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A Study on the Action of the Hypothalmic Feeding Centers During Insulin Stress

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A STUDY ON THE ACTION OF THE HYPOTHALAMIC FEEDING CENTERS DURING INSULIN STRESS IN INSULIN TOLERANT AND INTOLERANT MICE

BY

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ABSTRACT

Mice of the KC strain can survive injections up to 300 units of insulin as compared to the maximum 30-50 units tolerated by normal BUB mice. This resistance is inherited. The KC mice are able to survive the hypoglycemia by maintaining their blood sugar level just above that which produces convulsions. Previous work had suggested that an increased rate of food consumption supplied the carbohydrate necessary to overcome the hypoglycemia.

Current theories on the mechanism of hunger drive emphasize the role played by the hypothalamic feeding centers. The best understood centers are the lateral feeding center and the ventromedial satiety center. Evidence has accumulated to show that the ventromedial center is a glucoreceptor which inhibits the activity of the lateral nucleus during periods of high blood glucose.

To test the hypothesis that the hypothalamic feeding centers play a role in the insulin resistance, the ventromedial nucleus was destroyed by aurothioglucose. This compound has been demonstrated to be relatively specific for the satiety center, and its administration results in a hyperphagia and obesity due to the unchecked activity

of the lateral center.

The amount of food consumed was measured daily for a period of ten days, and as expected the aurothioglucose-treated mice exhibited a hyperphagia. Of the remaining groups the tolerant strain ate more per day than the intolerant. In the period after insulin injection, however, the intolerant mice ate at the fastest rate and the tolerant mice just slightly less. The tolerant mice rendered hyperphagic and obese by aurothioglucose exhibited a suppression of food consumption in response to insulin.

When these findings are related to the proposed mechanism of insulin tolerance, it becomes questionable that the amount of food consumed is the sole determinant of survival after insulin stress. Also discovered is the possibility that this procedure may be of value in investigating the role played by feeding centers not located in the ventromedial nucleus and not sensitive to glucose or aurothioglucose.

ACKNOWLEDGEMENT

With sincere gratitude I wish to acknowledge the help of the late Dr. Elizabeth B. Chase under whose guidance this work was conceived and continued.

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I INTRODUCTION

This investigation utilizes the effect of the falling blood glucose level on the activity of the hypothalamic satiety center to reveal the extent to which the hunger drive may produce increased eating sufficient to cause the survival of mice with severe insulin hypoglycemia. The glucostatic theory of hunger is used to explain this interplay. In this scheme hypoglycemia is a stimulus which causes inhibition of the satiety center and hence permits initiation of feeding by the activity of the lateral-hypothalamic nucleus. The more severe the hypoglycemia, the greater the resulting hunger drive is, and the larger the amount of food that is ingested. In the aurothioglucose-treated mice, the destruction of the satiety center and the lack of a monitor of the blood sugar level is reflected in unchecked activity of the feeding center which it inhibits.

The possibility that a difference exists in the food intake of insulin-tolerant mice of the KC strain as compared to the insulin-intolerant mice of the BUB strain was first suggested in the study of these strains conducted by B. Argyris (1960). With the development of theories explaining

the phenomena associated with hunger and the ingestion of food, the feeding behavior of these animals appears to present a unique opportunity for investigation. According to Argyris's hypothesis the tolerant mice maintained blood glucose levels above the minimum by eating. This behavior requires the involvement of the hypothalamic hunger centers. This investigation examines her hypothesis by measurement of food intake daily in tolerant and intolerant mice and also during the period of severe insulin stress.

Other aspects of the complicated hunger-satiety cycle can be explored more easily by combining insulin's effects with those of the hyperphagia-producing compound aurothioglucose. With the satiety center in the ventral medial hypothalamic nucleus destroyed by aurothioglucose and the stimulatory lateral nucleus in control of eating behavior, the hypoglycemic stress brought about by insulin is expected to be ineffective in increasing the ingestion of food as indicated above. With the hypoglycemia unable to increase food ingestion, other mechanisms for increasing tolerance if present can be revealed. Also open to investigation are any secondary controls of eating which are independent of the satiety center.

II REVIEW OF THE LITERATURE

The report of a strain of mice tolerant to the effects of insulin by Chase et al in 1948 started a long series of investigations into the mechanism of the tolerance. The trait has been found to be controlled by three inherited factors with no dominance (Chase, 1950). Though the search for a mechanism has proceeded along many lines, no conclusive evidence has been presented indicating the manner in which these KC mice survive insulin stress. The underlying basis for the ability to withstand high doses of insulin has been reported as the maintainance of blood sugar at a level above that which produces convulsions, coma and death (Argyris, 1959). Measurement of the blood sugar level showed that both tolerant and intolerant strains experienced an immediate fall in blood sugar during the first hour after insulin injection. The glucose levels fell from the normal seventy to eighty milligrams per cent to twenty-five milligrams per cent.

After this initial fall the blood glucose of the tolerant mice remained at this constant level for five hours and then rose returning within an hour to normal levels. In the intolerant mice, however, the blood glucose

continued to fall, though at a slower rate than originally; in two hours the level was twenty milligrams per cent -- the range that produced convulsions, coma and death.

These findings emphasized the need for investigating the means by which blood sugar is maintained and the mechanism by which insulin upsets these controls. The metabolic role played by glucose is quite varied and has anabolic and catabolic aspects. Moreover, insulin appears to play some part in controlling most of the pathways by which glucose is utilized.

After entering the body via the digestive system, glucose is transported by the blood to the liver. Here it may either be stored as glycogen or passed to the peripheral circulation. The organs of the body use the glucose as a source of energy, as an energy store in the form of glycogen and fat, or as a carbon skeleton for other compounds such as amino acids. Insulin acts to increase the rate of glucose entry into muscle cells and thereby decrease its level in the bloodstream. Also insulin has been shown to affect the utilization of glucose in the production of fatty acids and possibly amino acids.

Thus tolerance could be affected either by decreasing

the rate at which glucose leaves the blood or increasing the rate at which it enters. The possibilities to be investigated are -- differences in insulin action on glycogenolysis in the liver and on glycogen neogenesis in muscle; differences in the fate of insulin either in deactivation or degradation; differences in insulin induced hyperphagias between the tolerant and intolerant mice.

