THE BRAIN LESION OF GOLDTHIOGLUCOSE OBESITY*

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Plates 28 to 34

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During a toxologic study of gold compounds, it was noted that the survivors of midlethal dose of goldthioglucose gained weight excessively. About one-third of the mice became obese (1). Subsequent studies established that goldthioglucose produced lesions in the hypothalamus (2). Other gold compounds of closely related general structure, such as goldthiomalate, failed to produce either the hypothalamic lesions or the obesity (2–4). Goldthioglucose obesity appeared to be of the hypothalamic variety which can be produced both in rats and mice by stereotactic injury to the ventromedial hypothalamic nuclei (5, 6).

The apparent specificity of goldthioglucose in producing lesions in the hypothalamus was ascribed to a particular affinity of the gold compound for the ventromedial nucleus, which has been identified as the satiety center. This interpretation supported the glucostatic theory of regulation of food intake which asserted that the cells of the ventromedial nucleus are sensitive to an increasing arterio-venous difference in blood glucose. High blood levels of glucose would load the special receptor cells with glucose and activate discharge of impulses inhibiting further food intake. The presumed selectivity of goldthioglucose for the ventromedial nucleus was ascribed to the special affinity of these specialized nerve cells for the glucose moiety (4).

Recently, the specific affinity of the hypothalamic center for goldthioglucose was investigated by quantitative measurement and localization of the gold. This was made possible by activation analysis capable of detecting gold in tissues in micromicrogram quantities and by use of radioautography for localization (7, 8). The results clearly indicated that the obese animals had a higher concentration of gold in the hypothalamic area than similarly injected animals that had not become obese. It was, however, not possible to decide unequivocally

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whether more gold had been attracted initially to the hypothalamus. The alternative had to be considered: that the hypothalamic lesion itself resulted in greater retention of the initially uniformly distributed compound. Moreover, accumulations of gold could be demonstrated not only in the hypothalamic region, but also in other areas, confirming earlier evidence of extrahypothalamic lesions due to goldthioglucose (9). Finally, our investigations indicated that, contrary to some earlier statements, the final lesion produced by goldthioglucose is a fine glial scar and not merely a reduction in the number of neurons as depicted in the original report of the lesions. Consequently, the goldthioglucose lesion was reinvestigated with two purposes in mind. First, we wished to trace the entire development from the initial large necrotic lesion to the very small final scar and secondly, it was hoped, by accurate mapping of the area involved, to determine whether failure of obesity to develop in most of the survivors of a midlethal dose was due to failure of goldthioglucose to produce the initial lesion in two-thirds of the animals or whether the development of obesity depended on the extent of the lesion and its location.

Materials and Methods

Swiss albino mice of the NIH strain, weighing 17 to 20 gm, were injected intraperitoneally with 12.5 mg of goldthioglucose in 0.25 ml saline. This dose had been previously determined to kill 50 per cent of the animals within 1 to 3 days, with no late mortality ascribable to the gold compound. All animals were fasted 24 hours prior and 20 hours after injection, since this management had, in our experience, reduced mortality.

In a preliminary experiment, 10 mice surviving 24 hours were killed for histologic studies. In the main experiment 230 animals were randomized and 200 injected. Ten animals each of the injected group were killed 1, 4, and 14 days after injection. On day 76, the weight of the injected animals varied from 24 to 48, and that of the 30 controls from 24 to 29 gm. Three controls and 15 of the injected animals were killed, representing the entire weight range. Subsequently, weights of the controls increased to a maximum of 31, and those of experimental animals to 57. On days 205, 222, 228, 236, and 263, 2 animals each were killed, 1 of each group weighing in excess of 40 gm, the other 32 gm or less. In 2 supplementary experiments a total of 50 animals were injected and 2 each killed on days 3, 5, 7, 8, 9, 10, 11, 12, and 14.

At autopsy, the calvarium was removed, but the brain was left *in situ* and fixed together with the base of the skull in Bouin's fixative. After fixation, the brain was gently removed, embedded in paraffin and serial sections of the midbrain cut at 7μ. For visualization of the astroglia, an additional group of mice was injected with goldthioglucose and killed 5 to 15 days later. Fixation was by perfusion with Cajal's formaldehyde-ammonium bromide. Frozen sections were stained by Cajal's mercuric-gold technique (10).

RESULTS

A total of 50 brains were examined of animals that had been injected with goldthioglucose 1 to 14 days earlier. Hypothalamic lesions were found in 49. This included 23 animals killed 1 day after injection, 9 animals killed 4 days after injection, 2 animals each killed 5, 7, 8, 9, 10, and 12 days after injection, and 5 animals killed 14 days after injection. In 1 animal, killed 1 day after injection, no lesion was found.

