

THE EFFECT OF HYPERGLYCEMIA ON HYPOTHALAMIC GOLD UPTAKE AND HYPERPHAGIA IN GOLDTHIOGLUCOSE-TREATED MICE*

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The hypothalamic regulation of feeding behavior involves a continually active lateral feeding center which induces a sustained urge to eat and a normally quiescent ventromedial satiety center which, when activated, suppresses this urge (1-10). Mayer and associates (11-13) have suggested that the satiety signals originate as a result of the reaction of some component of cells of the ventromedial center to the attachment, uptake, or utilization of glucose. This glucostatic theory appeared to be supported by the finding (12) that a single injection of goldthioglucose was followed by the production of ventromedial hypothalamic lesions and the development of hyperphagia and obesity, whereas similar injections of a number of other organic gold derivatives (goldthiomalate, goldthioglycerol, goldthiocaproate, goldthiosorbitol, goldthiogalactose) did not produce these results. The glucostatic theory also appeared to be supported by our recent finding that the administration of goldthioglucose, but not goldthiomalate, is followed by a demonstrable localization of gold in the ventromedial hypothalamic lesion (14, 15).

In the studies to be described we have investigated the relationship of the extracellular glucose concentration to the deposition of gold in the mouse hypothalamus after a single intravenous injection of goldthioglucose. It was found that the concentration of goldthioglucose in the blood, the amount of hypothalamic gold deposition, and the degree of resultant hyperphagia varied directly with the concentration of glucose prevailing in the blood when the goldthioglucose was injected.

Methods

Experiments were conducted on Brookhaven National Laboratory white Swiss female mice weighing 25 to 30 gm. The animals were fed *ad libitum* with purina rodent feed¹ unless otherwise indicated.

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¹ Ralston Purina Company, St. Louis, Missouri.

Blood samples for gold, glucose, and mannose analyses were obtained by cardiac exsanguination. Four mice per group were bled at 5-minute intervals for the 1st hour and at 15-minute intervals for the 2nd and 3rd hours. Hyperglycemia was produced by the intraperitoneal administration of 0.5 ml of a 75, 50, or 25 mg glucose solution. Hypoglycemia was produced by an intraperitoneal injection of 0.05 units of regular insulin² per mouse. Goldthioglucose, 0.5 mg per gm of mouse weight, was administered intravenously *via* a tail vein from a solution containing 25 mg of goldthioglucose per ml of saline. Blood samples were obtained for analysis of gold concentration at the time intervals noted above. To determine the concentration of free and bound gold, aliquots of whole blood from goldthioglucose-treated animals were dialyzed overnight against distilled water and the dialyzable and non-dialyzable fractions were analyzed for gold.

Glucose determinations were carried out by automatic recording photometric equipment,³ utilizing a method in which the reduction of ferrocyanide is measured. The levels of blood mannose were determined by finding the difference between the total reducing sugar in the blood as determined by the autoanalyzer and the blood glucose levels determined by a glucose oxidase method (16).

Procedure.—

Insulin hypoglycemia: (Blood glucose concentration 25 to 35 mg per cent) 75 animals eating *ad libitum* were given 0.05 u of regular insulin intraperitoneally. Sixty minutes later when the blood glucose level was 25 to 35 mg per cent, they were given goldthioglucose, 0.5 mg/gm body weight.

Fasted animals: (Blood glucose concentration 75 to 85 mg per cent). Seventy-five mice fasted 24 hours were given goldthioglucose, 0.5 mg/gm body weight.

Non-fasted: (Blood glucose concentration 120 to 130 mg per cent). Goldthioglucose, 0.5 mg/gm body weight, was administered to 75 mice.

Hypermannosemia: Seventy-five mice were fasted for 24 hours and then 75 mg of mannose (Fisher reagent grade) in a 0.5 ml solution was administered to each intraperitoneally. Thirty minutes later, when the blood mannose level was 250 to 300 mg per cent and the blood glucose level was 180 to 220 mg per cent, goldthioglucose, 0.5 mg/gm body weight, was injected.

Hyperglycemia: Seventy-five mice were given 75 mg each of glucose (Fisher reagent grade) intraperitoneally. Forty-five minutes later when the blood glucose was 360 to 400 mg per cent, they were given goldthioglucose, 0.5 mg per gm body weight.

Another group of 75 animals each received the same treatment as above; however, 0.5 mg goldthioglucose per gm of body weight was given 60 to 75 minutes later when the blood glucose concentration was 250 to 275 mg per cent.

