THE ROLE OF THE VENTROMEDIAL HYPOTHALAMUS IN GLUCOREGULATION

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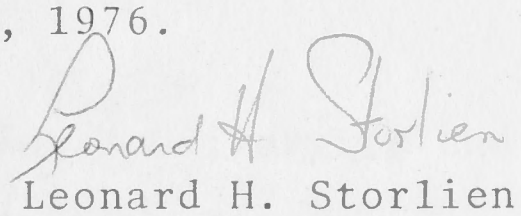

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LIST OF ABBREVIATIONS

mm	millimeter(s)
cm	centimeter(s)
mg%	milligrams per 100 milliliters
mg	milligram(s)
gm	gram(s)
kg	kilogram(s)
ml	milliliter(s)
ma	milliamp(s)
U	international unit(s)
i.m.	intramuscular
i.p.	intraperitoneal
°C	degree Centigrade
o.d.	outer diameter
SD	standard deviation

ABSTRACT

The study of central nervous system influences on energy balance has been dominated by the ventromedial hypothalamic (VMH) "satiety center" and lateral hypothalamic (LHA) "feeding center" concepts which arose largely from the dramatic effects on food intake and weight following electrolytic lesions of those structures. The aim of this thesis is to resynthesize the hypothalamic feeding data, concentrating on VMH functioning, within a framework of the role of that structure in glucoregulation.

In the introduction results are summarized which focus on the failure to show any deficit in "satiety" following VMH destruction. This is contrasted with the increasing evidence for autonomic and, in particular, VMH influence on visceral organs critical to glucoregulation. The reliance of the VMH syndrome on abnormal insulin secretion is highlighted.

In Chapter Two, three experiments explore the dependence of both VMH and LHA lesion syndromes on nervous connections with the viscera. VMH hyperphagia, hyperinsulinemia and accelerated weight gain are shown to depend on the vagus for their expression. The hypophagia, hypodipsia and weight loss of the LHA syndrome are shown not to depend, in any simple way, either on intact parasympathetic or sympathetic nervous connections with the viscera.

Chapter Three attempts to provide a link between studies which show that manipulations of the VMH have pronounced effects on the functioning of visceral structures critical to energy supply and those showing that alterations in insulin and glucose availability at the VMH influence firing patterns of certain neurons of that structure. In a series of 4 experiments it is shown that insulin has marked hypoglycemic effects when applied directly to the VMH; that this effect is not an artifact of diffusion into the ventricles; that the hypoglycemic response is even larger in diabetic animals and thus probably reflects an integrated metabolic pattern, including at least the pancreas and liver, facilitating glucose disposal; and, finally, that this series of metabolic events is at least partially mediated by the vagus.

The major findings of Chapters Two and Three are then: (1) the reversal of the VMH electrolytic lesion effect by vagotomy, and (2) the direct hypoglycemic effects of an increase in insulin levels within the VMH, together suggesting a glucoregulatory role for the VMH based on direct nervous innervation of the viscera. Based on these results and on the existing observations of VMH-insulin-glucose relations, the dynamics of hypothesized VMH insulin-glucoreceptors are specified. They are seen to be spontaneously active, suppressed by insulin, and activated by

glucose (but only in the presence of insulin). These receptors, in concert with others responsive to sensory information, are seen to stabilize blood glucose levels: (1) by initiating an integrated series of metabolic events which act to facilitate glucose disposal in anticipation of the initiation of a meal, and (2) by suppressing or reversing the same (or similar) set of metabolic events following the meal to avoid a hypoglycemic overshoot. The primary role of the VMH is thus seen as one of stabilizing glucose supply to the brain. It is hypothesized that the major "deficit" following a VMH lesion is the failure to suppress the reactive phase of insulin secretion, thus inducing a postprandial hypoglycemia and entraining the vicious circle of intake to counter the hypoglycemia which only serves again to overstimulate insulin. Arguments for a central VMH role in long-term regulation of body weight are countered, and it is suggested that the weight at which any particular VMH-lesioned animal stabilizes merely reflects the degree of impairment of VMH suppression of reactive insulin secretion in concert with the increasing insulin resistance attendant upon obesity. Finally, in this chapter, the relation of palatability to the VMH syndrome is discussed.

Chapter Four tests the predictions of the VMH insulin-glucoreceptor model in two areas which have been traditional

problems for the "glucostatic" theory of feeding: diabetic hyperphagia and adrenaline anorexia. The duration of the initial hypophagic period following induction of diabetes is seen to depend on the energy reserves of the animal and it is argued that: (a) hypophagia is the natural result of diabetes hyperglycemia, and (b) the onset of hyperphagia reflects the influence of a long-term energy depletion signal. Adrenaline anorexia follows as a natural consequence (in terms of the VMH insulin-glucoreceptor model) of the hyperglycemia-hypoinsulinemia. This view is reinforced in the first of a series of 3 experiments which demonstrate the absence of the anorexia phenomenon in diabetic animals. The second and third experiments rule out peripheral theories of adrenaline anorexia by demonstrating the phenomenon in both vagotomized and sympathectomized animals. It is suggested that feeding may be used as a glucoregulatory device in emergencies and, in such situations, the VMH is involved in controlling food intake. However, under normal circumstances neither the VMH nor blood glucose-insulin levels are likely to influence feeding.

Much of the theorizing about VMH function has been based on the crude electrolytic lesion technique. In Chapter Five an attempt is made to identify a naturally occurring "VMH syndrome". To this end the hyperphagia and increased weight gain attendant upon diestrus in the female

rat is studied. The results of a series of 4 experiments are negative. The estrus cycle is found not to parallel the VMH syndrome, in that insulin levels, high in VMH-lesioned animals, are found to be depressed during diestrus, the period of maximal weight gain. In addition, vagotomy, which reverses VMH hyperphagia and weight gain, does not affect the rhythmic weight fluctuations of the estrus cycle. The results not only disconfirm the relative hyperphagia and weight gain of the estrus cycle as a model of the VMH syndrome but call into question assertions of the relationship between insulin and weight regulation.

Finally, the glucoregulatory model of VMH function is seen to have direct relevance to three areas of clinical importance. In Chapter Six the implications of the model for diabetes mellitus are explored. The model predicts the combination of hyperglycemia and hypoinsulinemia in the diabetic will generate a high level of activity in the VMH insulin-glucoreceptors and result in a counterproductive set of metabolic reactions (i.e., suppression of any residual insulin secreting capacity, augmented hepatic glucose output and increased free fatty acid efflux). The contribution of a postulated chronic sympathetic nervous system (SNS) arousal to the metabolic disturbances of diabetes mellitus is judged in a series of 4 experiments. An increase in the efficiency of intake utilization, a decrease

in blood glucose, and reduced efflux of free fatty acids from fat are all seen following SNS destruction in diabetes. The results encourage further elucidation of the role of the SNS in diabetes.

In Chapter Seven (Discussion) two further matters of clinical importance are discussed in light of the glucoregulatory model of VMH functioning. These are: (1) neurogenic control of weight regulation, and (2) intravenous feeding and caloric regulation.

CHAPTER ONE

INTRODUCTION

Research concerned with the neural control of feeding behavior has, over the past 35 years, focussed in particular upon the ventromedial and lateral areas of the hypothalamus. The results obtained have been the subject of numerous extensive reviews [see Anand, 1961; Andersson, 1972; Grossman, 1966; 1975; Hoebel, 1971; Mogenson, 1974; Morgane and Jacobs, 1969; Rabin, 1972; Teitelbaum, 1961], but may be summarized as follows. Destruction of the ventromedial hypothalamic area (VMH), via either electrolytic [Brobeck, Tepperman and Long, 1943; Hetherington and Ranson, 1940] or radio frequency [Dahl and Ursin, 1969; Herrero, 1969; Hoebel, 1969] lesions, results in hyperphagia and gross obesity. This hyperphagia is the result, in free-feeding animals, of an increase in the size of the individual meals rather than an increase in their number [Thomas and Mayer, 1968]. Electrical stimulation of the VMH terminates ongoing feeding behavior [Anand and Dua, 1955; Larsson, 1954; Wyrwicka and Dobrzecka, 1960]. Electrical activity in the VMH increases during, and for a period after, feeding and decreases during deprivation [Anand, Subberwal, Manchanda and Singh, 1961]. Intravenous infusion, or direct application, of glucose increases VMH activity [Anand, Chhina,

Sharma, Dua and Singh, 1964; Oomura, Ono, Ooyama and Wayner, 1969; see also Oomura, 1973].

Conversely, it was noted that similar manipulation of lateral hypothalamic area (LHA) activity produces strikingly reciprocal results. Thus, lesions of the LHA result in anorexia and weight loss [Anand and Brobeck, 1951; Morrison, Barnett and Mayer, 1958], while electrical stimulation initiates feeding behavior [see Hoebel, 1969]. Changes in levels of electrical activity in relation to meals and following intravenous infusion or topical application of glucose generally were found to be the inverse of those observed in the VMH [Anand, Chhina, Sharma, Dua and Singh, 1964; Oomura, Ono, Ooyama and Wayner, 1969; Oomura, Ooyama, Yamamoto and Naka, 1967].

The concept which evolved from this work was of a VMH "satiety" center whose primary function was to terminate feeding in response to rising levels of certain blood-borne factors which correlated with a satisfactory nutritional state in the organism. The VMH was thought to accomplish this function via a system of inhibitory fibers projecting onto the lateral hypothalamic "feeding" center. In fact, placing parasagittal cuts in the region of the hypothesized, laterally coursing, inhibitory fibers did result in a syndrome much like that following VMH lesions [Albert and Storlien, 1969; Gold, 1970; Scalfani and Grossman, 1969].

The wide favor which this model received was due to the wealth of consistent data, its pleasing apparent simplicity, and, in no small part, to the enormity of the feeding and weight effects following electrolytic lesions.

The primary difficulty in the medial "satiety" area segment of this system has been the repeated failure to demonstrate any specific satiation deficit following destruction of the VMH. Thus, intraperitoneal injections of glucose are even more anorexigenic in VMH lesioned cats [Russek and Morgane, 1963]. Eating in response to insulin-induced hypoglycemia is normal [Epstein and Teitelbaum, 1967]. There is no impairment of postprandial satiety to gastric infusion of proteins, carbohydrates or fats following VMH lesions [Panksepp, 1971]; nor any effect of similar lesions on solely post-absorptive satiety [Booth, Toates and Platt, 1976]. Compensation is nearly perfect for the caloric value of glucose infused intragastrically [Liu and Yin, 1974] or intravenously [Rowland, Meile and Nicolaides, 1975]. Finally, the response to caloric dilution is unimpaired when palatability is controlled [Smutz, Hirsch and Jacobs, 1975].

By contrast with this negative evidence, data have steadily accrued in support of a central role for the VMH in control of visceral organs involved in the handling of ingested nutrients, particularly the stomach, liver and

pancreas. Among the most relevant of these changes to energy balance are the marked hyperinsulinemia in the face of only normal blood glucose [Hales and Kennedy, 1964], enlargement of pancreatic islets [Han, Yu and Chow, 1970], massive accumulation of fat [Hetherington and Ranson, 1940], increased gastric acidity [Ridley and Brooks, 1965], increased free fatty acid levels [Hales and Kennedy, 1964], enlarged, "fatty" liver [Brobeck, Tepperman and Long, 1943], and decreased growth [Reichlin, 1961] which follow VMH lesions. Conversely, electrical stimulation of the VMH increases blood glucose while suppressing insulin [Frohman and Bernardis, 1971] and depleting liver glycogen [Shimazu, Fukuda and Ban, 1966]. The failure of hypothysectomy to reverse the VMH effect [see Cox, Kakolewski and Valenstein, 1968] has moved attention away from pituitary hormones. While undoubtedly decreased growth and a reduced basal metabolic rate (possibly from a decrease in thyroid functioning) could contribute to the magnitude of the effect, it would appear that insulin is critical to the expression of hyperphagia and weight gain following VMH lesions. Certainly hyperinsulinemia has been consistently shown to accompany VMH obesity [see Frohman and Bernardis, 1968; Hales and Kennedy, 1964; Hustvedt and Løvø, 1972; York and Bray, 1972]. Electrical stimulation of the VMH results in an increase in blood glucose and a suppression of the

insulin rise which would normally occur in response to the induced hyperglycemia [Frohman and Bernardis, 1971]. This suppression of insulin may occur indirectly from stimulation of the adrenal medulla [Frohman and Bernardis, 1971] and/or directly via neural input to the pancreas [Miller, 1975]. In addition, the insulin levels seen 48 hours after VMH lesions with food intake restricted to normal levels correlate highly with the subsequent weight gain observed on free feeding [Hustvedt and Løvø, 1972].

However, a number of problems interfere with establishing the causality of this relationship. Both hyperphagia and obesity themselves result in increased insulin levels even in neurally intact animals [Sims, Horton and Salans, 1971]. Additionally, VMH-lesioned animals increase their food intake primarily by increasing meal size [Teitelbaum and Campbell, 1958; Thomas and Mayer, 1968]. Normal animals trained to consume their food in large meals (versus nibbling) also display increased insulin levels and increased fat deposition [Cohn, 1963; Fabry, 1967; Leveille, 1972]. These considerations have prompted studies in which insulin levels were controlled by induction of diabetes.

York and Bray [1972] and Young and Lui [1965] demonstrated the failure of VMH lesions to increase either food intake or body weight in animals previously made diabetic either with streptozotocin or alloxan. York and

Bray [1972] concluded that "an increased circulating level of insulin is required for the appearance of hyperphagia and for the progression of obesity resulting from hypothalamic damage of adult female rats". Their conclusion has been challenged by both Friedman [1972] and, more recently, Vilberg and Beatty [1975]. Both these latter studies, however, are marred by a failure to measure residual insulin-secreting capacities in their animals made diabetic by alloxan [Friedman] and streptozotocin [Vilberg and Beatty]. For instance, it should be noted in the Friedman study that his alloxan-diabetic rats had food intakes no higher than those of control animals, a finding distinctly at odds with earlier results [see Brodsky, Nelson and Guest, 1952]. Some recovery of insulin secreting capacity (due most probably to hypertrophy of surviving beta cells) occurs over time following induction of diabetes with streptozotocin. In particular, special care must be taken to assess residual insulin-secreting capacity following destruction of an area like the VMH which is closely identified with the sympathetic nervous system [Ban, 1964, 1966]. Sympathetic nervous system arousal is known to suppress insulin secretion [see Woods and Porte, 1974; Miller, 1975], and destruction of the primarily sympathetic VMH might act to remove inhibition of the remaining insulin-secreting capacity after drug-induced pancreatic beta cell

destruction. In this regard the data of York and Bray [1972, Table 4] are particularly interesting. They show insulin levels in two groups of animals, both made diabetic by identical means and both maintained on 2U insulin/100 grams/day. Assays done on blood sampled 24 hours after the last insulin injection showed that the group of animals with VMH lesions had insulin levels almost double those of the unlesioned control group.

It would thus seem that the conclusion reached by York and Bray [1972] and Young and Liu [1965] must be tentatively accepted until such time as hyperphagia and supranormal weight gain can be demonstrated in a group of diabetic VMH-lesioned animals whose insulin secretory capacity is shown to be equal to unlesioned diabetics.

In synthesis, insulin would appear to play a central role in the VMH syndrome, correlating with, and probably being necessary for, the hyperphagia and obesity. The primary focus of this thesis is on clarifying the relationship between insulin-glucose regulation and the more traditional role seen for the hypothalamus, particularly the ventromedial area, in feeding. The first experiments (Chapter Two) are aimed at establishing the role of the autonomic nervous system in the VMH and LHA syndromes. Chapter Three provides evidence for a primary VMH role in glucoregulation and a model is proposed. Chapter Four tests

certain predictions generated by the model. Chapter Five is an attempt to determine whether the estrus cycle of the female rat can be used as a "mini-VMH syndrome" unencumbered by the multitude of extraneous metabolic changes undoubtedly produced by a lesion in such a central subcortical area. Finally, in Chapter Six, the predictions generated by the model of Chapter Three are applied to the treatment of diabetes mellitus.

CHAPTER TWO

HYPOTHALAMIC-VISCERAL RELATIONSHIPS

2.1 Introduction

As summarized in Chapter One, there has been a substantial accumulation of data demonstrating marked changes in the functioning of visceral, energy-regulating organs with alterations in hypothalamic activity and quite convincing evidence that hypersecretion of insulin is critical to the expression of hyperphagia and weight gain following VMH lesions. The following chapter is aimed at exploring the role of autonomic nervous connections with the viscera in the alterations of body weight which result from VMH and LHA lesions.

2.2 Experiment 1: VMH-Vagotomy

There are numerous mechanisms by which the body maintains a basal level of insulin secretion [see Goodner and Porte, 1972]. The origin of the malfunction following VMH lesions is not apparent. The hyperinsulinemia is not a secondary result of hyperphagia or pituitary hormone changes. Increased plasma insulin levels and hypertrophy of pancreatic islets have been demonstrated in hypophysectomized, VMH-lesioned animals [Han and Frohman, 1970; Han, Yu, and Chow, 1970] and in VMH-lesioned animals whose food intake has been restricted to control levels [Han, Yu and Chow, 1970; Hustvedt and Løvø, 1972]. In the absence of altered pituitary hormone or of stimulation indirectly resulting from a superabundance of incoming nutrient, the most likely mediating systems for insulin release are the sympathetic and parasympathetic components of the autonomic nervous system.

While the mechanism by which the sympathetic nervous system modifies insulin release is obscure, a dual role has been established. The inhibitory effect of epinephrine on insulin release [Porte, Graber, Kuzuya, and Williams, 1966] would appear to result from alpha-adrenergic receptor activation (alpha-stimulants inhibit and alpha-blocking agents stimulate insulin release). The reciprocal relationship is true for the beta-adrenergic system with beta-

stimulants increasing and beta-blockers decreasing insulin release [see Porte, 1969, for review].

The parasympathetic system may, however, be of greater importance to hypothalamic hyperinsulinemia. A substantial body of data supports the importance of parasympathetic vagal fibers in neural control of insulin secretion. Malaisse, Malaisse-Lagae, Wright, and Ashmore [1967] and Kajinuma, Kaneto, Kuzuya, and Nakao [1968] demonstrated a rise in insulin levels following infusion of acetylcholine and metacholine respectively. Frohman, Ezdinli, and Javid [1967] reported a rise in plasma insulin following vagal stimulation which could be blocked by the parasympathetic blocking agent atropine. These same investigators reported a fall in plasma insulin following bilateral vagotomy. Conditioned hypoglycemia, which appears to be dependent on a conditioned insulin release [Woods, Alexander and Porte, 1972], is also abolished by both vagotomy and atropine but not by the adrenergic suppressant, guanethidine [Woods, 1972]. Finally, Larsson [1954] reported electrical stimulation of the brainstem in the area of the dorsal motor nucleus of the vagus produced massive hyperphagia.

If hypothalamic hyperinsulinemia is mediated directly via pancreatic vagal innervation, vagotomy should reduce insulin levels to control values in VMH-lesioned animals. The present experiment was designed to test this

possibility.

2.2.1 Method

Subjects were female albino rats of between 180 and 200 grams at operation obtained from the breeding colony of the John Curtin School of Medical Research (Canberra, Australia). Animals were divided into 3 groups. One group (VMH-Vag) received VMH lesions followed 3 weeks later by subdiaphragmatic vagotomy. A second group (VMH-Sham) received the VMH lesions followed by sham vagotomy. Animals in the control group (Sham-Vag) received sham VMH lesions followed by vagotomy. All subjects were weighed on the day of the VMH or sham lesion, 1 and 2 weeks later, on the day of the vagotomy or sham vagotomy, and 4, 7, 11 and 14 days following that operation. Blood samples were taken one day before the VMH or sham lesion, 1 and 2 weeks following this operation, and 1 and 2 weeks following vagotomy or sham vagotomy. Food intake was measured for the 24-hour period preceding the deprivation prior to each blood sampling. A weighed amount of pellets (Mecon Rat Chow) was placed in the cage and the remaining pellets weighed again at the end of the 24-hour period. Spillage, collected on paper towels placed under the individual cages, was air dried. The dry weight of spilled food was subtracted from the difference between the "in" and "out" pellet weights to give a final intake figure. Arbitrarily, only VMH-lesioned animals

gaining at least 5 times the mean control gain in the 2 weeks following placement of the lesion were continued into the vagotomy phase of the experiment.

Under Nembutal anesthesia (50 mg/kg) electrolytic lesions (2 ma for 15 seconds) were produced bilaterally through coated stainless steel electrodes insulated except for 0.3 mm at the tip. A tail cathode completed the circuit. Coordinates [de Groot, 1959] were anterior 6.0 mm, lateral ±0.5 mm, and 0.5 mm above the floor of the cranium. Operative procedures for control animals were identical except that the electrode was lowered only 5.0 mm from the dura and no current was passed. Penicillin (50,000 U/day) was injected i.m. for two days post-operatively.

Bilateral subdiaphragmatic vagotomy was also carried out under Nembutal anesthesia (50 mg/kg). An incision was made caudal from the xiphisternum approximately 2 cm in length. Under a 20 power dissection microscope, the esophagus was located and lifted from the body cavity on a pair of small forceps, care being taken to avoid damage to the liver. The vagi were located and at least 3 to 4 mm of each nerve was cut loose and removed. All traces of nerve tissue were cleared while avoiding damage to the musculature of the esophagus or blood supply to the stomach. The muscle wall was repaired with silk sutures and the wound closed with Michel wound clips. The wound area was liberally

covered with Cicatrin (Calmic Pharmaceuticals, Australia) and penicillin injected i.m. (50,000 U/day) for 3 days following the operation. Sham operations were identical with the exception of the actual severing of the vagi.

All blood sampling was done by slitting a tail vein under light ether anesthesia. The time from initiation of ether to completion of blood sampling was about 3 to 4 minutes. Blood sampling was done between 1400 and 1500 hours to avoid diurnal variation [Jolin and Montes, 1973] on 6- to 7-hour deprived animals. Following collection, all samples were immediately centrifuged and the plasma stored at -18°C for later insulin analysis.

Insulin was assayed by the double antibody radio-immunoassay technique. No rat standard was available so insulin is expressed in terms of human standard.

Following completion of the experiment, the animals were killed and the brains of experimental animals were removed, hardened in 10% formalin and sectioned at 50 microns. Every fourth section was stained with thionin to localize the lesion site. This was done only to confirm that the lesions included extensive bilateral damage to the VMH without substantial damage to surrounding structures.

In addition, the esophagus of each animal was also removed at sacrifice and examined under a microscope for evidence of intact neural tissue. This was the only test

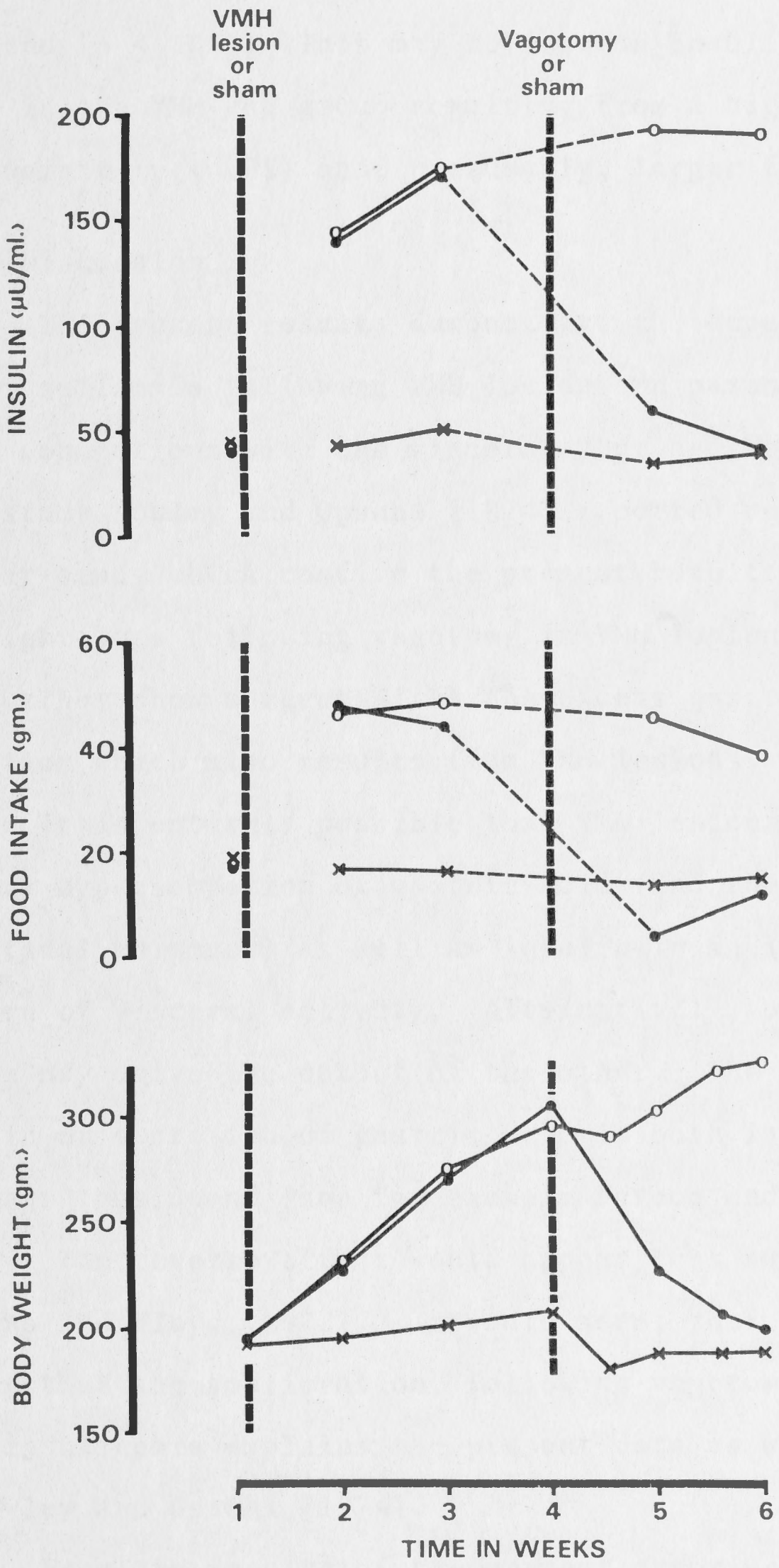
made for completeness of vagotomy and was done completely blind. Minimal regrowth had taken place and traces of intact tissue were found in only 3 animals. These were omitted from the analysis.

