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AN ABSTRACT OF THE THESIS OF Janet Lahti for the Master of Science in Psychology presented January 13, 1972.

Title: The Effect of Glucose on the Food Intake of Goldthiogluucose Injected Mice

APPROVED BY MEMBERS OF THE THESIS COMMITTEE:

Cord B. Sengstake, Chairman

Morris Weitman

Roger D. Jennings

Jean Mayer has postulated the presence of glucoreceptors located primarily in the ventromedial hypothalamic nuclei of the brain which are activated by glucose in the measure that they utilize it. According to Mayer, hunger occurs when glucose is not available for metabolic purposes and satiation supervenes when glucose is made available. Control of this mechanism rests with the glucoreceptors. These receptors are believed to be destroyed when an injection of the compound goldthiogluucose is given to mice. These animals subsequently become obese and are found to have gold deposits and lesions in the ventromedial area and also in other parts of the brain.

If the glucoreceptors are destroyed and cannot respond to the presence of glucose in the blood, it would be predicted that food intake

would not change with the glucose level. The purpose of this investigation was to see if the food intake of goldthioglucose injected animals is affected by a glucose injection.

One hundred and twenty mice were divided into four groups. One group, the controls, received no goldthioglucose. The other three groups received the following dosages: .5 mg. of goldthioglucose per gram of body weight, 1.0 mg. of goldthioglucose per gram of body weight and 1.3 mg. of goldthioglucose per gram of body weight. Fifty seven days after the goldthioglucose injection a training period began in which the mice were taught to consume their entire daily food intake during a 50 minute period. Then the experimental period began during which the animals were maintained on the 23 hour and 10 minute deprivation schedule. During this period the mice were given intraperitoneal injections of 60 mg. of glucose or normal saline on alternate days for ten days. The amount of food consumed in the 50 minutes beginning 15 minutes after the glucose or saline injection was determined.

Food intake of the goldthioglucose injected animals was not different than that of the controls under any factor of the experiment.

The results do not support Mayer's idea that the glucoreceptors as he describes them are destroyed by goldthioglucose.

THE EFFECT OF GLUCOSE ON THE FOOD INTAKE OF
GOLDTHIOGLUCOSE INJECTED MICE

by
JANET LAHTI

A thesis submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

in

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TO THE OFFICE OF GRADUATE STUDIES:

The members of the Committee approve the thesis of
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CHAPTER I

INTRODUCTION

Hunger and feeding are under the control of many sensory factors, both learned and unlearned. These contribute in an additive way to the arousal of hunger and to the development of satiation: Taste, smell, gastric contractions and gastric distension, to name some major ones. Added to these are powerful influences from the more internal environment, including both osmotic and specific chemical changes. It is the understanding of these internal physiological determinants of feeding behavior, and more specifically the influence of blood glucose on hunger and satiety which is of concern here.

I. HISTORY

Before the beginning of this century explanations of the origin of hunger were largely speculative. Early in the present century, Cannon (1912) demonstrated that deprivation of food and the subjective feeling of hunger are accompanied by rhythmic contractions of the stomach. Carlson (1916) confirmed this finding and made these gastric contractions the basis of a complete theory concerning the regulation of appetite. This theory was based on the idea that the control of food intake rests with the periphery of the body rather than with the central nervous system. With these efforts to explain empirically the mechanism of food intake, the study of hunger began in earnest.

Experimental evidence gradually accumulated against these theories. Adolph (1947) observed that if their food is diluted with inert material, animals quickly adjust for decreased caloric content per unit volume by consuming more of the diet. Grossman and Stein (1948) observed that denervation of the stomach in human subjects did not alter the appetite, as would have been anticipated from Carlson's theory. This kind of evidence led to the belief that the hunger mechanism was more complex than the theory of gastric contractions could explain.

A second view of the location of the physiological change which takes place to regulate food intake was advanced. This view holds that a complex regulatory mechanism is found in the central nervous system which matches energy intake to energy expenditure. Efforts were made by investigators to define this mechanism. Attention began to focus on the hypothalamic region of the central nervous system as a possible regulator of food intake. Hypothalamic obesity was first described by Mohr about 1840. A fifty-seven year old woman became obese within a year before her death. Autopsy showed a hypophysial tumor large enough to affect the hypothalamus (Brobeck, Tepperman and Long, 1943). A number of similar cases had already appeared when Frohlick (1902) described a syndrome with the essential features of adiposity and genital underdevelopment in patients with hypophysial tumors. Bailey and Bremner (1921) suggested that the extreme obesity observed in patients with Frohlick syndrome was due to hypothalamic lesions and not to damage to the hypophysis.

By the fourth decade of this century, researchers interested in the hunger mechanism had begun to focus their attention on the hypothalamic area of the brain.

CHAPTER II

REVIEW OF THE LITERATURE

I. VENTROMEDIAL HYPOTHALAMUS

Improved surgical techniques enabled Hetherington and Ransom (1942) to demonstrate that, in the rat, obesity regularly follows bilateral electrolytic destruction of the ventromedial nuclei of the hypothalamus. Brobeck, Tepperman and Long (1943) obtained similar results and found obesity due almost entirely to increased food intake and termed the syndrome hypothalamic hyperphagia. Like changes in the eating pattern have been produced experimentally by hypothalamic lesions in many species of animals including rats (Anand and Brobeck, 1952), mice (Marshall, Barnett and Mayer, 1955), cats and monkeys (Anand, Dua and Schoenberg, 1951).