One day after injection, the hypothalamic lesions could usually be seen with the unaided eye or a low magnifying lens in hematoxylin and eosin–stained sagittal sections as sharply circumscribed, symmetrical, pale, oval areas measuring 1 x 2 mm on either side of the ventral end of the third ventricle, often joined by a narrow bridge of similar pallor. On microscopic examination, the lesions extended occasionally as far forward as the optic chiasm, and they were often present in sections taken through the paraventricular and supraoptic nuclei. They were always present at the levels of the ventromedial hypothalamic nuclei and of the premammillary nuclei (Figs. 1 to 5). The bilateral lesions were often confluent in the midline or joined by a somewhat narrower bridge crossing the third ventricle. Posteriorly, the involved areas merged into a single midline lesion which occasionally extended as far as the posterior part of the medial mammillary nucleus. Both on day 1 and day 4 after injection the lesion consisted of a sharply circumscribed area of necrosis without cellular reaction. In a few animals killed as early as 12 to 24 hours after injection, a small number of pyknotic nuclei were still recognizable in the necrotic area, but after 24 hours only uniformly pink-staining material was visible. Occasionally a larger vessel traversing the necrotic lesion remained intact. No cellular reaction was present at the edge of the lesion. Where the third ventricle was involved, the ependyma had either disappeared or the markedly pyknotic nuclei of ependymal cells were still visible. Seven days after injection, the lesion had transformed itself, as it were by collapse of the broad area of necrosis, into a narrow band on either side of the ventricle of deeply eosinophilic staining material. Presumably this shrinkage of the lesion was primarily due to loss of fluid and diffusible products of autolysis, since no cellular infiltration was present at the line of demarcation of the lesion (Fig. 6). During the next 3 days, small round or oval cells infiltrated the lesion concomitantly with further shrinkage of the lesion. In addition to the microglia, fat-laden macrophages were present in varying numbers. Polymorphonuclear cells were seen only rarely (Fig. 7). By the 14th day after injection, cellular infiltration had again disappeared in 4 of the 5 brains examined, and the lesions had been transformed into a narrow scar, with only a few of either the microglia or the lipid laden cells remaining (Fig. 10). Transection, reduplication or herniation of the third ventricle was the only remaining indication of the substantial loss of brain tissue which was known to have occurred from the sequential examination of animals during the first few days after injection. The distortion of the normal configuration of the third ventricle and interruption of its ependymal lining was often visible only caudad to the ventromedial nucleus, while at the level of the nucleus itself no lesion was discernible in hematoxylin and eosin–stained sections (Figs. 8 and 9). The relative lack of residual anatomic distortion at the level of the ventromedial nucleus and the greater residual damage in the posterior hypothalamic area are of particular interest because this finding implies that the maximum ex-

tension of the lesion is not at the level of the ventromedial nucleus. This is supported by careful mapping of the acute lesion. As may be seen from Fig. 1, the ventromedial nucleus may be largely spared even though the necrotic lesion extends considerably ventrally, laterally, and caudally.

Of the 8 animals examined 76 days after injection, scars of the type just described were demonstrable in all. Only 1 still had some cellular infiltration in the involved area (Fig. 11). There was a distinct tendency for the obese animals, weighing in excess of 35 gm, to have more scarring than those with weights in the normal, *i.e.* below 30 gm (Fig. 12). This tendency was confirmed in the paired obese and non-obese animals examined 206 to 263 days after injection (Figs. 13 and 14). Indeed, in 2 non-obese injected animals examined 228 and 263 days after injection, no scar was found. Scars were found in all obese animals.

Animals killed 1 and 4 days after injection also had necrotic lesions in sites other than the hypothalamic area. In the hippocampus, symmetrical lesions were occasionally present extending from the midline and involving about one-third of the hippocampus (Fig. 15). In other animals only the hippocampal commissure was involved. Necrotic lesions were also found in the medulla oblongata (Fig. 16). These localizations corresponded to those reported by Perry and Liebelt (11) which had been found to be sites of concentration of gold-thioglucose in brains subjected to neutron activation and radioautography (7). These extrahypothalamic lesions have not been studied systematically. However, their occurrence appeared to be less constant than the hypothalamic lesions, and when present they were quite variable in extent. In none of the lesions did the areas involved correspond to recognizable units of nuclei and tracts, *e.g.* in the hippocampal commissure, an irregular area of white matter was often involved which clearly did not follow the distribution of commissural fibers. The involvement of the hippocampus, when present, appeared to be a simple extension of the necrosis of the hippocampal commissure, but was restricted to the median third and again did not follow known fiber tracts. The only regularity discernible in all lesions was their symmetrical distribution.

Brains perfused with Cajal's calcium-bromide fixative, from which frozen sections were cut for silver impregnation, indicated a marked degree of gliosis at a time when only a negligible scar was visible in hematoxylin and eosin–stained sections.

DISCUSSION

The presence of hypothalamic lesions in 49 of 50 mice killed during the first 2 weeks after goldthioglucose injection contrasts sharply with an incidence of only 30 per cent of clearcut obesity in the surviving animals. Obesity must therefore be correlated with the extent of the lesions, rather than with their presence. This notion receives support from the mapping of the extent of the

initial necrotic lesion, which is not centered in the ventromedial nucleus. Rather, the symmetrical necrotic area, which extends for a considerable distance rostral and caudad, is centered ventrally to the ventromedial nuclei. It may involve the nuclei only marginally or destroy them completely. Thus, depending on the extent of the necrotic lesion, part or all of the nucleus on each side may be involved. These observations are in keeping with the findings of Liebelt *et al.* (11) that in CAB mice low doses of goldthioglucose produce small lesions which are restricted to the acellular area between the arcuate and ventromedial nuclei. No obesity occurs in animals bearing these smaller lesions.