Statistical analysis was done using a one-way, fixed effects analysis of variance at selected time points followed, where appropriate, by Scheffé individual comparisons.

2.2.2 Results

Data are summarized in Figure 1 (see Appendix A for the F ratios). The major result is the effect of vagotomy on animals with prior lesions of the VMH (VMH-Vag group). These animals, selected on the basis of their post-lesion weight gain, all displayed, in addition, highly significant hyperphagia ($p < .001$, 1 and 2 weeks following the lesion) and hyperinsulinemia ($p < .05$ and $p < .01$, respectively, 1 and 2 weeks following the lesion). Weight, insulin level and food intake in this group all dropped markedly following vagotomy. By 2 weeks post-vagotomy, there were no significant differences on any measure between VMH-Vag ($n = 7$) and Sham-Vag ($n = 8$) groups ($p > .20$ in all cases) while both these groups differed on all measures from the VMH-Sham ($n = 8$) group ($p < .01$ in all cases). It is interesting to note that although food intake 1 week post-vagotomy in the VMH-Vag group was significantly less ($p < .01$) than that of the Sham-Vag group, insulin levels were still

Figure 1. The effect of VMH lesions followed by Vagotomy (●) on measures of plasma insulin, food intake and body weight. VMH lesion-sham vagotomy (o) and sham lesion-vagotomy (x) groups are included for comparison.


VMH lesion or sham
Vagotomy or sham
INSULIN (μU/ml.)
200
150
100
50
0
FOOD INTAKE (gm.)
60
40
20
0
BODY WEIGHT (gm.)
300
250
200
150
1
2
3
4
5
6
TIME IN WEEKS

elevated ($p < .05$). This may reflect an insulin resistance in the VMH-vag group resulting from a higher mean body weight ($p < .05$) and, presumably, larger fat cells.

2.2.3 Discussion

The present results demonstrate the dependence of the hyperinsulinemia following VMH lesions on parasympathetic vagal connections with the viscera. During the course of this study Powley and Opsahl [1974] reported results of a similar study which confirm the present results with respect to weight loss following vagotomy in VMH lesioned animals and further show a reversal of the excess gastric acid secretion which also results from VMH lesions.

It is entirely possible that VMH lesions result in primary hypersecretion of gastric acid (and then gastrointestinal hormones) as well as insulin in an integrated pattern of visceral activity. Alternatively, oversecretion of one may drive the output of the other. The effect of insulin on secretion of gastric acid is both large and the findings consistent [see for example Farooq and Isenberg, 1975]. The reverse effect would appear less supported [Fajans and Floyd, 1972]. It would seem, therefore, more likely that the amelioration, following vagotomy, of the hyperinsulinemia explains the present data as well as that of Powley and Opsahl [1974].

From the results of the present experiment nothing

can be said about exactly how the oversecretion of insulin comes about after VMH destruction. However there are a number of existing results which provide clues to this process and it may be worth examining them at this point. On available evidence it would seem that destruction of the VMH is not a sufficient condition for hyperinsulinemia. Frohman, Goldman, Schnatz and Bernardis [1972] were able to demonstrate normal insulin levels in VMH-lesioned animals following a 48-hour fast. Further, Martin, Konijnendijk and Bouman [1974] and Trevethan [1972] restricted both their VMH-lesioned and control animals to 67% [Martin, Konijnendijk and Bouman] and 80% [Trevethan] of normal control intake. These studies demonstrate normal basal insulin levels in VMH-lesioned animals thus maintained. Louis-Sylvestre [1971] reported normal glucose tolerance curves 24 and 48 hours following VMH lesions in animals totally deprived of food during this period. This contrasted with the elevated "K" values (indicative of facilitated glucose removal) of VMH-lesioned animals allowed access to food during this post-operative period. Steffens, Mogenson and Stevenson [1972] found a transient increase in basal insulin levels following VMH lesions which disappeared in their "non-hyperphagic" group (3 animals with histologically verified extensive bilateral VMH lesions but which, for whatever reason, failed to overeat in the immediate post-operative period).

Woods, Kaestner and Vasselli [1975] recently indicated insulin rises following local anesthesia of the VMH. Whether this effect would, as in the study of Steffens, Mogenson and Stevenson [1972], also disappear over time is unclear.

Basal hyperinsulinemia has certainly been shown to occur when VMH-lesioned animals are given amounts of food equal to normal consumption [Han and Frohman, 1970; Hustvedt and Løvø, 1972] and even when this food is tubed intragastrically to control completely for meal patterning [Han and Frohman, 1970]. Additionally, in a similar intragastric pair-feeding study, Han, Yu and Chow [1970] demonstrated increased pancreatic islet size in VMH-lesioned animals. It was, however, also demonstrated by Han and Frohman [1970] that on normal amounts of food, fed intragastrically, the VMH-lesioned animals accumulate significantly more fat than controls. This observation must mean larger fat cells and that may well account for the observed increase in basal insulin levels.

In summary, depriving VMH-lesioned animals for 48 hours [Frohman, Goldman, Schnatz and Bernardis, 1972]; restricting their food intake to 67% [Martin, Konijnendijk and Bouman, 1974] or 80% [Trevethan, 1972] of normal control intakes; or not allowing access to food for the 48-hour period following placement of the lesion [Louis-Sylvestre,

1971] all result in insulin levels in VMH-lesioned animals equivalent to similarly treated controls.

The primary defect following VMH lesions is then likely to be not a shift in basal insulin levels but a dis-inhibition of the stimulated insulin response. It can be noted in the study of Martin, Konijnendijk and Bouman [1974] that in spite of normal basal levels, stimulated insulin release was greater in their VMH-lesioned animals. The model which is formulated in the general discussion to Chapter Three (based largely on the results of this experiment and those of the following chapter) speaks further to this point.

2.3 Experiment 2: LHA-Vagotomy

The hypophagia and weight loss which result from electrolytic lesions of the LHA are often seen as reflecting changes along the same continuum as the hyperphagia and weight gain of the VMH syndrome [see Woods, Decke and Vasselli, 1974; Woods and Porte, 1976]. The results of Powley and Opsahl [1974] and of the previous experiment have demonstrated the importance of vagal connections with the viscera in the etiology of the VMH syndrome. The reciprocal effects of VMH and LHA stimulation on blood glucose [Frohman and Bernardis, 1971; Shimazu, Fukuda and Ban, 1966], liver enzyme activity [Shimazu and Ogasawara, 1975; Shimazu, Fukuda and Ban, 1966], and gastric secretion [Misher and Brooks, 1966] further reinforce the possibility that whatever the nature of the visceral change induced by VMH lesions, and reversed by vagotomy, a change in the opposite direction may result from LHA lesions.

Initial results appeared to support this stand. Snowdon and Wampler [1974] reported a reversal, by vagotomy, of the food intake depression which results from LHA lesions. This result, however, is in contrast to the more recent findings of Powley and Opsahl [1976]. In this study they observed the small weight loss normally seen following vagotomy in control animals. Vagotomy, in their LHA lesioned rats, not only failed to ameliorate the weight

reduction of this group but resulted in a further weight loss greater in magnitude than that observed in the control group. It is possible that the differences between the two studies can be accounted for in terms of the different measures (Snowdon and Wampler measured food intake, while Powley and Opsahl measured weight) used to assess the effect of vagotomy on the LHA syndrome.

The present experiment was designed to resolve the conflicting evidence by assessing the effect of vagotomy in normal and LHA lesioned rats on measures of food intake and weight gain. In addition, basal plasma insulin levels were measured largely because there is some indication that vagotomy may lower blood insulin levels [Frohman, Ezdimli and Javid, 1967; Kaneto, Kosaka and Nakao, 1967] and if LHA lesions result in a syndrome opposite to that produced by VMH lesions then the cumulative effect of both vagotomy and LHA lesions may be a detectable basal hypoinsulinemia.

2.3.1 Method and Results

Subjects were male, Wistar-derived rats of between 300 and 360 grams obtained from the breeding colony of the John Curtin School of Medical Research (Canberra, Australia). Animals were assigned randomly to 1 of 3 groups. One group (LHA-Vag) received LHA lesions followed 4 weeks later by bilateral subdiaphragmatic vagotomy. A second group (LHA-Sham) received the LHA lesions followed by sham vagotomy.

The control group (Sham-Vag) received sham lesions followed by vagotomy. All subjects were weighed on the day before LHA or sham lesion, daily until weight gain was seen on dry food, and weekly thereafter.

Blood samples were taken 1 day before LHA or sham lesion, 2 weeks following that operation and 2 and 4 weeks following vagotomy or sham vagotomy. Food intake was measured over a 24-hour period preceding the 6- to 7-hour deprivation prior to each blood sample.

The lesioning procedure for both experimental and control animals were as in Experiment 1. Coordinates [de-Groot, 1959] were anterior 6.0 mm, lateral ±1.8 mm, and 8.0 mm below the dura. A current of 1 ma was passed for 10 seconds.

Wet, sweetened food was given following the LHA lesions and the animal was hydrated if necessary. When weight gain was observed, the wet food was withdrawn and reinstituted if further weight loss occurred. All animals were gaining weight on dry rat chow and plain water by 3 weeks following the lesion.

Bilateral, subdiaphragmatic vagotomy, blood sampling and insulin assay were all carried out as in Experiment 1.

One week following the final blood sampling animals were deprived of food for 36 hours and tested for completeness of vagotomy. Basal and insulin-stimulated acid

secretion were assessed as follows. Animals were anesthetized with sodium pentobarbitol (40 mg/kg i.p.). A midline incision was made posterior from the xiphisternum. A small cut was made in the duodenum approximately 3 cm from the pylorus. A length of polyethylene tubing (P.E. 240), flared on the end to a diameter of 5 mm, was slipped into the duodenum. The flared end was pushed through the pylorus, drawn back to a snug fit and sutured into place. The stomach was flushed with physiological saline warmed to 37°C until draining clear. A small polyethylene tube (P.E. 60), also flared at the end, was then purse-sutured into the antrum of the stomach through a small puncture. Five mls of warmed physiological saline were then injected through the antral tube each 5 minutes. Fifteen minute flow periods were collected from the duodenal cannula and titrated with 0.005 N NaOH protected from the atmosphere with soda lime. Two 15-minute periods of baseline data were collected. Insulin (2 U/kg) was then injected subcutaneously and 2 further 15-minute periods of acid secretion were collected and titrated. A strict criterion for completeness of vagotomy was used; acid output of either 15-minute period following insulin injection was not allowed to exceed the output of the mean of the 2 pre-insulin periods. Of the 18 vagotomized animals tested, 1 LHA and 1 Control did not meet criterion and were discarded from the experi-

ment and 1 LHA animal died under the anesthetic.

The animals were then killed with ether and the brains of the experimental animals were removed, hardened in 10% formalin and sectioned at 50 microns. Every fourth section was stained with thionin to localize the lesion sites.

Statistical analysis was done using a one-way analysis of variance with fixed effects followed, where appropriate, by Scheffé individual comparisons.

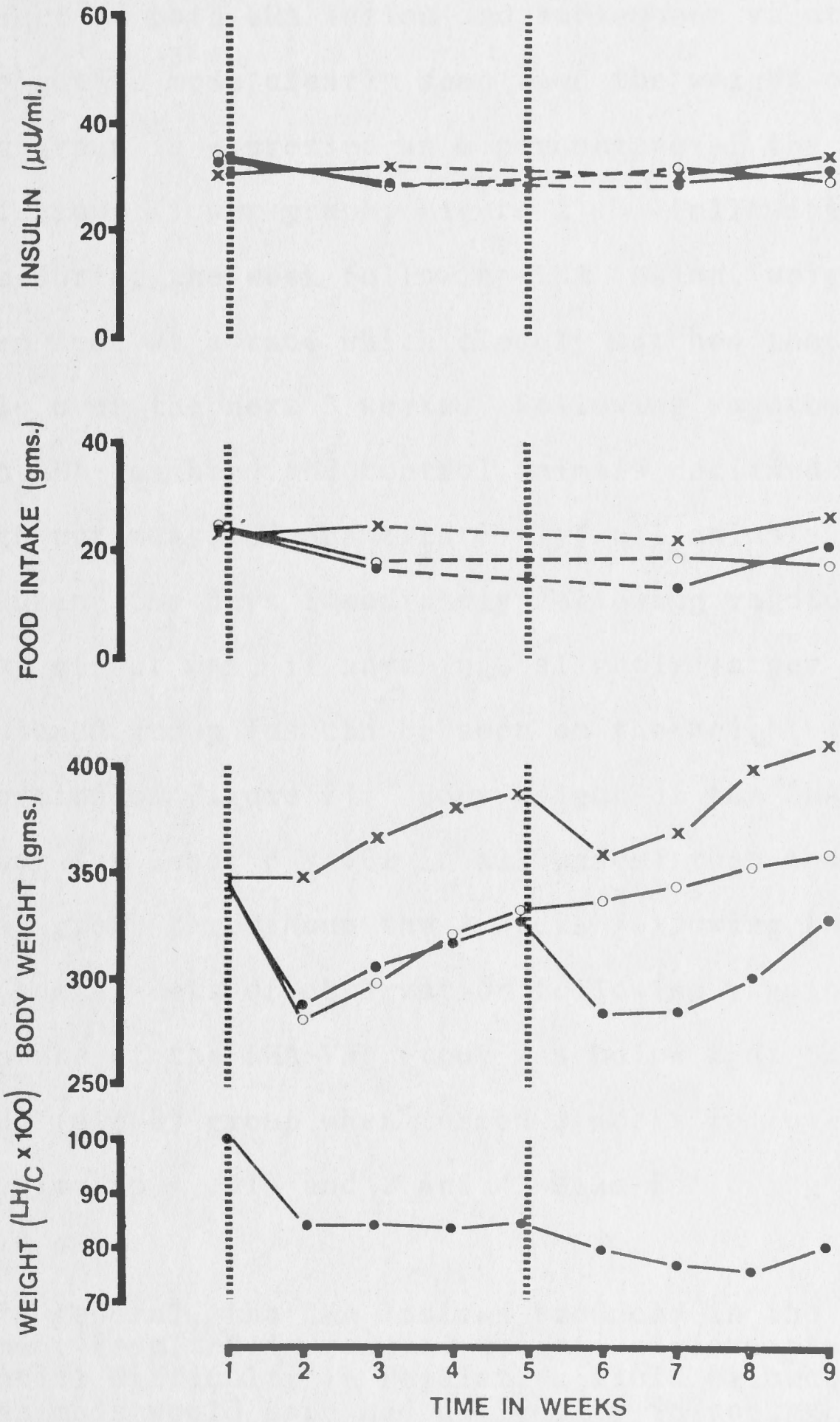
2.3.2 Results

Results are shown in Figure 2 (F ratios are summarized in Appendix B). Weight, in the LHA-lesioned animals (LHA-Vag, n = 7; LHA-Sham, n = 8), declined rapidly following the operation in the absence of either voluntary food or water intake. The period of anorexia lasted from 4 to 7 days and was followed by a gradual increase in intake of wet mash, and finally dry food,[1] to the point of sustaining

---

[1]There have been a number of reports focussing on various aspects of the LHA-lesioned animal's difficulty in regulating fluid balance [see Epstein, 1971; Kucharczyk and Mogenson, 1976; Rowland and Nicolaides, 1975; Stricker and Wolf, 1967]. In the present experiment, 30 animals sustained LHA lesions. Of those, 23 reacted with anorexia and substantial weight loss. Histology done on the 7 unsuccessful animals generally confirmed either asymmetrical or misplaced lesions. Of the 23 animals initially retained for the experiment, 19 made the transition from wet mash to dry food and water from tubes within a maximum of 4 or 5 exposures and without apparent undue adverse reaction. They maintained, on dry food, a near normal growth rate but at a reduced weight level (Figure 2). These results suggest

Figure 2. The effect of LHA lesions followed by vagotomy (●) on measures of plasma insulin, food intake and body weight. LHA lesion-sham vagotomy (o) and sham lesion-vagotomy (x) groups are included for comparison. Body weight of the LHA-Vag group is also expressed as a percentage of the Sham-Vag control group.


INSULIN (μU/ml.)
60
40
20
0
FOOD INTAKE (gms.)
40
20
0
BODY WEIGHT (gms.)
400
350
300
250
WEIGHT (LH/C x 100)
100
90
80
70
1
2
3
4
5
6
7
8
9
TIME IN WEEKS

weight gain in all animals by the third week post-lesion. The effect of both LHA lesion and subsequent vagotomy on body weight is most clearly seen when the weight of the LHA-Vag group is expressed as a percentage of the Sham-Vag control group (lower graph, Figure 2). Following the decline during the week following LHA lesion, weight gain was then seen at a rate which closely matched that of controls over the next 3 weeks. Following vagotomy, weight in both LHA-lesioned and control animals declined slowly. Although not measured quantitatively, all animals ate and drank during the days immediately following vagotomy. The vagotomy effect was, if anything, slightly larger in the LHA-lesioned group (as can be seen on the Weight (LH/C x 100) portion of Figure 2). Body weight of the LHA-Vag group was lower (at least $p < .01$ in all cases) than that of the Sham-Vag group throughout the 4 weeks following LHA or Sham lesion and 4 weeks of observation following vagotomy.

Food intake of the LHA-Vag group was below that of the Sham-Vag ($n = 8$) group when tested 2 weeks following LHA or Sham lesion ($p < .01$) and 2 and 4 weeks following vagotomy

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that, in general, the LHA lesions produced in the present experiment resulted in reduced weight and low intake without substantial difficulty in regulating fluid balance. Whether these animals would have had difficulty in coping with specific, induced hydroregulatory challenges is not known.

($p < .01$ and $p < .05$ respectively).

Basal gastric acid secretion did not differ between LHA-Vag (2.60±0.91 μE H+/15 minutes) and Sham-Vag (2.83±0.61 μE H+/15 minutes) groups ($p > .20$). The F ratio never reached significance at any of the 4 insulin measurement times (see Appendix B).

2.3.3 Discussion

These results demonstrate that an intact vagus is not necessary to sustain either the weight reduction or the hypophagia which results from electrolytic lesions of the LHA. This conclusion is in agreement with that reached by Powley and Opsahl [1976]. Also in agreement with these authors, basal gastric acid secretion did not differ between vagotomized animals who had previously either sustained an LHA lesion or a sham operation. A reduction in gastric acid secretion then appears not to be the cause of the reduced level of weight maintenance following LHA lesions.

Neither LHA lesions nor vagotomy had any effect on basal insulin levels. This is consistent with the results of Steffens, Mogenson and Stevenson [1972]. Both sets of results must be interpreted with caution, however, for two reasons. One obvious problem is the use of human insulin standard. This may tend to obscure differences depending on whether human insulin is more or less reactive than rat

insulin with the particular insulin binding agent used. Secondly, the differences between LHA-lesioned and control animals, if they exist, are obviously small and would require that the assay be carefully adjusted to maximal sensitivity at the rat's normal basal level of insulin if differences are to be detected.

It is perhaps surprising that in spite of the parallels frequently drawn between the VMH and LHA lesion preparations, little is know of insulin-glucose relationships following LHA manipulations. Electrical stimulation of the LHA has been reported to produce an increase in plasma insulin [Idahl and Martin, 1971; Kuzuya, 1962; Steffens, Mogenson and Stevenson, 1972] and, variously, an increase [Natelson, Stokes and Smith, 1972], no effect [Frohman and Bernardis, 1971], a decrease [Kurotsu, Tabayashi and Ban, 1953; Shimazu, Fukuda and Ban, 1966] and a triphasic (increase-decrease-increase) change [Booth, Coons and Miller, 1969] in blood glucose. No interpretable data exist regarding the effects of LHA lesions on insulin or glucose levels. As pointed out, it is perhaps unreasonable to expect a reduction in the already very low basal insulin values [present experiment; Steffens, Mogenson and Stevenson, 1972]. The stimulated insulin levels of this latter study are marred by the absence of appropriate control animals. Chlouverakis and Bernardis [1972] attempted to gather basal

data in lean and obese (obob) mice. Unfortunately, following the placement of LHA lesions all the lean mice died. Their surviving obese mice showed a pattern of weight maintenance at a reduced level for 2 months following the lesions and there is some suggestion of a reduced insulin level in these animals; especially in 2 whose weight had been reduced prior to surgery. The "obob" mouse does, however, present an obese-hyperglycemic syndrome and the reduction of insulin levels may be an indirect result of a decrease in glucose levels. Despite the absence of any relevant data, reduced insulin levels in LHA-lesioned animals have been used as a key argument for a causal relation between insulin and weight maintenance level [Woods, Decke and Vasselli, 1974; Woods and Porte, 1976].

Unfortunately, given access to only a human insulin assay set-up (requiring from each rat a substantial amount of blood) it was not possible to pursue this point. Proper insulin response—glucose tolerance curves are needed for both control and LHA-lesioned animals maintaining reduced weight levels. The oral procedure employed by Louis-Sylvestre [1971] as well as the procedure using an i.v. bolus of glucose would provide valuable information.

It was perhaps unrealistic to anticipate that a disruption of parasympathetic innervation of the viscera would ameliorate the weight reduction of the LHA syndrome.

The tonic effect of the vagus on weight and intake occurs over the first 2 weeks following vagotomy, probably due to gastric stasis and the "dumping" phenomenon well known from human work. However, intake and weight gain soon return to near normal. If the weight and intake reductions of the LHA syndrome were to depend solely on a reduction of vagal tone, then one would anticipate that the maximal effects on intake and weight which could result from LHA lesions would be equal to those generated by vagotomy.

2.4 Experiment 3: LHA-Sympathectomy

The failure of vagotomy to reverse the LHA syndrome in spite of the reciprocal effects of LHA and VMH stimulation of visceral function requires a reevaluation of the nature of the change induced by LHA lesions. Explicit in the classic notion of hypothalamic control of food intake has been the idea that the VMH functions by inhibiting LHA activity. The amelioration of the VMH syndrome by vagotomy (i.e., cutting of the parasympathetic output which has close anatomical identification with the LHA) further reinforces this supposition.

There would then seem two possibilities to account for the LHA effects. LHA lesions, unlike VMH lesions and in spite of the reciprocal effects of LHA and VMH stimulation, may not depend on modulation of visceral activity for their effect. The second alternative assumes, as the basis of the LHA effect, that the parasympathetic LHA inhibits the sympathetic VMH; in other words, there may be a symmetrical hypothalamic system whereby the VMH effect owes its existence to an ascendancy (i.e., disinhibition) of parasympathetic innervation of the viscera and the LHA to an overbalance in the direction of sympathetic innervation.

Consistent with the second hypothesis, Opsahl [1973] showed a mild amelioration of the LHA weight effect with adrenal demedullation. The results took the form of a

slight decrease in weight in the control animals which did not occur in the LHA group.

The anatomy of the sympathetic innervation of the viscera is not, however, as clearcut as in the case of the parasympathetic system which houses its efferent, and afferent, pathways in a single pair of vagal nerve trunks. Besides the adrenal medulla, recent results would seem to suggest that direct sympathetic nervous connections are capable of modulating visceral activity, and, in particular, endocrine pancreatic secretion [Miller, 1975]. If the LHA has its effect on intake and weight in a manner parallel to that of the VMH it may be via these connections. The following experiment examines the effect of destruction of the direct, nervous, sympathetic innervation of the viscera on the LHA syndrome.

Of the techniques available for sympathectomy, surgery is difficult on this rather diffuse system, and chemical destruction by either nerve growth inhibitory factor (immunosympathectomy,[see Levi-Montalcini and Angeletti, 1966]) or 6-hydroxydopamine [Angeletti and Levi-Montalcini, 1970] affects central and peripheral nervous tissue and requires that treatment be carried out in neonates in order to induce permanent destruction. Any later failure to observe an LHA lesion weight reduction could then be as much a result of ineffective lesions as of a positive sympathec-

tomy effect. Fortunately, a third chemical method of producing SNS destruction has recently been demonstrated. Guanethidine sulphate has been reported to produce widespread, essentially complete destruction (greater than 98% at the level of the cervical ganglion, according to Burnstock, Evans, Gannon, Heath and James,[1971]) of the SNS. It has the additional advantages of being effective in adult animals and of not crossing the blood-brain barrier and thus, when injected peripherally, of having no central effects.

The following experiment was therefore designed to examine the effect of peripheral sympathectomy induced by guanethidine sulphate (GS) on the food intake, water intake and weight gain of male rats with, and without, lateral hypothalamic lesions. In addition, since no data appeared available on the effects of peripheral sympathectomy on intake and weight gain in normal, adult animals, an appropriate control group was added to allow this comparison.

2.4.1 Method

Subjects were, as in the previous experiment, adult, male Wistar-derived rats from the breeding colony of the John Curtin School of Medical Research (Canberra, Australia). Animals were randomly assigned into 4 groups. One group (LHA-GS; n = 9) received LHA lesions followed 4 weeks later by initiation of GS (Ismelin, kindly supplied by CIBA-GEIGY) treatment daily (25 mg/kg i.p.) for 6 weeks. A second

group (LHA-Sham; n = 9) received LHA lesions followed by injections of physiological saline. One group of control animals (Sham-GS; n = 9) received sham lesions followed by GS. A second control group (Sham-Sham; n = 9) received sham lesions followed by saline.

Food and water intakes were measured in 24-hour periods before and 2 and 4 weeks following LHA or sham lesions, bi-weekly during GS or saline treatment, and weekly for 6 weeks following sympathectomy. Weight was measured on the day before LHA or sham lesion, daily until weight gain was seen following the lesions and recorded weekly thereafter.

The lesion, and sham lesion, technique was as described in Experiment 1 with coordinates and parameters and maintenance following the lesions as in Experiment 2.

Statistical analysis was done using a one-way, fixed effects analysis of variance at useful time points followed, where appropriate, by Scheffé individual comparisons.