Efforts were made to describe the syndrome in detail and it was found that rats with ventromedial lesions pass through two stages, the dynamic and the static. The dynamic phase lasts approximately two months. During this time the animal displays a voracious appetite and gains weight rapidly. As the animals enter the static phase, weight increase levels off and food intake decreases somewhat but remains at a higher level than with normal rats.

Research has not offered an explanation for this leveling off of food intake. The hypothalamic hyperphagic rats seem to eat the same number of times as controls but they eat more at a time. Obesity is

due primarily to increased intake and there is no major impairment of the metabolic process as a result of lesions (Miller, Bailey and Stevenson, 1950; Teitelbaum, 1955).

By implanting electrodes into the ventromedial area of rats, Kranse (1962) was able to show that stimulation of this area causes hungry rats to stop eating.

To test the idea that the metallic deposits left by the electrolytic lesions are the cause of the obesity, Reynolds (1963) produced lesions in the ventromedial hypothalamus in two different ways, radio frequency current and electrolysis. Radio frequency lesions are produced by a surgical procedure identical to that used for electrolytic lesions except that no residual deposit of metallic ions is produced. Radio frequency destruction failed to produce hypothalamic hyperphagia which follows the electrolytic lesions. Reynolds believes that scar tissue produced by the electrolytic lesions may have an irritative effect on surrounding tissue. Hyperphagia arises, according to Reynolds, from the chronic irritation of the ventromedial feeding center. Reynolds (1965) produced both electrolytic lesions and radio frequency lesions in the lateral hypothalamus, the area associated with the initiation of eating. He found no difference between the two lesions in producing aphagia which usually follows lateral hypothalamic damage. From his work Reynolds concluded that cells producing a cessation of eating are located at the periphery of the ventromedial hypothalamus rather than in its center and that the area for the initiation of eating is the nucleus of the lateral area.

Rabin (1968) investigated the electrical activity of the lateral hypothalamus in the rat following lesions of the ventromedial area. He used two kinds of lesions: the electrolytic and the radio frequency. The controls and the rats with radio frequency lesions had electroencephlogram recordings consisting of low voltage fast activity. The EEG following electrolytic lesions consisted of a pattern of large amplitude waves. It was concluded that the data are generally consistent with the irritative hypothesis proposed by Reynolds.

Hoebel (1968) has shown that some radio frequency lesions in the ventromedial hypothalamus do, in fact, produce hyperphagia and obesity. Reynolds' irritative hypothesis is an unlikely explanation for these data since it is based on the idea that only the electrolytic lesions cause the irritation which results in obesity.

Reynolds' idea that the satiety area is on the edge of the ventromedial hypothalamus can be questioned in view of a series of experiments by Grossman (1968) in which lesions were produced above and below as well as anterior and posterior to the feeding center. Grossman found no effect on food intake as a result of these lesions.

It is clear that electrolytic lesions in the ventromedial hypothalamus result in hyperphagia. The mechanism by which this takes place and the anatomical limits of the area are not understood. Reports concerning the radio frequency lesions seem inconclusive.

II. LATERAL HYPOTHALAMUS

Further exploration of the hypothalamus led to the demonstration that an increase in eating of as much as 100% could take place in rats and cats as a result of electrical stimulation of the lateral hypothalamus

(Delgado and Anand, 1953). The effect of this electrical stimulation resembles the hunger normally produced by deprivation of food (Coons, Levak and Miller, 1965). When the lateral area is destroyed bilaterally, complete aphagia and death due to starvation are the result in rats (Anand and Brobeck, 1951) and cats (Bach, Obrian and Cooper, 1957). A unilateral lesion has no such effect (Anand, 1961).

The syndrome resulting from lateral hypothalamic lesions has been described by Teitelbaum and his associates (1955, 1962). Rats with lesions in the lateral hypothalamus will eventually eat and drink if they are kept alive long enough by tube feeding. Four clear-cut stages in the recovery have been described. In stage one the animal is aphagic and adipsic and will die if not forced to consume food. During the second stage the animal will eat but not enough to maintain body weight. Rats will regulate their intake of a liquid diet during the third stage. They maintain their weight but are still adipsic. In the fourth stage the animal will drink water and will survive on dry food and water. In some cases there is no recovery from adipsia.

It is evident that the lateral area in some way influences the initiation of eating, since its destruction causes failure to eat and its stimulation causes overeating.

III. HYPOTHALAMIC FEEDING SYSTEM

A good deal of research has been devoted to finding the possible fiber connections between the inhibitory mechanism of the ventromedial hypothalamus and the excitatory feeding center in the lateral region.

Anand and his colleagues (1960) postulated a hypothalamic feeding system involving the ventromedial nucleus, the lateral area and nerve fibers connecting these two centers. In this system the lateral area would be the feeding center responsible for initiating the ventromedial would be the satiety center capable of exerting inhibitory control over the lateral feeding center.

Investigation of the concept that the ventromedial hypothalamic region could act as an inhibitor to the lateral hypothalamic feeding center has been conducted by several researchers.

Oomura and associates (1964) recorded spontaneous discharges from the ventromedial nucleus and the lateral area of the hypothalamus. The activity in one area was recorded while the other was stimulated. The results revealed that significant reciprocal relations do exist.

The possible route of these anatomical connections has recently been investigated. Destruction of neurons caused their axons to degenerate, a process that is believed to occur over a period of several days. Goldthiogluucose, which is believed to selectively destroy cells sensitive to blood glucose level changes, was given to mice and the degenerating axons stained by a method developed by Fink and Heimer (1967). By using this method, Aries and Mayer (1968) have found evidence for direct fiber connections from the medial to the lateral hypothalamus.

