly lower bone ash levels than did the control animals (Table I). Variation between bone ash values of the rachitic group was much greater than in the control group.

The high variation of bone ash levels of the rachitic animals corresponds to the variation in radium retention in this group, in contrast to the relatively small variations in the control and treated groups.

Discussion. Significantly more radium was retained by the animals receiving vit. D than by the untreated rachitic animals. Differences in mean radium retention between Groups I and II, and between Groups II and III, are highly significant, t values being 5.33 and 9.19 respectively. Even the difference between Groups I and III has a probability of only 1%, so is also significant. It was assumed, because of the previous studies by many other workers, that total body retention of radium 23 days after administration of the radium would be analogous to skeletal retention. The results of our experiments do not permit us to decide whether the effect of vit. D is on the initial deposition of radium in bones, or on rate of removal from the bones.

It would appear that retention of radium, therefore, is affected by the absence or presence of vit. D in the diet in the same manner as are the retentions of Ca^{45}, $Sr^{89,90}$, and Ba^{140}. Since it is impossible to demonstrate in rats a requirement for vit. D on diets with normal Ca and P contents, the high Ca-low P diet was used to demonstrate these effects on radium retention. There is of course no assurance that vit. D would affect Ra retention in rats on normal diets, or in humans.

Summary. Total body retention of injected radium was significantly less in rachitic rats than in vit. D-treated rachitic rats, or in non-rachitic control animals.

1. Evans, R. D., Harris, R. S., and Bunker, J. W. M., *Am. J. Roentgenol.,* 1944, v52, 353.
2. Norris, W. P., and Kisieleski, W., *Cold Spring Harbor Symposia on Quantitative Biol.,* 1948, v13, 164.
3. Greenberg, D. M., *J. Biol. Chem.,* 1945, v157, 99.
4. Copp, D. H., Hamilton, J. G., Jones, D. C., Thompson, D. M., and Cramer, C., *Conf. Metab. Interrelations, Trans.,* 1951, v3, 226.
5. Dudley, H. C., and Friedman, L., *Science,* 1952, v115, 146.

Received January 21, 1857. P.S.E.B.M., 1957, v95.

Hypothalamic Lesions Associated with Goldthioglucose-Induced Obesity.* (23361)

ROBERT A. LIEBELT AND JOHN H. PERRY (Introduced by Arthur Kirschbaum)
Department of Anatomy, Baylor University College of Medicine, Houston, Texas.

A massive and persistent obesity can be induced in various strains of mice with a single injection of goldthioglucose(1-4). Of those animals surviving the LD_{50} dose about one-third show varying degrees of fat deposition (5). This obesity is associated with a marked

* This investigation supported by Public Health Service Grants.

increase in spontaneous food intake; carcass analysis indicates that increase in body weight is essentially due to increase in body lipid(6).

Marshall, Barrnett and Mayer(7) described lesions in the ventromedial nuclei of the hypothalamus of animals injected with goldthioglucose 24 to 72 hours prior to autopsy. Neurones were scarce in the ventromedial nuclei of mice made obese by injection of gold thioglucose at least 3 months prior to investigation. Sections of brains of mice which failed to become obese after injection with goldthioglucose at least 3 months prior to autopsy revealed no distinct abnormalities. No lesions were observed in other areas of the brain.

Our observations indicate that 2 genetically unrelated inbred strains of mice (CBA and $C_{57}BL$) demonstrate differences in susceptibility to goldthioglucose-induced obesity. One hundred percent of CBA mice become obese with no mortality following a given dose of goldthioglucose which has no effect on weight gain of $C_{57}BL$ mice. However, larger doses of goldthioglucose, which are fatal for CBA mice, produce significant weight gain in $C_{57}BL$ mice. It was the purpose of this investigation to determine the relationship between weight gain following goldthioglucose and the nature of the lesions in the hypothalamus in these 2 strains of mice.