The work of Argyris (1959, 1960) has been the most extensive on the physiology of insulin-tolerant mice and has become the basis for this study. Her measurement of liver glycogen in the fed tolerant and intolerant mice showed a greater store of glycogen in the livers of the tolerant mice. The difference is insufficient to account for the survival of these mice since both intolerant and tolerant strains were found to deplete their glycogen reserves in the two hours after injection of forty-five units of insulin and with no access to food. She injected I^{131} insulin and measured the appearance of radioactive label in the urine; from this experiment she concluded that both strains degraded insulin at the same rate. Shanmugasundarem (1968) used more sophisticated labeling techniques to confirm this conclusion. Snedecor (1952) had

shown that insulin was not deactivated in the blood and therefore was not present in an inert complex. These investigations led to the conclusion that the mice are indeed able to utilize mechanisms to maintain blood sugar in the presence of large quantities of active insulin, but give no clue to the mechanism by which they accomplish it.

Evidence was found that the tolerant mice could not survive if given no food during the experimental period. Argyris found that powdered chow given ad libitum was quite effective in combating the insulin stress and even was capable of prolonging the survival time of the intolerant mice. She, therefore, investigated the rate of food absorption from the intestines of each strain. It was found that both strains absorbed carbohydrate at the same rate; seventy-five per cent of the carbohydrate being removed in forty minutes and eighty-six per cent within an hour from 0.5 milliliters of a thirty per cent glucose solution administered by stomach tube. High fat and high protein diets given to these mice during insulin stress were found to decrease the survival of the insulin-tolerant mice. From these data Argyris concluded that the increased amount of ingested carbohydrate in tolerant as compared to

intolerant mice played a predominant role in their survival after insulin injection.

The cause of the greater eating response which the tolerant mice exhibited was not investigated. The search for the mechanism of the tolerance must take into account the means by which hunger is controlled. The tolerance of these mice to insulin could reflect superiority in their ability to sense the requirement for exogenous carbohydrate and rapidly to initiate and maintain feeding. The resulting increase in carbohydrate ingestion could be sufficient to allow the tolerant mice to keep their blood sugar level just slightly above the level at which convulsions, coma and death occur.

The physiological investigation of the causes of hunger started with Cannon and Washburn in the early part of the twentieth century. They were able to show that gastric contractions cause subjective hunger pangs (Cannon and Washburn, 1912). Further observations indicated that satiety was not directly related to bulk consumed and could be shown to be influenced by nutrient content (Adolph, 1947, Janowitz and Grossman, 1949). These investigations as well as more psychological techniques brought the gastric theory

of hunger into question.

Frohlich's Syndrome of obesity and gonadal hypofunction in adolescents focused attention on the hypophysis (Bruch, 1939). Investigation of the hypophysis continued until it was shown that a hypophysectomy which did no damage to the brain did not result in obesity (Hetherington and Ranson, 1940, 1942). Failure of sexual development did occur after hypophysectomy removed the necessary trophic hormones. Using these surgical methods, specific areas in the hypothalamus separate from those involved in pituitary control were implicated, and the investigators were able to obtain the now classical example of hypothalamic obesity. Investigations showed that the obesity was accompanied by no metabolic or hormonal irregularity; the only observed difference between normal and hypothalamic obese animals is a hyperphagia in the obese animals (Anliker and Mayer, 1956, Brobeck, 1943, 1946, Tietelbaum and Campbell, 1958).

Electrophysiological investigations have demonstrated the relationship of two areas in the hypothalamus -- the ventromedial nucleus believed to be a satiety center, and the lateral nucleus thought to act as a feeding center (Anand and Brobeck, 1951, Anand, 1960, Delgada and Anand,

1953, Forsberg and Larsson, 1954). Electrical stimulation of the satiety center has been found to cause a cessation of eating while stimulation of the lateral feeding center causes its initiation. The controlling center of the two is the ventro-medial nucleus; upon stimulation it inhibits the lateral nucleus (Anand et al. 1961, 1962). The mechanism by which the ventromedial nucleus itself is controlled is under debate. Four theories of hunger have been proposed and each is based upon a different stimulus initiating the activity of the ventromedial nucleus.

Mayer (1953) has proposed a glucostatic theory of hunger. The basic principle of the theory is that animals eat to maintain their blood sugar level in a certain range. The centers which monitor the blood sugar are located in the ventromedial hypothalamic nucleus which according to this theory is stimulated by high blood sugar. In a series of papers Mayer and his associates have further elaborated and defended this theory (Mayer, 1957, 1960, 1963, Mayer and Bates, 1952, Mayer, Bates and Von Italie, 1952, Mayer and Sudsanek, 1959, Mayer, Vitale, and Bates, 1951). Among the refinements added was an explanation of the hunger of

diabetics as being caused by the inability of the ventromedial-glucose centers to be excited by the high blood glucose levels in the absence of insulin. Also added was evidence that the difference in arteriovenous glucose concentration might have greater importance than the absolute blood sugar levels. Figure I (Appendix) diagrammatically summarizes how the present theory utilizes evidence from various investigators to reach a comprehensive theory. The ventromedial nucleus is seen as influencing the stopping of feeding behavior not only by its direct action on the lateral nucleus but also indirectly through the inhibition of hunger pangs brought about by gastric contractions.

A second theory has been proposed by Brobeck (1948, 1955, 1957). This theory is based upon the supposition that animals eat to keep warm. The observation that animals eat less in a warm environment and more when subjected to cold forms the basis of this theory. A refinement added to this theory is the observation that the quantity of food eaten is related to the amount of energy obtainable from a food substance rather than the total caloric content of the food. This theory has failed to gain wide acceptance due

to the inability to demonstrate thermoregulatory activity in the ventromedial nucleus of the hypothalamus.

Two related theories are those of Tietelbaum and Kennedy; both these theories are based upon the "static phase" of hypothalamic obesity. In this static phase the animals' food ingestion ceases to be excessive and falls to that level (slightly higher than normal) required to maintain the obesity. Starvation of the animals causes a drop in body weight; access to food results in a resumption of the hyperphagia until the obesity is returned to the former level. Tietelbaum (1955, 1961) proposed some mechanism exists for checking body weight with which hypothalamic lesions interfere. Kennedy (1950, 1951) postulated the existence of a metabolite or hormone which builds up during the hyperphagic phase of the obesity and finally reaches a level at which it is capable of influencing hunger sensations. Two molecules proposed as candidates for this control are free fatty acid and insulin.