From the experiments of Mayer *et al.* (6), using a stereotactic instrument, it appears that obesity develops only when the ventromedial nucleus is destroyed bilaterally. There is, however, no sharp line between obesity and normal weight gain and there are varying degrees of obesity among the mice which exceed the control weights. Whether the minor degrees of obesity develop when the bilateral destruction of the nuclei falls short of being truly complete, or whether destruction of efferent tracts (12, 13) or other features of the lesions determine degrees of obesity is impossible to state from the data at hand. The basic notion, however, that the extent of the initial necrotic lesion determines the development of obesity is in accord with the known fact that obese animals have more prominent scars than those within the normal weight range and that accumulation of gold in the radioautographs of the hypothalamus is prominent only in the obese animals (7, 8).

One of the puzzling features of the goldthioglucose lesions is their distribution. It has been suggested that the localization in the hypothalamus, the hippocampal commissure and the dorsal midbrain denotes areas of a deficient or easily injured blood-brain barrier (9). The uniform distribution of gold throughout the brain at 1 hour after goldthioglucose injection, as determined by activation analysis, does not support this concept nor does the similar concentration obtainable with goldthiomalate (7, 8, 14). A special susceptibility of the hypothalamus and the other affected areas to damage by goldthioglucose must still be postulated to explain the localization of the lesion and the failure of goldthiomalate to produce either lesions or obesity. It is tempting to think of special chemoreceptors or a distinct metabolism in the areas involved (14). Such a mechanism has been postulated to explain the sharply circumscribed lesions which can be produced in the hypothalamus and the pyramidal layer of the hippocampus by 3-acetylpyridine and which can be prevented by nicotinamide (15). The concept was supported by distinctive staining with dithizone and other indirect evidence. Similarly, focal neurologic lesions were produced in dogs by monoamine oxidase inhibitors (16). In both instances, as in the case of goldthioglucose, the lesions were reproducible only in 1 or 2 species of animals (16, 17). The areas involved in goldthioglucose lesions may be postulated to be similarly distinctive. However, no relevant characterization of their metabolism

has been possible to date. In particular, the hypothalamic localization of the lesions cannot be accounted for by a specific affinity of the ventromedial nucleus for the glucose moiety, since this nucleus is frequently situated only at the margin and may be largely spared in the presence of a clearcut hypothalamic lesion.

The transformation of a relatively large initial necrotic lesion into a barely visible scar is one of the puzzling features of the goldthioglucose lesion. The occasionally complete disappearance of even this minute residuum may, however, be more apparent than real. In brains fixed and stained by Cajal's method gliosis was striking, and it is not unlikely that such preparations would have revealed the presence of a residual lesion in the rare cases in which hematoxylin and eosin–stained sections failed to do so. The reduction of the size of the lesion so that serial sections are needed for its demonstration presumably accounts for the failure to visualize the lesions in the original investigation of goldthioglucose obesity (1). Apparently Marshall *et al.* (2) who first described the acute necrotic lesion, also failed to observe the residual scar. Their legend accompanying the illustration of the hypothalamus 3 months after injection describes a reduction in the number of cells in the ventromedial nucleus. It is now apparent that the initial necrotic lesion does not result in a mere reduction of nerve cells with preservation of the outline of the nucleus, but rather in a residual scar which was not visualized in the particular section illustrated.