Murphy (1968) failed to find a powerful inhibitory pathway from the lateral hypothalamic area to the ventromedial hypothalamus. He suggested that one of the mechanisms by which experimental lesions or stimulation in the lateral hypothalamus influence feeding behaviour may

be by the interruption or activation of fiber systems from other sources such as the amygdala which terminate in a converging fashion at the lateral edge of the ventromedial hypothalamus.

Some connections appear to exist between the ventromedial hypothalamus and the lateral area. The mapping of these routes is far from complete and the manner in which they are related to the feeding system is not understood.

In summary of the evidence concerning the location of the hunger center it seems that the ventromedial area of the hypothalamus controls the cessation of eating while the lateral area controls the initiation of eating. This evidence is based on lesions produced electrolytically. Four stages have been described in the recovery from lesions in the lateral area progressing from complete aphagia to complete recovery in some cases. Hypothalamic hyperphagia, resulting from lesions in the ventromedial area, produces a dynamic phase, characterized by a voracious appetite; and a static phase, in which food intake levels off. Some connections have been found between the two areas but they are not well defined. Radio frequency lesions seem to produce a syndrome similar to electrolytic lesions in the lateral area but reports are conflicting concerning their effect on the ventromedial area.

IV. BEHAVIORAL EVIDENCE

In an early application of operant techniques to the study of feeding, Anliker and Mayer (1957) trained both normal mice and those made hyperphagic as a result of lesions of the ventromedial hypothalamus to obtain food pellets by pressing a lever. Bilateral destruction of

the ventromedial area did not result in an increase in the rate of lever pressing, a generally reliable correlate of hunger, but hyperphagic mice spent more time pressing the lever at the same rate as the control animals. So these hyperphagic animals were eating at a normal rate but spending more time eating than the animals who were not hyperphagic.

Miller, Bailey and Stevenson (1950) found that a decreased motivation for food in hyperphagic rats is evident not only from a relatively low rate of bar pressing for food, but also from inferior performance on other behavioral tests. Their interpretation is that hypothalamic lesions cause the mechanism regulating hunger to stick at a relatively constant low level. While food intake remains excessive throughout the development of obesity the strength of motivation underlying the feeding response of the hyperphagic animal is not greater than that of a normal animal.

It appears from this research that electrolytic lesions in the ventromedial area do not increase hunger motivation but rather interfere with the mechanism which stops eating behavior.

Teitelbaum (1955) demonstrated that the experimental animals in the initial phase of hyperphagia, like normal animals, maintain appreciable food intakes despite adulteration of their diet with cellulose or quinine, which makes it much less palatable. After becoming obese, animals show almost complete rejection of food adulterated with such substances. It appears that once the rat with ventromedial hypothalamic lesions gets obese, he becomes a finicky eater and sensitive to the negative stimulus aspects of the diet.

Since these hyperphagic animals are sensitive to the negative stimulus aspect of their diet, it is reasonable to question whether the type of stimulus (positive or negative) provided by taste is the basic motivational factor underlying excessive food intake which follows lesions of the ventromedial area. Teitelbaum (1963) indicates that this is not the case. When required to feed themselves by bar pressing for food ingested through permanently implanted gastric tubes, animals with ventromedial lesions eventually overeat and become obese in spite of the absence of oropharyngeal sensation. Thus it appears that excessive responsiveness to highly palatable food is not the cause of hyperphagia associated with hypothalamic lesions.

So behavioral evidence indicates that animals with ventromedial hypothalamic damage eat longer rather than faster and that responsiveness to food palatability is not the cause of obesity.

V. HYPOTHALAMIC ACTIVATION

After the establishment of the two hypothalamic areas as the centers for the regulation of food intake, attention was given to investigating possible explanations for the activation of these centers.

Three main theories have been advanced to account for this activation: the thermostatic, the lipostatic and the glucostatic. The lipostatic theory was proposed by Kennedy (1953, 1966) who suggested that fat stored in the body in some way regulates long term food taking perhaps through biochemical alterations which occur in the contents of its circulation as fat depots enlarge. He suggested that the animal inhibits feeding when further intake would be expected to threaten the

fat stores. He also states that an animal begins eating when the body's metabolism threatens to take from its fat stores and an animal stops eating when additional food would add to the fat stores.

Brobeck (1955, 1957, 1960) outlined the thermal hypothesis which is based on the idea that cells in the anterior hypothalamus and the preoptic area are sensitive to temperature change. It can be shown that body temperature rises after the ingestion of food, the amount of rise differing with various dietary constituents. Brobeck called this the "specific dynamic action" of a food and observed that eating is depressed by high temperatures and enhanced by low temperatures. Support for the thermostatic theory has been given by Andersson and Larsson (1961). They found that local cooling of the preoptic area and rostral hypothalamus induces eating in the satiated goat. On the other hand, warming of the same area reduces drinking and eating. However, shifts in temperature required to obtain these effects are very large. It is possible that temperature can affect eating and drinking but it does not seem to be the exclusive regulator.

The third and the most widely investigated theory, the glucostatic, (Mayer, 1953) will be considered in more detail here. Research reported in this paper is concerned with effects of variations in blood glucose levels on food intake in animals with lesions in the satiety area and those without. It is a test of the glucostatic theory.

VI. GLUCOSTATIC THEORY

In the early 1950's Jean Mayer postulated the presence of chemoreceptors, located in the ventromedial hypothalamic nuclei and perhaps other central and peripheral areas as well, which have a special affinity for glucose and are activated by this metabolite in the measure that they utilize it (Mayer, 1953). This glucostatic hypothesis was based in part on the observation that carbohydrate reserves are proportionately much more depleted between meals than are reserves of protein and fat. Mayer recognized that the regulation of food intake has both a short term and long term aspect. He suggests the glucostatic mechanism to account for short term regulation but seems to favor the lipostatic hypothesis as the basis for long term regulation.