The favored theory at the present time is the glucoreceptor theory. The cells in the ventromedial nucleus are supposed sensitive to changes in the blood sugar level.

Anand *et al*. (1964) has recorded the activity of single neurons in the area, and he has found that they respond to changes in the glucose level of an infusing medium. The application of other solutions to this area caused no response.

Thus the ventromedial nucleus is seen as influencing hunger (the drive to eat) directly via the lateral nucleus. At the same time, however, appetite (the determination of which foods are acceptable to decrease the hunger drive) is also affected. In a series of investigations (Bruce and Kennedy, 1951, Corbit and Stellas, 1964, Kennedy, 1950, Tietelbaum, 1955, 1957) it has been demonstrated that the hyperphagic animals show less acceptance of food which is adulterated in any way. Also found by these investigators is a low motivation of the hyperphagic animals to run mazes or push levers to obtain the reward of food. The glucoreceptor theory's position is strengthened by the inclusion of a means by which the ventromedial nucleus can affect the appetite centers of the cerebral cortex utilizing ascending pathways from the hypothalamus.

Another point in favor of the glucoreceptor theory

is the action of aurothioglucose. This compound causes hyperphagia and obesity in mice indistinguishable from that caused by electrolytic lesions in the ventromedial nucleus of the hypothalamus (Brecher and Waxler, 1949, 1950). Since the compound was first reported to cause lesions in only the ventromedial nucleus, its action was cited as evidence that the glucoreceptors were located in that area (Drachman and Tepperman, 1954, Marshall et al., 1955, Mayer and Marshall, 1956). The mechanism of the action of aurothioglucose is thought to be its accumulation in the glucoreceptor cells instead of glucose. These cells are postulated as having a high specificity for glucose molecules, and by accepting the aurothioglucose molecules they are presumed to poison themselves when the gold moiety reaches a high concentration. An important basis for this conclusion was the inability of other aurothio compounds including the similar aurothiosorbitol to elicit the hyperphagia supposedly due to their inability to overcome the specificity of the glucoreceptive cells.

Proponents of other theories were quick to point out

that the action of aurothioglucose could be explained as merely being a reflection of the blood-brain barrier with aurothioglucose leaving the blood more easily in the region of the ventromedial nucleus than in other organs (Perry and Liebelt, 1961). The absence of the hyperphagic effect in any other species of animals investigated as well as certain strains of mice is interpreted by some investigators as an indication of the lack of specificity of aurothioglucose for glucoreceptor cells (Liebelt et al., 1957, 1960, Wagner and deGroot, 1963). Evidence has also accumulated that the lesions are not confined to the ventromedial nucleus as originally reported (Brecher et al., 1965). Perry and Liebelt (1961) found lesions outside the ventromedial nucleus when mice were treated with aurothioglucose. These lesions were found in the visual sensory nucleus of the vagus, the dorsal motor nucleus, the hypoglossal nucleus, the dorsal hippocampal formation, the anterior hypothalamic nucleus, and parts of the premamillary arcuate. Debons et al. (1962) found similar results by investigating the areas of the brain which accumulated radioactive gold from the compound. Rather than concluding

as did Perry and Liebelt, that this involvement of cells in other areas called into question the specificity of aurothioglucose, these investigators proposed the idea that the other areas also contained glucoreceptors and therefore were sensitive to the action of the chemical. The fact that even with these other areas destroyed by the aurothioglucose all of the observable changes in the metabolism and behavior of the mice could be explained as resulting from the hyperphagia tends to support this conclusion of Debons.

III EXPERIMENTAL DESIGN

The experiment was undertaken to provide additional information about the mechanism by which food ingestion differs in the insulin tolerant and intolerant mice. It is taken as a working hypothesis that insulin injection by lowering the blood glucose concentration reduces the activity of the satiety center and causes a hyperphagia. It is further hypothesized that this hyperphagia is greater in the tolerant than intolerant mice.

It is presumed from previous work that aurothioglucose will produce lesions that damage the glucoreceptors of the satiety center selectively and also produce a hyperphagia. It is therefore hypothesized that the survival of the aurothioglucose-treated mice would reflect an eating rate which maintained blood glucose above the level producing convulsions and death. Furthermore, it is my hypothesis that aurothioglucose-treated mice would be unable to adjust their feeding rate to the insulin hypoglycemia. The above hypotheses have been tested in appropriate experimental conditions.

The experiment's design is based upon the primary

role of blood glucose levels in inducing hunger via the ventromedial hypothalamic nucleus. Any change in the rate of food ingestion in insulin-injected aurothioglucose mice would be an indication that other physiological factors besides blood sugar level played a role in the determination of feeding behavior. No attempt was made to locate the centers in the ventromedial nucleus of the hypothalamus. The level of hyperphagia necessary to bring about a rise in blood sugar capable of preventing a fatal hypoglycemia is revealed by these experiments. The techniques utilized to test these hypotheses are summarized in Table 1.

Table 1

Summary of Experimental Design

Group	N	Pre-Treatment	Post-Treatment	
			Insulin Stress	Food Allowance
I Intolerant BUB	10	Placebo[1]	250 U	ad lib.[2]
II Tolerant KC	10	Placebo[1]	250 U	ad lib.[2]
III Tolerant KC	10	Aurothio-glucose[1]	250 U	ad lib.[2]
	4	Aurothio-glucose[1]	6.8 U/gm body wt.	ad lib.[2]
	4	Aurothio-glucose[1]	500 U	ad lib.[2]
IV Tolerant KC	17	None	250 U	None
	3	None	250 U	ad lib.

1. Food consumption was recorded for ten days prior to insulin stress.
2. Food consumption was recorded for six hours (at thirty minute intervals) immediately following insulin injection.

IV MATERIALS AND METHODS

Selecting the Animals

Animals used in this study were obtained from the inbred strains of KC and BUB mice maintained at the University of Rhode Island by Dr. Elizabeth B. Chase. Mice selected were males between two and three months of age.