2.4.2 Results

The data are summarized in Figures 3, 4, and 5 (see Appendix C for the F ratios). Mean body weight, food intake and water intake of both LHA-lesioned groups remained significantly ($p < .01$ in all cases) below that of their appropriate control groups from the time of the first measurement following the lesion through the duration of the

Figure 3. The effect of guanethidine sulphate sympathectomy on the mean food and water intakes of groups of LHA lesioned (---o---) and sham lesioned (---●---) male rats. LHA lesioned-saline injected (——o——) and sham lesioned-saline injected (——●——) groups are included for comparison. The dotted line under the abscissa indicates the 6 weeks of injection of either guanethidine or saline.

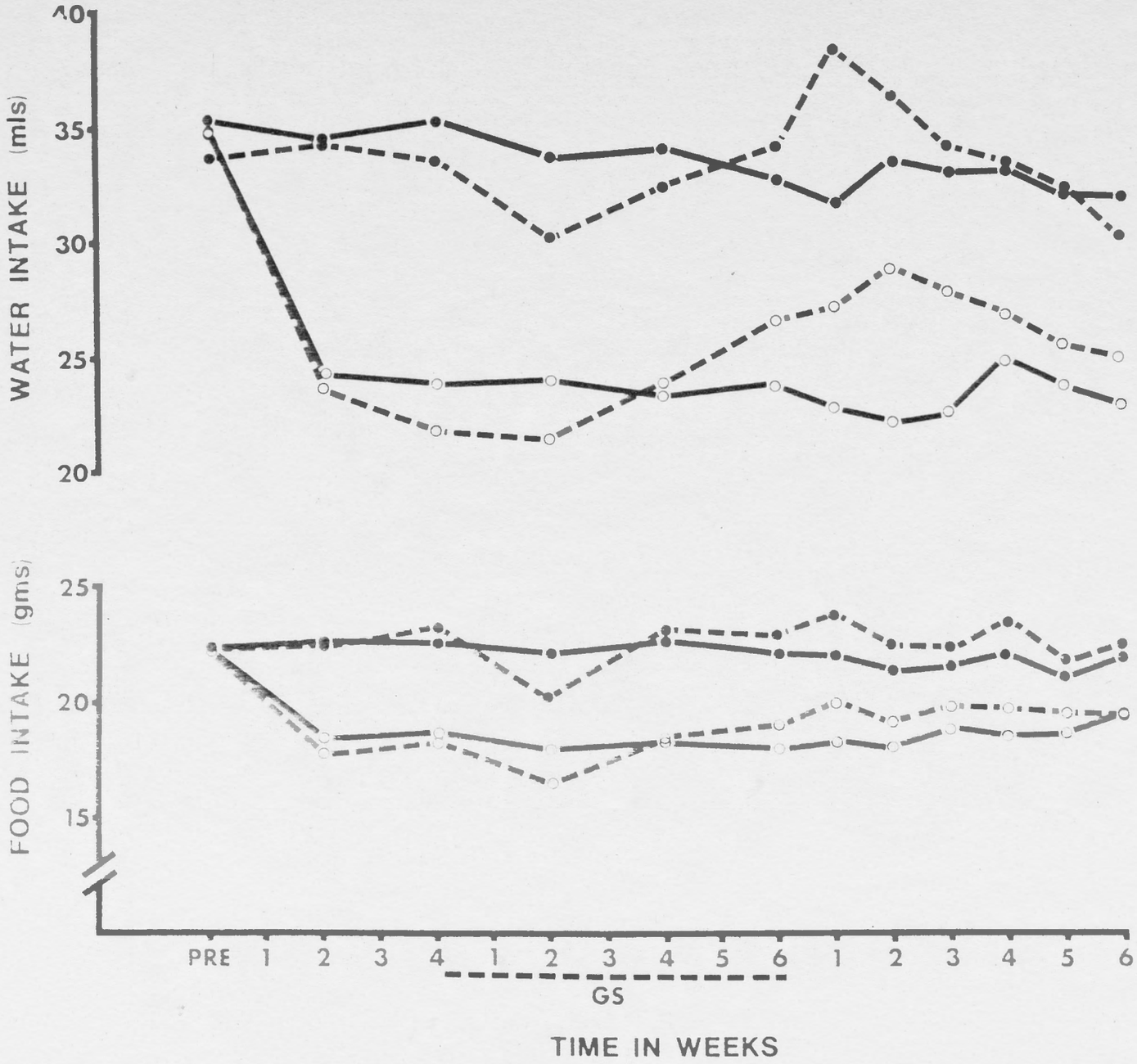

WATER INTAKE (mls)
40
35
30
25
20
FOOD INTAKE (gms)
25
20
15
PRE 1 2 3 4 1 2 3 4 5 6 1 2 3 4 5 6
GS
TIME IN WEEKS

Figure 4. The effect of guanethidine sulphate sympathectomy on the mean body weights of groups of LHA lesioned (---o---) and sham lesioned (---●---) male rats. LHA lesioned-saline injected (——o——) and sham lesioned-saline injected (——●——) groups are included for comparison. The dotted line under the abscissa indicates the 6 weeks of injection of either guanethidine or saline.

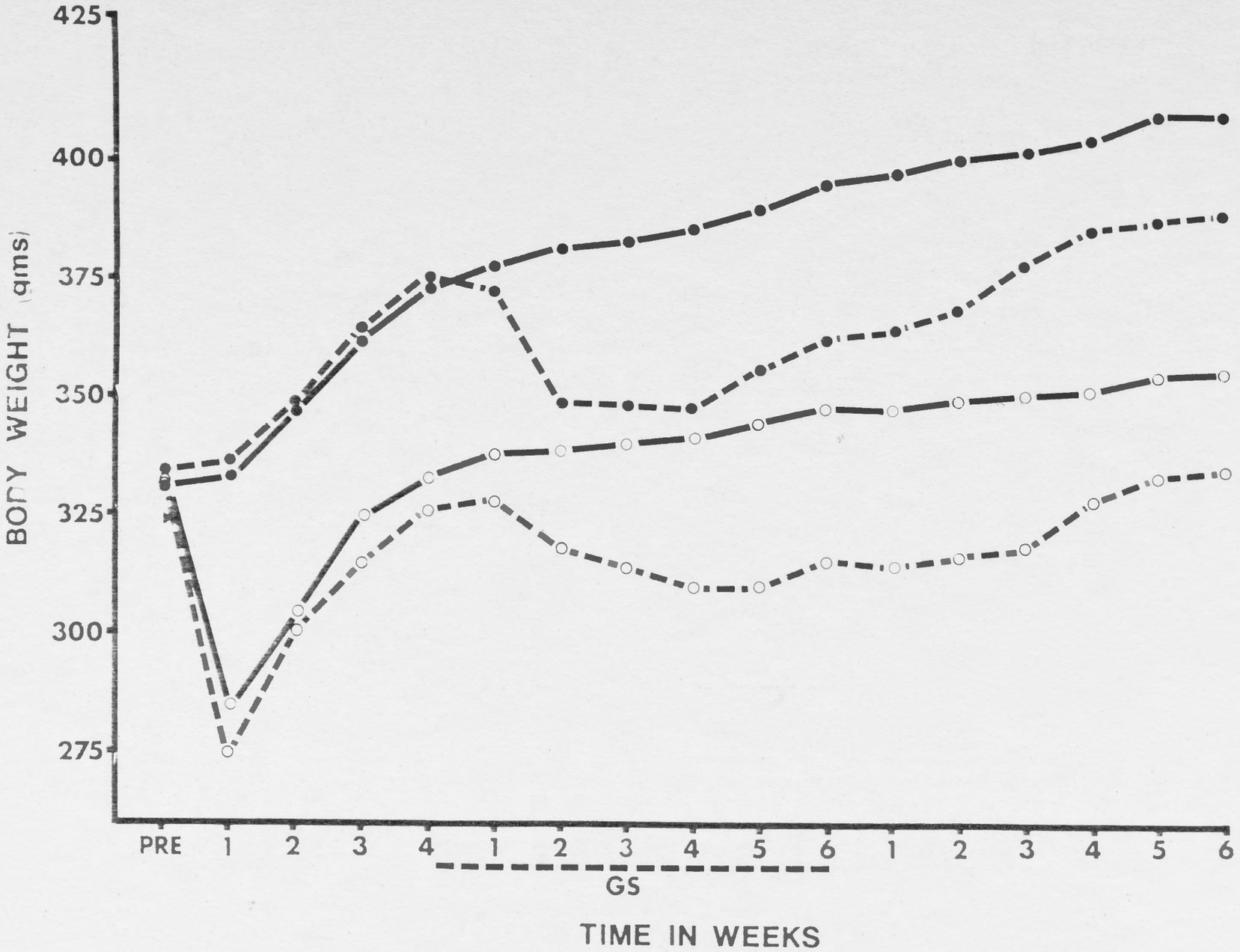
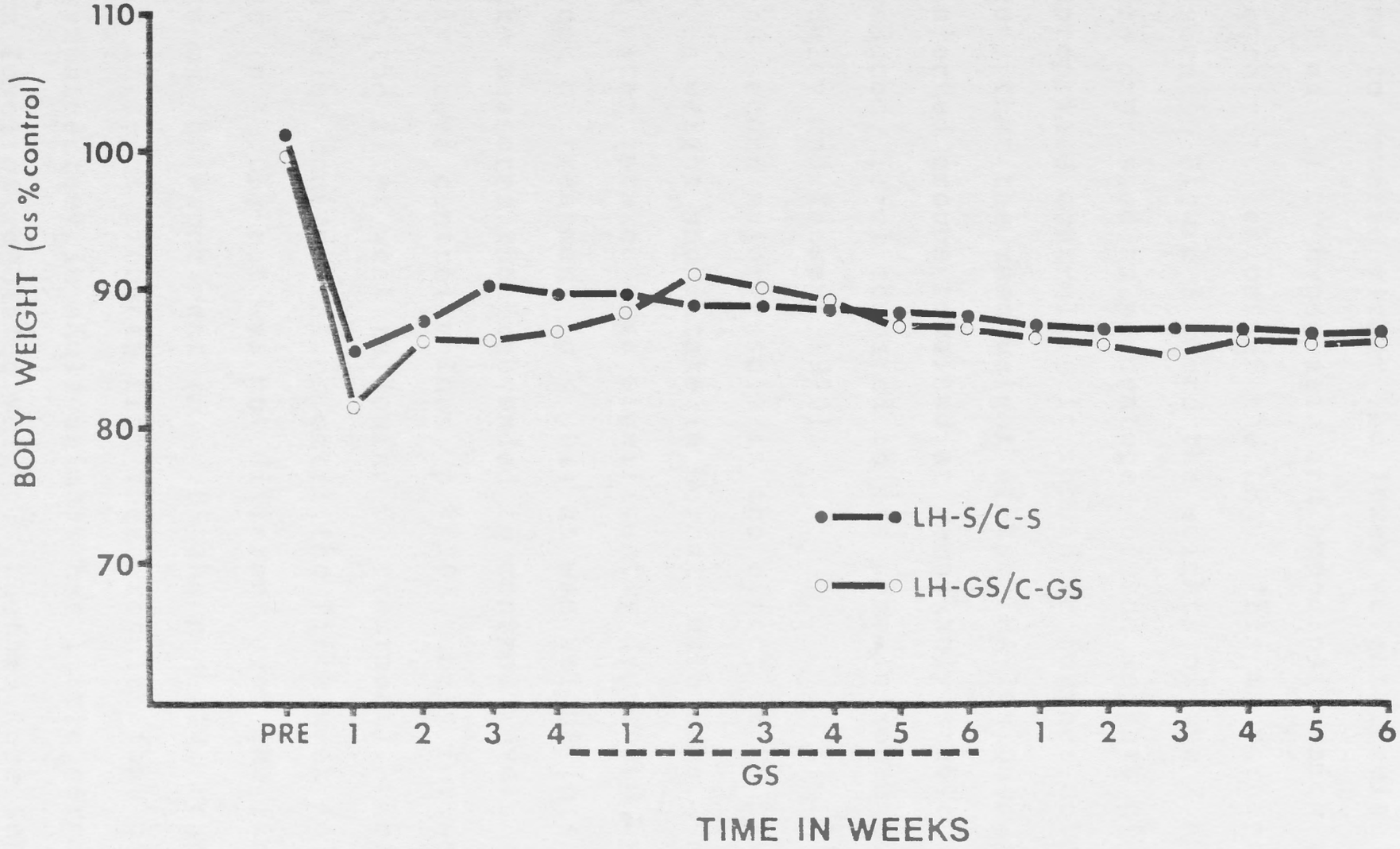

425
400
375
350
325
300
275
BODY WEIGHT (gms)
PRE 1 2 3 4 1 2 3 4 5 6 1 2 3 4 5 6
GS
TIME IN WEEKS

Figure 5. Body weights of Figure 4 with the LHA lesioned groups expressed as a percentage of their appropriate controls.


BODY WEIGHT (as % control)
110
100
90
80
70
PRE 1 2 3 4 1 2 3 4 5 6 1 2 3 4 5 6
GS
TIME IN WEEKS
LH-S/C-S
LH-GS/C-GS

experiment. Thus the major finding is the failure of sympathectomy to reverse either the lower weight levels (Figures 4 and 5) or hypophagia and hypodipsia which result from electrolytic lesions of the LHA. This is perhaps most clearly seen in Figure 5 where the weights of the 2 LHA groups are expressed as percentages of the weights of their appropriate controls. It should be further noted from this figure that the mean weight of the LHA-lesioned, saline-injected groups remained at remarkably stable, although reduced, level compared to its sham-lesioned control group [Powley and Keesey, 1970].

The second major result is the effect of GS sympathectomy on weight and intake in normal, male rats. Both food and water intakes were significantly depressed by the second week of treatment ($p < .01$) as was weight ($p < .01$). The intake measures then rebounded to control levels (significantly above control values, $p < .05$, only for water intake in the first week following GS treatment). Weight remained below control values until the fifth week following treatment ($p < .05$) but was not different over the final two weeks of the experiment ($p < .05$ and $p < .10$, respectively). It seems probable from these results that peripheral sympathectomy in adult animals has little permanent effect on level of regulated weight. Whether more subtle feeding effects occur is a matter for further experimenta-

tion.

2.4.3 Discussion

The result of this and the previous experiment suggest that whatever the nature of the change following LHA destruction, which is reflected in a level of weight significantly below that of control animals, the change does not depend on either direct neuronal input from, or output to, the autonomic nervous system. Thus the LHA syndrome is not a perfect reciprocal of that condition observed following VMH destruction.

There is the possibility of a complex interaction between the normally short-duration actions of the direct nervous sympathetic innervation of the relevant visceral organs and the longer term results of adrenal medulla activation. Alternatively, some recovery of function following destruction of one or the other segments of this complex system may be possible, presumably due to a combination of hypertrophy in the remaining part of the system and a post-denervation, post-synaptic hypersensitivity [Evans, Iwayama and Burnstock, 1973]. At present too little of these mechanisms is known to comment meaningfully on these possibilities.

CHAPTER THREE

VMH INSULIN-GLUCORECEPTORS

3.1 Introduction

The major result of Chapter Two was the reversal by vagotomy of both the hyperinsulinemia and increased weight resultant upon electrolytic lesions of the VMH. While this finding certainly does not constitute proof, it is consistent with the notion that VMH hyperphagia and obesity are the result of a primary oversecretion of insulin. Thus we have the following two sets of overlapping results. First, electrical stimulation of the VMH yields hyperglycemia and a suppression of insulin secretion [Frohman and Bernardis, 1971; Miller, 1975]. VMH lesions lead to hyperinsulinemia [Hales and Kennedy, 1964] which seems dependent upon increased vagal activity [Experiment 1; Frohman, Ezdenli and Javid, 1967; Kaneto, Kosaka and Nakao, 1967]. Secondly, glucose infused i.v. or applied locally effects an increase in the firing rate of certain VMH neurons while insulin results in a decrease [Anand, Chhina, Sharma, Dua and Singh, 1964; Oomura, Ono, Ooyama and Wayner, 1969; Oomura, 1973]. Both sets of results are consistent with the notion of a primary role for the VMH in glucoregulation. The critical missing result is the one linking the two sets of data. That is, changes in the

levels of VMH insulin or glucose have been shown to effect changes in VMH activity and changes in VMH activity have been shown to result in alterations in glucose and insulin secretion. However, the VMH activity changes induced by insulin or glucose have not been shown to have direct glucoregulatory effects. The experiments described in the following chapter focus upon this point.

3.2 Experiment 4: VMH Insulin Injections

Numerous studies have established the existence of CNS insulin-sensitive receptors whose outputs are involved in peripheral regulation of glucose [Anderson and Hazelwood, 1969; Chowers, Lavy and Helpern, 1961; Szabo and Szabo, 1972; 1974; Woods, Chen and Porte, 1974]. Insulin, in these studies, has either been injected into the cerebral ventricular system or, in the cases of Szabo and Szabo [1972; 1974], into the carotid artery. Hypoglycemia consistently results from both methods but using these procedures the dynamics and location of insulin receptor sites cannot be established. The ventromedial nuclei of the hypothalamus, however, offer a most likely locus. Insulin itself is known to effect a decrease in activity of spontaneously discharging VMH neurons [Anand, Chhina, Sharma, Dua and Singh, 1964; Oomura, Ono, Ooyama and Wayner, 1969; see also Oomura, 1973]. While insulin generally is not thought to facilitate glucose uptake in the brain [Crone, 1965], the VMH appears to be an exception [Debons Krimsky, Likuski, From and Cloutier, 1968; Smith, 1972]. The VMH is part of the sympathetic nervous system [Ban, 1964; 1966] and electrical stimulation results both in an increase of plasma glucose and a suppression of insulin release which would otherwise normally accompany the rise in systemic glucose levels [Frohman and Bernardis, 1971]. The

following experiments were designed as an attempt to localize the brain area and outflow pathways of the central insulin receptors.

3.2.1 Method

Experiments were performed on male, Wistar-derived rats of 300 to 350 grams, obtained from the John Curtis School of Medical Research (Canberra, Australia). They were 4 hour fasted. Anesthesia was induced with sodium pentobarbital (50 mg/kg i.p.), and the animal was placed in a stereotaxic unit. A microsyringe was mounted directly on the stereotaxic electrode carrier and the microsyringe needle (o.d. 0.40 mm) was used for the direct, unilateral injection into the brain. Only one injection was made per animal. The needle was positioned 1 mm above the injection sites 5 minutes before injection. The first blood sample was then taken, the needle lowered into position, 1 microlitre of either insulin (40 milliunits - crystalline zinc insulin) or denatured insulin injected over 30 seconds, and the needle removed one minute later. The original blood sample and samples at 5, 10, 20, 30 and 60 minutes post-injection were taken from the cut end of the warmed tail and assayed for glucose by the ortho-toluidine method [Hyvarinen and Nikkila, 1962]. In these and all subsequent glucose assays the blood was taken directly into small tubes into which 2 to 3 drops of 4% sodium

fluoride and 4% potassium oxalate had previously been placed and allowed to dry. In all normal animals of the following four experiments the initial pre-injection range of blood glucose was 95-107 mg% and all post-injection values are expressed as either an increase or a decrease relative to each animal's own initial value. Following the last sample the animal was killed with ether and the brain removed and hardened in 10% formalin. Sections were cut at 30 microns and every second section through the injection site stained with thionin.

3.2.2 Results

In this initial experiment insulin injections were made into the VMH, LHA and cortex.

Figure 6 shows the injection sites. Insulin was injected into 8 sites in the VMH, 5 in the LHA, and 4 in the cortex. Denatured insulin was injected into 4 VMH locations. Figure 7 shows the changes in blood glucose. One-way, fixed effects analyses of variance showed a significant effect of injection area at 5, 10 and 20 minutes following insulin injection (F ratios are given in Appendix D). Scheffé comparisons revealed a significant drop in blood glucose following insulin injection into the VMH (compared to denatured insulin) at 5 ($p < .01$), 10 ($p < .01$), and 20 ($p < .05$) minutes. No other individual comparisons were

Figure 6. Sections redrawn from the brain atlas of Pellegrino and Cushman [1967]. Numbers near the top of the sections denote the anterior-posterior coordinates of the sections in relation to the interaural line. Open circles indicate insulin injection sites where the blood glucose had changed less than 5 mg% by the 5 minute sample; half-filled circles indicate a drop of 5-10 mg%; and filled circles a drop of greater than 10 mg%. Open squares show injections of denatured insulin none of which produced a change larger than 3 mg%. Two extra sites are shown but not included in the analysis, one in the AHA and one ventral to the VMH.

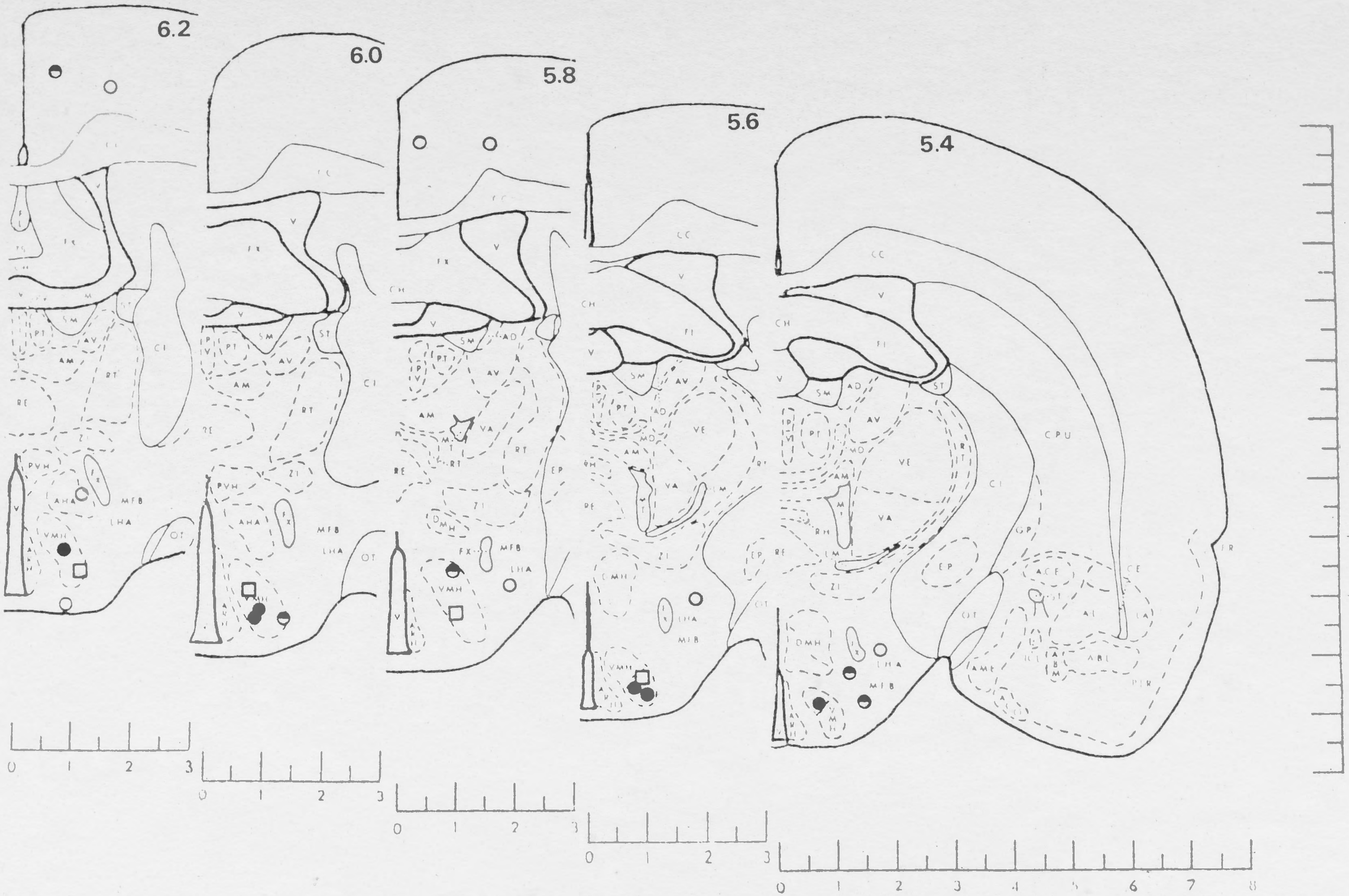
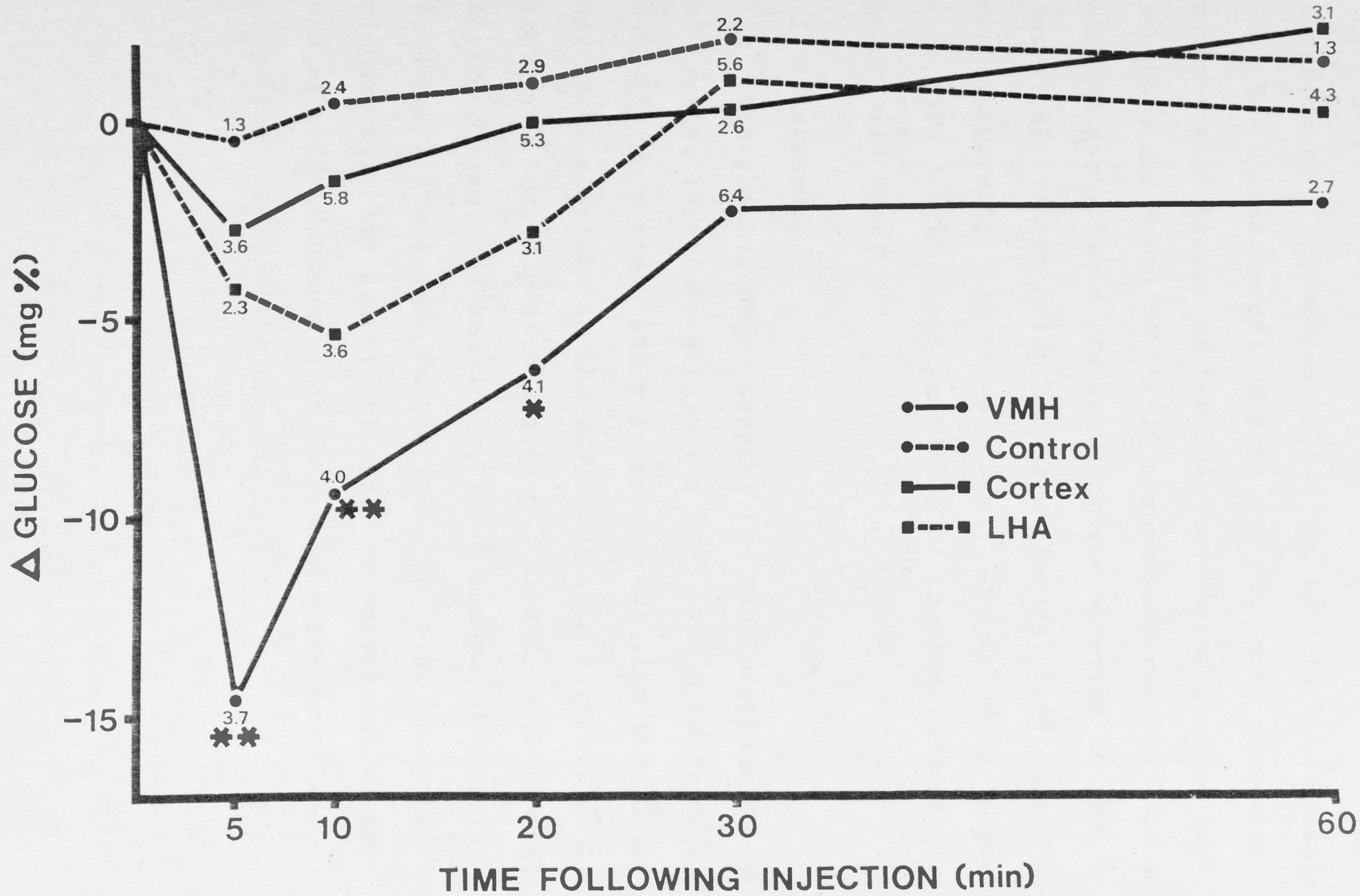

6.2
6.0
5.8
5.6
5.4
CC
FX
SM
AV
AM
RT
CI
PVH
MFB
LHA
VMH
AHA
OT
VA
ZI
VE
EP
DMH
MFB
CPU

Figure 7. Changes in blood glucose in the hour following injection of insulin into the VMH (n = 8), LHA (n = 5), or cortex (n = 4) and denatured insulin into the VMH (Control; n = 4). Standard deviations are given by the number above or below the data points. Double asterisks indicate significant difference between VMH and Control groups at the 0.01 level and the single asterisk at the 0.05 level.