Each series was then divided. Forty-three animals in each group of mice were observed for weight gain and food and water intake. Thirty-two animals were killed by decapitation at 24 hours. The brains were carefully removed from the skull and placed on a slide, which in turn was placed on dry ice. When the brains acquired a rubbery consistency, the hypothalamic area and bilateral contiguous cortical areas (the control areas) were dissected under threefold magnification, weighed, separately placed in an aluminum boat, and then in a numbered nylon cassette. Fifteen cassettes were stacked with a goldthioglucose standard and an aluminum boat blank. The choice of standards and methods of preparation used were those previously detailed (15). The cassette stacks were then wrapped in aluminum foil 0.001 inch thick, placed in a cassette carrier and introduced into a water-cooled port (temperature 60–80° C) of the Brookhaven National Laboratory Graphite Reactor to be irradiated for 9.5 hours at a flux of 7.5×10^{12} neutrons/cm² second. After activation the tissue specimens were al-

² U40 insulin, Eli Lilly and Co., Indianapolis, Indiana

³ Autoanalyzer, Technicon Corporation, Chauncey, New York

lowed to "cool" for 48 hours and removed from the cassettes, mounted on a 3 x 5 inch card and covered with mylar.

To measure the gamma emission spectrum, the counting cards were placed in a penco 100 channel pulse-height analyzer 14.5 cm from a 3 inch sodium iodide (T1) crystal. A 97.5 mg/cm² aluminum absorber was interposed between the sample and the detector to diminish the beta interference. A cesium¹³⁷ standard was used to calibrate the gamma spectrometer. (98.6 per cent of the gamma emission is at 0.411 Mev; the average beta energy is 0.32 Mev.)

To determine the amount of gold in the brain samples, the area under the gold¹⁹⁸ photopeak was determined, corrected for background, and compared with standards which had been carried through the activation and counting procedure in an identical manner.

All animals were weighed daily and comparisons among the different groups were made on the basis of absolute weight change for the 2-week period following goldthioglucose administration.

Histological studies were performed on groups of mice under the same experimental conditions. The brains of these animals were removed and fixed in 10 per cent buffered formalin for 24 hours, washed in tap water, and imbedded in paraffin. Transverse serial sections of 7 μ thickness were prepared and stained with hematoxylin and eosin for histological study.

RESULTS

Gold levels in the hypothalamus and control lobes of mice given goldthioglucose at different levels of blood glucose concentration are summarized in Table I. At all levels gold deposition, measured as neutron-induced radioactivity, was significantly greater in the hypothalamus than in the adjacent control lobe ($p < 0.001$). The hypothalamic sections taken from fasted animals (blood glucose concentration 70 to 80 mg per cent) had significantly less ($p < 0.001$) gold than similar hypothalamic sections taken from the non-fasted animals (blood glucose concentration 120 to 130 mg per cent). Non-fasted animals in turn had significantly less ($p < 0.001$) hypothalamic gold accumulation than the hyperglycemic group (blood glucose concentration 360 to 400 mg per cent). The hypermannosemic animals (blood glucose concentration 180 to 200 mg per cent; blood mannose concentration 250 to 300 mg per cent) had hypothalamic gold accumulations which fell between the non-fasted and hyperglycemic groups. There was no statistically significant difference ($p = 0.4$) in gold accumulation in the hypothalamus between fasted and insulin-treated animals (blood glucose concentration 25 to 35 mg per cent).

Gold accumulation in the control brain sections varied between 34 and 43 per cent of that in the hypothalamus for the different groups of animals. As in the hypothalamic area, gold accumulation in the control brain sections was lowest in the fasted and insulin-treated animals, significantly higher in non-fasted animals, and highest in the mannose and glucose-treated animals. No statistically significant difference was noted in comparing the control sections of brain tissue from the glucose-treated and mannose-treated groups of animals; however, as noted above, there was a significant difference ($p = 0.004$) in the gold content of the hypothalamic areas of the two groups.