Before use in these experiments mice from the KC strain were tested for insulin tolerance by the administration of two hundred units of insulin at forty days of age. BUB animals obtained from that sensitive inbred strain were assumed to be intolerant due to the pedigree of this phenotype in that strain. This assumption was found to be justified since all mice from this strain died after receiving an insulin injection of 250 units.

Preliminary preparation of the animals for insulin stress

The animals for preliminary preparation were divided into three groups. Two of these groups were composed of KC tolerant mice and the third of BUB intolerant mice. All three groups were deprived of food for four hours and

then injected according to varying procedures. One of the tolerant groups and the intolerant group were injected intraperitoneally with a placebo (0.02 ml. of physiological saline per gram of body weight); the remaining KC tolerant group was injected i.p. with 0.02 ml. of aurothioglucose solution per gram of body weight. The concentration found to produce the most consistent hyperphagia contained fifty milligrams of aurothioglucose in one milliliter of physiological saline. Thus the dose of aurothioglucose was one milligram per gram of body weight. Twenty hours after the various injections the food was replaced and the mice that survived were permitted to eat ad lib. The toxicity of aurothioglucose is sufficient to kill about one-third of the animals injected in the KC strain. Of the surviving mice approximately one-half became hyperphagic and these animals were selected for inclusion in the insulin trials.

Food consumption measurements

Animals were then placed in individual pens. Food hoppers, modified by enclosing their bottoms with aluminum

sheeting to reduce food loss, were employed in the determination of weight of mouse chow consumed. Purina mouse chow was given ad libitum to all animals, and the weight of pellets in the hopper was kept between one hundred and one hundred fifty grams. Weights of hoppers and contents were taken at ten o'clock in the morning. Before replacement of the hoppers, loose powder and small pellets were removed to prevent their removal by the mice. Hoppers and contents were reweighed before replacement. Measurements were taken for ten days. Records of weight gain were taken to aid in the selection of the hyperphagic obese mice from those treated with aurothioglucose.

To measure food consumption during the subsequent insulin stress runs described below a pellet of chow was attached by wire to a holder constructed of aluminum sheeting bent into a five by eight centimeter tray. This holder was quite effective in retaining the powder produced by the animals as they nibbled at the pellet. Few animals overturned the tray and little of the waste produced was lost. In a dry run with no insulin injection the overturning of the tray was more of a problem with the untreated

mice than with the insulin-injected mice. The tray and its contents were weighed periodically during the insulin stress runs.

Insulin stress

Insulin was administered intraperitoneally in a dosage of two hundred fifty units or one half milliliter of U 500 insulin between eleven and eleven thirty in the morning to cause a hypoglycemic stress. The animals subjected to this stress had been pretreated as indicated above. Ten animals were taken from each of the first three groups indicated in Table 1.

Insulin was also administered in doses proportional to body weight to four obese hyperphagic-tolerant mice and a five hundred unit injection was given another group of four obese hyperphagic-tolerant mice as variations of the method of inducing the stress. The amount of insulin in the proportional dosage trial was determined to be equal in number of units per gram of body weight to the dose received by the placebo-treated intolerant mice or 6.8 units of insulin per gram of body weight. Since none

of these injections totaled more than four hundred ten units, the injection of five hundred units (or double the usual) was used to insure adequate dose even if the fat tissue was diluting the effects of the insulin.

A final test for the necessity of exogenous carbohydrate utilized a group of twenty tolerant (KC) mice with no pretreatment (Group IV). Of these mice seventeen were injected with two hundred fifty units of insulin and placed in pens with no access to food. Three animals injected with two hundred fifty units and allowed food ad lib. served as controls. As this test was run to demonstrate the necessity for carbohydrate ingestion by the tolerant mice to survive the hypoglycemia, no records were taken of the amount eaten by the controls; feeding was observed, however, to insure that the controls availed themselves of the chow present.

V RESULTS

The data presented, obtained from the three groups of mice over a period of ten days when the mice were between three and four months old, revealed differences in the amount of food eaten daily. The groups differed as follows: the tolerant KC selected for their obvious hyperphagia ate significantly more than either of the other two groups. The intolerant (BUB) mice ate the least. The data are summarized in Table 2. (Appendix Tables I, II, III contain a complete presentation of data.)

The differences between the various means were tested using Students t test, and all differences are highly significant with a p value of less than 0.001.

Table 2

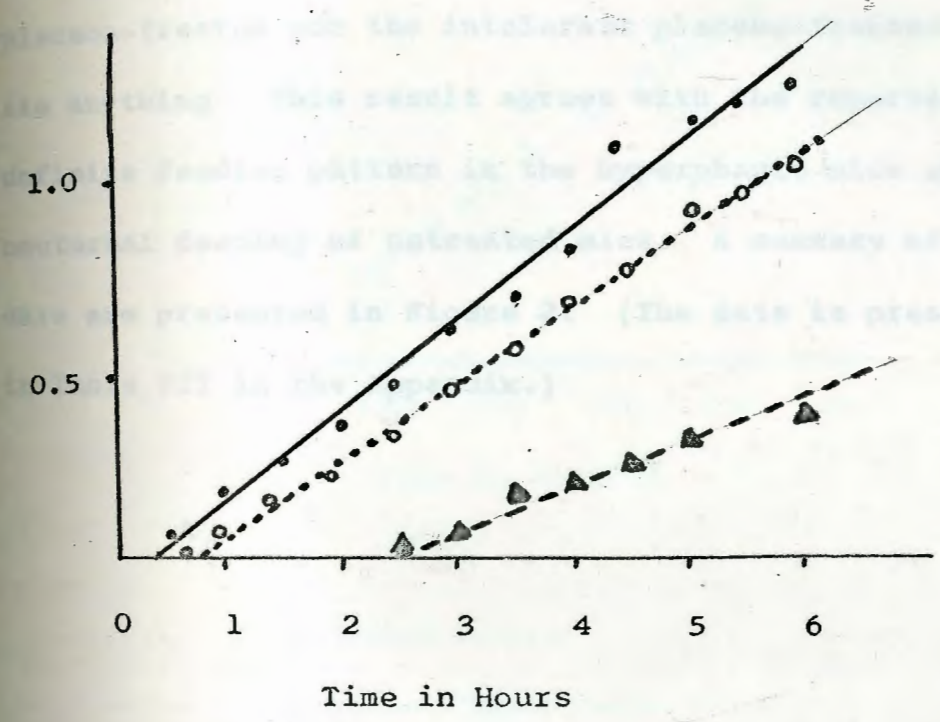
Mean Daily Food Consumption

Group	Treatment	Mean ± s.e. (grams)
I Intolerant (BUB)	Placebo	3.84 ± 0.05
II Tolerant (KC)	Placebo	4.09 ± 0.05
III Tolerant (KC)	Aurothioglucose	5.34 ± 0.06