0
−5
−10
−15
Δ GLUCOSE (mg %)
1.3
2.4
2.9
2.2
3.1
1.3
5.6
4.3
2.6
5.3
5.8
3.6
2.3
3.6
3.1
2.7
6.4
4.1
4.0
3.7
VMH
Control
Cortex
LHA
5
10
20
30
60
TIME FOLLOWING INJECTION (min)

significant. The decrease following injection of insulin into the LHA was largely due to two rats with placements on the medial border of the LHA whose blood glucose had dropped 8 and 9 mg% by the 10 minute sample. The 14.6 mg% mean VMH group blood glucose decrease observed in the present experiment using only a unilateral injection compares favourably with the 27-28 mg% drop found by Szabo and Szabo [1972] with total perfusion of insulin induced via the carotid artery.

3.2.3 Discussion

The results offer support for the localization of CNS insulin receptors within the VMH. The hypoglycemia found in the present study is consistent with the notion that insulin uptake in the VMH results in a reduction in electrical activity of certain specialized cells [Anand, Chhina, Sharma, Dua and Singh, 1964; Oomura, Ono, Ooyama and Wayner, 1969] and, further, is the first evidence that this activity has direct relevance to peripheral glucoregulatory mechanisms. The following experiments are designed to clarify the present result.

3.3 Experiment 5: Ventricular Insulin Injections

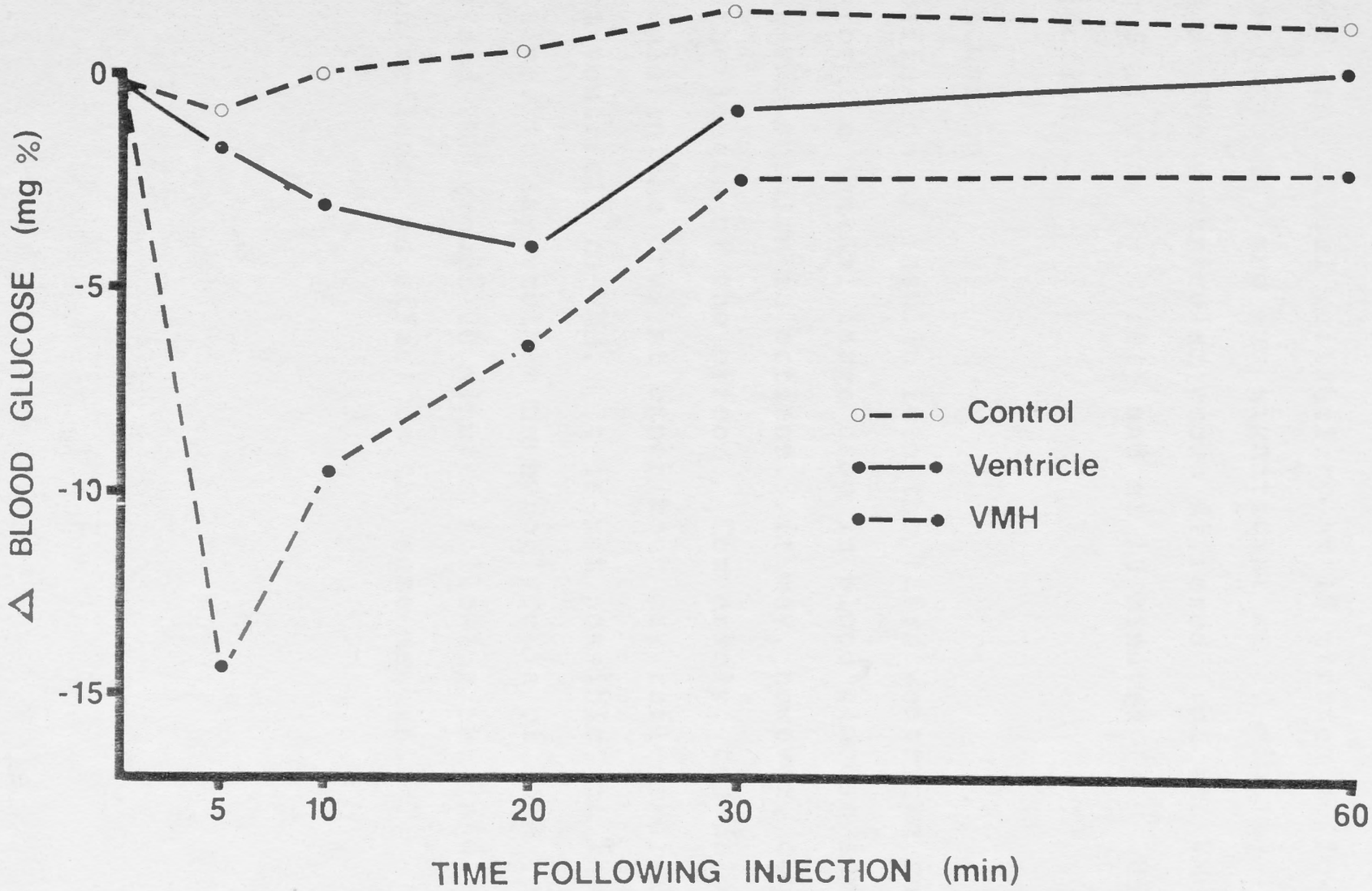
Most of the studies which have demonstrated the hypoglycemic effect of centrally applied insulin have used injections of insulin into the cerebral ventricular system [Anderson and Hazelwood, 1969; Chowers, Lavy and Helpern, 1961; Woods, Chen and Porte, 1974]. The VMH lies in close proximity to the third ventricle. Hence, the hypoglycemia observed in Experiment 4 may have been due to spread of insulin into this ventricle. This possibility was explored in the following experiment.

3.3.1 Method and Results

Subjects and methods were as in the previous experiment with the following alterations. A larger, elliptical hole was created in the skull on the midline and the sagittal sinus was partially exposed. The microsyringe was tilted just off vertical and entered the brain along side the sinus. The needle was then lowered to within 3 mm of the injection site. The first blood sample was then taken and the remainder of the procedure was identical to that of the preceeding experiment.

The results of injection of insulin into the third ventricle of 4 animals are shown in Figure 8. The VMH and control groups of Experiment 4 are shown for comparison and used for the statistical comparisons by one-way, fixed effects analysis of variance (see Appendix E for the $\underline{F}$

Figure 8. Changes in blood glucose in the hour following injection of insulin into the third ventricle (n = 4). The VMH-insulin and VMH-denatured insulin groups of Experiment 4 are included for comparison. Injection of insulin into the ventricle caused a significant depression ($p < .05$) in blood glucose only at 20 minutes following injection.


0
-5
-10
-15
Δ BLOOD GLUCOSE (mg %)
5
10
20
30
60
TIME FOLLOWING INJECTION (min)
Control
Ventricle
VMH

ratios) followed by Scheffé comparisons. Ventricular injection of insulin caused a decrease in blood glucose which approached statistical reliability at 10 minutes following injection ($p < .10$) and was significant at 20 minutes ($p < .05$). The ventricular group differed from the VMH group at 5 minutes ($p < .01$) and at 10 minutes ($p < .05$) post-injection.

3.3.2 Discussion

Diffusion of insulin into the third ventricle cannot account for the initial large drop in blood glucose seen following VMH insulin injections. It may, however, contribute to the length of the effect. Conversely, the delayed hypoglycemia in the present experiment may reflect diffusion from ventricle to VMH. It is not possible, at the present time, to say whether the hypoglycemia of both ventricular and VMH groups 20 minutes following the insulin injection reflects an effect on the same mechanism.

3.4 Experiment 6: VMH Insulin in Diabetics

The emphasis thus far has been on the role of the VMH in glucoregulation via its control of insulin secretion. Glucoregulation, however, seems most reasonably viewed as integrated patterns of visceral activity initiated to facilitate either increased availability or increased disposal of glucose. The diabetic animal presents a preparation with exaggerated rates of both hepatic glucose output and free fatty acid efflux but only very limited capacity to alter insulin secretion in the face of a glucoregulatory challenge. If mechanisms other than altered insulin contribute to the hypoglycemia following insulin injections into the VMH, then they may be sufficiently visible to result in hypoglycemia following VMH insulin in the diabetic rat.

3.4.1 Method and Results

Subjects were male rates of the same age and origin as those of Experiment 4. Diabetes was induced with a single i.p. injection of streptozotocin (60-65 mg/kg in citrate buffer pH 4.5) at least 3 weeks before the experiment. Pilot work had shown severely diabetic rats (blood glucose >350 mg%) react badly to the stress of anesthesia and blood loss. Animals were therefore screened for degree of diabetes 1 week before the present experiment. After a 6 hour fast they were lightly anesthetized with ether and a blood sample obtained from the tail. Blood glucose was analyzed and animals whose blood glucose levels

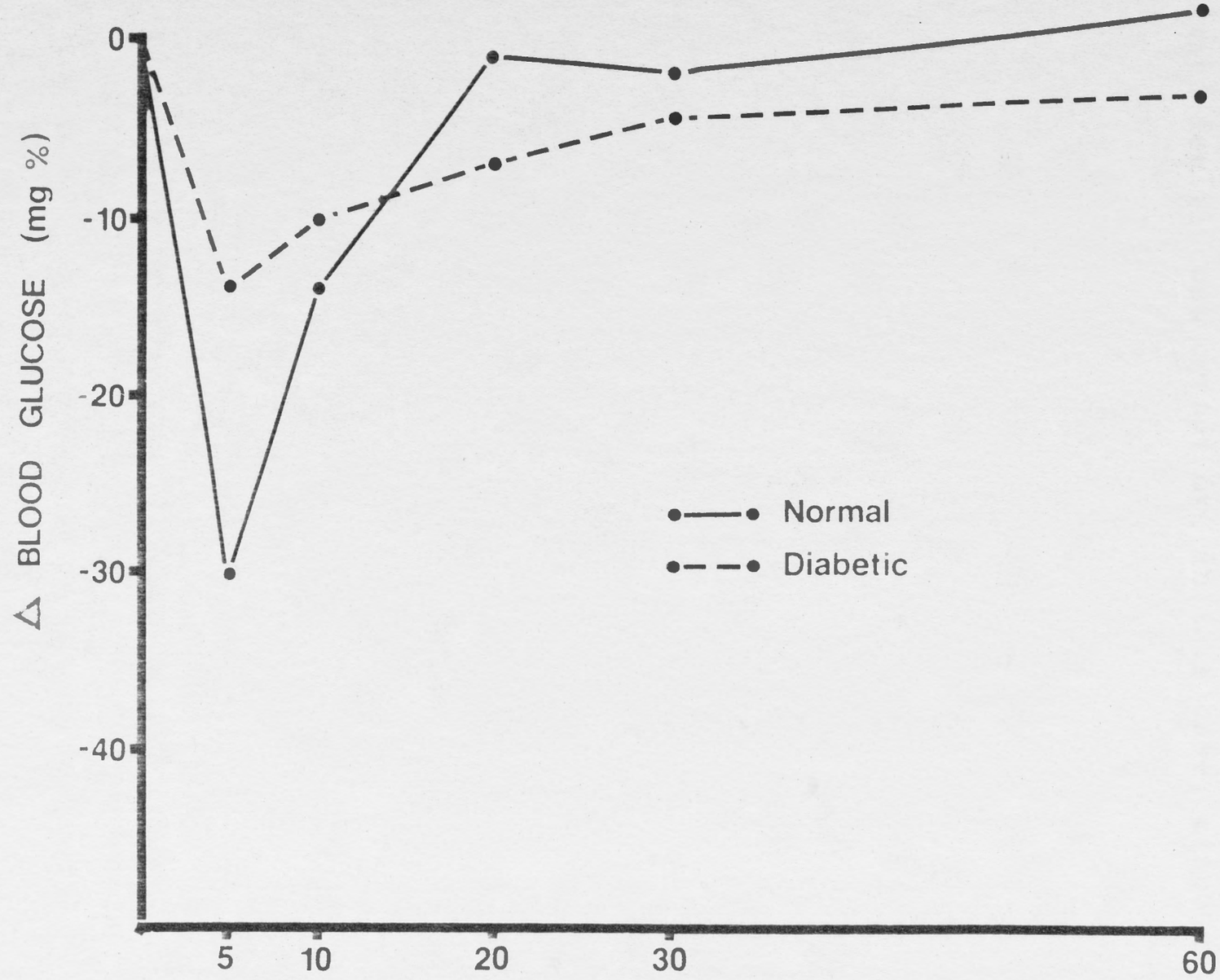
were between 250 and 350 mg% were retained for use in the present experiment. All other procedures were as in Experiment 4.

Results are shown in Figure 9. Since anesthetic and blood sampling tended to reduce the blood glucose of diabetics a certain amount over time, the 2 lines represent blood glucose changes of the diabetic animals of the present experiment and the VMH animals of Experiment 4 (for comparison), both corrected for appropriate control group changes (the blood glucose of each experimental animal was adjusted for the mean change in its control group at each time point). Four diabetic animals were found to have injection sites within the VMH. Their blood glucose drop (adjusted relative to 3 denatured-insulin diabetic controls) was significantly larger at 5 minutes compared to the VMH group ($t = 2.74$; $p < .05$ for a two-tailed test) but tended to be less severe at 20 minutes ($t = 1.83$; $p < .10$ for a two-tailed test). If this 20 minute difference were found to be reliable with a larger group of animals, it might be interpreted as reflecting the contribution to the hypoglycemia of a slower pancreatic component.

3.4.2 Discussion

This experiment suggests that reduction in hepatic glucose output and/or decreased FFA efflux are significant contributors to the initial, marked hypoglycemia which

Figure 9. Changes in blood glucose in the hour following injection of insulin into the VMH of streptozotocin diabetic rats (n = 4). The VMH-insulin group of Experiment 4 is included for comparison. The blood glucose drop of both groups is corrected for changes in their appropriate VMH-denatured insulin controls. The diabetic group displayed a greater hypoglycemia at 5 minutes following injection ($p < .05$).


0
-10
-20
-30
-40
Δ BLOOD GLUCOSE (mg %)
Normal
Diabetic
5
10
20
30
60
TIME FOLLOWING INJECTION (min)

results from insulin injection into the VMH. This is consistent with the notion that it is an integrated series of metabolic events which accomplish the centrally-generated command for glucose output or, in this case, disposal.

3.5 Experiment 7: VMH Insulin following Vagotomy or Sympathectomy

The speed of the initial hypoglycemia following insulin application to the VMH suggests a neural, rather than hormonal, mechanism. Both sympathetic and parasympathetic nerves have been shown capable of modifying both hepatic glucose and pancreatic insulin secretion. Szabo and Szabo [1974] have already observed a partial reduction, following vagotomy, in the magnitude of the hypoglycemia attendant upon perfusion of the brain with insulin. Adrenergic alpha and beta block with phentolamine and propanolol, respectively, had no effect.

The following experiment looked at the effect on blood glucose of direct application of insulin to the VMH in vagotomized animals. In addition, the data of a single sympathectomized animal are presented.

3.5.1 Method and Results

Vagotomy was performed as in Experiment 1 and approximately 1 month was allowed for recovery. The vagotomozed animals were then at the age of the animals of Experiment 4 but weighed slightly less. The insulin injection procedure was identical to that described for Experiment 4 with the following exceptions: (1) no 60-minute sample was taken, (2) after the 30-minute sample the skull incision was sewn up and the animal was allowed to recover,

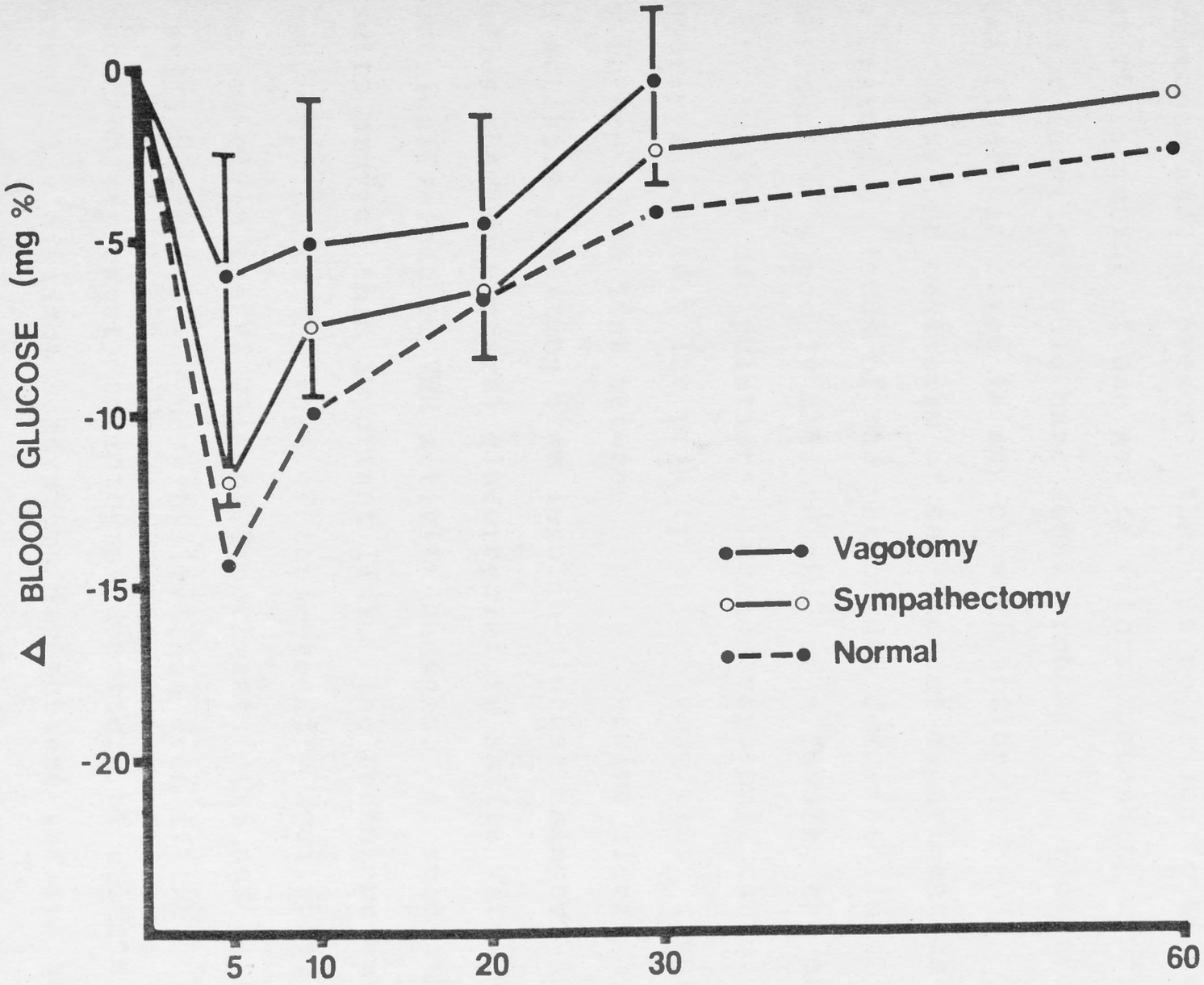
and (3) access to water, but not food, was allowed for the next 24 hours. An acid secretion test for completeness of vagotomy was then carried out as described in Experiment 2.

The brain was removed and the injection site localized as in Experiment 4. Five animals met the dual criteria of an insulin injection site within the VMH and complete bilateral vagotomy. Three vagotomized, denatured-insulin-injected animals served as controls. Only 2 sympathectomized animals were available and one had an accurate VMH insulin placement. The results, shown in Figure 10, are equivocal. Because of the rather large variability, mean glucose changes, corrected for control, are plotted for the vagotomized group and the range, at each time point, of individual blood glucose values is shown. The blood glucose drop in the vagotomy group is not significantly smaller than that of the VMH-insulin normals of Experiment 4 ($t = 1.18$; $p > .20$ for a two-tailed test). The single sympathectomized animal did not appear to react differently from a normal animal.

3.5.2 Discussion

The results support the view that the vagus plays a substantial role in the hypoglycemia which results from central application of insulin. The distressingly large variability in both the present data and those of Szabo and Szabo [1974] may again be due to the use of acid secretion

Figure 10. Means and corresponding ranges of variation in the 30-minute period following injection of insulin into the VMH of a group of vagotomized animals (n = 5) and a single guanethidine-sympathectomized animal. The VMH-insulin group of Experiment 4 is included for comparison.


0
-5
-10
-15
-20
Δ BLOOD GLUCOSE (mg %)
5
10
20
30
60
TIME FOLLOWING INJECTION (min)
Vagotomy
Sympathectomy
Normal

in response to hypoglycemia as the test for completeness of vagotomy. It is almost certain that the fibers subtending this test function are not the ones involved in the present phenomenon and, at present, there is no reason to believe that the severing of one set of fibers subtending a particular function would have equal probability of destroying vagal fibers involved in any other function.

A second criticism of the present experiment is that the anatomical locus of the phenomenon described in Experiment 4 is poorly defined and, as a result, the effect of any further manipulations, such as vagotomy, cannot be properly assessed. The primary objective of this chapter was to provide a link between studies showing alterations in VMH activity resulting from insulin-glucose changes, and studies showing visceral glucoregulatory shifts with electrically-induced VMH activity changes. As such, the results provide this important link. The technique employed represents the best of those readily available. However, given the results of Experiments 4, 5 and 6, there is still certainly the possibility that even if the phenomenon represents an integrated pattern of effects, anatomically distinct subsystems may subtend various aspects. Thus a reasonable mapping study would necessitate, at very least, insulin injections or glucose injections in separate groups of animals with controlled hepatic glucose output,

pancreatic alpha and beta output, or free fatty acid efflux. Such a study, given the tedious glucose assay available, was beyond the scope of this thesis. It may be also argued that direct application of insulin (or any drug) could provoke entirely abnormal tissue reaction. Thus, having demonstrated the phenomenon with one technique, employing a combination of other techniques, such as that of Szabo and Szabo [1972; 1974] and of knife cuts of specific pathways (the more likely ones could be severed without serious interruption of blood supply to critical areas), may be best to provide further confirmatory data.

3.6 Model of VMH Function and Discussion of the Model

The results presented in this chapter provide the necessary link between the studies showing that manipulations of hypothalamic activity have marked effects on blood glucodynamics and those showing that insulin and glucose have substantial effects on the firing patterns of certain hypothalamic neurons. If these data provide the basis for hypothesizing a central VMH role in glucoregulation, then there is need for a model which can explain the dynamics of this glucoregulatory system. An attempt to provide such a model is presented below. In overview, an insulin-glucoreceptor system is first devised to describe a group of VMH neurons, and the electrical activity of these cells is discussed in terms of their glucoregulatory effects. It is postulated that the VMH normally acts to stabilize glucose supply and that an oversecretion of insulin in reaction to the ingestion of food can account for the hyperphagia and weight gain resultant upon VMH lesions. Finally, the role of the VMH in long-term weight regulation and the effects of palatability on the final weight achieved are discussed in light of the model.

The following receptor system is then generated as a model of certain neurons within the VMH area whose activities are directly relevant to glucoregulation. It is given first, not because these particular neurons are

necessary to the conclusions reached, but because the receptor model does describe the existing data parsimoniously and will help in understanding how the proposed model of VMH functioning might work.

The following 4 rules are seen to describe the activity of these specialized cells:

(1) Insulin must be present at the receptor sites in order for them to be responsive to glucose. This proposal relies on the studies showing destruction of the VMH following goldthioglucose to depend on the presence of insulin [Debons, Krimski, Likuski, Cloutier and From, 1968; Smith, 1972].