An effort to test this theory was made by Mayer and Von Itallie (1967). They found a correlation between overall glucose utilization, measured through arteriovenous differences in glucose concentration, and the presence or absence of gastric contractions and sensations of hunger.

Another attempt to test the relationship between gastric contractions and blood glucose levels involved the injection of the hormone glucagon which promotes both the formation of glucose, and the utilization of glucose by the cells. Injection of this hormone checked gastric contractions and reports of hunger (Stunkard, Von Itallie, Reiss, 1955). Glucagon injections over a two week period reduced significantly the amount of food eaten (Stunkard and Wolf, 1954).

By placing cannulas in the veins so that blood could be withdrawn at any time, Steffans (1967) demonstrated that, in an ad lib situation, blood glucose concentration rises rapidly within a few minutes after the start of a meal, whereas the between meal glucose concentration is fairly constant. The results seem compatible with the view that changes in the blood glucose level set up signals causing termination of feeding. It thus appears that the ventromedial area may exercise a measure of control over gastric hunger contractions and in response to an increase in glucose utilization.

Additional evidence concerning the influence of a blood factor on food intake was demonstrated in an experiment (Davis and Miller, 1967) in which the blood of two rats, one satiated, and one deprived, was mixed 30 minutes prior to the time the deprived animal was usually fed. Immediately after this procedure, members of the pair were given access to milk for 30 minutes. Food intake of the deprived rat was reduced 50% below his normal level after the blood had been mixed. The study indicates that the blood of a satiated animal contains a factor inhibiting food intake.

To summarize the explanations for the activation of the hypothalamic area, the glucostatic, the lipostatic and the thermostatic hypotheses each offer tenable explanations for the variation in hunger. The glucostatic theory is the most widely accepted but it appears that each of these three can have some influence.

From the investigations reported above it might be concluded that the blood contains some factor or factors important in the regulation of food intake. Evidence that this factor is glucose appears somewhat circumstantial.

In 1964 Anand and colleagues tried to determine whether any differences in firing frequency could be detected in neurons of the satiety and feeding center as a result of procedures which alter the blood glucose level. Recordings of the unit activity from neurons of the hypothalamic feeding and satiety mechanisms and from adjacent hypothalamic regions were made in anesthetized dogs. In starved animals the unit activity in the ventromedial hypothalamic satiety center neurons was slower than that obtained from the lateral hypothalamic feeding center neurons. Intravenous glucose and insulin or a combination of both were given and changes in spike activity noted. Frequency of spikes recorded from the satiety center neurons increased and that of feeding center neurons significantly decreased after glucose was given intravenously. The spike activity pattern reversed in response to intravenous insulin injections. Oomura and associates (1964) also found that spontaneous unit discharges of the ventromedial nucleus were affected by intravenous injections of glucose.

So it appears that the satiety center is activated by blood glucose levels in the body. There are other changes produced by feeding which may also stimulate these neurons. One possibility is that the specific dynamic action of foods may increase the heat stress of the body (Strominger and Brobeck, 1953). Another change which may stimulate hypothalamic neurons is the concentration of certain circulating metabolites influenced by fat depots (Kennedy, 1953) and water concentration and its shifts among compartments of the body (Lepkorsky, Lyman, Flemming, Nagumo and Dimick, 1957).

From these investigations by Anand and Oomura it is evident that the glucose level of the blood affects spike activity, even though there are several explanations of how this may take place. These studies do not explain how changes in spike activity in the ventromedial area are related to inhibition of feeding.

Glucoreceptor Mechanism

Russek and Morganne (1963) questioned the fact that the signals provided by the blood are always channeled through the ventromedial area. Cats with ventromedial hypothalamic lesions were injected with glucose, and it was found that sensitivity to glucose does not differ from those animals not deprived of the ventromedial area. Russek and Morganne believe that these results provide evidence for the existence of glucoreceptors outside the ventromedial area.

Intraperitoneal injections of glucose and normal saline were given to rats with electrolytic lesions in the ventromedial hypothalamus and to normal controls maintained on a $23\frac{1}{2}$ hour deprivation schedule. Food intake was depressed even more in the hypothalamic hyperphagic rats than in the controls (Reynolds and Kimm, 1965). These researchers also believe that glucoreceptors could be located outside the ventromedial hypothalamus. Another alternative offered by these researchers is one advanced by Kranse (1962). According to Kranse, electrical stimulation of the ventromedial hypothalamic region depresses food intake not as a primary effect on hunger, but as a general distracting or disrupting effect. Reynolds and Kimm believe it is possible that the electrolytic lesions sensitize other neural structures either through release from

inhibition or by irritation. If these sensitized structures include some cells which are glucoreceptive, and if activation of these cells leads to some behavior incompatible with eating, the exaggerated effect would be explained.

These latter observations question the role of the ventromedial hypothalamus in the regulation of food intake. Several investigators have recently suggested that the changes in food intake which are commonly seen following ventromedial damage might be secondary to hormonal effects and/or a general change in affect. Valenstein (1969) has recently shown that the very pronounced hyperphagia-obesity syndrome can be seen only in the female of the species and appears to be attributable to hormonal mechanisms.