The test situation of the insulin stress revealed a reverse in the relative rates of food ingestion. As indicated in Tables and Figure 1, the intolerant BUB animals reacted to the insulin injection by consuming the chow at a high rate. The tolerant (KC) non-obese mice were found to ingest the food at the next highest rate. Surprisingly, the tolerant hyperphagic animals ate at the lowest rate of t he three groups. The rates (co-efficient of regression assuming a straight line) were 0.003 grams of chow per minute for the tolerant animals, 0.004 grams per minute for the intolerant, and only 0.001 grams per minute for the tolerant hyperphagic mice. Since some of the intolerant mice died during the insulin stress, points in the latter hours of test are based upon fewer mice than those at the start. The rates are significantly different with a p value of less than 0.001.

Figure 1

Cumulative Food Consumption of Mice
During Insulin Stress



——— = Intolerant mice (BUB)

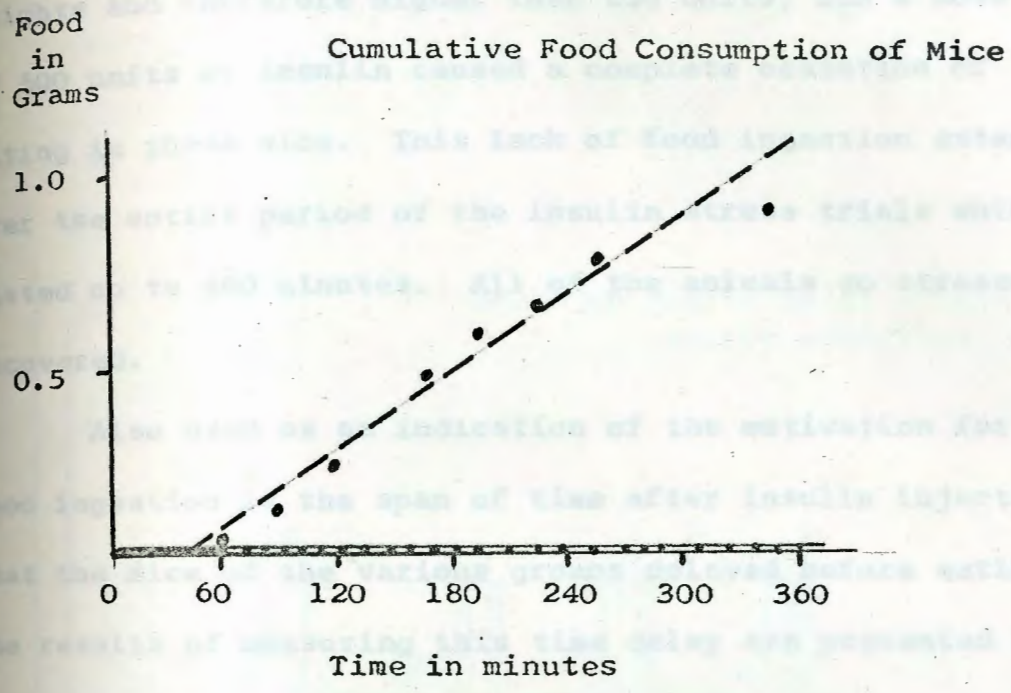
····· = Tolerant mice (KC)

- - - - - = Tolerant hyperphagic mice (KC)

● O ▲ = Respective means

During a test of the equipment in which no insulin was administered, the results were similar to those obtained from measuring the amount of food consumed daily. The tolerant hyperphagic mice ate at the highest rate, 0.003 grams of chow per minute, while neither the tolerant placebo-treated nor the intolerant placebo-treated mouse ate anything. This result agrees with the reported lack of definite feeding pattern in the hyperphagic mice and the nocturnal feeding of untreated mice. A summary of these data are presented in Figure 2. (The data is presented in Table VII in the Appendix.)

Figure 2



..... = Tolerant mouse

---------- = Tolerant hyperphagic mice

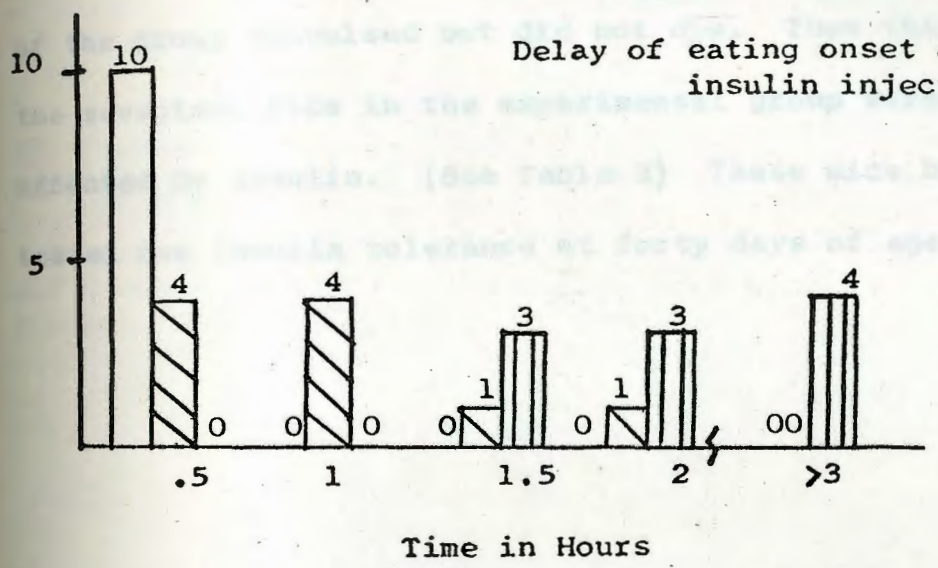
_____ = Intolerant mouse

● = Mean

The test of eating rates of tolerant hyperphagic animals using an amount of insulin proportional to body weights and therefore higher than 250 units, and a dose of 500 units of insulin caused a complete cessation of eating in these mice. This lack of food ingestion extended over the entire period of the insulin stress trials which lasted up to 480 minutes. All of the animals so stressed recovered.