(2) Activity in receptor sites not occupied by insulin is spontaneously high, irrespective of glucose levels.

(3) Glucose-free, insulin-occupied sites are quiescent. These latter 2 rules depend upon the studies of Anand, Chhina, Sharma, Dua and Singh [1964] and Oomura [1973] showing that an increase in circulating insulin levels or direct application of insulin reduces the electrical activity of certain VMH neurons.

(4) Glucose reactivates the insulin-occupied sites [Anand, Chhina, Sharma, Dua and Singh, 1964; Oomura, 1973].

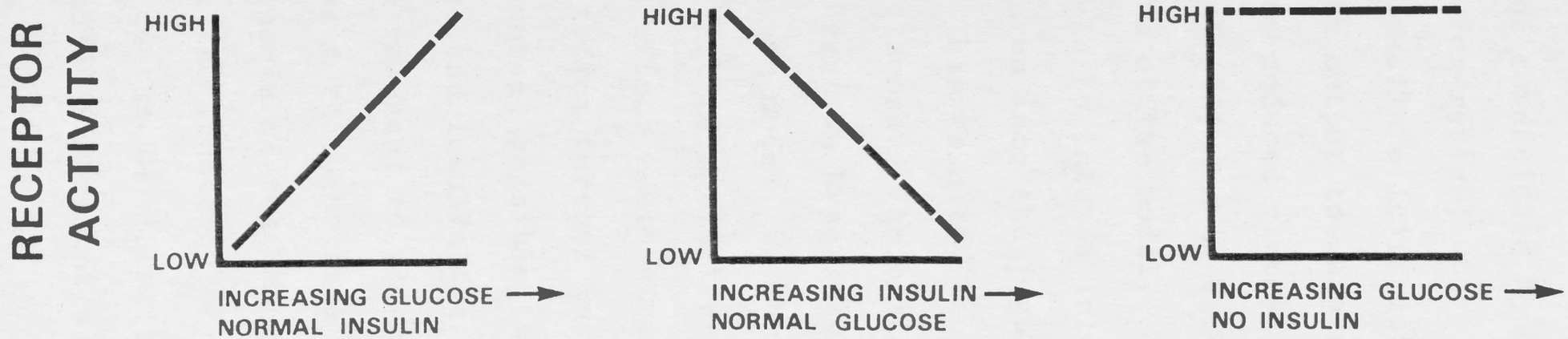
That is, empty or insulin-and-glucose-occupied sites are firing at maximal rate, but insulin-occupied sites are inactive. Figure 11 contains 3 examples of theoretical receptor activity graphs.

The model is interesting in that it predicts maladaptive reactions in the face of either no insulin or, at the opposite end of the scale, high or rising insulin levels in the absence of high blood glucose. Such reactions have been observed, however, under a number of circumstances.

With low insulin, glucose levels are less effective at modulating the activity of the VMH which is at a high level. This should produce further increases in blood glucose while further suppressing insulin output [Frohman and Bernardis, 1971; Shimazu, Fukuda and Ban, 1966]. Such anomalous behavior is, in fact, observed. Following injections of anti-insulin serum there is a prompt rise in blood glucose [Armin, Grant and Wright, 1960; Stern, Wagle, Sweeney and Ashmore, 1963] and in diabetes unrestrained hepatic glucose production is well documented [see Anderson, 1974].

Rising central levels of insulin (without concomitant rise in blood glucose) should diminish VMH activity thereby reducing glucose production. This anomaly also has been observed [Storlien, Bellingham and Martin, 1975, based on Experiment 4; Szabo and Szabo, 1972] and is particularly

Figure 11. Three examples of theoretical changes in insulin-glucoreceptor activity of the VMH with changes in the plasma concentration of insulin and glucose.


RECEPTOR ACTIVITY
HIGH
LOW
INCREASING GLUCOSE
NORMAL INSULIN
HIGH
LOW
INCREASING INSULIN
NORMAL GLUCOSE
HIGH
LOW
INCREASING GLUCOSE
NO INSULIN

clear in the case of conditioned hypoglycemia [see Woods and Kulkosky, 1976 for review] where exogenous (or stimulated endogenous) insulin elicits not the adaptive response of increased glucose output to maintain normoglycemia, but, in fact, causes reduced glucose production. The data of Szabo and Szabo [1972] are in excellent accord with the post-hoc predictions of the model. They used a preparation whereby insulin could be infused into the carotid artery and anti-insulin serum into the jugular vein to avoid systemic effects. This results, in the normal animal, in a decrease in blood glucose. The effect is much greater in diabetics and is absent in hyperinsulinemic animals. According to the present model, in the diabetic glucose output is maximal and subject to maximal decrease. In hyperinsulinemic animals, the effect would already be minimal and no change would result from further insulin.

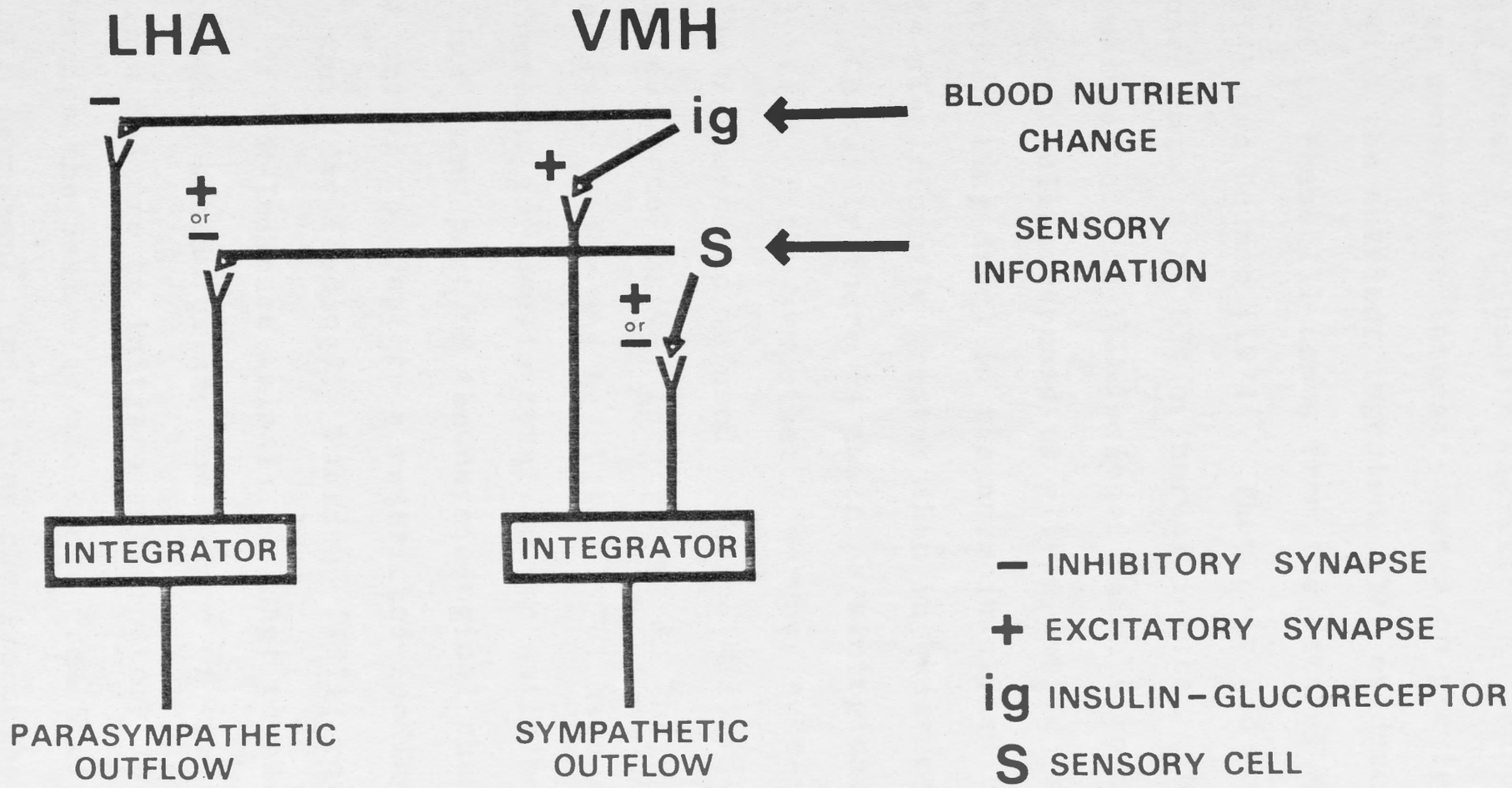
It is, of course, possible that the VMH neurons affected by glucose and insulin are independent of the neurons of passage relevant to glucoregulation, or inhibitory with respect to these same fibers. The reversal of the hyperinsulinemia of the VMH syndrome by vagotomy (Experiment 1) and the induction of hypoglycemia by application of insulin to the VMH [Storlien, Bellingham and Martin, 1975, based on Experiment 4) make these possibilities unlikely.

The postulated VMH insulin-glucoreceptors are excitatory with respect to the glucoregulatory system, which passes through (or is generated in) the VMH area, and extend inhibitory collaterals to the LHA [Tannenbaum, Paxinos and Bindra, 1974]. This is represented graphically in Figure 12. The model also includes provision for influences based on the sensory qualities of food [Campbell, Bindra, Krebs and Ferenchak, 1969] which would be of prime importance if the VMH is shown to play a central role in the glucoregulatory adjustments which anticipate a meal.

In the normal animal there would appear to be three possible phases of insulin release. Phase I occurs completely in anticipation of the act of ingestion [Parra-Covarrubias, Rivera-Rodriguez and Almarez-Ugalde, 1972; Wiley and Leveille, 1970]. Phase II occurs during the act of ingestion and before the actual rise in blood nutrients [Strubbe and Steffens, 1976]. This readily available insulin pool may normally be released by the same brain mechanism that drives the first phase or may be under control of a gastric-originated peripheral vago-vagal reflex or a combination of both. Phase III of insulin release would appear to be an outcome of the actual rise in blood nutrients. As we shall see, however, the magnitude of its expression seems capable of modulation by cephalic input.

There are data which suggest the role of the VMH is

Figure 12. The theoretical relationship of the insulin-glucoreceptors of the VMH to the sympathetic and parasympathetic components of the autonomic nervous system.


LHA
VMH
ig
S
BLOOD NUTRIENT CHANGE
SENSORY INFORMATION
+ or −
+ or −
INTEGRATOR
INTEGRATOR
PARASYMPATHETIC OUTFLOW
SYMPATHETIC OUTFLOW
− INHIBITORY SYNAPSE
+ EXCITATORY SYNAPSE
ig INSULIN-GLUCORECEPTOR
S SENSORY CELL

to initiate Phase I of insulin secretion and suppress Phase III with an appropriate intensity based on previous experience with the nutrient ingested. The evidence for VMH involvement in Phase III comes from the data of Martin, Konijnendijk and Bouman [1974]. They pair-fed control and VMH-lesioned animals at 67% of normal intake. This reduces basal insulin levels in VMH-lesioned animals to normal. However, the insulin response to glibenclamide (a synthetic insulin-stimulating drug) in the normoinsulinemic VMH animals is significantly greater than in their comparable controls. Certainly there is ample physiological evidence that activation of the sympathetic nervous system can reduce the insulin response to infused glucose [Miller, 1975].

The evidence for VMH involvement in Phase I is more circumstantial. Wiley and Leveille [1970] have demonstrated that an increase in insulin level to the anticipation of feeding time forms part of the physiological change which allows an animal to adapt to a restricted feeding schedule (i.e., 2 hours/day feeding). Panksepp [1971] revealed the inability of VMH-lesioned animals to adapt to such a restricted feeding schedule, and the nature of their deficit may lie in a failure to initiate anticipatory insulin secretion. Perhaps the best evidence comes from Steffens [1969]. He devised a permanent, indwelling cardiac catheter from which repeated blood samples could be taken and blood

glucose determined. After observing the normal pattern of both behavior and blood glucose surrounding a spontaneous meal he measured the effect on blood glucose of denying access to food at the moment it appeared an animal was about to begin a subsequent meal. In normal animals a steady decline in blood glucose occurred following the denied "meal", presumably due to anticipatory insulin release. No such decline was observed in VMH-lesioned animals similarly denied meals.

It seems quite plausible, then, that the primary role of the VMH is to stabilize energy supply in two ways: firstly, by initiating insulin output and suppressing glucose (and most likely free fatty acid efflux) production in anticipation of the rise in blood nutrients which rapidly occur following food intake, and, secondly, by initiating glucose output and suppressing insulin in the reactive phase following food ingestion to avoid a hypoglycemic overshoot.

It should be reemphasized at this point that glucoregulation is most realistically seen as changing patterns of integrated metabolic events whose total result is a shift towards increasing or decreasing blood glucose levels. Perhaps the preeminent glucoregulatory control is food intake. Whether the VMH acts directly to influence this aspect of glucoregulation is not presently clear. Data generated in the next chapter may provide some clues towards

answering this question and it will be discussed in their light.

VMH hyperphagia and obesity are then seen to be the primary result of the failure to suppress the reactive phase of insulin secretion (proportionally, the major contributor to total insulin output) and to initiate glucose output following a meal. This would lead to an abnormally large diversion of incoming nutrients away from availability for short-term energy usage (i.e., hypoglycemia) and into long-term body stores (i.e., fat deposition). The immediate result would be a dearth of immediate energy for ongoing activity. Consistent with this view is the prediction that the initial result of a VMH lesion would be a shorter meal-to-meal interval. However, as LeMagnen [1959] has so elegantly shown, given time to adapt, rats eat to anticipate an energy deficit. Thus the VMH-lesioned animal may learn to increase the size of its meal in order to avoid the rapid recurrence of an available-energy deficit. The increased meal size observed in, for example, the Thomas and Mayer [1968] study may then only reflect the amount of food necessary both to exhaust the readily available insulin reserve and to provide a "residue" of nutrient capable of meeting energy requirements over a reasonable post-prandial period.

Panksepp and co-workers, in a series of papers [Nance and Panksepp, 1975; Panksepp, 1971a; 1971b; 1974;

Panksepp and Nance, 1972], have strongly suggested a critical role for the VMH in long-term body weight regulation. They base this conclusion largely on the observations that VMH-lesioned animals: (a) apparently fail to adapt to a restricted feeding schedule (i.e., 1 hour access to food per day,[see Panksepp, 1971]), (b) become obese on palatable diets [Corbit and Stellar, 1964], (c) fail to increase food intake as much as controls to injections of protamine zinc insulin, and show smaller reductions in food intake following cessation of treatment [Panksepp and Nance, 1972], and (d) attenuate the vigor of refeeding after deprivation in diabetic rats [Panksepp and Nance, 1972]. Taking these points in turn, the present model of VMH function suggests that the VMH is important in the initiation of the metabolic adjustments to the anticipation of food intake. As suggested, these pre-ingestive reflexes may become increasingly important in the restricted feeding situation (1 hour/day access to food) employed by Panksepp. The deficit in the VMH animal may be the inability to make such adjustments. It should also be noted that Panksepp [1971a] reduced the weight of his VMH animals only to the weight of controls. The unwillingness of VMH animals to overcome any interference with their food ingestion at high body weights is well known. The present argument suggests that the vicious circle of hypersecretion of insulin re-

quiring more intake, which in turn results in further insulin output, accounts for the observation of obesity without resorting to the postulate of a long-term body stores deficit. The data showing "deficits" following insulin injections are particularly weak. Against a background of already very high circulating insulin levels and undoubted obesity-induced insulin resistance one could hardly expect insulin injections (or the withdrawal of these injections) to result in similar intake adjustments to those observed in control animals. Finally, I would agree with Panksepp that the hyperphagia which results from induction of diabetes almost certainly reflects a long-term body stores deficit signal. However, the very fact that VMH lesions do not cause the death of diabetic animals due to inanition seems to argue against the notion that the VMH is critical to monitoring such a body stores signal. The reduction in re-feeding vigor following deprivation in VMH lesioned diabetics may only reflect some amelioration of the diabetic syndrome due to VMH destruction [York and Bray, 1972: Table 4].

In summary, there appears little evidence that the VMH is involved in long-term adjustments in feeding behavior. Indeed, there is now quite good evidence that the VMH-lesioned animal defends the lower end of his body weight continuum with a vigor at least comparable to that of normal

animals [Beatty, 1973; Ferguson and Keesey, 1975; Franklin and Herberg, 1974; Kent and Peters, 1973; Marks and Remley, 1972; Peters, Sensenig and Reich, 1973; Porter and Allen, 1972; Wampler, 1973]. The upper end asymptote of weight for any particular VMH-lesioned animal would appear to reflect the degree of over-secretion of insulin which may, in turn, quantitatively reflect the amount of destruction of the relevant glucoregulatory VMH neurons and/or, as obesity ensues, increasing insulin resistance and the capacity of the pancreatic beta cell to secrete.

Why palatability should have such a profound effect on this upper limit is unclear. There are perhaps 3 potential explanations.

First, it may be an artifact of the lesion technique. The early report of Graff and Stellar [1962] offered preliminary evidence for a dissocation between weight gain and exaggerated response to the gustatory qualities of food. They concluded that "there are separate, but overlapping, neural mechanisms for hyperphagia and for finickiness." Their conclusion is, however, based on a very limited number of animals in post-hoc groupings. The anatomical distinction suggested by Graff and Stellar has, nonetheless, apparently found at least some support in recent unpublished work by Bevan [quoted in Hoebel, 1976].

Second, in each of the repeated observations that

palatability is a critical variable determining the magnitude, and even the existence of VMH hyperphagia and obesity [see, for examples: Brooks, Lockwood and Wiggins, 1946; Corbit and Stellar, 1964; Ferguson and Keesey, 1975; Franklin and Herberg, 1974; Strubbe and Steffens, 1975; Teitelbaum, 1955], there exist potential confounds. Quinine, used almost exclusively as the negative taste adulteration, has recently been shown to affect directly in vitro secretion of insulin [Henquin, Horemans, Nenquin, Verniers and Lambert, 1975], a particularly critical point if the present analysis of VMH functioning is correct. On the "positive" taste side, each alteration of palatability invariably also involves both a shift in caloric density and a probable shift in rate of ingestion and/or absorption. For example, the apparent increase in palatability of wet mash over powdered chow over chow pellets may merely reflect a decreasing rate of ingestion and absorption. Although such data are not yet available, the ultimate peak of blood glucose for a given meal size most likely depends on the rate of ingestion/absorption and the amount of insulin secreted depends on the glucose peak. Correspondingly, any manipulation which increases the glucose peak in the VMH animal will also increase dramatically the magnitude of the insulin hypersecretion.

The third possibility is that palatability would

continue to have a marked effect on VMH obesity even if caloric density and texture were controlled. Interactions between palatability and weight maintenance levels have not, as far as I am aware, been fully explored even in the intact animal. Certainly the short-term effect on consumption has been demonstrated for quinine and, on the positive side, for adulterations of diet involving addition of glucose, sucrose or fat. These latter diets, however, also involve changes in caloric density. There is no convincing evidence that adulteration with non-nutritional sweeteners (e.g., saccharin) have any short- or long-term effects on body weight maintenance level in spite of increased palatability (as judged largely by subjective evaluation in human subjects [see Mook, 1974]). The case for initial metabolic alterations based on palatability is much stronger. Increasing salivation [Wooley and Wooley, 1973], gastric secretion [Pavlov, 1902] and pancreatic exocrine secretion [Behrman and Kare, 1968] with increasing palatability (without ingestion) have all been demonstrated. This is not unreasonable, as in the natural setting bitter substances are often poisonous as well, and sweetness is likely to correlate with caloric density. If we can assume that the metabolic shifts in response to palatability changes would extend to insulin, then by the argument of the previous paragraph even a pure increase in palatability

(without accompanying caloric density or texture changes) would create, in the VMH-lesioned animal, a condition once again of massive oversecretion of insulin with its obesity-generating consequences. In the intact animal the initial increase in insulin secretion would be smaller (because of VMH inhibition) and once the caloric consequences were judged to be the same as the original, less palatable diet, insulin secretion would be suppressed to "normal".

Finally, this analysis presumes two things. First, that the normal animal would react to the aversive consequences of oversecretion of insulin to a novel, palatable diet not by overeating the next time the diet was presented, but by suppressing insulin appropriately. The fact that long-term weight gain is not generally seen on palatable diets would support this assumption. Second, it assumes the physical substrate for generating these metabolic alterations exists and is outside the VMH. Burton, Mora and Rolls [1975] have recently reported preliminary evidence of certain cells in the LHA whose firing rates to the presentation of given visual or taste stimuli are dependent upon the nutritional status of the animal. Given that result, it seems reasonable that firing rates in these cells would also change appropriately if nutritional state were held constant and palatability were varied. Such cells could

easily function as the necessary physical substrate for generating metabolic adjustments to changes in palatability.

CHAPTER FOUR

DIABETIC HYPERPLAGIA AND ADRENALINE ANOREXIA

4.1 Introduction

Diabetic hyperphagia and adrenaline anorexia have been repeatedly seen as phenomena critical to the glucostatic theory of feeding. The characteristics of these preparations, and/or the interpretation of them, offer potential problems for the model of VMH function proposed in the preceding chapter. This chapter is devoted to a reanalysis of: (a) diabetic hyperphagia, and (b) adrenaline anorexia.

4.2 Experiment 8: Diabetic Hyperphagia

The hyperphagia which results from induction of chronic diabetes by sub-total pancreatectomy, alloxan, or streptozotocin has been used as an argument against the role of blood glucose in satiety under normal feeding conditions. As initially seen, "satiety" was directly correlated with blood glucose level. Thus the diabetic presented a problem case where abnormally high levels of blood glucose coexisted with hyperphagia. Mayer [1955] brought his theory in line with this observation by assuming that glucose utilization was the appropriate correlate of "satiety". The lack of insulin would then impede the uptake of glucose in the diabetic and little would be utilized in spite of the high circulating levels. With the confirmation of apparent insulin-facilitated glucose uptake in the VMH [Debons, Krimsky, Likuski, From and Cloutier, 1968; Smith, 1972] the "glucostatic" theory was further refined to an approximation of its present form.

Diabetic hyperphagia, however, again becomes something of a problem for the model proposed in the preceding chapter. In the normal animal high activity in the insulin-glucoreceptor VMH neurons is principally generated by high glucose levels in the presence of "normal" insulin, an event correlated with meal offset. While that relation may be spurious, it is perhaps more reasonable to assume that

there is some force in the direction of suppression of feeding associated with high levels of activity in those particular VMH neurons. The problem arises in the diabetic case where very high levels of receptor activity are generated for an entirely different reason, that of theoretically zero insulin (which eliminates any effect of the abnormally high blood glucose). However, despite their entirely different origins, there is no a priori reason to suggest that the high VMH insulin-glucoreceptor activity of the immediate post-prandial normal animal is seen as reflecting any different phenomenon from the high activity generated by the insulin-deficient diabetic. Thus a prediction which may be rightly forced from the present position is that diabetes should be associated with a reduction in food intake.

It has been repeatedly demonstrated that various techniques for producing acute onset of diabetes all result in an initial hypophagia which is followed by a gradual increase in food intake over the two weeks following induction of diabetes. With one notable exception [Booth, 1972], the significance of this pattern appears to have been either ignored or tacitly attributed to the debilitating effects of the diabetes-inducing treatment [Brodsky, Nelson and Guest, 1952; Kumaresan and Turner, 1965; Mayer and Bates, 1952].

Malaise arising indirectly as a side effect of drugs has been ruled out by Brodsky, Nelson and Guest [1952] who maintained animals with insulin following induction of diabetes with alloxan. Offset of insulin therapy in these animals was also followed by a hypophagic to normophagic period of four to five days before onset of hyperphagia. A similar pattern has been observed with the streptozotocin diabetic (pilot experiment observations). Acute forms of diabetes induced by either anti-insulin serum [Anderson, Kilbourn, Robinson and Wright, 1963] or mannoheptulose [Panksepp, Tonge and Oatley, 1972] are also associated with an immediate reduction in food intake reversible, in the latter study, by 2-deoxy-D-glucose.

An alternative to the malaise explanation assumes that animals initially eat less than normal either for the simple reason that their blood glucose levels are far above normal or because some correlate or offshoot of the high VMH insulin-glucoreceptor activity is read as reflecting the immediate post-prandial state.

The gradual onset of hyperphagia would then represent an over-ride arising from a long-term control system responsive to depletion of body tissue. If such an explanation is appropriate, then reducing body weight prior to induction of diabetes should result in a shorter period of post-induction hypophagia and increasing body weight should

prolong this hypophagic period. The present experiment is designed to test this hypothesis by measuring the food intake of fat and thin rats following the onset of diabetes induced by streptozotocin.