Methods. The strains of mice used had been inbred in this laboratory for more than 20 generations; mice were 3 months of age when goldthioglucose was administered. Purina lab chow was fed *ad libitum;* mice were housed in groups of 4-6 animals per cage (6 x 12 inches). The goldthioglucose suspension was prepared by adding 100 mg to 2 cc of peanut oil which was vigorously agitated prior to each injection. (Our recent studies show that goldthioglucose dissolved in saline is as effective in producing obesity.) Most injections were made intraperitoneally; previous experience indicated no difference between the intramuscular and intraperitoneal routes. A total of 100 CBA mice were injected with 0.18 to 0.75 mg† of goldthioglucose, compared

† Dosage of goldthioglucose is expressed in mg/g body weight.

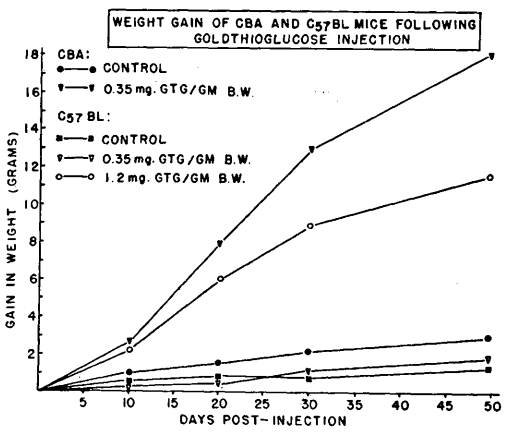

FIG. 1. Response in terms of wt gain to varying doses of goldthioglucose in CBA and $C_{57}BL$ inbred mice. Each group represents 15-25 mice.

to the LD_{50} dose of 0.80 to 1 mg per gram used by others(1-4); a total of 50 $C_{57}BL$ mice were injected with 0.18 to 1.2 mg of goldthioglucose. Body weights were taken at weekly intervals for a period of 50 days post-injection. The response in terms of weight gain to different amounts of goldthioglucose was consistently reproducible in both strains of mice. Another group of mice was killed at intervals varying from 2-19 days post-injection. Stained serial sections of the brains of 23 CBA and 19 $C_{57}BL$ mice were studied. Twenty CBA and 16 $C_{57}BL$ had received goldthioglucose and 3 animals from each strain served as controls. The usual amount of drug was 0.35 mg, but a few animals received smaller (0.18 mg) or larger doses (0.75 to 1.2 mg).

Results. The average weight gain of CBA mice injected with 0.35 mg of goldthioglucose was 19 g with no mortality at 50 days post-injection (Fig. 1). The control animals during this time gained 2 g. Control $C_{57}BL$ mice as well as those animals injected with 0.35 mg of goldthioglucose showed a 1 to 1.5 g gain in weight at 50 days post-injection. However, animals of this strain receiving 1.2 mg of goldthioglucose gained an average of 11 grams during this same period. Both strains of mice had approximately the same body weight (23-25 g) prior to injection.

Although in $C_{57}BL$ mice 0.35 mg of goldthioglucose caused no significant weight increase, bilateral lesions (Fig. 2) appeared in

PLATE I. Photomicrographs of sections through infundibular region of hypothalamus of goldthioglucose treated mice. ME, median eminence; VM, nucleus ventromedialis; A, nucleus arcuatus; III, third ventricle; L, lesion.