The intraperitoneal administration of either glucose or mannose (75 mg in

TABLE I
*Gold Levels in the Hypothalamus and Control Lobes Following Goldthioglucose Administration at Various Blood Glucose Levels**

Experimental group	Blood glucose concentration	Brain tissue			
		Hypothalamus†	Control lobe†	Hypothalamus mean values and standard deviation	Control lobe mean values and standard deviation
	<i>mg per cent</i>	<i>CPM/mg</i>	<i>CPM/mg</i>	<i>CPM/mg</i>	<i>CPM/mg</i>
Insulin-treated	25-35	99	31	94 ± 15	30 ± 3
		115	33		
		94	31		
		105	28		
		72	30		
		103	33		
		85	26		
		76	28		
Fasted	70-80	99	36	88 ± 12	31 ± 3
		90	31		
		95	31		
		70	28		
		86	35		
		99	29		
		70	28		
		95	32		
Non-fasted	120-130	122	52	114 ± 17	49 ± 7
		101	49		
		137	50		
		100	42		
		105	50		
		97	44		
		109	41		
		138	64		
Mannose-treated	180-220	209	98	195 ± 37	83 ± 17
		226	91		
		215	100		
		201	98		
		146	69		
		148	59		
		245	63		
		169	85		
Glucose-treated	360-400	315	87	277 ± 58	92 ± 24
		250	84		
		252	87		
		207	69		
		238	100		
		321	111		
		384	138		
		251	63		

* The Table represents the data from a typical experiment.

† Each value is derived from 4 pooled brain sections included in a single cassette for neutron exposure.

0.5 ml) not only produced hyperglycemia or hypermannosemia or both, but contracted the blood volume, as evidenced by changes in the hematocrit. The animals which received no intraperitoneal hexose had a hematocrit of $44 \pm$

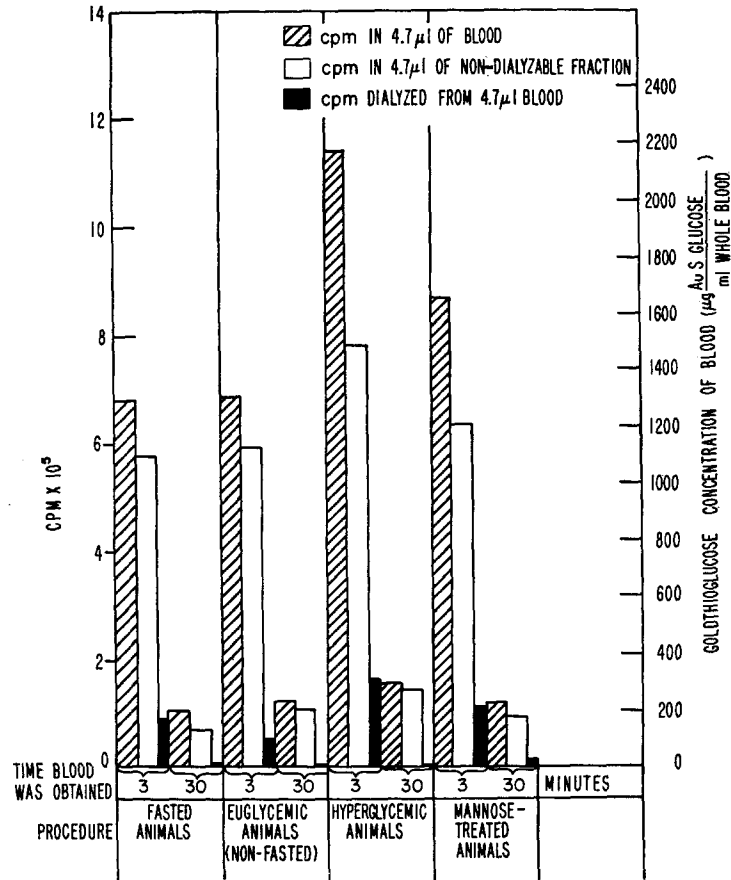


FIG. 1. Gold concentration of blood following intravenous goldthioglucose. Blood concentrations of total and bound gold, 3 and 30 minutes after intravenous administration of goldthioglucose (0.5 mg/gm mouse) to fasted, non-fasted, hyperglycemic, and hypermannosemic mice.

1; those which received the intraperitoneal injection of mannose (75 mg in 0.5 ml) had a hematocrit of 51 ± 1 , 20 to 30 minutes following the injection. The hyperglycemic animals (blood glucose concentration = 400 mg per cent) had a hematocrit of 55 ± 1 , 45 minutes after the intraperitoneal administration of 75 mg of glucose in a 0.5 ml solution. Thus the decreased intravascular space may be a factor contributing to the elevation of the goldthioglucose concentration in the blood of the hyperglycemic animals (*vide infra*).