A closer look at the feeding behavior of the male rat with such lesions (Grossman, 1966) suggests that the relatively small changes in food intake in the male might be due to a general change in affect rather than influences specific to feeding behavior. Male rats with ventromedial lesions overeat only when the food is highly palatable and are hypophagic or even aphagic when the diet is made slightly unpleasant by adulteration with roughage or quinine. Even when the food is palatable, such animals do not work for food as well as normal rats (Grossman, 1966) and similar deficits become apparent when saccharin or quinine is added to the ventromedial animals' water supply or when these animals are required to work for water rewards (Corbit, 1965; Grossman, 1966).

It seems evident that electrolytic lesions in the ventromedial hypothalamus result in an animal's overeating and becoming obese. The

manner in which hyperphagia comes about is not understood. It is possible that the lesions produce irritation, a hormonal change, release of certain neurons from inhibition or a general change in affect. Some blood factor seems to effect food intake and evidence that it is glucose comes from: changes in the activity of neurons in the ventromedial hypothalamus as the blood glucose level changes, influence of glucose on gastric contractions, and evidence that blood glucose levels rise at a time food intake is stopped. A more clear understanding of the relationship between the changes in neuronal activity in the ventromedial area as a result of changes in the blood glucose level and the manner in which the electrolytic lesions in the ventromedial area produce obesity is needed. The work of Reynolds and Kimm and Russek and Morgagne offer evidence against the hypothesis that glucoreceptors are located in the ventromedial hypothalamus.

Goldthioglucose Induced Obesity

During a toxological study of gold compounds, it was noted that a number of albino mice receiving goldthioglucose showed a marked increase in size. Subsequent experiments resulted in the finding that mice showed marked weight gains by a single injection of goldthioglucose (Waxler and Brecher, 1949).

Mayer and Marshall (1956) demonstrated that the injection of compounds in which the gold thiomoiety is linked to metabolites other than glucose (goldthiomalate, goldthiogalactose, goldthiosorbitol, goldglycerol) produced neither ventromedial damage nor obesity.

A direct demonstration of the special affinity of the ventromedial nuclei for goldthioglucose was provided when Marshall, Barnett and

Mayer (1955) showed that in mice, injections of goldthioglucose sufficient to produce obesity selectively destroy cells in this region. Liebelt and Perry (1957) confirmed these findings in experiments with several different strains of mice. It is believed that it is the affinity of the cells in the ventromedial area for glucose that leads them to take on the goldthioglucose.

Recently the specific affinity of the hypothalamic center for goldthioglucose was investigated by quantitative measurement and localization of the gold deposits in the brain (Debons, Silver, Cronkite, Johnson, Brecher, Tenzer and Schwartz, 1962). The results indicated that the obese animals had a higher concentration of gold in the hypothalamic area than similarly injected animals that had not become obese. Accumulations of gold could be noted not only in the hypothalamic region, but also in other areas, confirming earlier evidence of extra-hypothalamic lesions due to goldthioglucose (Perry and Liebelt, 1961). Brecher, Laqueur, Cronkite, Edelman and Schwartz (1965) investigated the goldthioglucose lesion in order to trace its entire development from a large initial necrotic lesion to a barely visible scar. They found that the ventromedial nucleus is not the center of the lesion since it is frequently situated only at the margin. The ventromedial nucleus may be largely spared in the presence of a clear-cut hypothalamic lesion produced by the goldthioglucose. The more prominent scar in the obese animals correlates with the larger initial lesion necessary for complete bilateral destruction of the ventromedial nucleus which is known to be a prerequisite to hypothalamic obesity.

Goldthioglucoase seems unique among gold compounds in that it produces lesions in the ventromedial hypothalamus and other areas of the brain.

Of mice surviving a dose of goldthioglucoase which is lethal to 50% (LD 50) approximately 30% become obese (Mayer, 1954). The increase is due primarily to an increase in adipose tissue (Waxler and Brecher, 1950). The weight gain reaches its peak between 11-15 weeks after injection with a total gain of about four times the original body weight (Mayer, 1960). In general there is a greater increase in weight when larger doses of goldthioglucoase are administered. The toxic effect of goldthioglucoase varies with the different strains of mice. For example, the mid-lethal dose for the C₅₈ strain is .4 mg/gram body weight, whereas the mid-lethal dose for Balb/C mice is 1.5 mg/gram (Leibelt, Kaoru, Leibelt and Perry, 1960).

Efforts to describe the goldthioglucoase obese animal have resulted in the finding that obese mice eat more each time they eat but eat fewer meals than controls. No damage to the water intake regulatory mechanism was found (Friedman, Wayne and Janowitz, 1962).

Some researchers have investigated the relationship of the variation in blood glucose at the time of injection to the lesions produced by goldthioglucoase. Comparison was made of the gold accumulation in the satiety center of goldthioglucoase injected mice with that of diabetic mice which have a high blood glucose level. In this study (Debons, 1968) it was found that diabetes inhibited the deposition of gold and the formation of lesions whereas the controls injected with goldthioglucoase showed gold deposition and lesions. The insulin deficiency directly or through the effects of circulating metabolites

decreases uptake of goldthioglucose by the satiety center. Likuski and colleagues (1968) administered two glucose analogues at the same time that goldthioglucose was given. Among animals receiving this treatment, less obesity and less gold deposition at the site of the lesion was observed. Mice made diabetic and subsequently injected with goldthioglucose showed a decreased uptake of gold in the ventromedial hypothalamus. These researchers attributed their results to competition between the glucose inhibitor and goldthioglucose for the glucoreceptor sites.