Also used as an indication of the motivation for food ingestion is the span of time after insulin injection that the mice of the various groups delayed before eating. The results of measuring this time delay are presented in Figure 3. The intolerant animals are shown as beginning to ingest food immediately while the tolerant animals delayed for a short period. As mentioned above, the tolerant hyperphagic obese animals displayed a surprising apathy toward food and were quite variable in starting to eat.

Figure 3



□ = Intolerant (BUB) Mice

▨ = Tolerant (KC) Mice

▥ = Tolerant hyperphagic (KC) Mice

In the test to ascertain the importance of food to the survival of tolerant non-obese mice, it was found that the three controls which were given food ad libitum experienced no convulsions and none died. In the experimental group which was not permitted access to food over half of the mice experienced convulsions and died; several of the group convulsed but did not die. Thus thirteen of the seventeen mice in the experimental group were seriously affected by insulin. (See Table 3) These mice had been tested for insulin tolerance at forty days of age.

Table 3

Survival of Insulin-Stressed Tolerant Mice

Treatment	Number	Survived without Convulsions	Survived after Convulsions	Died
250 U Insulin Food ad lib.	3	3	0	0
250 U Insulin Starved	17	4	3	10

VI DISCUSSION

Daily food consumption

The data on daily eating rates during the period before insulin stress demonstrated the expected high intake of the aurothioglucose-treated mice resulting in their obesity. As noted above, the hyperphagia has been described as the direct cause of the obesity in these mice.

The results from the sham injected mice were opposite to those expected since Argyris (1960) had reported that the intolerant mice ate more than the tolerant. Both investigations revealed that the difference between the tolerant and intolerant strains was small. Though no relationship between daily eating rates and those of stress was proposed by Argyris, this investigation started with the assumption that the difference was related to innate characteristics of the feeding centers of these mice. The data from daily-food intake observations were interpreted as indicating that Argyris' conclusions were incorrect and the tolerant mice had a greater response to hypoglycemia -- either from fasting or insulin stress. Thus the hypothesis that tolerance was a direct consequence of the greater

feeding center activation during stress in tolerant mice as compared to intolerant was apparently supported by these data.

Food consumption during insulin stress

That hypothesis immediately became untenable since the greatest response to the hypoglycemia came from the intolerant mice both in speed at which ingestion began and rate at which it was maintained. The data clearly show that the intolerant mice maintain a small but significant increase in the rate of food ingestion over that exhibited by the tolerant mice during insulin stress.

If the glucoreceptor mechanism of Mayer is operant this result is as would be expected for animals experiencing a severe hypoglycemic incident. That is, in this situation with its rapidly falling blood sugar levels, the glucoreceptor in the hypothalamic ventromedial nucleus would act to permit behavior leading to food ingestion at a rate proportional to the degree by which the feeding center is released from its inhibitory influence. The expectation that this rate is less than that of the tolerant mice is not supported

by the evidence. The attempt to theorize a glucostatic mechanism as the basis for insulin tolerance was not demonstrated by these experiments. The requirement for a supply of exogenous carbohydrate is confirmed, however, by the death of tolerant mice not given access to food during the insulin stress situation.

None of the above discussion is based on any principles more complex than those derived from the glucostatic theory of Mayer. The fact that the aurothioglucose-treated hyperphagic mice stopped eating cannot be explained as being due to any action of the no longer existent glucose centers. The survival of these mice even though most of them did not begin to eat until well after the end of the critical first two hours also poses a problem to the postulated means by which they overcome the hypoglycemia. The ability of the hyperphagic tolerant mice to survive even though they fail to ingest any food for up to eight hours cannot be explained by any interpretation of the glucostatic theory.

The possibility that the large amount of fat present in the hyperphagic mice was decreasing the effect of insulin

was the first explanation suggested. A report by Tucker et al. (1965) that the effect of insulin was decreased in proportion to the amount of fat present led to the experiments with doses proportional to body weight. As noted previously these experiments revealed a decrease in food ingestion when the amount of insulin administered was increased.

The hypothesis was proposed that the obese animals became too comatose as a result of the high insulin dose to avail themselves of the food present. The hypothesis was shown to be false when careful observation indicated the mice to be almost as active as the mice in the other experimental groups. Though sleeping a little bit more than animals in the other test groups, they roused themselves readily, walked about the cage, and even sniffed at the chow pellet and played with it, but they did not eat.

The remaining possibility is that the insulin level itself plays a role in the gross intake of food; the mechanism of this action has been proposed by Hales and Kennedy (1964). The blood insulin level has been found to increase in obese hyperphagic animals and these investigators feel that it is this hormone that causes the stopping of the hyperphagia. The tolerant hyperphagic mice, therefore, could be considered

to have been inhibited from eating by the extraordinarily high insulin levels present during periods following the injection. The normal action of insulin is to lower the blood glucose level, and this hypoglycemia is the stimulus to food ingestion according to the glucostatic theory. In the Hales and Kennedy theory insulin functions as a weight regulator, and high insulin serves as the stimulus which regulates food ingestion to maintain a constant body weight. Thus the difference found between those animals with intact hypothalamic centers and those whose satiety centers had been damaged can be explained by postulating that the glucoreceptance centers are dominant to any insulin receptors that might exist. With this mechanism the results in the normal animals are seen to reflect the activity of the glucoreceptor in the intolerant and tolerant mice while the proposed insulin-receptor dominates in the tolerant mice whose ventromedial nucleus has been damaged or destroyed.

The insulin-glucoreceptor mechanism for the regulation of food intake does not explain the cause of insulin tolerance. Though the results in insulin-stressed tolerant

mice under starved conditions lead to the conclusion that exogenous carbohydrate plays some role in the animals' survival, the fact is that the aurothioglucose-treated tolerant mice survived without eating. The lack of convulsions in these animals may be related to the lesions in the ventromedial hypothalamic nucleus as Spirtos and Halmi (1959) report that electrically produced lesions also prevent convulsions in rats not specifically tolerant to insulin. The manner of this phenomenon's occurrence and the importance it may have for the understanding of the causes of convulsions deserve further investigation.