As can be seen in Fig. 1, the concentration of free as well as bound goldthioglu-
 cose in the blood varied in the same direction, and was roughly proportional
 to the blood glucose concentration. Accordingly the animals injected with gold-
 thioglu-
 cose during maximal hyperglycemia exhibited the highest concentrations
 of both total and unbound gold; the ratio of free to bound gold in the blood 3
 minutes after intravenous injection of goldthioglu-
 cose varied directly with the
 level of blood glucose concentration prevailing at the time of the goldthioglu-
 cose injection (Table II). It is also noteworthy that within 3 minutes after the
 intravenous injection of goldthioglu-
 cose, approximately 50 per cent of the
 total amount administered had been removed from the circulating blood. In
 30 minutes less than 10 per cent of the administered goldthioglu-
 cose remained

TABLE II
*Percentage of Free Goldthioglu-
 cose in the Blood at 3 Minutes after Intravenous Goldthioglu-
 cose Administration under the Various Experimental Conditions*

Experimental group	Blood glucose concentration	Free goldthioglu- cose*
	<i>mg per cent</i>	<i>per cent</i>
Fasted animals	70-80	15
Non-fasted animals	120-130	15
Hypermannosemic animals	180-220	27
Hyperglycemic animals	360-400	31

* Calculated as: $\frac{\text{Conc. of total Au} - \text{conc. of bound Au}}{\text{Conc. of total Au}} \times 100$ for any given blood sample

in the circulating blood, and at least 80 per cent of this was bound to the blood
 constituents (Fig. 1).

Animals given goldthioglu-
 cose gained significantly more weight ($p = 0.0035$)
 than control animals which received an intraperitoneal solution of glucose
 (75 mg in 0.5 ml) alone. Those animals given goldthioglu-
 cose, when their blood
 glucose concentration was 120 mg per cent, gained more weight ($p = 0.02$)
 than fasted animals given goldthioglu-
 cose when their blood glucose concen-
 tration was 80 mg per cent. The hyperglycemic and the hypermannosemic-
 hyperglycemic animals which were treated with goldthioglu-
 cose in the same
 manner all gained significantly ($p = 0.002$) more weight than the similarly
 treated non-fasted euglycemic animals (Fig. 2).

Fig. 3 shows the conversion of mannose to glucose following the intraperito-
 neal injection of 75 mg of mannose. Figs. 4 and 5 illustrate that the simultane-
 ous administration of goldthioglu-
 cose (intravenously) and glucose (intraperi-
 toneally), and the administration of goldthioglu-
 cose when blood glucose concen-
 tration is maximal have only minor effects on the glucose tolerance curve.

Microscopic examination revealed lesions in the hypothalamus in all of the

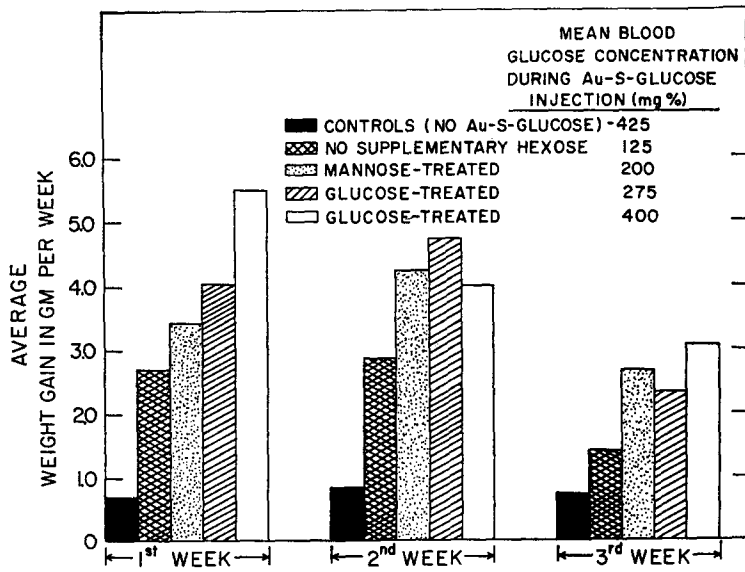


FIG. 2. Weight gain of untreated and various groups of mice given goldthioglucose with or without supplementary hexose.