The accumulation of gold in the hypothalamus and the development of hyperphagia and obesity were studied in mice given a single intravenous injection of goldthioglucose at various levels of blood glucose (Edelman, Schwartz, Cronkite, Brecher and Livingston, 1965). Glucose concentration at the time of the goldthioglucose injection was correlated directly with the level of goldthioglucose in the blood three 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. This finding that as the glucose concentration of the blood is increased, the severity of the reaction to the goldthioglucose increases is different from those of Likuski and Edelman. There is no obvious explanation for these opposing results but it is possible that cells respond differently to different types of glucose and so would take on more or less goldthioglucose depending upon the manner in which the glucose level of the blood was raised.

Unlike electrolytically-induced ventromedial lesions, goldthioglucose-caused lesions seem to affect the food intake regulation with little impairment shown in other functions. For example, unlike mice

made obese by electrolytic lesions, goldthioglucose obese mice do not show the usual: rage response, gonadal atrophy (they can mate and rear young) and disturbances in water balance (Anderson, 1953; Friedman, 1962; Ingram, 1952, 1956; Katsuki, Hevonto, Hotino, Ito, Ishimoto, Makino, Hososake, 1962; Mayer and Marshall, 1956; Montreul-Langlois, 1956).

In summary it might be said that goldthioglucose produces lesions in the ventromedial hypothalamus as well as in other areas of the brain. The level of obesity varies a great deal. Reasons for this variation are not completely understood but it seems to depend in part on the amount of goldthioglucose given, and the strain of mice used. The blood glucose level at the time of injection has been demonstrated to influence the uptake of gold, the extent of the lesion and the severity of the obesity, but the direction of this difference is not clear. Functions other than food intake are not affected by goldthioglucose.

CHAPTER III

DESCRIPTION OF THE PROBLEM

Mayer (1953) has suggested that hunger occurs when glucose is not available for metabolic purposes and that satiation supervenes when glucose is made available. According to Mayer, the control of this mechanism rests with the ventromedial glucoreceptors. If cells sensitive to blood glucose levels are destroyed by a goldthioglucose injection, food intake should no longer be affected by changes in the blood glucose level. So the food intake of animals who have been made obese by a goldthioglucose injection should not change as blood glucose utilization changes.

The purpose of this investigation is to examine the effect of intraperitoneal injections of glucose on the food intake of mice who have been previously injected with goldthioglucose.

If the glucoreceptors in any area of the body are destroyed by the goldthioglucose it would be expected that food intake would not change with the blood glucose level.

CHAPTER IV

METHOD

The Goldthioglucose Injection

The subjects were 120 female mice of the Swiss-Webster strain obtained from Simonsen Laboratory. The animals initial body weight was 20-21 grams.

It has been demonstrated that as the dosage of goldthioglucose is increased the drug is lethal to a larger proportion of the animals (Leibelt, Kaoru, Leibelt and Perry, 1960). In an effort to keep the experimental groups as nearly the same size as possible, a disproportionately large number of animals were assigned to groups receiving the higher dosage levels.

At injection the subjects were separated into four groups: Group I, controls, receiving no treatment (19 animals); Group II given .5 mg. of goldthioglucose per gram of body weight (25 animals); Group III given 1.0 mg. of goldthioglucose per gram of body weight (33 animals) and Group IV given 1.3 mg. of goldthioglucose per gram of body weight (40 animals).

The goldthioglucose used was Solganol (Schering Corporation) and was administered by intraperitoneal injections from a solution containing 100 mg. of goldthioglucose per cc of sesame oil.

All animals were fasted 24 hours prior to injection since this management reduces mortality without decreasing the incidence of obesity (Owens, Parsons and Crispell, 1953).

Two to five mice were housed in each cage. The subjects were fed ad lib food and water. The weight of the food intake pooled for all mice in each cage and the body weight of each animal was recorded weekly.

Deprivation

Fifty-seven days after being injected with goldthioglucose, the animals were put on a food deprivation schedule. This schedule was designed to allow the animals to adjust to getting their daily food intake during a 50 minute period. This was done by shortening the feeding periods in incremental steps from 90 minutes to 50 minutes. The fifty minute eating period was arrived at by way of observation that the animals could maintain between 80% and 91% of their pre-deprivation body weight by eating for 50 minutes a day. At the end of the deprivation period the mice were eating once a day from 6:15 p.m. to 7:05 p.m.

The Experimental Period

The deprivation schedule was continued during the ten day experimental period. The mice were transferred to individual cages for this phase of the experiment so that individual food intake could be measured.

On the first day of the experimental period all animals were given an intraperitoneal injection of 0.3 ml of normal saline containing 60 mg. of glucose (20 mg. per ml. of normal saline) 15 minutes before their daily feeding time. Prior to the feeding period the plastic cages were emptied of litter so that any scattered food could be collected after the feeding period to permit the total food consumption to be calculated.

A similar procedure was followed on the second day with the exception that the injection given was 0.3 ml of normal saline containing no glucose.

The procedures of the first two days were repeated on alternate days for the remainder of the 10 day period.

The mice were weighed following each feeding period. During this experimental period it became apparent that the animals could not maintain an adequate weight on this schedule. It is possible that the trauma of the injection was responsible for this. Supplementary food was provided prior to day four (saline), day five (glucose), day seven (glucose) and day eight (saline). On these days six grams of lab chow was given to each animal at 8:00 p.m. and the extra food, if not eaten by 10:00 a.m. the next day, was removed. Thus on these days the animals were deprived for 8 hours and 15 minutes instead of 23 hours and 10 minutes.

During the ten day experimental period data was obtained for three days in each of the following conditions: glucose with no supplementary feeding and saline with no supplementary feeding and two days each of saline with supplementary feeding and glucose with supplementary feeding. To simplify the analysis, the data was reorganized. Data for the last two days, one a glucose injection and the other saline were not included. Individual food intake means were found for the remaining like conditions described above and these means were used for the analysis of variance.