VII CONCLUSIONS AND SUMMARY

1. Placebo-pretreated insulin-tolerant (KC) mice had a lower daily food consumption than placebo-pretreated insulin-intolerant (BUB) mice.

2. Aurothioglucose produced a hyperphagia and obesity in about one half of the tolerant (KC) mice into which it was injected.

3. Insulin injection stimulated the ingestion of food in placebo pre-treated mice of both tolerant and intolerant strains.

4. Insulin injection decreased food consumption in aurothioglucose-treated tolerant mice, stopping consumption completely at highest levels injected.

5. Latency of feeding initiation after insulin injection reflects the rate of eating with the delay exhibited by the mice in the order obese tolerant, tolerant, intolerant.

6. The tolerant mice of the KC strain were shown to depend upon eating for their survival after insulin stress.

7. Aurothioglucose pre-treatment protected tolerant (KC) mice from convulsions and death when deprived of food

after insulin injection.

8. The results of experiments with aurothioglucose-treated tolerant mice are consistent with the existence of an insulin sensitive center as proposed by Hales and Kennedy which is capable of reducing rate of food ingestion.

9. The survival of obese tolerant mice without eating during insulin stress has revealed the importance of acquiring a better knowledge of the mechanism of the production of insulin-induced convulsions.

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APPENDIX

Scheme for Hypothalamic Control of Hunger

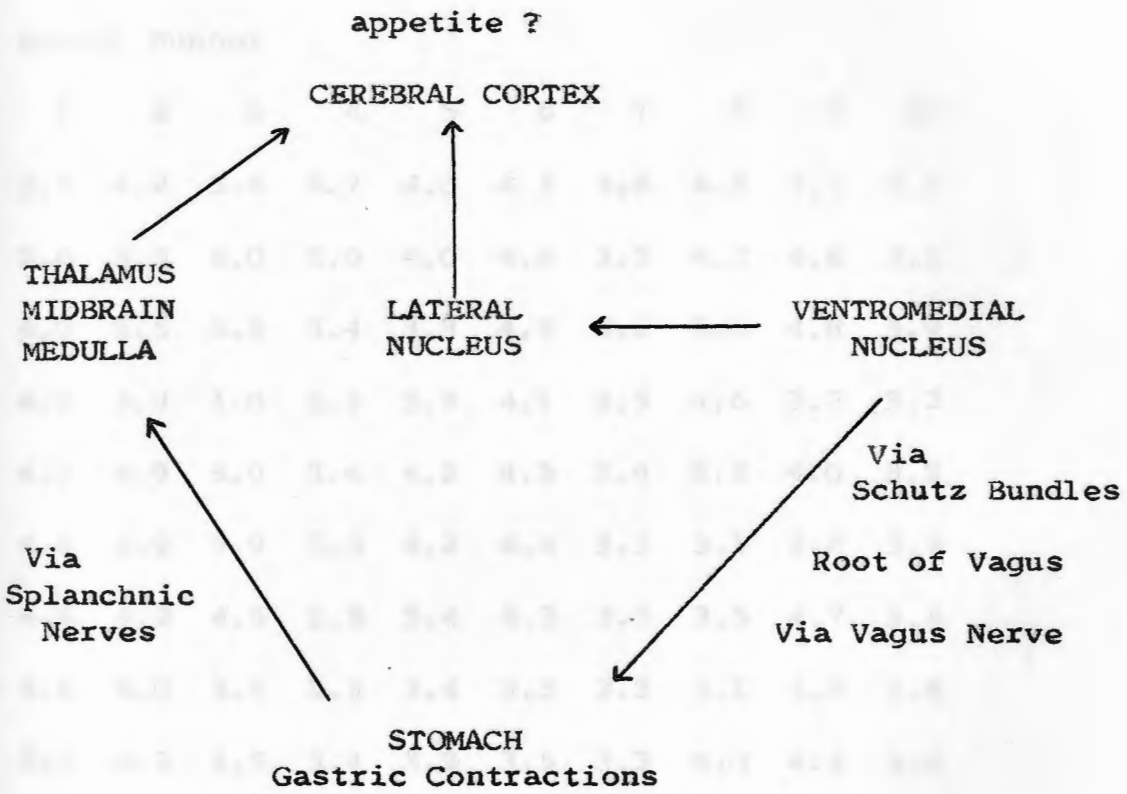

Figure I.

Table I

Daily Food Consumption

of Intolerant (BUB) Non-obese Mice (grams)

Animal Number									
1	2	3	4	5	6	7	8	9	10
3.6	4.2	3.8	3.7	4.0	4.5	3.6	4.3	5.3	3.9
3.6	3.5	4.0	3.6	4.0	4.4	3.5	4.2	4.8	3.2
4.0	3.5	3.8	3.4	3.3	4.5	3.6	3.0	4.8	3.9
4.0	4.9	4.0	3.3	3.3	4.1	3.5	4.6	3.2	3.2
4.0	4.9	3.9	3.4	4.2	4.5	3.4	3.2	4.0	3.2
4.6	4.9	3.9	3.3	4.2	4.4	3.3	3.1	4.8	3.2
4.6	4.2	4.5	3.3	3.4	4.3	3.3	3.5	4.7	3.4
4.6	4.0	3.9	3.3	3.4	3.5	3.3	4.1	4.4	3.4
3.3	4.2	4.5	3.4	3.3	3.5	3.3	4.0	4.4	3.4
3.3	4.0	4.5	3.4	3.3	3.6	3.3	3.0	4.3	3.0

Total chow consumed = 384.1 gms

mean $\pm$ se = 3.84 $\pm$ 0.05 gms/day

Table II

Daily Food Consumption

of Tolerant (KC) Non-obese Mice (grams)