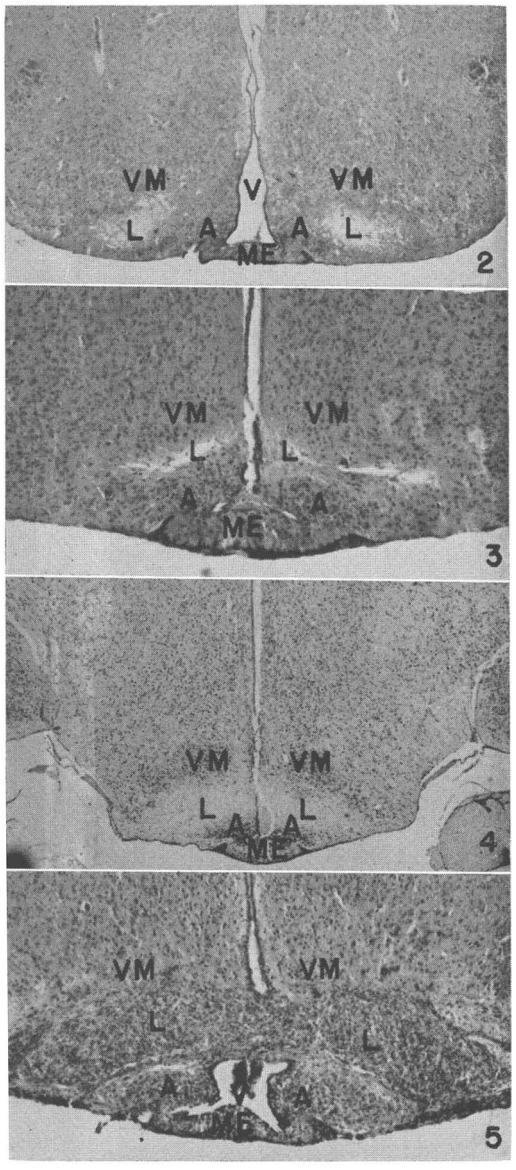

FIG. 2. Bilateral hypothalamic lesions *un*associated with wt gain in C_{57}BL. Dose: .35 mg/g body wt. Duration of experiment: 2 days. Stain: Romanes Silver. ×23.

FIG. 3. Bilateral hypothalamic lesions *un*associated with wt gain in CBA. Dose: .18 mg/g body wt. Duration of experiment: 19 days. Stain: Hematoxylin and eosin. ×23.

FIG. 4. Hypothalamic lesion associated with marked wt gain in CBA. Dose: .35 mg/g body wt. Duration of experiment: 2 days. Stain: Toluidin Blue. ×45.

FIG. 5. Hypothalamic lesion associated with moderate wt gain in C_{57}BL. Dose: 1.2 mg/g body wt. Duration of experiment: 19 days. Stain: Hematoxylin and eosin. ×45.

the hypothalamus. These extended from the mammillary bodies to the optic chiasma and were localized in the cell-poor interval between the ventromedial nuclear complex and the arcuate (infundibular) nucleus. *Similar lesions* (Fig. 3) *occurred in CBA mice with a dose of 0.18 mg. CBA mice receiving this low dose showed no significant weight gain.*

On the other hand, doses of 0.35 mg in CBA mice produced large lesions (Fig. 4), which were correlated with a great increase in body weight. In the C_{57}BL strain lesions of comparable magnitude (Fig. 5) resulted only when the dose of goldthioglucose was increased 3 to 4 times (1.2 mg). As indicated above, weight gain in the C_{57}BL strain was not as great as in the CBA, although the hypothalamic damage was similar (Fig. 4,5). CBA mice which receive more than 0.75 mg of goldthioglucose usually die within 3 to 4 days post-injection, and autopsy reveals gastric ulcers and stomachs overdistended with food. The brains of these animals show destruction of all of the nuclei of the hypothalamus except the supraoptic, dorsal parts of the filiform group, and the most ventral cells of the arcuate nuclei.

Lesions associated with significant increase in body weight differed from those unattended by weight increment, in that there occurred considerable destruction of the arcuate nucleus and ependymal cells of the third ventricle in the former (Fig. 4,5). Differences in rostral and caudal limits of the lesions in the obese and non-obese animals were not great.

All CBA mice with significant increase in body weight showed lesions in the limbic system (Fornix, ventral psalterium, and primordial hippocampus), (Fig. 7,8), in addition to hypothalamic damage. The histopathologic features of limbic and hypothalamic lesions were similar. Alteration of background staining, edema, and hemorrhage predominated in cases with 2- to 3-day experimental intervals. Macrophages were abundant in 5- and 9-day

PLATE II. Photomicrographs of sections through fornix and ventral psalterium of goldthioglucose treated CBA mice. CC, corpus callosum; LV, lateral ventricle; F, fornix; VP, ventral psalterium; AH, Ammon's horn; PH, primordial hippocampus; L, lesion.