4.2.1 Method

Subjects were female, Wistar-derived rats obtained from the breeding colony of the John Curtin School of Medical Research (Canberra, Australia). All groups were equated for weight before treatment and individual animals ranged from 180 to 220 gms. Two groups were starved until their weight had reached 90% and 80% of pre-starvation levels and then maintained at those percentage levels (relative to control) with appropriate amounts of food given daily at the beginning of the dark period (1900 hours). Another group of animals received insulin injections of 8-12 U/kg daily until reaching 110% of control weight and were maintained at the 110% level with appropriate amounts of insulin. After 2 weeks at maintenance all animals were deprived of food at the beginning of the light period (0700 hours) and injected at 1300 hours with 65 mg/kg i.p. of streptozotocin (kindly supplied by Upjohn Corporation of Kalamazoo, Michigan) in citrate buffer (pH 4.5). Control animals received an equivalent volume of buffer. Food and water were then continuously available for the remainder of the experiment and measured in 24-hour periods from 1900 hours starting the evening of

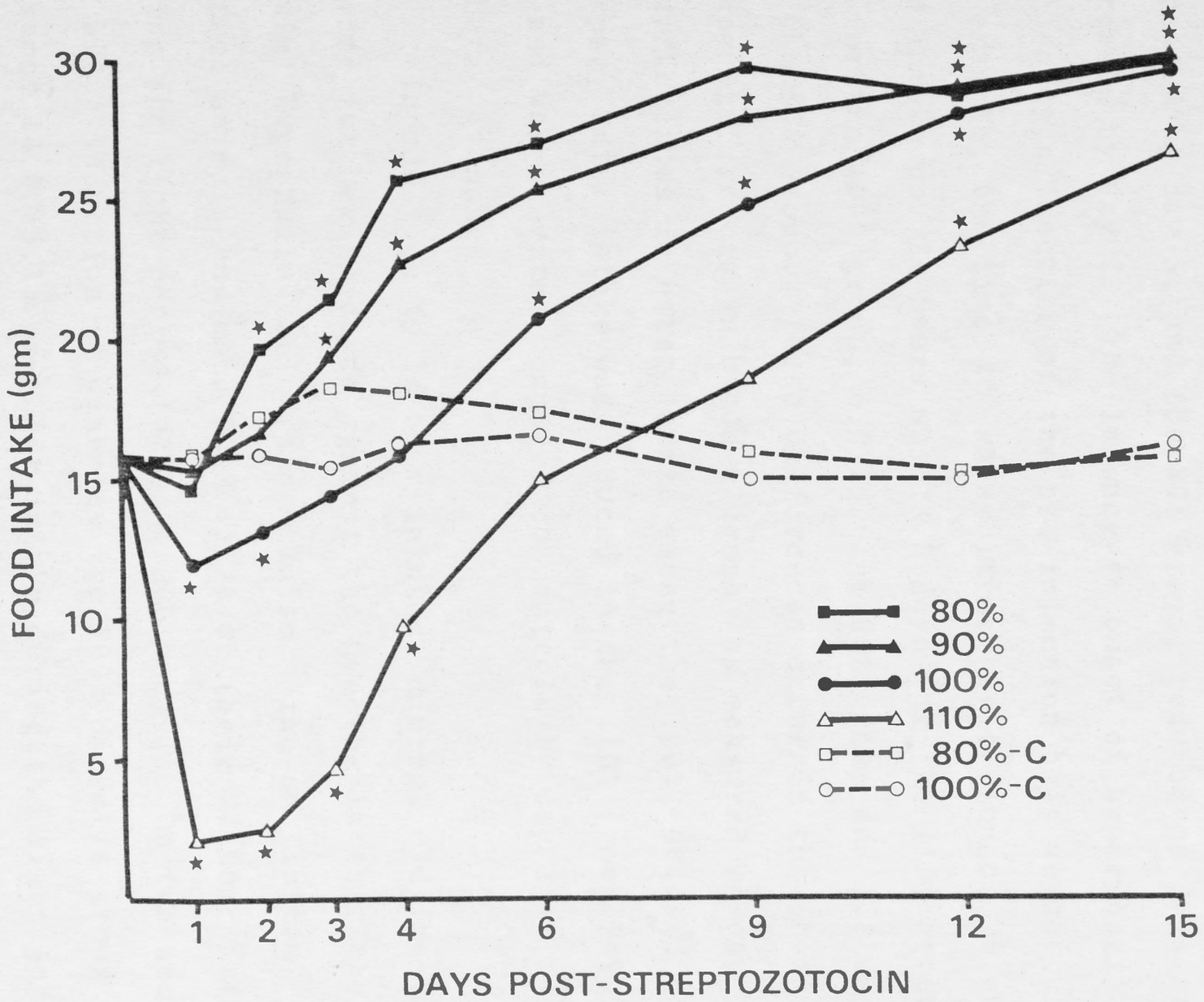
the injection. A weighed amount of pellets (Mecon Rat Chow) was placed in the cage and those remaining weighed again at the end of the 24-hour period. Spillage, collected on paper towels placed under the individual cages, was air dried. The spilled amount was subtracted from the difference between the initial food weight and weight remaining at the end of the 24-hour period to give a final intake figure. On the sixteenth day post-injection, all animals were deprived for 6 hours, lightly anesthetized with ether and blood sampled from a tail vein for glucose analysis by the ortho-toluidine method [Hyvarinen and Nikkila, 1962]. Blood glucose of the diabetic animals ranged from 330 to 500 mg% and analysis of variance revealed no differences between diabetic groups. Controls ranged from 87 to 109 mg%.

4.2.2 Results

Figure 13 gives the food intake results in the 15 days post-injection in terms of consumption per 24-hour period for the 4 diabetic groups, for normal controls and for controls treated identically with the 80% group except for actual administration of the streptozotocin. Day by day, one-way analysis of variance with fixed effects was done on the data displayed in Figure 13 (see Appendix F for a summary of the F ratios) followed by Scheffé individual comparisons where appropriate. Differences at the

Figure 13. The effect of increased (110% group, n = 6), normal (100% group, n = 8) or decreased (90% group, n = 9; 80% group, n = 6) body weight on food intake over the 15 days following injection of streptozotocin. Food intake of normal (100%-C group, n = 8) and decreased (80%-C group, n = 5) body weight controls are included for comparison.

*differs from normal controls, $p < .01$.


30
25
20
15
10
5
FOOD INTAKE (gm)
1
2
3
4
6
9
12
15
DAYS POST-STREPTOZOTOCIN
80%
90%
100%
110%
80%-C
100%-C

0.01 level were considered significant.

In summary, when compared to controls, food intake in the 110% group was reduced for 4 days following injection and increased by day 12; 100% group, reduced for 2 days and increased by day 6; 90% group, reduced no days and increased by day 3; and the 80% group, reduced no days and increased by day 2. The latency to onset of hyperphagia is clearly a function of the pre-injection body weight. For example, the time to an arbitrary criterion of 25 gms food intake per 24 hours was 13.8 days for the 110% group, 9.4 for the 100% group, 6.0 for the 90% group and 3.8 days for the 80% group. These differences occur in the absence of any differences in blood glucose (as measured on day 16) or differences in water intake among the 100%, 90% and 80% groups. Water intake was reduced in the 110% group for 1 day and was elevated compared to controls by day 2 in all diabetic groups.

In addition to 24-hour intakes, diurnal cycling data provide further evidence against the hypothesis that the initial hypophagia reflects a malaise. The ad libitum control animals consumed 16.8 ±1.7% of their 24-hour intake during the light period (0700 - 1900 hours). On the second day after induction of diabetes the 100% diabetic group consumed 14.0 ±3.1% of their intake during the light period. It seems unlikely that an animal who was hypophagic because

it was ill would maintain such an accurate diurnal intake rhythm. By day 15, when the hyperphagia of the 100% diabetic group was at asymptote, the animals of that group were still consuming only some 28% of their food during the light period.

4.2.3 Discussion

The results argue strongly against the notion that the initial 4 to 5 day hypophagic to normophagic period following induction of diabetes reflects a debilitation of the animal. By the second day post-injection, diabetic animals of the 80% group are already markedly hyperphagic relative to initially comparable control animals. The time of onset of hyperphagia is clearly a function of depletion of body tissue.

An argument which can be made against the present results is that there is most likely a positive correlation between initial body weight and ketosis and, thus, by reducing body weight, ketotic malaise might be reduced. Three points are relevant to this argument. First, tests for urine ketones by Ames Lab-Stix on the second and third days post-streptozotocin registered only + or ++ for the 100% diabetic group (on a scale of 0 to +++), a relatively mild ketosis. Second, as pointed out, hypophagia due to illness and accurate diurnal fluctuations in food intake seem to be an unlikely combination. Finally, there are no data which

demonstrate that mildly elevated ketone levels would result in a reduction in food intake. Indeed, there is reason to believe that such elevations might increase food intake. The very survival of an animal made diabetic by, for example, alloxan would appear to depend on the animal's ability to overeat [Brodsky, Nelson and Guest, 1952].

The hyperglycemia and depleted pancreatic insulin of the streptozotocin diabetic syndrome is fully developed, and stable, by 24 hours post-injection [Junod, Lambert, Orci, Pictet, Gonet and Renold, 1967]. Thus, the effects of a carbohydrate-based satiety system should be stabilized from 24 hours to 2 weeks post-injection. Any change in food intake may then be reflecting a relatively pure change in the power of signals generated in the long-term system. Although the nature of this system is in dispute, the present syndrome may offer an excellent experimental model with which to study long-term energy control unencumbered by fluctuations in short-term signals.

Finally, Le Magnen, Devos, Gaudilliere, Louis-Sylvestre and Tallon [1973] have suggested the neuroendocrine basis for diurnal intake rhythm may be a greater insulinosecretory responsiveness to hyperglycemia at night compared to responsiveness during the day. The existence of normal diurnal cycling in the diabetic animal whose

insulinosecretory capabilities are insufficient to control its blood glucose levels would seem to argue against Le Magnen's suggestion.

4.3 Experiment 9: Adrenaline Anorexia and Diabetes

The anorexia which results from i.p. injections of adrenaline has been used as an argument against the traditional "glucostatic" theory on the basis of the following considerations. In order to produce anorexia via a "glucostatic" mechanism, adrenaline would either have to increase Δ-glucose directly or do so indirectly by increasing both insulin and glucose levels. Any indirect effect is likely to be marginal as adrenaline, while known to increase blood glucose, in addition, suppresses insulin. Its direct effect, at least peripherally, is to decrease, rather than increase, Δ-glucose [Somogyi, 1951]. It is questionable whether adrenaline crosses the blood-brain barrier [Weil-Malherbe, Axelrod and Tomchick, 1958]; however, even when applied centrally it causes an increase, rather than decrease, in food intake [Grossman, 1967].

Russek [1971; 1976] has synthesized a good deal of the literature on adrenaline anorexia within his own framework of peripheral control of food intake. In essence, Russek suggests that the adrenaline anorexia observed depends on the glycogenolytic effect of adrenaline (i.e., rate of glucose transport across liver membrane).

On the other hand, the interpretation from the insulin-glucoreceptor argument proposed here would be that the hyperglycemia and suppression of insulin secretion following

adrenaline injection result in a high level of VMH activity (i.e., large number of empty and insulin-and-glucose-occupied receptors) and that this high level of activity results both in a potentiation of the hyperglycemia and, either directly or indirectly, a cessation of feeding.

Two demonstrations were then aimed at defending this viewpoint. The first attempted to demonstrate that the hyperglycemia was the relevant stimulus for food intake suppression following adrenaline. It was presumed that adrenaline-induced hypercglycemia would be absent in a diabetic but that if the anorexia were due to any other effects of adrenaline these would still be present. Secondly, since the diabetic is also deficient in liver glycogen, Experiment 9 does not differentiate between the present proposal and that of Russek. However, Russek's framework requires neural pathways from the liver to the brain. Two experiments looked at adrenaline anorexia in vagotomized (Experiment 10) and sympathectomized (Experiment 11) rats.

In this first experiment adrenaline anorexia was compared between normal and diabetic rats.

4.3.1 Method

Diabetes was induced by a single, i.p. injection of streptozotocin (65 mg/kg in citrate buffer of pH 4.5) at least 3 weeks before the beginning of the experiment. It

was initially intended to assess the anorexic effects of adrenaline on animals deprived of food for 24 hours. However pilot work revealed that diabetic animals, already lighter than controls, lost even more weight in absolute terms over the 24-hour fast (i.e., a much larger percentage of body weight). Three groups were eventually formed: (1) control animals (n = 9) 24-hour deprived, (2) diabetic animals (n = 9) 24-hour deprived, and (3) diabetic animals (n = 9) 11-hour deprived (a period that resulted in approximately the same percentage body weight loss as that seen over 24 hours deprivation in controls). All diabetic animals had blood glucose levels at least 280 mg%.

Treatments were separated by 4 days and run as follows. All animals were deprived for their appropriate lengths of time on two occasions with no injection to acclimatize them to the deprivation procedure. Following the third, fourth and fifth deprivations, animals received either physiological saline (10 ml/kg i.p.), a low dose of adrenaline hydrochloride (0.12 mg/kg i.p. in a volume of 10 ml/kg) or a high dose of adrenaline hydrochloride (0.18 mg/kg in a volume of 10 ml/kg). Order of treatments was completely counterbalanced. Food (Mecon rat cubes) was made available immediately after injection and food intake was measured one-half hour, 1 hour and 2 hours later. Spillage was collected on paper toweling under the cages, air dried

and corrected for when making intake calculations. A two-way analysis of variance (3 x 3) was carried out treating the repeated measures on the Treatment factor (saline, adrenaline-low and adrenaline-high) as a random effect and the Groups factor (Control, Diabetic-24 and Diabetic-11 as a fixed effect. Data for food intake after 1 hour and 2 hours are also shown in Table 1. Scheffé individual comparisons were used for individual comparisons.

4.3.2 Results

The data are summarized in Table 1 (see Appendix G for F ratios).

The results demonstrate that minimizing the changes in blood glucose and insulin minimizes the anorexic effects of adrenaline.

TABLE 1

Food intake (gms) over the 2 hours following injection of either physiological saline or adrenaline hydrochloride (low dosage: 0.12 mg/kg; high dosage: 0.18 mg/kg) in normal animals deprived 24 hours (Control) and diabetic animals deprived either 24 hours (Diabetic-24) or 11 hours (Diabetic-11). There were 9 animals in each group.

GROUP	TREATMENT	FOOD INTAKE (gms)		
		1/2 hour	1 hour	2 hours
Control	Saline	3.14	1.26	0.42
	Adrenaline-low	0.93	1.51	1.88
	Adrenaline-high	0.29	0.91	2.03
Diabetic-24	Saline	2.62	2.78	1.54
	Adrenaline-low	2.27*	2.41	1.89
	Adrenaline-high	2.12*	2.59	1.88
Diabetic-11	Saline	2.41	1.93	1.07
	Adrenaline-low	2.00*	1.88	0.93
	Adrenaline-high	1.93*	1.69	1.54

*indicates significant difference from appropriate Control condition ($p < .05$) on comparisons made using the one-half hour intake data.

4.4 Experiment 10: Adrenaline Anorexia and Vagotomy

Russek [1971; 1976] has presumed the vagus to be the likely neural pathway carrying glycogen depletion signals from the liver to the brain. This would seem reasonable from the data of Niijima [1969] showing a good relationship between vagal firing rate and concentration of glucose perfusing the liver. The present experiment looked at the effect of chronic, bilateral subdiaphragmatic vagotomy on food consumption following adrenaline.

4.4.1 Method and Results

Vagotomy or sham vagotomy was performed as described in Experiment 1. At least 4 weeks were allowed between vagotomy and the beginning of the adrenaline injection phase of the experiment.

The experiment was run according to the method of Experiment 9 with all animals deprived 24 hours at the onset of each treatment. Five deprivations (each separated by 4 days) were instituted, the first 2 acclimatized animals to the procedure, and the following 3 preceded injection with either physiological saline, a low dose of adrenaline hydrochloride (0.12 mg/kg) or a high dose of adrenaline hydrochloride (0.18 mg/kg). All solutions were given i.p. in a volume of 10 ml/kg. Counterbalancing of treatment order was incomplete in the vagotomy group due to elimination of a single animal on the basis of the acid secretion

in response to insulin test (see Experiment 2) carried out following the treatment phase of the experiment. A two-way analysis of variance with mixed effects of vagotomy and sham-vagotomy groups by treatment (2 x 3) was done on the one-half hour data. The data on which the analysis was based, as well as data regarding food intake after 1 and 2 hours are shown in Table 2 (F ratios are contained in Appendix H). Scheffé comparisons were carried out to determine which one-half hour intake differences were significant.

TABLE 2

Food intake (gms) over the 2 hours following injection of either physiological saline or adrenaline hydrochloride (low dosage: 0.12 mg/kg; high dosage: 0.18 mg/kg) in 24-hour deprived sham-vagotomized (n = 6) or vagotomized (n = 8) animals.

GROUP	TREATMENT	FOOD INTAKE (gms) 1/2 hour	1 hour	2 hours
Sham-vag.	Saline	2.89	1.20	0.55
	Adrenaline-low	1.03*	1.33	1.90
	Adrenaline-high	0.34*	0.72	2.63
Vagotomy	Saline	3.41	0.96	0.41
	Adrenaline-low	1.46*	1.41	1.76
	Adrenaline-high	0.57*	1.09	2.95

*indicates significantly different ($p < .05$) from appropriate saline control on comparisons made using the one-half hour intake data.

The anorexic effect of adrenaline was the same in both vagotomized and sham-vagotomized animals thus offering no support for Russek's hypothesis. While again the presently employed test for completeness of vagotomy may be questioned, it seems unlikely that destruction of the vagus sufficient to eliminate both hypoglycemic acid secretion and any visual remnants of esophagal nervous tissue would be insufficient to affect significantly vagal fibers subtending a glycogen depletion signal.

4.5 Experiment 11: Adrenaline Anorexia and Sympathectomy

The sympathetic nervous system is an alternative substrate which could mediate Russek's "glycogenostat". Because surgical interruption of this system is difficult in the rat, chemical destruction with guanethidine sulphate was employed. This, as previously pointed out, offers the advantages of essentially complete peripheral SNS destruction with no central effects.

4.5.1 Method and Results

Sympathectomy was performed as in Experiment 3 (6 weeks of daily, i.p., 25 mg/kg injections of guanethidine sulphate) while the control group received the same number of injections of an equivalent volume of physiological saline. Approximately 1 month was allowed beyond cessation of treatment with GS before the experiment was initiated. The method of Experiment 9 was again followed. Five 24-hour deprivation periods were imposed, each separated from the other by 4 days. The last three deprivations each preceded injection with either physiological saline or one of two adrenaline hydrochloride dosages (low: 0.12 mg/kg; high: 0.18 mg/kg) in a counterbalanced design. As in Experiment 9 and 10, a two-way mixed effects analysis of variance was performed on the one-half hour intake data, followed by Scheffé comparisons.

Table 3 summarizes the data (Appendix J contains the $\underline{F}$ ratios).

TABLE 3

Food intake (gms) over the 2 hours following injection of either physiological saline or adrenaline hydrochloride (low dosage: 0.12 mg/kg; high dosage: 0.18 mg/kg) in 24-hour deprived sympathectomized (n = 6) or sham-sympathectomized (n = 6) animals.

GROUP	TREATMENT	FOOD INTAKE (gms) 1/2 hour	1 hour	2 hours
Sham-sympathectomy	Saline	3.29	1.14	0.62
	Adrenaline-low	1.10*	1.48	2.00
	Adrenaline-high	0.22*	0.83	2.41
Sympathectomy	Saline	3.06	0.99	0.69
	Adrenaline-low	1.03*	1.66	2.13
	Adrenaline-high	0.41*	0.92	2.17

* indicates significantly different ($p < .05$) from appropriate saline controls on comparisons made using the one-half hour intake data.

As with vagotomy, sympathectomy has no significant effect on the degree of anorexia induced by i.p. adrenaline.

4.6 Discussion of Experiments 9, 10 and 11

Taken together, the results of these three experiments provide evidence favoring a central, rather than peripheral, basis for adrenaline anorexia and suggest the phenomenon relies on changes in blood glucose and insulin for its expression.

However, while finding no support in the present results, Russek's arguments do focus on an important issue. Russek [1976] suggests a direct role in feeding for his peripheral "glycogenostat" but only an indirect role for the central glucoreceptors via their effects on blood glucose. Certainly this latter position is very much in sympathy with the analysis of VMH function made in this thesis. The data thus far available have not been directed towards determining whether the changes in food intake following manipulation of VMH activity are a direct result or indirectly come about from changes in glucoregulation. However, it seems reasonable to hypothesize that the VMH would have limited direct effect on feeding within a fairly broad, but normally occurring, range of insulin and glucose levels and of body energy reserves just as it is reasonable that an animal eats to anticipate deficits rather than as an emergency reaction to such depletion. As Fitzsimons [1972] concluded in his comprehensive review of thirst: "In normal circumstances drinking is largely anticipatory of future needs

for water . . .". The same is probably true for hunger [Le Magnen, 1959; see also Oatley, 1973]. It does seem unreasonable that an animal would wait, following a meal, for the declining level of some metabolite signalling a deficit before initiating a further meal (especially a metabolite as critical to brain function as glucose).

At the extremes of the blood glucose range (in emergency conditions), the VMH may play an increasingly large, and direct, role in feeding (even though its signals might be overridden by long-term influences as in the case of the diabetic). Thus while Russek pays less attention to differentiation between normal and emergency conditions, I would suggest that the levels of hyperglycemia produced by the dosages of adrenaline normally used to induce anorexia would constitute an "emergency" for the animals and result in direct glucoregulatory depression of feeding.

Mayer's "glucostatic" theory still receives much attention. If the present analysis is correct then it should be limited to those feeding responses generated in emergency hypoglycemic or hyperglycemic states. In the normal, free-feeding animal the "glucostatic" metabolic patterns are probably just that, changes induced to stabilize blood glucose availability and are not likely to influence feeding directly.

CHAPTER FIVE

THE FEMALE ESTRUS CYCLE AS A MODEL FOR VMH OBESITY

5.1 Introduction

The thesis, to this point, has been directed at developing, and justifying empirically, a model of VMH function which sees the predominant role of that structure as one of maintaining stable glucose supply to the brain. In order to proceed with this analysis it is of obvious value to identify a preparation in which varying levels of VMH activity lead to significant shifts in energy balance and which is of a more physiological nature than the electrolytic lesions upon which so much of the theorizing about VMH function has been based. Electrolytic lesions are, at best, a crude disruption of normal neural functioning. This is particularly true in the case of a lesion of the VMH whose anatomical location places it in close proximity to numerous fiber tracts, especially those subserving a multitude of hormonal functions. In fact, so complicated is the VMH lesion preparation that of the thousands of studies employing the technique, not one has convincingly located that anatomical locus (in or around the ventromedial area) from which a maximal weight effect may be expected.

Of the weight and food intake fluctuations which

naturally occur in animals, those accompanying the estrus cycle in female rats have been seen as phenomena in whose origin the VMH played a major role. The following chapter explores the possibility that the rhythmic energy balance fluctuations of the estrus cycle are driven by changes in VMH activity and hence that those fluctuations can be used to further the investigation of the VMH's role in energy balance.

5.2 Experiment 12: Estrus Cycle and Insulin

The rhythmic changes in food intake and weight accompanying estrus cycling in female animals are well documented [see Wade, 1972]. In intact animals, conditions of high estrogen (proestrus, estrus) are associated with low levels of food intake and weight loss while diestral, pregnant, or pseudopregnant animals (low estrogen) have relatively high rates of weight gain. Food intake rises in ovariectomized females to stabilize weight at approximately 20% higher levels than normal. Food intake then reduces to a level which sustains that weight [Tartellin and Gorski, 1971]. Single injections of estrogen generally produce a decrease in food intake, although there is some controversy over a longer series of injections [see Wade, 1972]. The mechanism of the inhibitory effect of estrogen on food intake and weight is, however, obscure. The possibility of a complex interaction of estrogen and other hormonal mechanisms (especially prolactin) or a direct effect of estrogen on visceral functioning certainly cannot, at present, be dismissed. However a somewhat simpler system, involving direct autonomic innervation of the viscera, has received little attention.

The similarities between ovariectomized and VMH-lesioned female animals may provide an interesting clue to the estrogen effects on food intake and weight. Following

both ovariectomy and VMH lesions the animal increases food intake in order, apparently, to increase her body weight to a new, higher, level. When this point is reached food intake drops back to the level necessary to retain the new weight.

Further, Wade and Zucker [1970] have demonstrated a depression of eating following implants of estrogen into the VMH in ovariectomized females. Estrogen injected i.p. is known to affect electrical activity in a number of hypothalamic nuclei but according to Faure and Vincent [1971], "The 'specific' estrogen-sensitive neurons were located strictly in the ventromedial nucleus and the posterior part of the lateral hypothalamus." Six of 11 VMH neurons which they studied showed accelerated firing after estrone sulphate injections. Twelve of 16 posterior lateral hypothalamic neurons reduced firing rates in response to estrogen.

On the basis of somewhat different analyses of the above data, very recent studies have looked directly at the weight effect of estrogen in VMH-lesioned animals. These studies are difficult to analyze, but Nance and Gorski [1973] appear to have eliminated the estrogen weight effect in VMH-lesioned animals while King and Cox [1973] and Reynolds and Bryson [1974] showed only a partial reduction in the estrogen weight effect. The lack of adequate localization studies of either the area responsible for VMH

weight effects or of the areas responsive to estrogen make difficult interpretation of a partial reduction in the effect of estrogen on weight.

A large part of the normal suppressive effects of the VMH appears to be related to autonomic control of visceral (especially stomach and pancreas) function via the vagus. Hyperacidity and/or hyperinsulinemia may, in fact, account for the hyperphagic effects of VMH lesions [Powley and Opsahl,1974; Experiment 1]. Recently Omole [1972] has provided data which may suggest the effects of estrogen on food intake are of a similar origin. Omole demonstrated increased gastric acidity following ovariectomy and during diestrus, periods during which there is a high level of food intake and low level of estrogen. Unfortunately, from Omole's data it is not possible to determine whether increased acidity causes, or is caused by, the hyperphagia. Herner and Caul [1972], however, found higher ulceration in 24-hour-deprived diestrus animals compared to similarly deprived ones in estrus. This may suggest that the increased acidity is independent of food intake.

The following mechanism of the effect of estrogen on food intake and weight is then possible. During periods of high plasma estrogen, estrogen-sensitive neurons in the area of the VMH are stimulated, and LHA neurons suppressed. High levels of VMH activity result in high blood glucose [Frohman

and Bernardis, 1971] and in low levels of both gastric acid [Misher and Brooks, 1966] and insulin [Frohman and Bernardis, 1971]. During periods of low estrogen the reverse is true. That is, during diestrus a condition of depressed VMH activity exists which mimics qualitatively the absence of firing occasioned by VMH destruction.

With this mechanism in mind, the following demonstrations are necessary. A relative hyperinsulinemia should accompany diestrus. Vagotomy should eliminate the cyclical weight changes as well as the basal insulin and acid differences.

The first experiment assessed the possibility of insulin and glucose level changes paralleling the estrus cycle.