Some research indicates that the more obese animals tend to have more extensive lesions with a higher concentration of gold than the nonobese animals (Brecher, Laqueur, Cronkite, Edelman, and Schwartz,

1965; Debons, Silver, Cronkite, Johnson, Brecher, Lenzer and Schwartz, 1962). If larger lesions result in more damage to glucoreceptors, the food intake of the extremely obese animals would be affected less as the blood glucose level changes than that of the less obese or the controls. To investigate this possibility data for the animals in the experiment were regrouped according to body weight of the mice. The body weights for this regrouping were obtained two weeks after the end of the experimental period. During this two week period the animals were fed ad lib food and water. All animals which had received goldthioglucose and weighed more than the heaviest control were classified obese and divided in two groups: the moderately obese, weighing between 37 and 44 grams and the extremely obese weighing more than 44 grams.

CHAPTER V

RESULTS

I. THE GOLDTHIOGLUCOSE INJECTION

Table I shows the number of animals surviving the goldthioglucoase injection at each dosage level, the number who became obese, and the number of animals included in the experiment.

TABLE I

NUMBER OF EXPERIMENTAL ANIMALS

	<u>Mg. Goldthioglucoase per gram Body Weight</u>			
	<u>0</u>	<u>.5</u>	<u>1.0</u>	<u>1.3</u>
Number of animals in the group	19	25	33	40
Number surviving goldthioglucoase injection	19	25	11	4
Number of animals in experiment	18	22	11	-
Total number of obese animals	0	7	7	-
Moderately Obese	0	4	1	-
Extremely Obese	0	3	6	-

Inspection of Table I indicates that approximately one-third of those mice receiving .5 mg. of goldthioglucose per gram of body weight became obese and more than half of those in the 1.0 mg. per gram group became obese.

Data for one animal in Group I (controls) and three animals in Group II (.5 mg. per gram) were not used since these mice lost so much weight during the first two days of the experimental period that supplementary glucose injections had to be given. Since so few of the animals in Group IV (1.3 mg. per gram) survived the goldthioglucose injection data for this group were not included.

II. THE EXPERIMENT

With the animals grouped according to the injection received, an analysis of variance for three factors with repeated measures on two factors was performed on the data (Weiner, 1962). The summary of this analysis is presented in Table II.

Inspection of Table II reveals that the dosage level of goldthioglucose given made no difference in the food intake of the mice under any condition of the experiment. This is indicated by the significant main effect for the glucose saline factor and the supplementary feeding factor in the absence of significant interaction effects.

TABLE II

ANALYSIS OF VARIANCE

SUMMARY TABLE

Source of Variation	Sum of Squares	d.f.	Mean Square	F
<u>Between Subjects</u>		50		
A. Goldthioglucose	3.4732	2	1.7366	-
Error (a)	870.5661	48	18.1368	-
<u>Within Subjects</u>		153		
B. Glucose, Saline	11.2774	1	11.2774	42.2375*
AB. Goldthioglucose and glucose, saline	.4724	2	.2362	-
Error (b)	12.8164	48	.2670	-
C. Supplementary food	7.7762	1	7.7762	24.4075*
AC. Goldthioglucose and sup. food	.4491	2	.2245	-
Error (c)	15.2932	48	.3186	-
BC. Glucose, Saline and sup. food	.6822	1	.6822	11.7823*
ABC. Goldthioglucose; glucose and saline and sup. feeding	.2082	2	.1041	1.7979
Error (bc)	2.7802	48	.0579	-

* p .001

It can be seen, however, that for all levels of the goldthio-glucose injection the animals' food intake was different when they were given glucose than when they were given saline.

This is indicated by the absence of a significant main effect and of an interaction for the goldthiogluucose factor and the presence of a main effect for the glucose saline factor.

The analysis also reveals that providing supplementary food resulted in a difference in food intake which was consistent among other factors.

A significant interaction was observed between the glucose factor and the supplementary feeding factor. It can be seen by examining the table of means (Table III) that the difference in food intake between days in which glucose was given and days in which saline was given was greater when supplementary food was offered than when it was not.

The table of means (Table III) shows that at all levels of obesity, the mean food intake for the mice was less when they were given a glucose injection than when they were given saline.

TABLE III

MEAN FOOD INTAKE IN GRAMS BASED UPON THE DOSAGE LEVEL OF
GOLDTHIOGLUCOSE

Goldthioglu- cose Injection	Supplementary Food		No Supplementary Food	
	Food Intake in Grams		Food Intake in Grams	
	Glucose	Saline	Glucose	Saline
Group I Controls (no injection)	1.46	1.92	2.02	2.32
Group II .5 mg. per gm.	1.77	2.31	2.27	2.68
Group III 1.0 mg. per gm.	1.48	2.32	1.96	2.36

It can also be seen that the mean food intake was less on days when supplementary food was given than when it was not.

Table IV shows the mean food intake based on the regrouping of the animals according to their level of obesity.

TABLE IV

MEAN FOOD INTAKE IN GRAMS BASED UPON THE LEVEL OF OBESITY

Level of Obesity	Supplementary Food		No Supplementary Food		Number of Mice
	Glucose	Saline	Glucose	Saline	
Controls (Not Obese)	1.46	1.92	2.02	2.32	19
.5 mg. per gm. (Not Obese)	1.72	2.27	2.19	2.55	15
1.0 mg. per gm. (Not Obese)	1.32	2.24	1.90	2.54	5
Moderately Obese	2.18	2.76	2.49	2.90	5
Extremely Obese	1.76	2.43	2.03	2.45	9

Food intake of animals given a glucose injection seems to be less at all levels of obesity than when they were given saline. The extent of glucose-saline difference appears to be similar in all conditions. It also appears that food intake is less when the subjects were given supplementary food than when they were not.