SUMMARY

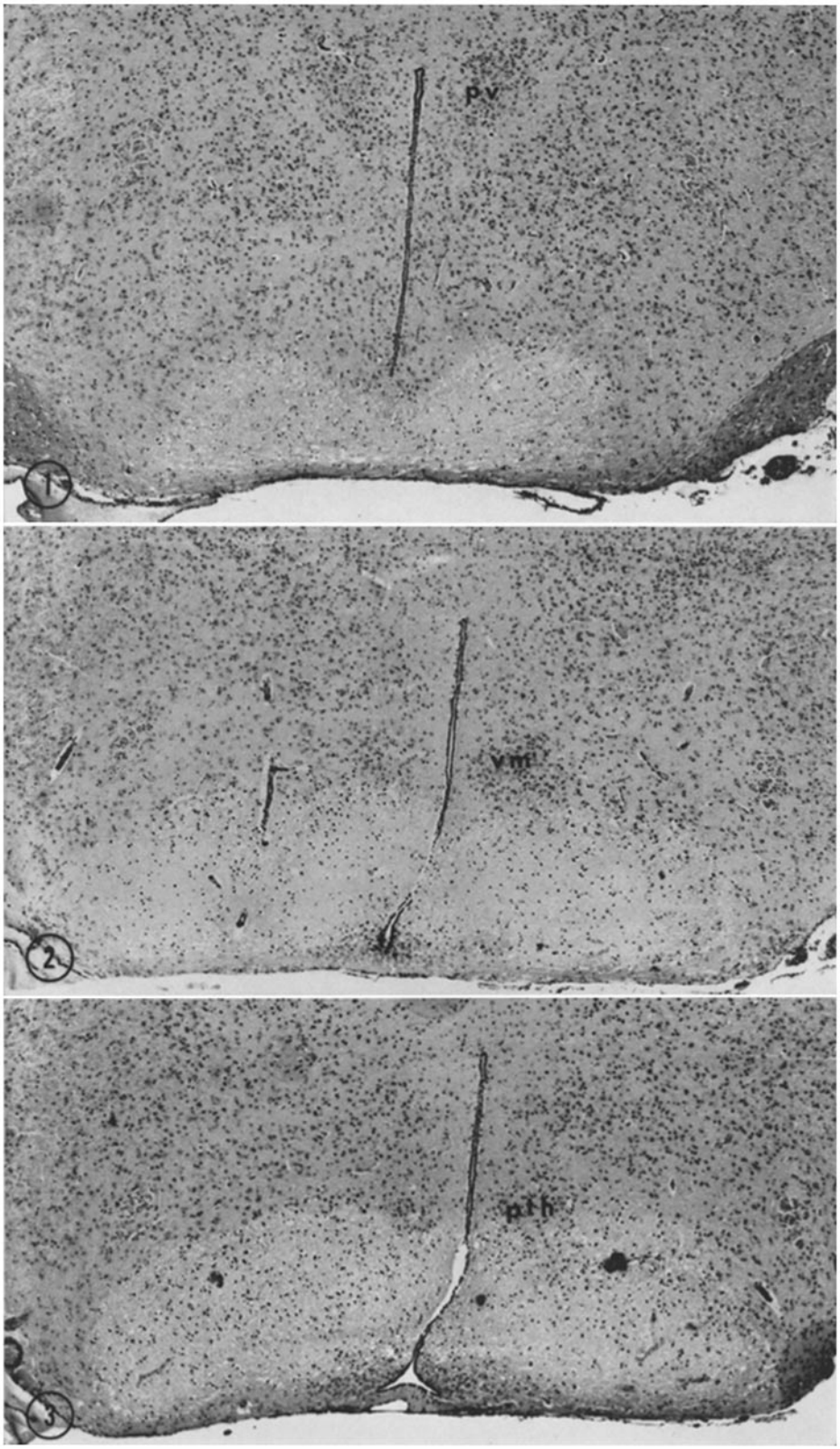
The development of hypothalamic lesions due to goldthioglucose are described. The initial extensive necrotic lesion occurs in close to 100 per cent of animals injected with LD_{50}. Within 2 weeks the necrotic material has been removed and a narrow scar results. After a lapse of several months, the scar is often difficult to visualize, especially in animals that have not developed obesity. The ventromedial nucleus is not the center of the lesion. The nucleus is preserved in some instances, and partially or completely destroyed in others, depending on the extent of the lesion. The more prominent scar in the obese animals correlates with the larger initial lesion necessary for the complete bilateral destruction of the ventromedial nucleus which is known to be a prerequisite for the development of hypothalamic obesity.

Thus, contrary to earlier suggestions, goldthioglucose does not localize specifically in the cells of the ventromedial nucleus.