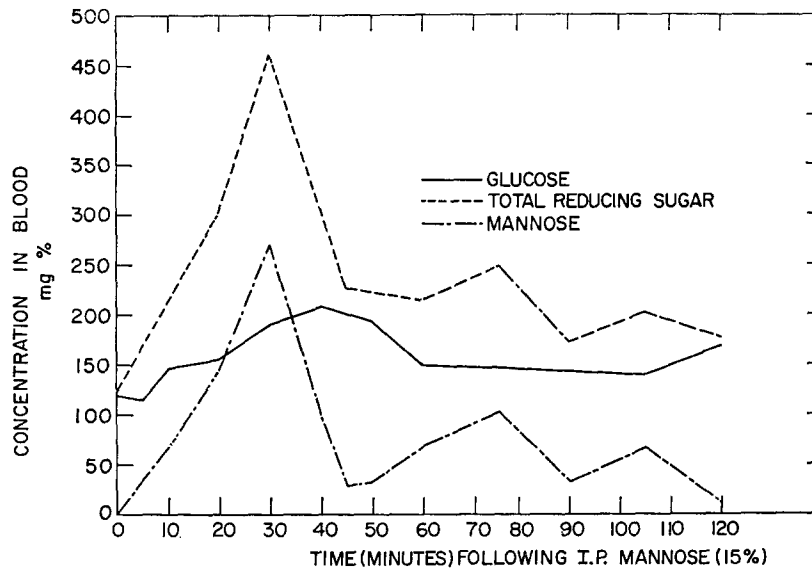


FIG. 3. Conversion of mannose to glucose following intraperitoneal administration of 75 mg of mannose.

animals given goldthioglucose, but no lesions were seen in the control cortical brain tissue of any animal in any of the experimental groups. The lesions occasionally extended as far forward as the optic chiasma, and were often present

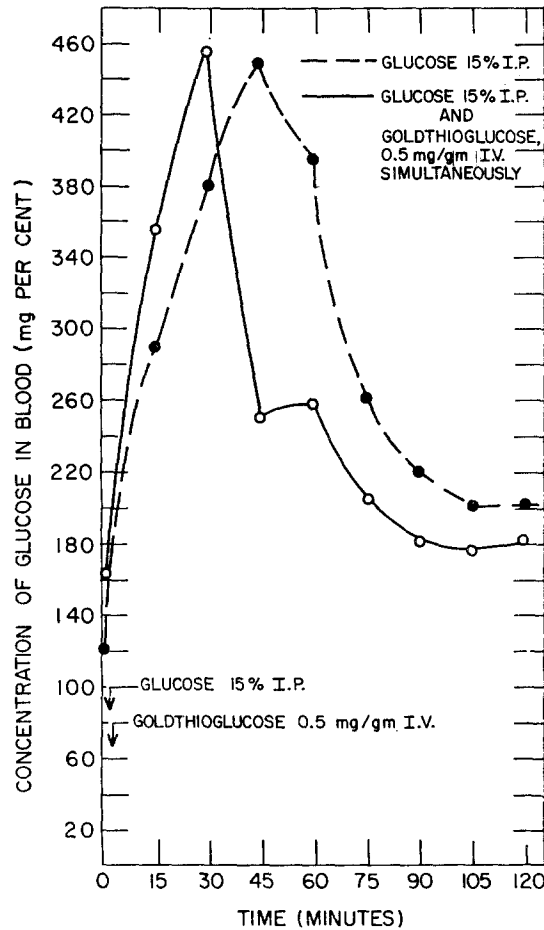


FIG. 4. Effect of goldthioglucose on glucose concentration I. Broken line represents blood glucose concentration at time intervals following $\frac{1}{2}$ ml 15 per cent intraperitoneal glucose at time = 0. Solid line represents blood glucose concentration at time intervals following $\frac{1}{2}$ ml 15 per cent intraperitoneal glucose plus intravenous goldthioglucose (0.5 mg/gm mouse) at time = 0.

in the sections taken through the paraventricular and supraoptic nuclei. Posteriorly the involved areas merged into a single midline lesion which occasionally extended as far as the posterior part of the medial mammillary nucleus. In general the higher the blood glucose concentration, the more ex-

tensive were the lesions and the greater was the magnitude of the weight gain. The histopathology is presented in detail elsewhere (17).