5.2.1 Method

Subjects were virgin, Wistar-derived, female rats obtained from the breeding colony of the John Curtin School of Medical Research (Canberra, Australia). The animals were housed individually in wire cages and maintained on free access to food and water. The colony temperature was 22 ±1.5°C and the lights were on from 0800 to 2000 hours. Smears were taken each day between 1000 and 1030 hours by insertion of a small, saline-wetted cotton bud into the vagina. The smears were then wiped onto clean slides, allowed to dry and stained with methylene blue. At least 5

complete cycles were followed before blood sampling and all animals showed regular 4- or 5-day cycles. To avoid conditioning effects only one blood sample was taken per animal. On the sample day food was removed from the cages at 0800, the smear taken at the normal time, and the samples obtained at approximately 1300 hours. The animal was removed from the colony room, quickly anesthetized with ether and blood withdrawn from the abdominal aorta. Total elapsed time between removal from cage to completion of sampling was never more than 3 minutes. An aliquot of the blood sample was immediately frozen for later insulin assay using the double-antibody radioimmunoassay technique. Owing to a lack of rat standard, values are reported in terms of human standard. Blood glucose was analyzed immediately using the ortho-toluidine method [Hyvarinen and Nikkila, 1962].

Four day cycles were designated as follows: proestrus, estrus, metestrus, and diestrus. Five day cycles had a second day of diestrus. Samples were taken at proestrus, estrus and the first day of diestrus. Statistical comparisons were made using a one-way, fixed effects analysis of variance on both plasma insulin and plasma glucose data followed, where appropriate, by Scheffé tests.

5.2.2 Results

The data are summarized in Table 4. (Appendix K contains the F ratios.) Completely contrary to prediction,

TABLE 4

Plasma insulin (μU/ml) and glucose (mg%) levels (mean ± S.D.) of animals killed at 3 stages of the estrus cycle.

Group	N	Plasma Insulin	Plasma Glucose
Diestrus	12	61.0 ± 16.3	96.4 ± 3.1
Proestrus	18	94.1 ± 20.3	100.0 ± 4.0
Estrus	10	90.8 ± 22.0	99.2 ± 2.7

animals in diestrus showed significantly ($p < .02$) lower insulin levels than those of both proestrus and estrus groups, which did not differ from each other ($p < .20$). Blood glucose did not change over the estrus cycle.

5.2.3 Discussion

The results of this experiment demonstrate that not only does the cycling female rat fail to display a relative hyperinsulinemia during diestrus, but that during this phase of high food intake and weight gain insulin levels are actually depressed. This result can be interpreted in two

ways. Either this preparation is not parallel to the VMH lesion, or hyperinsulinemia is not a necessary factor in VMH obesity (or, for that matter, in intake in the normal animal). It may be that hyperacidity alone is sufficient to drive the hyperphagia and obesity which follow VMH lesions. The following experiments explore this possibility.

5.3 Experiment 13: Estrus Cycle and Gastric Acid - A Replication

Omole [1972] reported a relative hypersecretion of gastric acid at diestrus. The interpretation of this result is, however, obscured by a failure to control food intake. Thus, the high level of intake at diestrus may precede, rather than progress from, increased gastric acidity. The present experiment was designed essentially as a replication of the data of Omole [1972] under conditions of controlled food intake.

5.3.1 Method

Subjects and housing were as in Experiment 12. At least 5 complete cycles were followed before acid secretion testing and all animals showed regular 4- or 5-day cycles. Animals were deprived of rat cubes 24 hours before the acid secretion test but had free access to water. The procedure for collection of gastric acid was as in Experiment 2 with the exception that no insulin was injected and only two 15-minute periods were observed. Groups of animals were tested, as in Experiment 12, at proestrus, estrus and the first day of diestrus.

5.3.2 Results

A one-way, fixed effects analysis of variance was carried out on the 30-minutes acid secretion data shown in Table 5. (Appendix L contains the F ratios.) In essence

the results confirm those of Omole [1972]. Scheffé comparisons showed total acid secretion was significantly elevated at diestrus compared to both proestrus and estrus ($p < .01$ in both cases), and the latter two groups did not differ

TABLE 5

Basal acid secretion (μEq H^+; mean over 30 minutes ± S.D.) of groups of animals tested at 3 stages of the estrus cycle.

Group	N	Acid secretion
Diestrus	7	13.7 ± 2.7
Proestrus	8	4.6 ± 1.6
Estrus	7	4.2 ± 1.4

significantly from each other.

5.3.3 Discussion

These results are, at minimum, consistent with the notion that fluctuations in gastric acid secretion may be responsible for the parallel changes in food intake and weight. Following this result it becomes important to demonstrate that vagotomy eliminates both the intake-weight and acid changes associated with the estrus cycle.

5.4 Experiment 14: Estrus Cycle and Vagotomy

The following experiment looked at the effect of subdiaphragmatic vagotomy on the estrus cycle weight, insulin and gastric acid secretion changes.

5.4.1 Method

Animals and housing were as in Experiment 12. Cycling was followed for at least 3 complete cycles before vagotomy or sham vagotomy was performed. Surgery was carried out as in Experiment 1. Since vagotomized animals tended to continue a slight weight loss over 1 to 2 weeks following the operation, 4 weeks were allowed before initiation of testing. By this time all animals had regained preoperative weight. Completeness of vagotomy was initially assessed using the method of Snowdon and Epstein [1970]. The liquid diet used was similar to that of Teitelbaum and Epstein [1962] and water was available ad libitum. Intake of the liquid diet was measured using a drinkometer circuit [Zucker, 1969] whose output fed into a Grass polygraph. A meal consisted of at least 15 seconds of sustained licking separated by at least 10 minutes from other bouts of licking. Drinkometer testing was over 3 consecutive days and meal frequency was assessed on the last of these 3 days. The schedule employed was as follows: operation, 4 weeks of recovery, 3 days in the drinkometer cages, 3 days to re-acclimatize to their home cages and normal diet, weight

changes and smears over 5 to 6 cycles per animal, and finally blood sampling and acid secretion vagotomy testing.

A number of measures of cyclical weight changes were assessed in pilot experiments. The simplest and most reliable proved to be weight change from the afternoon of diestrus (smears were taken between 1500 and 1600 hours) to the afternoon of estrus versus the weight change from estrus on to the first day of diestrus. This measure was consistent both for groups of females and for each individual tested over at least 5 complete cycles and would thus seem preferable to invasive techniques such as ovariectomy and replacment therapy.

Blood samples were taken and treated as in Experiment 12 for determination of plasma insulin with the exception that blood was removed from the cut tip of the warmed tail under light ether anesthesia and the animal was allowed to recover. Two or 3 days following the blood sampling the animals were deprived of food cubes and treated as in Experiment 2. Two 15-minute periods of baseline data were collected. Insulin (2U/kg) was then injected subcutaneously and a further two 15-minute periods of acid secretion were collected and titrated. Half the vagotomized and half the controls were blood-sampled at estrus and acid-tested at diestrus; with the other half of the animals in both groups being treated in the reverse order.

5.4.2 Results

Drinkometer and weight data are shown in Table 6. Only vagotomized animals whose meal frequencies were at least double that of the control mean and whose acid responses to insulin were absent are included in the table (see Experiment 2 for the criterion used to evaluate the

TABLE 6

Number of drinkometer meals (mean/24 hours, range in brackets) and weight changes (mean ±SD) over the estrus cycle for normal and vagotomized female rats.

Group	N	Drinkometer Meals	Weight Change (gms) Diestrus-to-Estrus	Estrus-to-Diestrus
Control	7	12.9 (11-15)	-0.58 ± 0.27	2.01 ± 0.39
Vagotomized	14	34.6 (26-41)	-0.86 ± 0.17	2.25 ± 0.26

acid secretion test). No differences existed between control and vagotomized groups on either weight change from diestrus to estrus ($t = 1.13$; $p > .20$ for a two-tailed test) or from estrus to diestrus ($t = 0.63$; $p > .20$ for a two-tailed test). If anything, the weight change spread over these 2 segments of the estrus cycle tended to be slightly larger in the vagotomized group (3.11 gms versus 2.59 gms for controls).

Of the 16 vagotomized animals which initially

satisfied the drinkometer criterion, 14 also had no acid response to insulin. It is possible that some regeneration may have occurred in the other 2 animals between the time of the meal frequency and the insulin tests. Meal frequency would then still seem a reasonably good, atraumatic test to screen initially for completeness of vagotomy.

The important result is the failure of vagotomy to affect the cyclic weight changes of the female rat. Thus, whatever the nature of these changes, they are not mediated by parasympathetic innervation of the viscera. Insulin and basal acid secretion are shown in Table 7. The insulin values are in line with those reported in Experiment 12

TABLE 7

Means and standard deviations of plasma insulin (μU/ml) and basal gastric acid secretion (μEq H+/30 minutes) for vagotomized female rats at either diestrus or estrus.

Group	N	Plasma Insulin	Acid Secretion
Diestrus	7	79.9 ± 9.8	4.9 ± 1.6
Estrus	7	57.5 ± 13.1	3.8 ± 2.0

(Table 4). The insulin level at estrus is significantly greater than at diestrus ($t = 2.62$; $p < .05$ for a two-tailed test). The mean insulin level for vagotomized animals was slightly lower than the controls of Experiment 12, the difference being more noticeable at estrus. The differences in basal acid secretion between estrus and diestrus are, however, eliminated by vagotomy ($t = 1.00$; $p > .20$ for a two-tailed test).

5.4.3 Discussion

Taken together, the results of this experiment: (a) demonstrate that vagal innervation of the viscera is not involved in maintaining the oscillations in weight and insulin which accompany the estrus cycle in the female rat, and (b) eliminate changes in basal acid secretion as responsible for these cyclical weight fluctuations. The possibility still remains that the fluctuations in insulin secretion are related to the rhythmic changes in intake and weight.

5.5 Experiment 15: Estrus Cycle and Diabetes

The fourth experiment in this chapter was designed to assess the effects of controlled insulin levels on the weight and food intake changes during the estrus cycle. It has previously been noted that induction of diabetes by alloxan interferes with estrus cycling [Davis, Fugo and Lawrence, 1947]; however, Prager, Abramovici, Liban and Laron [1974] reported no disruptive effect of diabetes induced by streptozotocin.

5.5.1 Method and Results

Subjects and housing were as in Experiment 12. From 4 to 5 cycles were followed before induction of diabetes. Animals were deprived overnight and injected with streptozotocin (65 mg/kg i.p.). Food and water were then continuously available and smears were taken over the 2 weeks necessary for food and water intake and weight to stabilize.

Contrary to the observations of Prager, Abramovici, Liban and Laron [1974], persistent diestral smears were observed in the diabetic animals beginning 2 to 13 days (mean 5.6) following streptozotocin injection. During the 2 weeks following induction of diabetes, the animals lost from 4% to 11% (mean 8.1%) of their pre-injection body weight. To determine if this were the cause of the failure to cycle, a control group was deprived to 80% body weight over a 2-week period. This treatment failed either to

disrupt or lengthen the estrus cycle in these animals, results in agreement with those of Rosen and Petty [1974], who found no disruption even with deprivation down to 70% of body weight.

5.5.2 Discussion

This experiment fails to shed light on the problem for which it was originally designed. It is important for two reasons. First, it offers results not in agreement with those of Prager, Abramovici, Liban and Laron [1974]. Their dosage is below that normally used for induction of diabetes by streptozotocin and the form of diabetes thus induced may be peculiarly mild.

More importantly, the cessation of cycling caused by the induction of diabetes is not dependent on weight loss. The results demonstrate that the diabetic animal is capable of a cessation of cycling to only marginal weight loss (some 8%) while no disruption is observed in the normal animal reduced in weight as much as 30% [Rosen and Petty, 1974]. This difference may provide an important clue to the nature of protective metabolic adaptations available to the normal animal in the face of limited access to food, but apparently denied the insulin-deficient animal.

5.6 Discussion of Chapter Five

The research reported in this chapter was originally undertaken with the hope of establishing the estrus cycle of the female rat as a preparation which displayed the hyperphagia and obesity characteristic of the VMH syndrome (and for the same metabolic reasons) without the multiple problems associated with brain damage. As such the results are negative. Two experiments are critical in eliminating the diestral hyperphagia and weight gain of the female rat estrus cycle as a model of VMH damage. Experiment 12 showed that not only are insulin levels not elevated at the time of relative hyperphagia (diestrus) but are actually significantly depressed compared to the periods of low intake and weight loss (proestrus and estrus). In addition, while vagotomy reverses the obesity and hyperphagia of the VMH preparation (Powley and Opsahl, 1974; Experiment 1), Experiment 14 showed that vagotomy has no effect on the relative hyperphagia and weight gain of the diestral phase of the estrus cycle in female rats.

On the positive side there are several encouraging findings. The present results demonstrate that the weight fluctuations of the estrus cycle do not come about as a result of brain mechanisms acting via parasympathetic connections with the viscera. Thus despite the existence of "specific" estrogen-sensitive neurons in the two

hypothalamic areas most closely identified with energy balance, their activity would not appear to be related to regulation of food intake and weight in the same vagus-dependent manner as the classic VMH syndrome. Whether these estrogen-sensitive VMH cells have any relevance to weight regulation is still unclear. They may act via medullary or nervous sympathetic activity or indirectly via hormonal mechanisms. Alternatively, the estrogen effects may be of totally peripheral origin.

A second important point is with respect to the data of Experiment 12. The instability of the human diabetic during pregnancy and over the estrus cycle is well documented. Spellacy, Carlson and Schadé [1967] investigated the basal insulin levels in human females over the estrus cycle and concluded that there were no changes in basal insulin which could account for this instability. The present data call for a re-examination of this conclusion. A species difference is possible, however, the results of Spellacy, Carlson and Schadé cannot be accounted for in terms of suppression, due to social factors, of the expected weight increment at menstruation. Weight increases tend to raise basal insulin levels and thus would tend to obscure the relative hypoinsulinemia found at diestrus in the present experiments.

This raises the third point. Recently a good deal of

attention has focussed on the role of insulin in the regulation of body weight. Thus Woods and Porte [1976; see also Bernstein, Kulkosky, Lotter, Porte and Woods, 1975] have pointed out the positive relationship between weight levels and basal insulin, with the implication being that these two measures not only covary, but that changes in basal insulin are causal in changing regulated weight levels. The results of Experiment 12 stand in direct contrast to the positive relationship described by Woods and co-workers and thus question the nature of that relationship.

The low plasma levels of insulin at diestrus, while unexpected, may throw interesting light on the relationship between insulin levels and short- and long-term energy balance mechanisms. With repeated daily injection of insulin hyperphagia results [Kumaresan and Turner, 1965; MacKay, Callaway and Barnes, 1940]. The overeating continues until a new level is reached and then food intake reduces to maintain this weight [Panksepp, Pollack, Krost, Meeker and Ritter, 1975]. This pattern is very similar to that seen following VMH lesions where hyperinsulinemia is seen to be correlated with the hyperphagia [Hustvedt and Løvø, 1972]. With the artificial induction of diabetes by streptozotocin, the interesting pattern of Experiment 8 develops. The animal first reduces food consumption for a period of 2 days following injection. This, rather than

being due to illness because of the induction of diabetes, would seem to be an active suppression of intake. The induced diabetic, however, follows this hypophagic period with a steadily increasing hyperphagia which asymptotes at a chronically maintained high level. This occurs after obvious wasting of body tissue and would seem to reflect an imposition of the long-term control mechanisms based on some facet of this tissue loss.

The insulin-food intake relationship could then be synthesized as follows. During both short (single injection) and long (repeated injections and following VMH lesions) periods of very high insulin levels, hyperphagia and obesity result. During short-term induction of low insulin (anti-insulin serum, mannoheptulose, and the first 2 days of induced diabetes) food consumption is low. However, with a longer period of low insulin (chronic diabetes mellitus) hyperphagia occurs, most likely as a long-term system's response to depletion of particular body tissues or critical rate of their breakdown, but with no need for insulin.

It may be that the estrus-cycling female animal has adapted to fluctuating insulin levels (and persistent accumulation and degradation of tissue) by, for instance, anticipatory hyperphagia during diestrus to minimize tissue breakdown much in the manner of the hyperphagia of the chronic diabetic.

CHAPTER SIX

SYMPATHETIC NERVOUS SYSTEM CONTRIBUTIONS TO DIABETES MELLITUS

6.1 Introduction

The proposed model of VMH function may provide insights of practical, clinical significance in three major areas of treatment of human disease. Of these, applications to diabetes mellitus are seen as perhaps the most important and are examined in this chapter. The possibilities of applying the model to treatment of human obesity and to parental infusion of nutrient supply are considered in the discussion (Chapter Seven).

Historically, the similarities between diabetes mellitus and sympathetic nervous system arousal have been well noted. Both involve abnormal pancreatic alpha and beta cell function (hypoinsulinemia and hyperglucagonemia) and adjustment of body energy reserves and liver function in the direction of increased glucose manufacture and output (excessive lipolysis, proteinolysis, glycogenolysis, and gluconeogenesis with resulting hyperglycemia). Stress-induced hyperactivity of the SNS exacerbates the diabetic syndrome.

In spite of this, little attention seems to have been

paid to the possibility that the diverse metabolic abnormalities of diabetes mellitus owe their origin as much to a chronic high level of SNS activity as to a lack of insulin.

Recently a number of lines of investigation have given an excellent basis for a closer look at this question. There is now ample evidence for neural control of pancreas, liver and fat deposits. The demonstrations exist at 3 levels: anatomical evidence of neuronal investature of the organs themselves, the results of stimulation of the major nerves at points between the brain and their entrance to the organs, and, finally, results from direct stimulation of brain (particularly hypothalamic) sites associated with autonomic outflow. Prominent among these sites are the predominently sympathetic VMH and the parasympathetic LHA [Ban, 1964; 1966]. Interestingly, the activities in these two hypothalamic areas are often seen to be reciprocal [see Oomura, 1973].

The basis for autonomic nervous system control over pancreas has been generously established over the past 10 years. There is ample evidence for a rich nervous supply, both adrenergic and cholinergic, to the alpha and beta cells [see Woods and Porte, 1974]. Miller [1975], using his "support-dog" preparation, has shown the overall effect of sympathetic nerve stimulation to be suppression of insulin

secretion, a conclusion supported by the results of Frohman and Bernardis [1971] with stimulation of the VMH. Frohman and Bernardis did, however, ascribe the whole of this suppression effect to the adrenals. The effect of parasympathetic (vagal) stimulation is to increase insulin output [Frohman, Ezdinli and Javid, 1967; Kaneto, Kajinuma, Kosaka and Nakao, 1968].

Sympathetic stimulation increases glucagon output [Marliss, Girandier, Seydoux, Willheim, Kanazawa, Orci, Renold and Porte, 1973]. There is some disparity in the parasympathetic stimulation results. Kaneto, Miki and Kosaka [1974] reported an increase in glucagon following dorsal vagus stimulation; however Marliss and co-workers [1973] were unable to block, with atropine, the glucagon increase they observed following stimulation of the mixed autonomic nerves.

Though a direct nervous supply to the hepatocyte has only been demonstrated more recently [Tanikawa, 1968], Shimazu, Fukudu and Ban [1966] earlier demonstrated almost complete disappearance of liver glycogen following prolonged electrical stimulation of the VMH. Recently Shimazu and Ogasawara [1975] have shown that stimulation of the VMH results in an increase in phosphoenolpyruvate, a key gluconeogenic enzyme, and a decrease in pyruvate kinase, a key glycolytic enzyme. Sympathetic stimulation would then

appear to increase glycogen breakdown and gluconeogenesis while inhibiting glycolysis. The sum of these changes would be high blood glucose levels. Completing this picture is the finding that stimulation of the predominantly parasympathetic LHA results in a decrease in gluconeogenesis [Shimazu and Ogasawara, 1975]. In experiments concerning liver metabolism, the present issue is often complicated by differences between in vivo and in vitro preparations. For example, one can compare the differing conclusions about insulin effects on liver arrived at by Madison [1969] and Haft [1968]. Mortimore [1972] further reviewed these differences and pointed out the permissive effect of acetylcholine on glycogen deposition in the liver in the presence of insulin.

The role of free fatty acids (FFAs) in diabetes is also one of controversy. Sympathetic innervation of adipose tissue has long been known [Boeke, 1933]. FFAs certainly influence the rate of gluconeogenesis and their release is particularly sensitive to neural control [Penick, Prince and Kinkle, 1966]. Using the alloxan diabetic as a preparation exhibiting a high rate of FFA efflux from adipose tissue, Buckle [1963] was unable to show any effect of insulin on this rate of FFA release while Wertheimer and Shafrir [1960] reduced FFA release to normal with the autonomic blocking agent "dibenzyline". In concert with

this latter finding, Correll [1963] demonstrated release of FFA upon sympathetic nerve stimulation.

The second important series of results comes from work with the VMH. As pointed out previously, this structure is apparently unique (at least in the CNS) in that glucose uptake is insulin-mediated [Debons, Krimsky, Likuski, Cloutier and From, 1968; Smith, 1972]. Additionally, destruction of the VMH results in a syndrome that is characterized by metabolic abnormalities which are chronically uncompensated, which depend on the vagus for their expression, and which are fairly strikingly reciprocal to the metabolic abnormalities of diabetes. Two reports have now demonstrated an amelioration of certain diabetic syndromes with alpha-adrenergic blocking agents [Cegrell, 1972; Robertson, Brunsell, Hazzard, Lerner and Porte, 1972]. Finally, the postulated insulin-glucoreceptor model of Chapter Three suggests a mechanism by which inappropriately low insulin levels would result in a chronically high level of VMH-generated SNS activity. To review, the basis of the proposed system is the insulin-mediated uptake of glucose in specialized VMH cells relevant to glucoregulation. In essence this means that the brain perceives its normal energy supply as "available" glucose, this being determined by both blood glucose and the insulin required for its uptake. This is quite reasonable under normal circumstances

since glucose is by far the most potent stimulus for insulin secretion. However, in the diabetic case where insulin is only poorly secreted in response to glucose, the VMH uptake of glucose would be low and the CNS would "perceive" a much lower level of glucose than is existant. According to the model, SNS activity would remain high and the pattern of metabolic activity aimed at increasing blood glucose supply would be sustained.

If this system is correct, then a reduction in activity and/or destruction of the peripheral sympathetic nervous system should relieve some of the metabolic problems of diabetes. The following experiments were designed as an initial assessment of this argument.

Guanethidine sulphate (Ismelin, kindly supplied by CIBA-GEIGY) was chosen for treatment for several reasons. It is first of all a sympathetic blocking agent. As such it has a long history of human use for hypertension. As previously pointed out, in addition to its blocking action, it has recently been found to effect near complete destruction of the peripheral sympathetic nervous system with chronic, high dosage treatment. This treatment appears to be without serious side effects. Finally, as it does not cross the blood-brain barrier, it has no central effect.

6.2 Experiment 16: Sympathectomy and Diabetic Intake Utilization

This experiment was designed to look at one very general aspect of diabetes, the efficacy of utilization of intake. Diabetes mellitus induced with either streptozotocin or alloxan is characterized by polydipsia, hyperphagia and some weight loss. The polydipsia is thought to reflect the amount of fluid necessary to avoid renal collapse due to the hypertonicity of the glucose-laden blood. The hyperphagia would seem necessary to counteract the excessive tissue breakdown (which results in weight loss) and wastage of nutrients through urinary and fecal output. It was reasoned that if guanethidine sulphate sufficiently ameliorated any of the number of metablic abnormalities of diabetes, then this improvement would be expressed as a decrease in the amount of food and water needed by the diabetic animal to maintain a particular weight.

6.2.1 Method

Animals used were female, Wistar-derived rats obtained from the breeding colony of the John Curtin School of Medical Research (Canberra, Australia) weighing approximately 200 gms at the beginning of the experiment. Diabetes was induced in 24 animals by a single injection of streptozotocin (65 mg/kg i.p. in citrate buffer pH 4.5) following an overnight fast. Sixteen control animals received

only buffer. All animals were then maintained on ad libitum access to food and water for approximately 2 months to ensure stable intake and weight. From gross observation of the diabetic animals it was apparent that the severity of diabetic effects covered an appreciable range. It was therefore decided to create 2 groups (1 group to receive guanethidine sulphate and the other physiological saline) matched on the basis of blood glucose levels. Following a 4-hour fast animals were lightly anesthetized with ether and blood was taken from a cut at the tip of the tail. Blood glucose was analyzed by the ortho-toluidine method of Hyvarinen and Nikkila [1962]. On the basis of the glucose levels 2 matched groups of 7 diabetic animals each were formed. Guanethidine sulphate (GS) treatment was initiated one week following the blood glucose sampling. Treatment consisted of 6 weeks of daily injection with GS (25 mg/kg i.p.) or saline [Burnstock, Evans, Gannon, Heath and James, 1971]. Four groups were initially treated: diabetic animals given guanethidine sulphate (D-GS), diabetic animals given physiological saline (D-S), control animals given guanethidine sulphate (C-GS), and control animals given physiological saline (C-S). The original water intake, food intake and weight measures were based on the mean of the 3 days prior to the initiation of treatment. Water intake was measured every fifth day, food intake every 2 weeks,

and weight daily for the 6 weeks of treatment and 3 weeks following cessation of injections.

A fifth group of diabetic animals of comparable blood glucose level was added at the end of the first week when it was realized that water intake had dropped substantially in the D-GS group. This additional group (D-WR) had water intake restricted in the following manner. As each fifth day's intake was measured for the D-GS group, the intake was converted to a mean percentage of its original intake. Each animal in the D-WR group was then allowed an amount of water equal to this mean percentage of the individual's original intake, the percentage being updated each 5 days. Water was available to the D-WR group throughout the dark period (1900-0700 hours), removed from 0800 to 1200 hours and then replaced at 1200 hours. The bottle was invariably empty at 1900 hours when a new ration was given. The D-WR group was designed to assess the effect of a reduction in water intake on weight in diabetics not treated with guanethidine sulphate.

Multigroup statistical comparisons were made using one-way, fixed-effects analyses of variance at particular time points followed by Scheffé tests.

6.2.2 Results

The results are shown in Figures 14, 15 and 16 (F ratios are summarized in Appendix M). The important

Figure 14. The effect of guanethidine sulphate sympathectomy on the mean body weights of normal (C-GS; n = 8) and diabetic (D-GS; n = 7) female rats compared to normal (C-S; n = 8) and diabetic (D-S; n = 7) animals receiving only physiological saline. A diabetic group (D-WR); n = 5) was included whose water consumption was paired to that of group D-GS. The dotted line beneath the abscissa indicates the 6 weeks of daily injections.