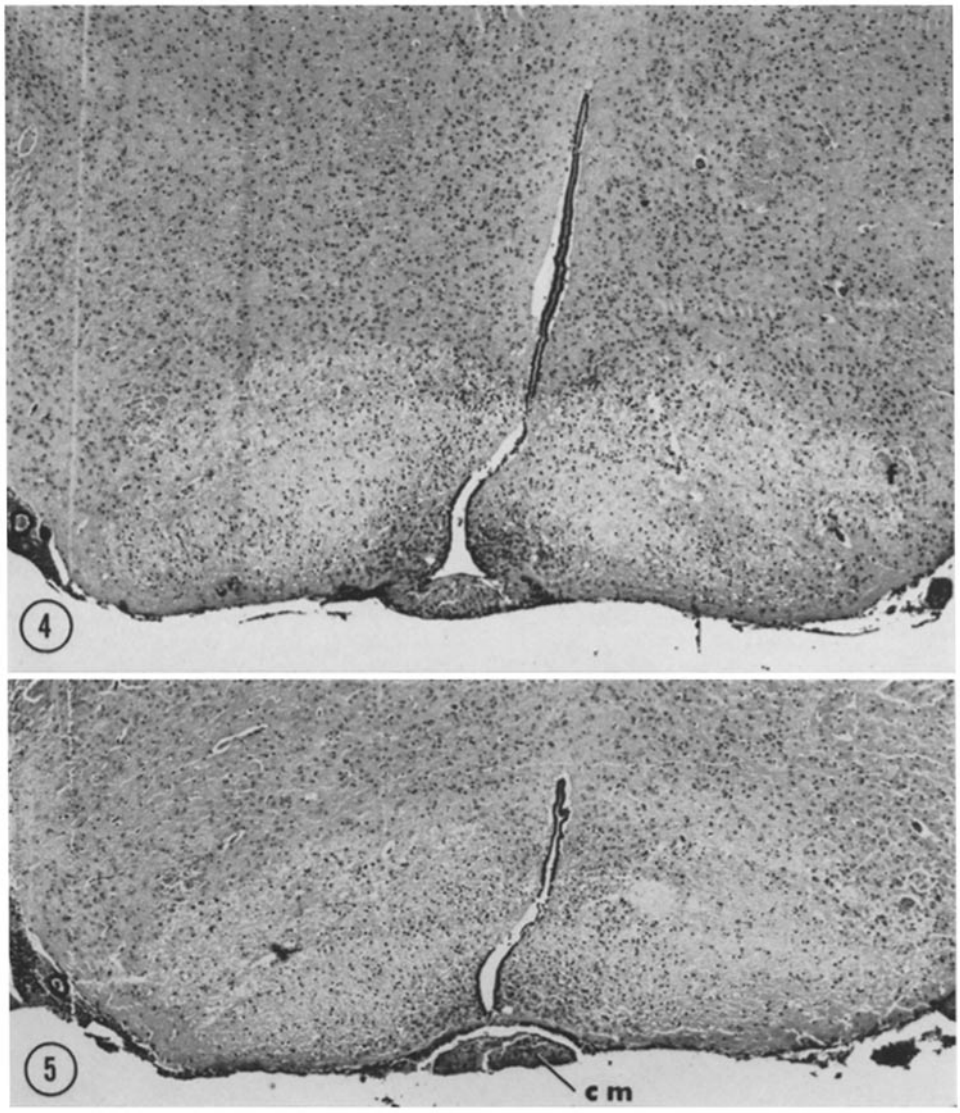
BIBLIOGRAPHY

1. Brecher, G., and Waxler, S. H., Obesity in albino mice due to single injections of goldthioglucose, *Proc. Soc. Exp. Biol. and Med.*, 1949, **70,** 498.
2. Marshall, N. B., Barrnett, R. J., and Mayer, J., Hypothalamic lesions in goldthioglucose injected mice, *Proc. Soc. Exp. Biol. and Med.*, 1955, **90,** 240.

3. Mayer, J., and Marshall, N. B., Specificity of goldthioglucose for ventromedial hypothalamic lesions and obesity, *Nature*, 1956, **178,** 1399.
4. Mayer, J., Some advances in the study of the physiologic basis of obesity, *J. Clin. Endocrinol. and Metab.* 1957, **6,** 435.
5. Brobeck, J. R., Tepperman, J., and Long, C. N. H., Experimental hypothalamic hyperphagia in the albino rat, *Yale J. Biol. and Med.*, 1943, **15,** 831.
6. Mayer, J., French, R. G., Zigherra, C. Y., and Barrnett, R. J., Hypothalamic obesity in the mouse; production, description and metabolic characteristics, *Am. J. Physiol.*, 1955, **182,** 75.
7. Schwartz, I. L., Cronkite, E. P., Johnson, H. A., Silver, L., Tenzer, D., and Debons, A. F., Radioautographic localization of "satiety center." *Tr. Assn. Am. Physn.*, 1961, **74,** 300.
8. Debons, A. F., Silver, L., Cronkite, E. P., Johnson, H. A., Brecher, G., Tenzer, D., and Schwartz, I. L., Localization of gold in mouse brain in relation to goldthioglucose obesity, *Am. J. Physiol.*, 1962, **202,** 743.
9. Perry, J. H., and Liebelt, R. A., Extra-hypothalamic lesions associated with goldthioglucose-induced obesity, *Proc. Soc. Exp. Biol. and Med.*, 1961, **106,** 55.
10. Lillie, R. D., Histopathologic technic and practical histochemistry, the Blakiston Company, New York, 1954.
11. Liebelt, R. A., Sekiba, K., Liebelt, A., and Perry, J. H., Genetic susceptibility to goldthioglucose-induced obesity in mice, *Proc. Soc. Exp. Biol. and Med.*, 1960, **104,** 689.
12. Morgane, P. J., Alteration in feeding and drinking behavior of rats with lesions in the globi pallidi, *Am. J. Physiol.*, 1961, **201,** 420.
13. Morgane, P. J., Electrophysiological studies of feeding and satiety centers in the rat, *Am. J. Physiol.*, 1961, **201,** 838.
14. Edelman, P. M., Livingston, L., Rahm, L., and Schwartz, I. L., The hypothalamus as a sensing center. A study of glucose in goldthioglucose obese mice, *Fed. Proc.*, 1964, **23,** 304.
15. Coggeshall, R. E., and MacLean, P., Hippocampal lesions following administration of 3-acetylpyridine, *Proc. Soc. Exp. Biol. and Med.*, 1958, **98,** 687.
16. Highman, B., and Maling, H. M., Neuropathologic lesions in dogs after prolonged administration of phenylisopropyl hydrazine and phenylisobutyl hydrazine, *J. Pharmacol. and Exp. Therap.*, 1962, **137,** 344.
17. Coggeshall, R. E., and MacLean, P. D., Cerebral effects of 3-acetylpyridine in the squirrel monkey, *Tr. Am. Neurol. Assn.*, 1959, **84,** 169.



(Brecher *et al.*: Brain lesion of goldthioglucose obesity)



