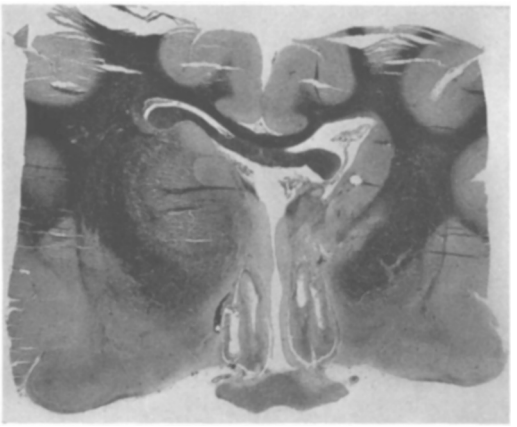

FIG. 1. Photomicrograph showing the greatest dimensions of satisfactory lesions in a typical animal. Weil stain.

and they extended caudally through the whole of the anterior hypothalamic area. Ventrally they reached the level of the optic chiasm and dorsally the roof of the third ventricle. However, when obvious microscopical differences were sought between the group of 7 cats that declined and the 4 that showed no change, no variations were readily apparent.

Discussion. Lesions located in presumably similar areas in the cat and rat appear to be without comparable effects on the sleep-waking cycle in the 2 species. The unexpected pattern of a sudden physical decline, beginning about the eighth postoperative day in a large percentage of cats, was interesting, but unexplained by such metabolic disturbance as might be indicated by the chemical methods herein used.

Without going into a minute analysis of fiber and nuclear damage and/or volumetric estimates of tissue damage, there appears to be no ready anatomical evidence for the difference in outcome of the 2 groups. Perhaps the eventual gathering of specifically meaningful knowledge of the area may help to account for these findings.

Summary. Eleven cats were subjected to lesions which were thought to correspond to Nauta's preoptic brain transections in rats. Seven of these exhibited a brief period of hyperactivity from the fourth through the seventh postoperative day, whereas 4 showed no change. Seven also declined physically, ceased drinking and eating, and died(2) or approached death before sacrifice. This decline could not be correlated directly with the periods of hyperactivity. There was no persistent alteration in sleep-wakefulness cycles. The only gross effect on serum electrolytes was concentration concomitant with dehydration. Potassium levels fell corresponding to a decrease in intake.

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Goldthioglucose-Induced Hypothalamic Lesion and ACTH Release.* (26944)

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Goldthioglucose (GTG) when administered to mice in appropriate concentrations will produce localized destruction in the hypothalamus(1,2,3) and other areas of the

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TABLE I. Levels of Plasma Compound B in Animals of Various Groups.

	Controls		5 days post-GTG		Controls		18 days post-GTG	
Resting levels	[a] ♂ 8n*	18.1 ± 1.9†	[c] ♂ 6n	56.8 ± 7.2	[e] ♂ 10n	14.6 ± .7	[g] ♂ 6n	14.4 ± 1.8
	[b] ♀ 7n	24.5 ± 1.7	[d] ♀ 6n	47.7 ±11.2	[f] ♀ 8n	23.3 ± 1.6	[h] ♀ 6n	25.5 ± 3.6
Stress: Ether	[i] ♂ 4n	35.6 ± 2.1	[k] ♂ 4n	46.2 ± 7.4	[m] ♂ 4n	64.3 ± 7.8	[p] ♂ 4n	68.3 ± 9.3
	[j] ♀ 4n	87.6 ± 2.1	[l] ♀ 4n	97.3 ± 7.4	[n] ♀ 4n	89.3 ± 5.9	[q] ♀ 4n	97.4 ± 1.9
Stress: Ether + fracture	[r] ♂ 4n	35.6 ± 1.3	[t] ♂ 4n	50.3 ± 3.9	[v] ♂ 4n	69.5 ± 7.5	[x] ♂ 4n	71.2 ± 4.3
	[s] ♀ 4n	64.1 ± 4.4	[u] ♀ 4n	78.6 ± 2.1	[w] ♀ 4n	91.3 ± 1.5	[y] ♀ 4n	112.5 ± 6.3

Statistical analysis: [a]/[b], $P < .05$; [i]/[j], $P < .01$; [r]/[s], $P < .01$; [a]/[c], $P < .01$; [k]/[l], $P < .05$; [c]/[m], $P < .01$; [f]/[n], $P < .01$; [k]/[k], N.S.; [j]/[l], N.S.

* This figure followed by n indicates No. of animals in the group.