A comparison of Table III with Table IV reveals that there is no striking difference in food intake between animals grouped according to the goldthiogluucose dosage given and those grouped according to the level of obesity reached. So, the level of obesity reached by an animal does not seem to influence his food intake under the conditions of this experiment.

CHAPTER VI

DISCUSSION

According to Jean Mayer "...the regulation of food intake is dependent on ventromedial nucleus glucoreceptors which can be destroyed by goldthioglucose." (1968, p. 1345). Mayer has also said that the receptors "...have a special affinity for glucose and are activated by this metabolite in the measure that they utilize it." (1967, p. 331). If these receptors in an animal are destroyed by goldthioglucose the animal should no longer respond to a change in blood glucose utilization by varying food intake.

A test of Mayer's idea that glucoreceptors in the ventromedial area respond to blood glucose utilization was made by Reynolds and Kimm (1965) using rats and by Russek and Morganne (1963) using cats. Both of these experiments involved the destruction of the ventromedial hypothalamus with bilateral electrolytic lesions. The lesions were of the same type as those which produce the hypothalamic hyperphagia syndrome described earlier in this paper (see page 4). In both cases the food intake of the hypothalamic hyperphagic animals was depressed after the animals had been given a glucose injection. If the glucoreceptors in the hypothalamic hyperphagic animals had been destroyed food intake should not have been depressed by the glucose injection.

In both studies, Russek and Morganne (1963) and Reynolds and Kimm (1965) explained their findings by saying that glucoreceptors

might be located outside the ventromedial hypothalamic area destroyed by the electrolytic lesions.

The goldthioglucose-induced lesions differ from those produced electrolytically in that the lesions are not necessarily restricted to one area of the brain and they seem to produce no behavioral change other than food intake. Except that the lesions were produced in a different manner the procedure reported here is similar to that of Reynolds and Kimm (1965). The results are similar to those of both Reynolds and Kimm and Russek and Morganné (1963). That is, goldthioglucose treated animals reduce their food intake as much as do normal animals, when given a glucose injection prior to feeding. While the goldthioglucose induced lesions differ from electrolytic lesions in several respects neither lesion seems to effect the animals' ability to respond to blood glucose level changes by altering food intake.

It would be expected, from Mayer's description of the glucoreceptors, that the destruction of these receptors by the goldthioglucose should cause some difference in food intake between the lesioned animals and the controls after glucose injections. Clearly no difference was observed so it might be concluded that (1) glucoreceptors do not exist or (2) that the mechanism by which these cells operate is more complex than has previously been postulated.

An explanation of the results of this experiment might be that the glucoreceptors were destroyed by the goldthioglucose lesions and the decreased food intake among all the animals after a glucose injection could be the result of discomfort or illness felt by the animals as a result of a sudden rise in the glucose level. This

explanation is questionable because on days in which the animals had been fed supplementary food prior to their daily feeding period they would eat less than when supplementary food had not been provided.

There is no reason to believe that the discomfort or illness would have been different on days when the animals were fed supplementary food than when they were not.

A tenable explanation of the results of this experiment can be based on an idea advanced recently by Grossman (1968). He has stated that the changes which are commonly seen following ventromedial damage might be secondary to hormonal effects and/or a general change in affect. Thus, in the present study, the goldthioglucose lesions might produce such a change which would result in the animal becoming obese. This could leave the mechanism which is sensitive to the blood glucose level intact and the animal could still respond to a rise in the glucose level by decreased food intake. This explanation also leaves some unanswered questions. Why does goldthioglucose and no other gold compound tested result in the lesion and subsequent obesity? How does the presence of glucose in the blood at the time of the goldthioglucose injection change the gold uptake and the extent of the lesion?

It is possible to explain the results of the present experiment by positing a mechanism by which the glucoreceptors operate. Cells in such a mechanism might be differentially sensitive to various levels of glucose.

Activity of the individual neurons within the system would increase in relation to the presence of that level of glucose. As a threshold of neuronal activity is reached food intake is stopped.

When goldthioglucose is administered, cells sensitive to that particular level of glucose are destroyed. So part of the glucoreceptor system is gone and these cells cannot contribute to the threshold. For a threshold now to be reached the remaining cells must be more active. This activity can increase only if that level of glucose increases. In an ad lib feeding situation the goldthioglucose injected animal becomes obese because he must eat more food in order for the remaining cells in the system to increase their activity enough to reach a threshold and stop food intake.

The size of the glucose injection used during the experimental period in this study could have increased the activity of those glucoreceptors which were not destroyed enough to reach a threshold and stop food intake among the goldthioglucose obese animals as well as the controls.

The interaction observed between the supplementary food and the saline glucose factor can also be explained by postulating a threshold for stopping food intake. The difference in food intake between days in which glucose was given and days in which saline was given was greater when supplementary food was offered than when it was not. It is possible that on the days when saline was given and no supplementary food provided the threshold for stopping food intake was not reached at all during the 50 minute feeding period. Thus the animals receiving saline and no supplementary food had not reached a threshold at which food intake would stop at the end of the eating period.

It is possible to test this idea by using a design similar to the one reported here with the exception that several types of glucose would be used during the experimental period instead of just one.

No matter what explanation is offered for the results of this experiment it is clear that Mayer's idea that the glucoreceptors, as described by him, are destroyed by goldthioglucose has not been supported by this study.

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