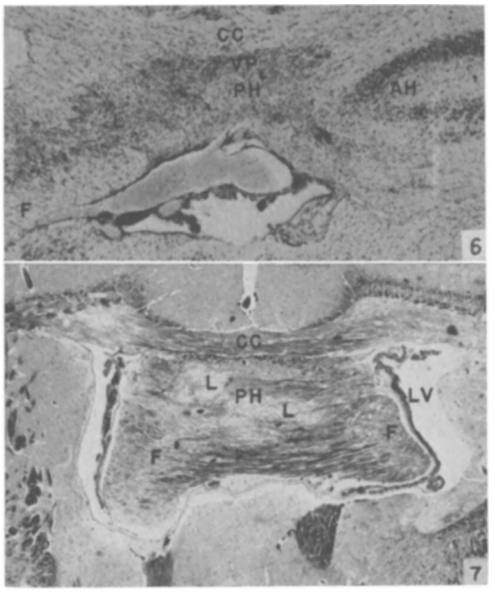

FIG. 6. Lesion of fornix and ventral psalterium associated with marked wt gain. Dose: .35 mg/g body wt. Duration of experiment: 15 days. Stain: Toluidin Blue. ×50.

FIG. 7. Lesion of primordial hippocampus, ventral psalterium and fornix associated with marked wt gain. Dose: .35 mg/g body wt. Duration of experiment: 2 days. Stain: Weil's myelin. ×30.

specimens. Gliosis was characteristic in animals allowed to survive 15 and 19 days after injection of goldthioglucose.

Discussion. It appears that the difference in response of CBA and C_{57}BL mice to goldthioglucose in terms of weight gain is not determined by mere presence or absence of lesions in the hypothalamus, but by extent of involvement of critical hypothalamic areas. Further investigation is needed to determine whether the "critical areas" are the postulated glucoreceptor center(7) or other regions. It is possible that the limbic lesions, which are being studied further in this laboratory relative to induced obesity are related to the greater increase in weight gain shown by CBA mice.

Since lesions of variable and predictable size can be induced in the hypothalamus by altering the dose of injected goldthioglucose, opportunity is provided to study localization of hypothalamic function.

Summary. The response to goldthioglucose-induced obesity differs in two genetically independent inbred strains of mice (CBA and C_{57}BL). This difference in response to a specific dose is associated with degree of hypothalamic damage, more extensive in CBA than in C_{57}BL mice, the former becoming more obese than the latter. Even when hypothalamic lesions comparable to those induced in very obese CBA mice are obtained by relatively large doses of goldthioglucose in C_{57}BL mice, weight gain is approximately one-half of that appearing in CBA's. Lesions of the fornix also occurred in CBA but not in C_{57}BL mice; these lesions may be related to the greater weight increment in CBA mice. A further possibility is a genetically controlled (peripheral) metabolic difference in reaction to hypothalamic lesions in these 2 inbred strains.

The goldthioglucose used was obtained through the courtesy of Dr. Robert W. Burlew and the Schering Corp., Bloomfield, N. J.

1. Brecher, G., and Waxler, S., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 498.

2. Parson, W., Camp, J. L., and Crispell, K. R., *Metabolism,* 1954, v3, 351.

3. Drachman, R. H., and Tepperman, J., *Yale J. Biol. and Med.,* 1954, v26, 394.

4. Mayer, J., and Zighera, C., *Science,* 1954, v119, 96.

5. Waxler, S., and Brecher, G., *Am. J. Physiol.,* 1950, v162, 428.

6. Waxler, S., and Enger, M., *J. Nutrition,* 1954, v54, 209.

7. Marshall, N. B., Barrnett, R. J., and Mayer, J., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 240.

Received February 18, 1957. P.S.E.B.M., 1957, v95.