† Concentrations of plasma-free corticosteroids expressed in μg Compound B/100 ml plasma ± S.E. (The superscript letter in front of the set of figures of each group permits rapid identification of the group for expressing the statistical analysis: [a]/[b] = figures of group [a] compared with those of group [b], etc.)

brain(4). Animals with such lesions show an increase in food intake(5,6) and subsequently obesity(7,8,9,10). In animals of the CBA strain, extent of the lesion in the diencephalon as well as the course of the ensuing obesity, are predictable for a given dose of GTG, in contradistinction to variability of results observed in other strains after injection of the drug(11). The hypothalamic lesion produced by GTG destroys areas of the ventral hypothalamus which in many reports (review in 12) have been implicated in control of the secretion of adrenocorticotropin (ACTH). The purpose of these experiments was to investigate whether the hypothalamic lesion regularly produced in CBA mice by GTG would inhibit the release of ACTH that ensues upon exposure to stress. In view of the simplicity of producing such a lesion, as compared to methods that use stereotaxic technics, our primary interest was in establishing whether GTG injection could provide an easy assay animal for studies on corticotropin releasing factor (CRF). Effects of the GTG-induced hypothalamic lesions on the resting pituitary-adrenal function, *i.e.*, in absence of stress, were also studied.

Methods and materials. One hundred twenty-one male and female mice of the CBA inbred strain§ were used. Animals were housed, 4-6 per cage (6″ × 12″ × 6″) and fed Purina laboratory chow *ad libitum*. Levels of pituitary-adrenal function were assessed by measurement of plasma free corticosterone (Cpd. B) levels(13,14). This was done on a 0.2 ml plasma sample from individual animals(13). Resting levels were obtained in animals sacrificed by instantaneous decapitation. All blood samples were obtained by decapitation, collecting the blood in heparinized tubes. The experimental protocol shown in Table I was designed to study in males and females the effects of goldthioglucose-induced hypothalamic lesions on the functional status of the pituitary-adrenal system during "resting" conditions and in response to acute stress as compared to untreated, control animals. It was decided to study the animals at 5 days and 18 days after injection of GTG. Previous experiments have shown(2) that at 5 days post GTG, the hypothalamic lesion is fully developed (Fig. 1). With the schedule at 18 days post GTG it was proposed to study pituitary-adrenal function in these animals when in the "active phase" of the weight gain leading to obesity (6). Two types of stress were used: ether anesthesia alone, or combined with fracture of one femur. In both cases, blood samples for plasma Cpd. B measurements were obtained exactly 15 minutes after application of the stress. All animals were sacrificed at the same time of day (between 1:00 p. m. and 3:00 p. m.), to minimize the 24 hour variation in plasma corticoid levels which has been reported in mice(15) and rats(16).

GTG‡ was administered as a single, i. p. injection of 0.4 mg/g body weight in animals

§Obtained from Kirschbaum Memorial Research Laboratory, Dept. of Anatomy, Baylor Univ. College of Medicine.

‡ Courtesy of Schering Corp., Bloomfield, N. J.

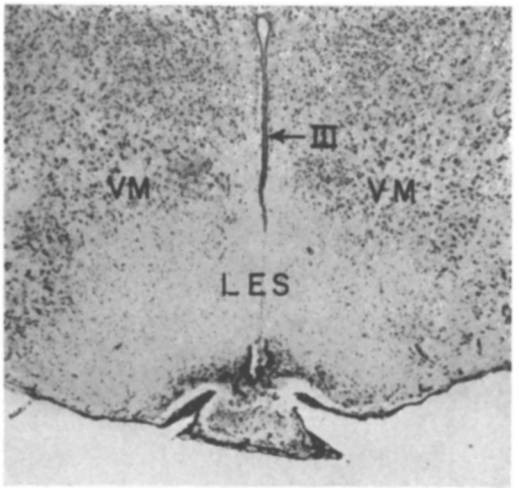

FIG. 1. Goldthioglucose-induced lesion in ventral hypothalamus of CBA mouse 5 days post-injection (V.M.—ventromedial nucleus; LES—lesion; III—third ventricle). Dose—0.4 mg/g body weight. Stain—thionine. x 45.

of 90-120 days of age(2). The various groups of animals shown in Table I were studied at either 5 or 18 days after injection of GTG. Brains of all GTG-treated animals killed at 5 days post-injection were fixed in 10% formalin. Midline sagittal reconstructions (frozen sections, 50 μ, thionin) were made for 8 brains to determine size and location of the hypothalamic lesion, and the stained sections of the remaining brains were examined histologically.