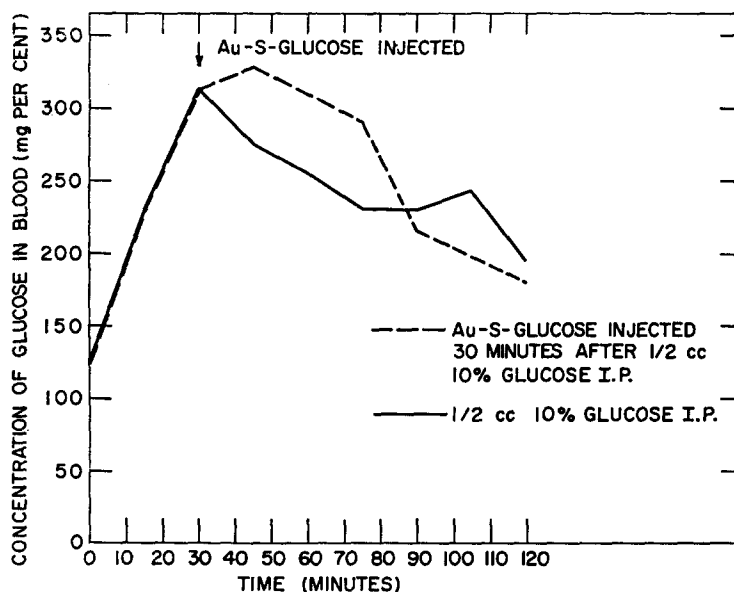


FIG. 5. Effect of goldthioglucose on glucose concentration II. Solid line represents blood glucose concentration at time intervals following $\frac{1}{2}$ ml 10 per cent glucose intraperitoneally at time = 0. Broken line represents blood glucose concentration at time intervals in animals treated identically as those represented by solid line; however, at time = 30 minutes they received intravenous goldthioglucose (0.5 mg/gm mouse).

DISCUSSION

The foregoing findings indicate that the weight gain, the extent of the hypothalamic involvement, and the degree of hypothalamic gold accumulation generally reflected the blood goldthioglucose concentration in the first few minutes following the injection. The same is true of the gold deposition in the control brain lobe, the ratio of gold content in the hypothalamic tissue to the gold content in the control cortical tissue being fairly uniform (Table I). The differential accumulation of goldthioglucose in the hypothalamus compared with other (control) areas of the brain may be due to differences in the permeability to goldthioglucose of various zones of the blood-brain barrier, or to differences in the metabolism of goldthioglucose in various parts of the brain. Alternatively, the necrotic goldthioglucose lesion itself may inhibit the egress of gold from the hypothalamic area and thus account for the higher concentration there.

The fact that hyperglycemia fails to diminish the relative deposition of gold in the hypothalamic area does not in itself exclude the possibility that glucose and goldthioglucoase compete for attachment at glucoreceptor sites, (11, 12) inasmuch as saturation of such sites with glucose need not have been achieved at the highest level of hyperglycemia attained in our experiments.

The gold content in the control brain sections of the maximally hyperglycemic animals ($92 \pm 24 \mu\text{g}$ wet tissue) was similar to the gold content in the hypothalamus of the fasted animals ($88 \pm 12 \mu\text{g}$ wet tissue); however, lesions were always present in the hypothalamus whereas no lesions were found in control areas in any group (17). This suggests that regions in the hypothalamus are more susceptible to damage by goldthioglucoase than other brain areas.

The findings that low levels of blood goldthioglucoase concentration produced small focal lesions in the ventromedial area whereas higher levels of blood goldthioglucoase concentration produced broader lesions of similar density suggest that there may be significant differences in the metabolism and/or supply of goldthioglucoase to various regions of the ventromedial hypothalamic area. One factor that may account for differences in the rate at which goldthioglucoase is supplied to various subdivisions of the ventromedial area is variability in the degree of vascular-glial contact.

In general, the lesions are most extensive and the rate of weight gain greatest in those animals which exhibit the highest goldthioglucoase level in the blood and the greatest gold concentration in the ventromedial area. Clearly the larger gold deposit is associated with a more extensive destruction of neurones concerned with appetite inhibitory function.

SUMMARY

The accumulation of gold in the hypothalamus and the development of hyperphagia and obesity were studied in mice given a single intravenous injection of goldthioglucoase at various levels of blood glucose concentration. It was found that the glucose concentration prevailing at the time of goldthioglucoase injection was correlated directly with the level of free and bound goldthioglucoase in the blood 3 minutes later, with the hypothalamic uptake of gold, with the extent of the hypothalamic lesion, and with the severity of the subsequent hyperphagia and obesity.

Hyperglycemia was associated with an increased gold deposition throughout the brain.

A gold content of $88 \pm 12 \mu\text{g}/\text{mg}$ wet tissue in the hypothalamus of fasted animals was associated with clearcut lesions in all animals studied, whereas a similar gold content in the control brain lobes of hyperglycemic animals was not associated with lesions in any animal. This finding indicates that some regions in the brain (*e.g.* the ventral hypothalamus) are more susceptible than others to damage by goldthioglucoase.

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