EXPLANATION OF PLATES

PLATES 28 AND 29

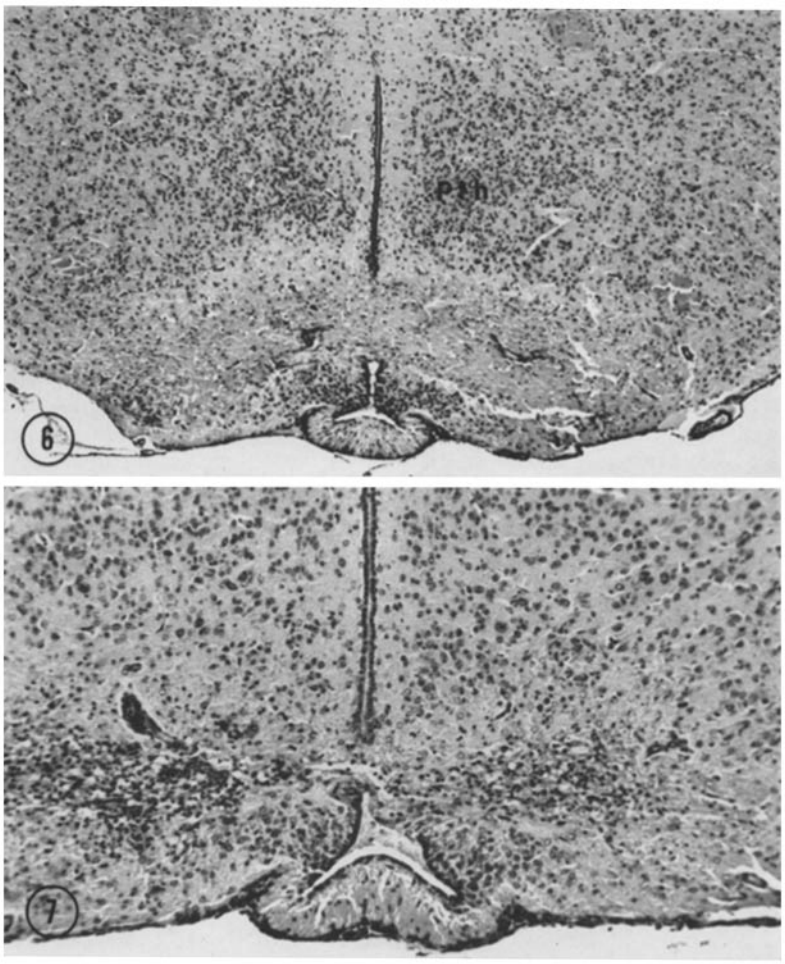
FIGS. 1 to 5. One day after injection of goldthioglucose. Selected serial sections of the same brain. The cuts illustrated in Figs. 2 to 5 are 0.8, 0.9. 1.3, and 1.5 mm caudad of that in Fig. 1. Symmetrical necrotic lesion extends from level of the paraventricular nuclei (*pv*) in Fig. 1 to that of the mammillary body (*cm*) in Fig. 5. Note partial preservation of ventromedial nuclei (*vm*), persistence of some pyknotic cells within the necrotic areas, and destruction of the ependymal lining of the third ventricle. *pth*, posterior thalamic nuclei, *f*, fornix. Hematoxylin and eosin stain. × 38.

(Brecher *et al.*: Brain lesion of goldthioglucose obesity)

PLATE 30

FIG. 6. Seven days after goldthioglucose injection. Collapse of the necrotic area. Hematoxylin and eosin Stain. × 38.

FIG. 7. Ten days after goldthioglucose injection. Infiltration of the collapsed area by small round cells and foam cells. In this particular lesion polymorphonuclear cells are also present. Hematoxylin and eosin stain. × 100.



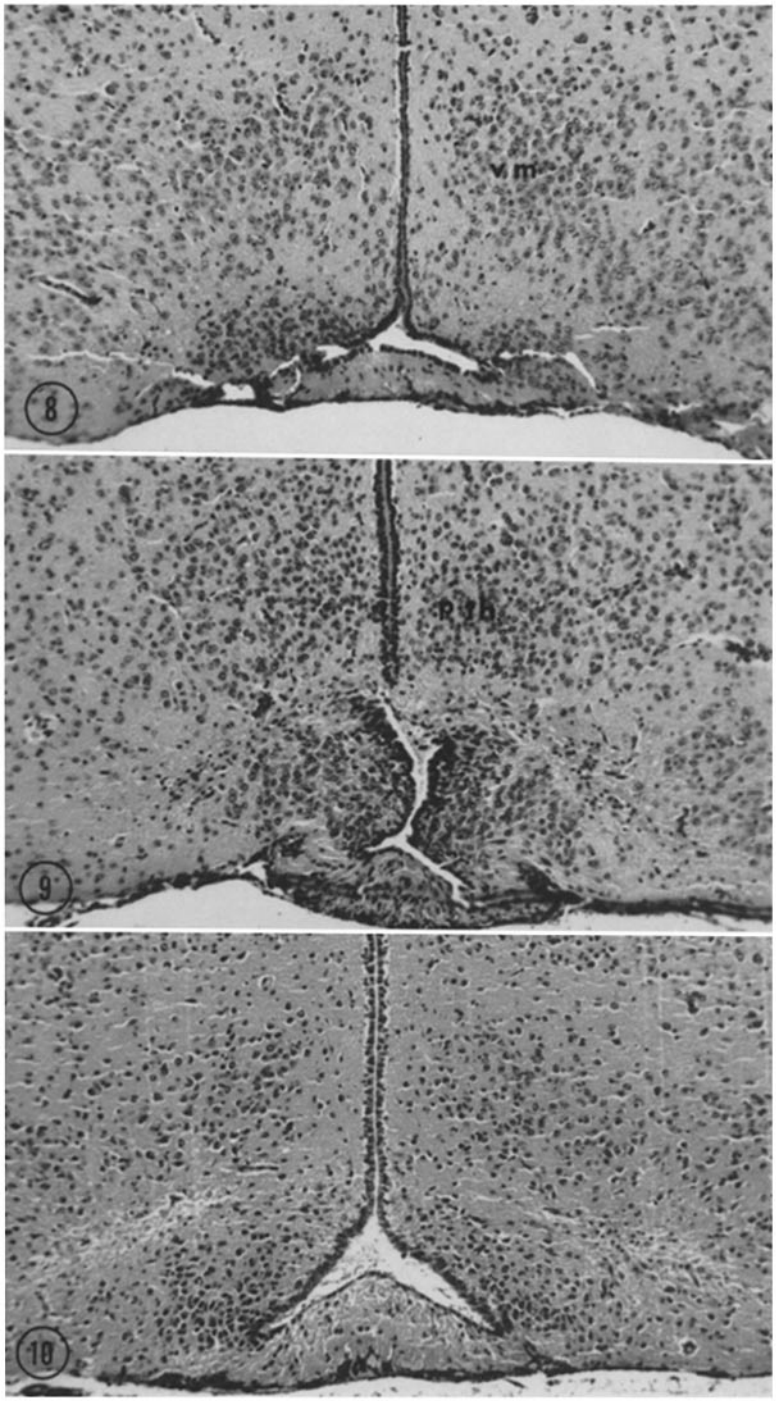
(Brecher *et al.*: Brain lesion of goldthioglucose obesity)