Animal Number									
1	2	3	4	5	6	7	8	9	10
5.0	4.1	4.4	4.3	3.5	4.1	3.3	4.4	4.3	4.1
4.8	4.1	4.3	3.7	3.2	4.7	4.0	4.1	3.5	3.6
4.8	4.8	4.2	3.6	3.5	4.7	4.0	4.1	3.5	3.6
4.5	4.7	3.6	4.0	4.0	4.7	4.0	4.1	3.7	3.4
4.5	4.8	3.5	4.4	3.9	4.6	3.8	4.2	3.8	4.9
4.4	4.7	3.3	4.4	2.8	4.4	3.8	4.5	4.0	3.4
4.3	4.2	4.0	3.6	3.9	4.5	3.6	6.6	3.9	3.5
4.4	4.2	3.6	4.1	3.9	4.4	3.6	6.6	3.4	3.8
4.8	4.2	5.0	4.2	3.9	4.4	3.6	3.5	3.2	3.8
4.7	4.2	4.2	3.9	3.2	4.4	3.9	4.4	3.3	3.7

Total Chow Consumed = 409.3 gms

mean $\pm$ se = 4.09 $\pm$ 0.05 gms/day

Table III

Daily Food Consumption

of Tolerant (KC) Obese Hyperphagic Mice (grams)

Animal Number									
1	2	3	4	5	6	7	8	9	10
6.2	5.0	5.7	6.7	6.0	5.2	5.8	5.8	6.2	5.7
6.0	5.0	5.8	5.8	5.0	5.5	5.4	5.1	5.7	5.6
5.2	5.3	4.2	5.9	5.0	5.5	5.4	4.9	6.2	5.7
6.1	4.6	4.1	5.3	5.5	5.5	5.4	5.1	5.7	5.5
5.1	5.7	4.2	5.5	4.2	5.5	5.4	4.8	6.2	5.7
4.9	5.2	5.2	5.5	4.2	5.2	5.6	5.1	6.3	5.3
5.1	5.1	5.1	5.6	4.2	5.1	5.7	4.8	6.2	5.3
5.1	5.7	5.9	6.8	4.5	4.6	5.5	5.1	5.7	5.3
5.5	4.7	5.0	4.5	5.1	4.6	5.5	4.9	6.2	5.7
5.1	5.5	4.7	6.0	4.9	4.6	5.5	4.9	6.3	5.6

Total chow consumed = 534.2 gms

mean $\pm$ se = 5.34 $\pm$ 0.06 gms/day

Table IV

Cumulative Food Consumption Insulin Stress Run:

Data for Intolerant Non-obese Mice

Time in Hours	Animal Number 1	2	3	4	5	6	7	8	9	10
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.09	0.04	0.13	0.22	0.08	0.04	0.07	0.05	0.04	0.04
1.0	0.10	0.24	0.24	0.26	+	0.09	0.21	0.16	0.12	0.27
1.5	0.18	0.32	0.33	0.28		0.27	0.34	0.24	0.13	0.27
2.0	0.25	0.38	0.39	0.37		0.31	0.43	+	0.13	0.34
2.5	0.36	0.43	0.54	0.44		+	0.50		+	0.36
3.0	0.40	0.54	0.67	--			0.63			+
3.5	0.58	0.57	0.85	0.55			0.64			
4.0	0.70	+	1.01	0.55			+			
4.5	0.75		1.42	+						
5.0	0.81		1.43							
5.5	0.93		1.43							
5.6	1.01*		1.61*							

-- = no reading taken

\+ = died

* = died after recording period

Table V

Cumulative Food Consumption for Insulin Stress Run:

Data for Tolerant Non-obese Mice

Time in Hours	Animal Number 1	2	3	4	5	6	7	8	9	10
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.07	0.00	0.00	0.0	0.04	0.00	0.12	0.00	0.00	0.06
1.0	0.10	0.01	0.04	0.0	0.13	0.00	0.28	0.02	0.02	0.11
1.5	0.19	0.08	0.16	0.03	0.23	0.00	0.39	0.15	0.14	0.31
2.0	0.25	0.13	0.23	0.07	0.32	0.00	0.61	0.27	0.20	0.37
2.5	0.34	0.14	0.38	0.15	0.39	0.04	0.84	0.36	0.22	0.46
3.0	0.50	0.18	--	--	0.51	0.08	1.10	0.49	0.29	0.49
3.5	0.58	0.24	0.47	0.24	0.73	0.17	1.25	0.63	0.47	0.64
4.0	0.74	0.32	--	--	0.81	0.20	1.44	0.79	0.47	0.77
4.5	0.74	0.42	0.66	0.46	1.01	0.29	1.45	0.87	--	0.91
5.0	0.80	0.52	--	--	1.15	0.44	1.49	1.03	--	0.97
5.5	0.93	0.72	--	--	--	--	--	--	--	1.26
6.0	0.99	0.72	--	--	1.46	0.52	--	--	--	1.32

-- = no reading taken

Table VI

Cumulative food Consumption for Insulin Stress Run:

Data for Tolerant Obese Mice

Time in Hours	Animal Number 1	2	3	4	5	6	7	8	9	10
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.5	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2.0	0.03	0.03	0.00	0.00	0.00	0.04	0.00	0.02	0.00	0.02
2.5	0.04	0.13	0.00	0.00	0.00	0.09	0.00	0.04	0.00	0.04
3.0	--	--	0.00	0.00	--	0.20	0.00	0.05	0.00	0.09
3.5	0.07	0.29	0.00	0.00	0.02	0.28	0.00	0.05	0.00	0.10
4.0	--	--	0.00	0.00	--	0.43	0.01	0.10	0.00	0.10
4.5	0.17	0.29	0.08	0.00	0.07	0.62	0.08	0.20	0.00	0.14
5.0	--	--	--	--	--	0.77	0.08	0.20	0.00	0.14
5.5	--	--	--	--	--	--	--	--	--	0.17
6.0	--	--	--	--	--	1.00	0.12	0.38	0.00	0.17

-- = no reading taken

Table VII

Cumulative Food Consumption

No Insulin Stress Run

Animal	KC	BUB	KC-HH[1] 1	KC-HH 2	KC-HH 3
Time in Minutes					
0	0.00	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00	0.00
60	0.00	0.00	0.13	0.00	0.05
90	0.00	0.00	0.22	0.00	0.14
120	0.00	0.00	0.36	0.19	0.24
165	0.00	0.00	0.67	0.36	0.35
195	0.00	0.00	0.78	0.53	0.48
225	0.00	0.00	0.93	0.54	0.48
255	0.00	0.00	1.08	0.54	0.67
345	0.15	0.00	1.30	0.70	0.67

1 KC-HH = KC tolerant hypothalamic-hyperphagic from aurothioglucose treatment