Results. Results are summarized in Table I. The normal control females had higher levels of plasma Cpd. B than the males under "resting" and stress conditions as previously reported by others(15,17). Five days after injection of GTG, at a time when the hypothalamic lesion was fully developed, levels of circulating plasma Cpd. B in both males and females were higher than in control animals, when measured in resting conditions, *i.e.*, in absence of stress. Exposure to stress led to no increase of these high levels in the male whereas in the female further increases were seen. Eighteen days after injection of GTG, resting levels of plasma Cpd. B in males or females had returned to normal levels as compared to normal control animals; exposure to stress was followed by significant elevations of plasma Cpd. B levels, similar to those observed in the normal control animals subjected to stress.

Fig. 1 shows a typical hypothalamic lesion as seen in an animal sacrificed 5 days after administration of GTG. This lesion was a constant finding in *all* animals injected with GTG and corresponds to what has been reported previously(2,4). A midsagittal diagram of the hypothalamus shows the anterior and posterior extent of the lesion (Fig. 2). The only minor variations observed were in the size of the lesions. All animals 18 days after GTG showed considerable increase in body weight when compared to their controls (males, -+20.6%; females, +29.6%).

Discussion and conclusions. The increase in plasma corticosterone resting levels seen in the first few days after injection of GTG may be due to one or several mechanisms: alteration of the conjugation capacity of the liver(18) due to the drug itself or accompanying the diminished food intake that immediately follows GTG injection(6), thus

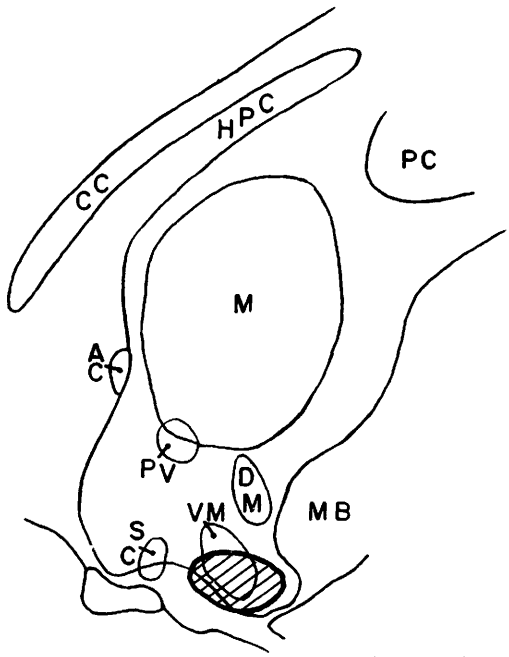

FIG. 2. Midsagittal diagram of the hypothalamus localizing a goldthioglucose-induced lesion in a CBA mouse at 5 days post-injection. Abbreviations used: CC, corpus callosum; DM, dorsomedial nucleus; M, massa intermedia; MB, mammillary body; PV, paraventricular nucleus; VM, ventromedial nucleus; SC, suprachiasmatic nucleus.

leading to increased plasma concentrations of *free* Cpd. B; or actual stimulation of the hypothalamic centers related to ACTH release by development of the hypothalamic lesion.

These results show, at any rate, that the hypothalamic lesion produced in CBA mice by the dose of GTG used in this study does not inhibit release of ACTH normally produced by exposure to stress. Animals with this type of hypothalamic lesion, therefore, cannot be used for an assay of CRF.

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Extension of Fertilizability in the Sand Dollar Egg with Vitamin B_{12}.* (26945)

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Unfertilized eggs of the sand dollar, *Dendraster excentricus*, lose the capacity to be fertilized within 48 hours when allowed to stand uncrowded in fingerbowls at 18°C. Addition of cobaltous chloride to the sea water will increase the fertile life of the eggs 4-8 days. When sulfhydril compounds are added to the proper concentrations of cobalt in sea water the fertile life may be extended to as much as 25 days(1,2). In view of these findings it seemed desirable to determine the effect of cyanocobalamin (Vit. B_{12}) on fertilizability.

Methods. Mature sand dollars were taken from Monterey Bay and maintained in aquaria at the Hopkins Marine Station, Pacific Grove, Calif. Ova were removed from ripe females and washed several times before being placed in various concentrations of the vitamin. Since the crystalline form of the vitamin was not available at the time of experiments a sterile aqueous solution (Squibb's Rubramin–containing 1,000 μg Vit. B_{12} per cc in isotonic NaCl with 1.5% benzyl alcohol as a preservative) was diluted with sea water. As simple dilution with sea water also diluted the benzyl alcohol it was necessary, in some cases, to add benzyl alcohol to the diluent to maintain a constant concentration of the preservative while varying the concentration of the vitamin. All cultures were kept in covered fingerbowls, out of direct sunlight and at 18 $\pm$ 1°C. Fertility was determined at 24-hour intervals by addition of fresh

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