PLATE 31

FIG. 8. Twelve days after goldthioglucose injection. Note preservation of ventromedial nucleus and absence of discernible lesion at this level. Hematoxylin and eosin stain. × 100.

FIG. 9. Section of same brain, immediately caudad to ventromedial nucleus. Obvious lesion: transection of third ventricle presumably occasioned by collapse of initial necrotic lesion and developing scar. Hematoxylin and eosin stain. × 100.

FIG. 10. Fourteen days after goldthioglucose. The symmetrical fine glial scar indicates the maximal extent of the residual lesion in this animal. Hematoxylin and eosin stain. × 100.

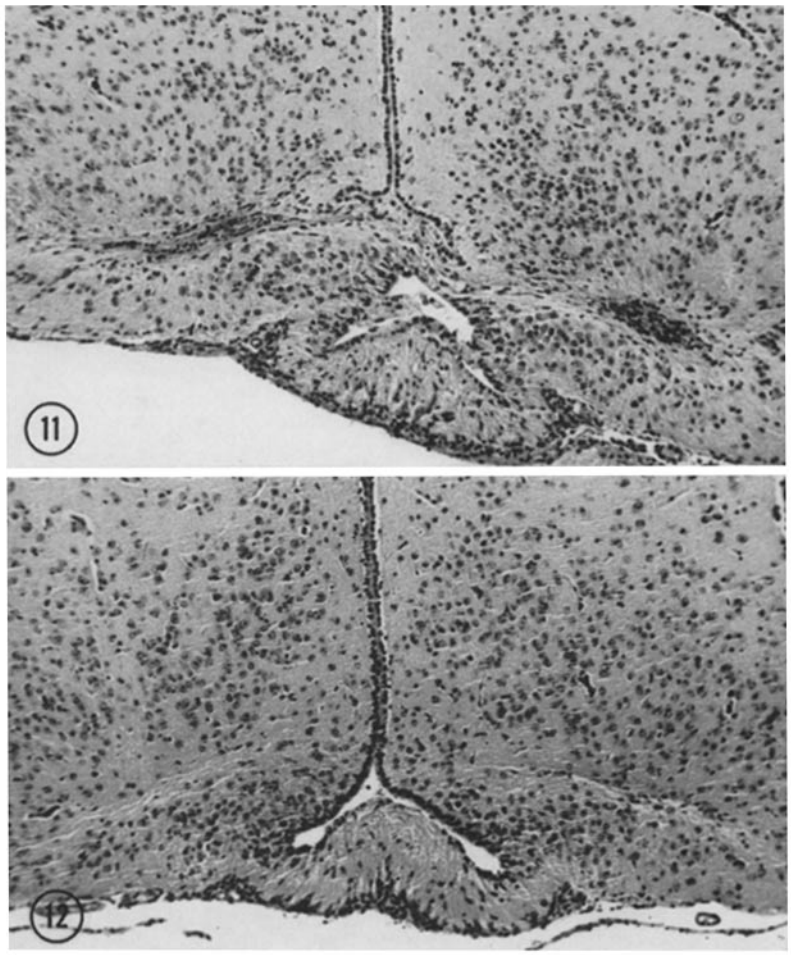


(Brecher *et al.*: Brain lesion of goldthioglucose obesity)

PLATE 32

FIG. 11. Seventy-six days after goldthioglucose. Brain of heaviest animal in the group (48 gm). Some round cell infiltration still present in large scar. Hematoxylin and eosin stain. × 100.

FIG. 12. Seventy-six days. Brain of animal weighing 24 gm. Minimal lesion. Hematoxylin and Eosin stain. × 100.

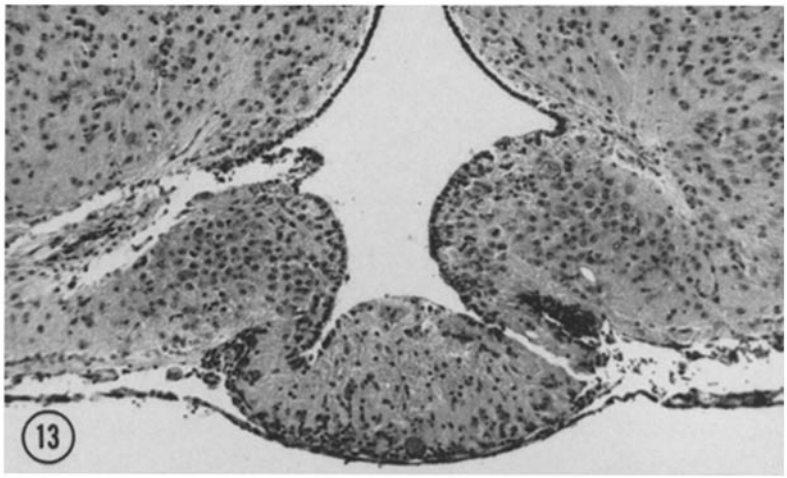


(Brecher *et al.*: Brain lesion of goldthioglucose obesity)

PLATE 33

FIG. 13. Two hundred and forty-two days. Brain of animal weighing 55 gm. Scar distorting 3rd ventricle. Hematoxylin and eosin stain. × 100.

FIG. 14. Two hundred and forty-two days. Brain of animal weighing 28 gm. Distortion of ventricle and probable gliosis in adjacent area is only indication of a residual lesion. Hematoxylin and eosin stain. × 100.



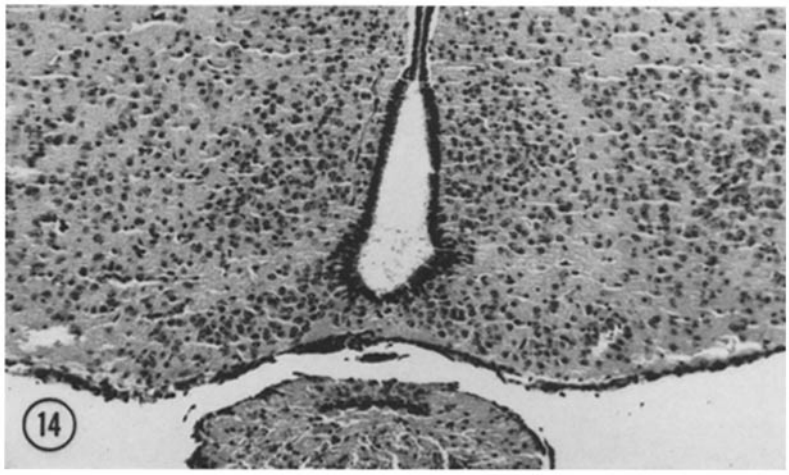


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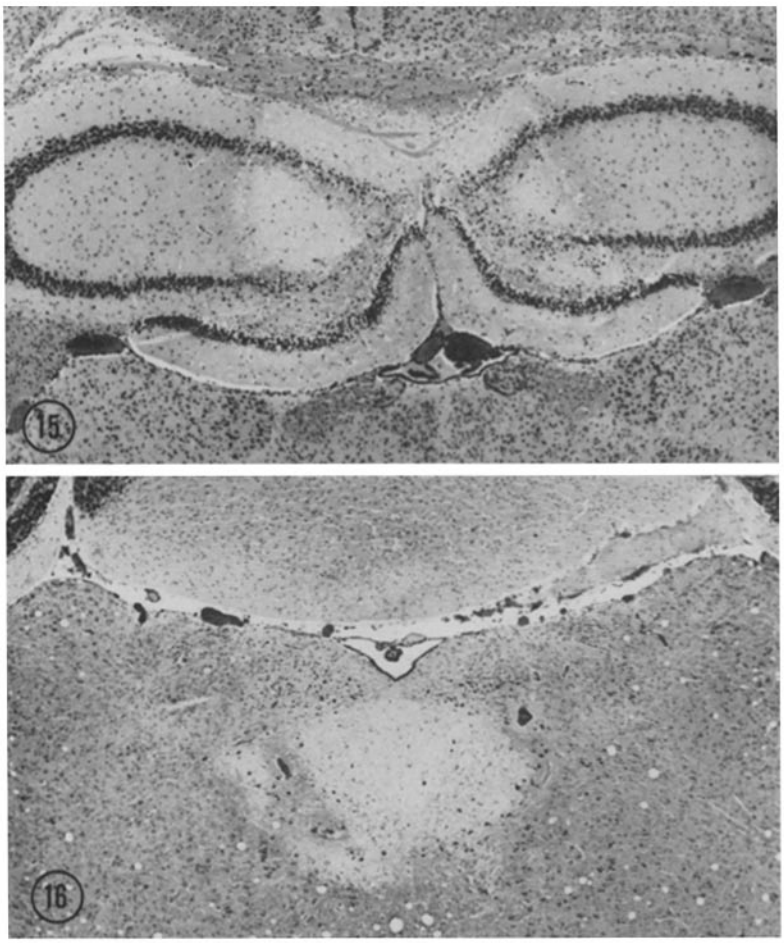
PLATE 34

FIG. 15. One day after injection. Hippocampal necrotic area. Hematoxylin and and Eosin stain. × 63.

FIG. 16. One day after goldthioglucose. Necrotic area in the medulla oblongata. Hematoxylin and eosin stain. × 63.



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