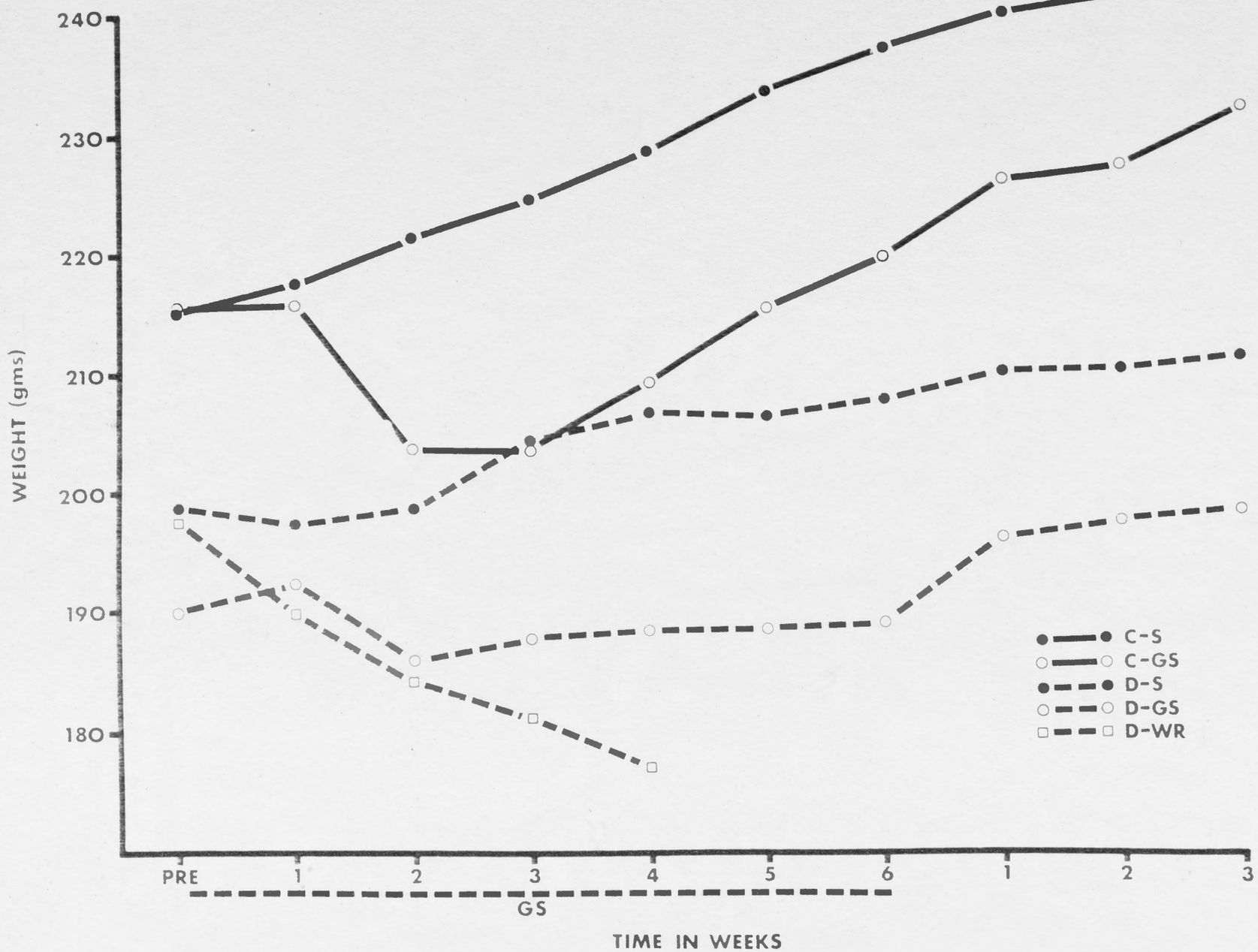

240
230
220
210
200
190
180
WEIGHT (gms)
PRE
1
2
3
4
5
6
1
2
3
GS
TIME IN WEEKS
C-S
C-GS
D-S
D-GS
D-WR

Figure 15. The effect of guanethidine sulphate sympathectomy on the mean food intake of groups normal (C-GS) and diabetic (D-GS) female rats compared to normal (C-S) and diabetic (D-S) groups receiving only physiological saline. A diabetic group (D-WR) was included whose water consumption was paired to that of group D-GS. The dotted line beneath the abscissa indicates the 6 weeks of daily injection.

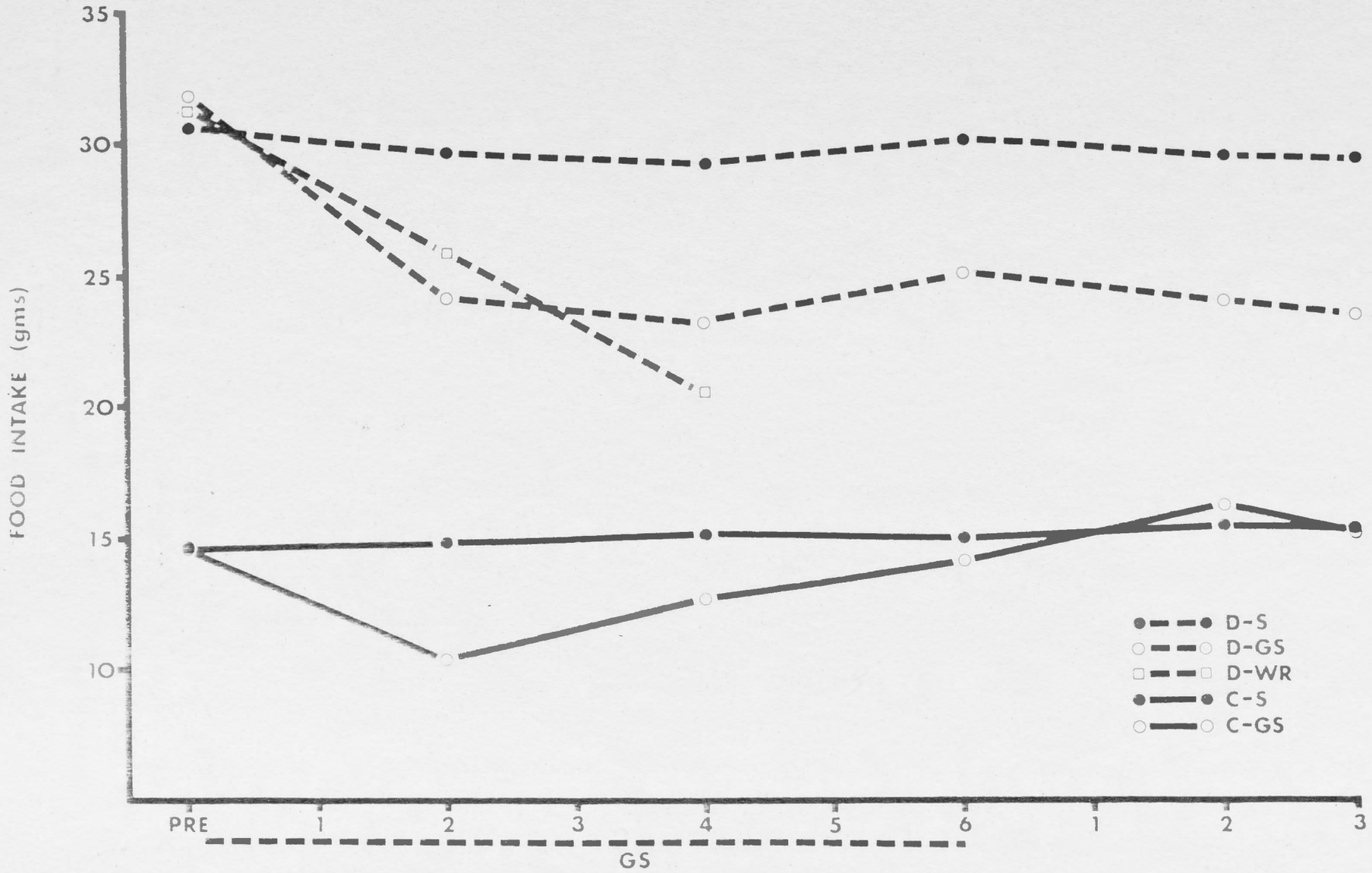
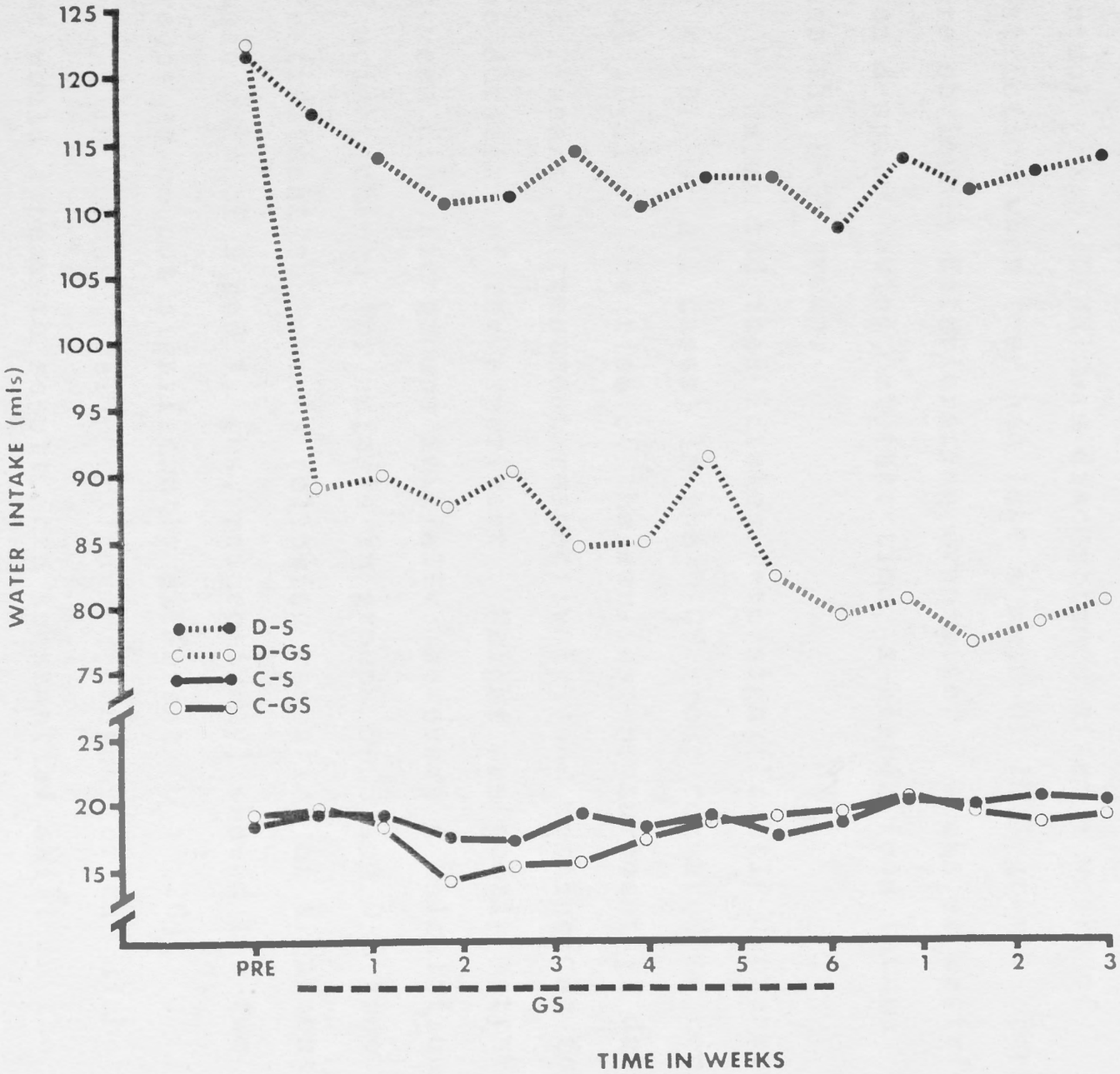

35
30
25
20
15
10
FOOD INTAKE (gms)
PRE
1
2
3
4
5
6
1
2
3
GS
TIME IN WEEKS
D-S
D-GS
D-WR
C-S
C-GS

Figure 16. The effect of guanethidine sulphate sympathectomy on the mean water intake of groups of normal (C-GS) and diabetic (D-GS) female rats compared to normal (C-S) and diabetic (D-S) groups receiving only physiological saline. The dotted line beneath the abscissa indicates the 6 weeks of daily injection.


WATER INTAKE (mls)
125
120
115
110
105
100
95
90
85
80
75
25
20
15
D-S
D-GS
C-S
C-GS
PRE
1
2
3
4
5
6
1
2
3
GS
TIME IN WEEKS

finding is a drop in food and water intake in the guanethidine sulphate-treated diabetic animals to a level some 70% to 75% of pre-treatment without loss of weight. This reduction in intake continued for the 3 weeks of observation following cessation of treatment. The water-restricted control group (D-WR) was discontinued after 4 weeks of restriction when they had lost a mean of 20.8 grams. They were obviously deteriorating even after 2 weeks of restriction despite having, at that time, a higher food intake than the D-GS group.

Water and food intakes were significantly depressed ($p < .01$ in all cases) in the D-GS group compared to control (D-S) at the time of the earliest measurement (3 days and 2 weeks of treatment, respectively) and remained so for the duration of the experiment. Weight was poorly matched between diabetic groups initially; however, a calculation of weight change for animals in groups D-GS and D-S from pre-treatment to 3 weeks following cessation of treatment (mean gain of 9 and 12 gms, respectively) showed the two groups to be not significantly different ($p > .20$).

In summary, peripheral sympathectomy of the diabetic rat would appear to result in a substantial shift in the direction of more efficient utilization of incoming nutrients. This point is further examined and discussed in the following experiments of this chapter.

The effect of sympathectomy on intake and weight in the normal, adult animal does not appear to have been previously reported in detail. The control data may then be of interest. A significant depression of both intake measures and weight was evident by the second week of treatment in the C-GS group compared to the C-S group (weight and food intake, $p < .01$; water intake, $p < .02$). This is approximately the time when guanethidine sulphate would begin to accumulate and initiate significant destruction of the sympathetic nervous system. Whether or not the depression in intake and weight was the result of this destruction or a side effect of the drug, intake measures returned to normal by the end of treatment, and weight, while still reduced ($p < .05$), appeared to be returning to control levels. Peripheral sympathectomy would then seem to have little long-term effect on intake or body weight in the adult, female rat, a conclusion in concert with that reached for the male rat (Experiment 3).

6.3 Experiment 17: Sympathectomy, Ganglionic Block and Diabetic Blood Glucose

As mentioned in the introduction to this chapter, guanethidine sulphate produces a relatively short-term ganglionic block following a single injection as well as eventual SNS destruction. This experiment looked specifically at blood glucose changes as a function of these two actions.

6.3.1 Method

The effect of SNS destruction was assessed on the animals of Experiment 16. Blood glucose was originally determined 1 week before treatment and a second sample was taken 4 weeks after treatment. No serial determinations throughout treatment were attempted, as diabetic animals react badly to the stress of blood sampling and it was felt this would seriously confound the intake and weight measures.

Blood glucose changes induced as a result of the ganglionic blocking action of GS were determined on separate groups of animals made diabetic as in Experiment 16 and closely matched for blood glucose levels. Two short-term experiments were run. Blood glucose was measured 6, 24 and 72 hours following a single injection (100 mg/kg i.p.) of GS in one group of diabetic animals. A further group of diabetic rats were given daily injections (100 mg/kg

i.p.) for 4 days and glucose was again measured 6, 24 and 72 hours after the last injection. The control group was given 4 days of saline injections and therefore is most strictly comparable to this latter group. In all groups food was not available for 4 hours before each blood sampling. Blood was always withdrawn from the cut tip of the tail under light ether anesthesia and glucose analyzed by the ortho-toluidine method of Hyvarinen and Nikkila [1962].

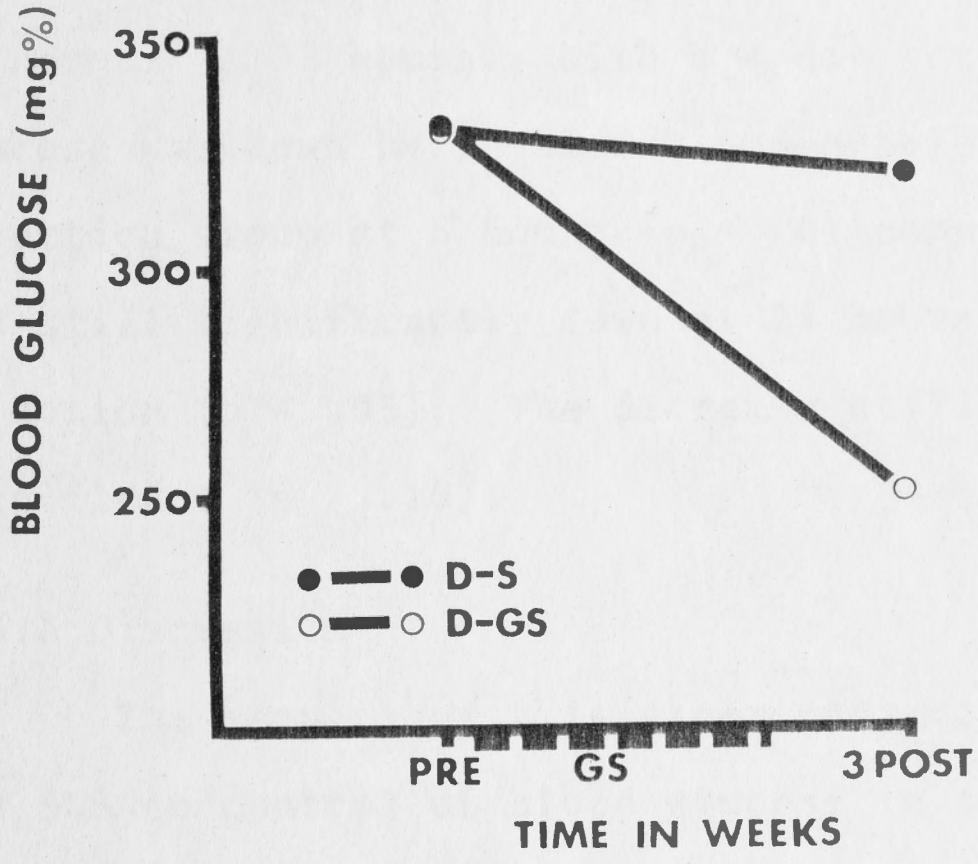
6.3.2 Results

The effect of SNS destruction on diabetic blood glucose levels is shown in Figure 17A. Blood glucose of sympathectomized diabetic rats was reduced by 22.3% 4 weeks following cessation of treatment while controls were not significantly affected. The post-treatment difference was significant at the 0.01 significance level.

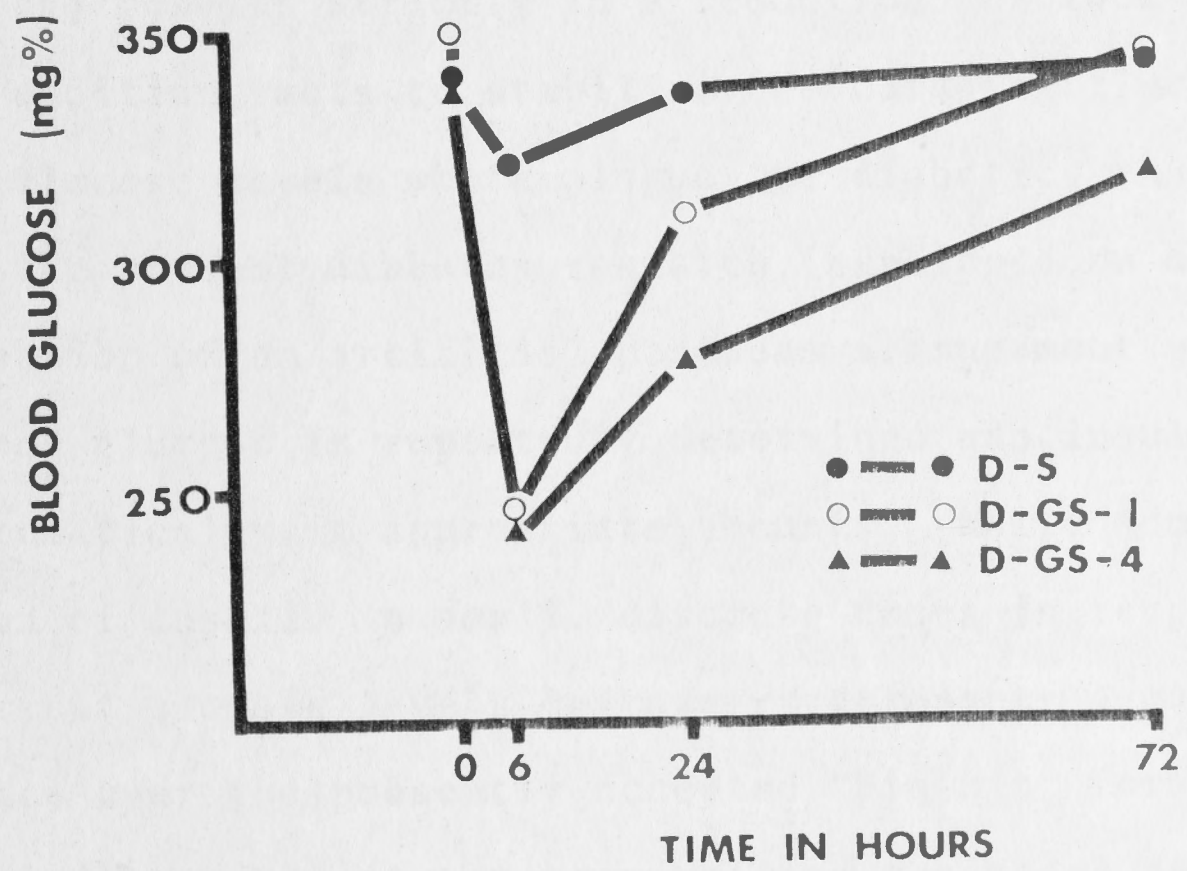
The data from the short-term experiments are shown in Figure 17B (see Appendix N for the F ratios). A single injection of GS (100 mg/kg) caused a significant drop in blood glucose of 28.3% within 6 hours ($p < .01$ compared to control). As judged by ptosis in mice, a single injection of GS should still be maintaining maximal ganglionic block at that time [Fielden and Green, 1965]. A slight reduction in blood glucose was still evident 24 hours following injection but, due to a large variability, failed to reach

Figure 17A. Plasma blood glucose of diabetic, sympathectomized (D-GS); n = 7) and diabetic, physiological saline-injected (D-S; n = 7) groups prior to, and 3 weeks following, treatment.

Figure 17B. The effect of a single 100 mg/kg injection of guanethidine on blood glucose of diabetic animals 6, 24 and 72 hours following that injection (D-GS-1; n = 6) compared both to a control, saline-injected group (D-S; n = 4) and a group whose blood glucose was measured 6, 24 and 72 hours following the last of a series of 4 daily 100 mg/kg guanethidine injections (D-GS-4; n = 6).


BLOOD GLUCOSE (mg%)
350
300
250
D-S
D-GS
PRE
GS
3 POST
TIME IN WEEKS

A


BLOOD GLUCOSE (mg%)
350
300
250
D-S
D-GS-1
D-GS-4
0
6
24
72
TIME IN HOURS

B

statistical significance ($p > .10$). Glucose had returned to normal by 72 hours. With a 4-day series of injections, glucose was down by an amount comparable to the single injection group at 6 hours ($p < .01$ compared to control) and still significantly down at 24 hours following the last injection ($p < .05$). The decrease at 72 hours was not significant ($p > .10$).

6.3.3 Discussion

The results of this experiment confirm the role of the SNS in control of blood glucose in the diabetic, both via acute ganglionic block and chronic destruction.

It is of great interest whether sympathectomy (or SNS block) results strictly in a reduction in blood glucose or, in addition, acts to stabilize the damaging fluctuations in glucose levels which plague the diabetic. A good deal of the present diabetes research is being done on the creation of an artificial pancreas arrangement whereby blood glucose is repeatedly determined and insulin injected automatically in appropriate amounts. While administration of insulin in small, discrete doses in response to current glucose levels certainly represents a marked advance over the presently accepted "big hit" form of insulin administration, it may be, recalling the model generated in the discussion of Chapter Three, that the anticipatory nature of the cephalic portion of insulin secretion will frustrate this approach.

6.4 Experiment 18: Sympathectomy and Diabetic Plasma FFAs

There are numerous mechanisms by which ganglionic block or sympathectomy induced by guanethidine sulphate might reduce blood glucose. As pointed out previously, the availability of free fatty acids (FFAs) certainly influences, as substrate, the rate of gluconeogenesis in the liver. It has long been known that ganglionic block induced by "dibenzyline" reduces the efflux of FFAs from the adipose tissue of diabetics [Wertheimer and Shafrir, 1960]. The present experiment extended this finding to guanethidine sulphate (GS).

6.4.1 Method

Animals and induction of diabetes were as in Experiment 16. Blood was collected from a tail vein under light ether anesthesia, the lipid portion of the blood was extracted with chloroform-methanol, and FFAs were analyzed by the method of Duncombe [1963]. All blood sampling was done between 1600 and 1700 hours on 9- to 10-hour deprived animals. Four groups of diabetics were run. In 2 groups food was removed at 1700 hours of the second day following induction of diabetes, and either GS (100 mg/kg i.p.; n = 4) or physiological saline (n = 6) injected. Blood sampling was done the afternoon of the same day. In the other 2 groups either GS (100 mg/kg i.p.; n = 7) or an

equivalent volume of saline (n = 6) was injected each day at 1600 hours for days 8, 9, 10 and 11 following induction of diabetes. Food was removed at 0700 hours on day 12 and blood sampled that afternoon.

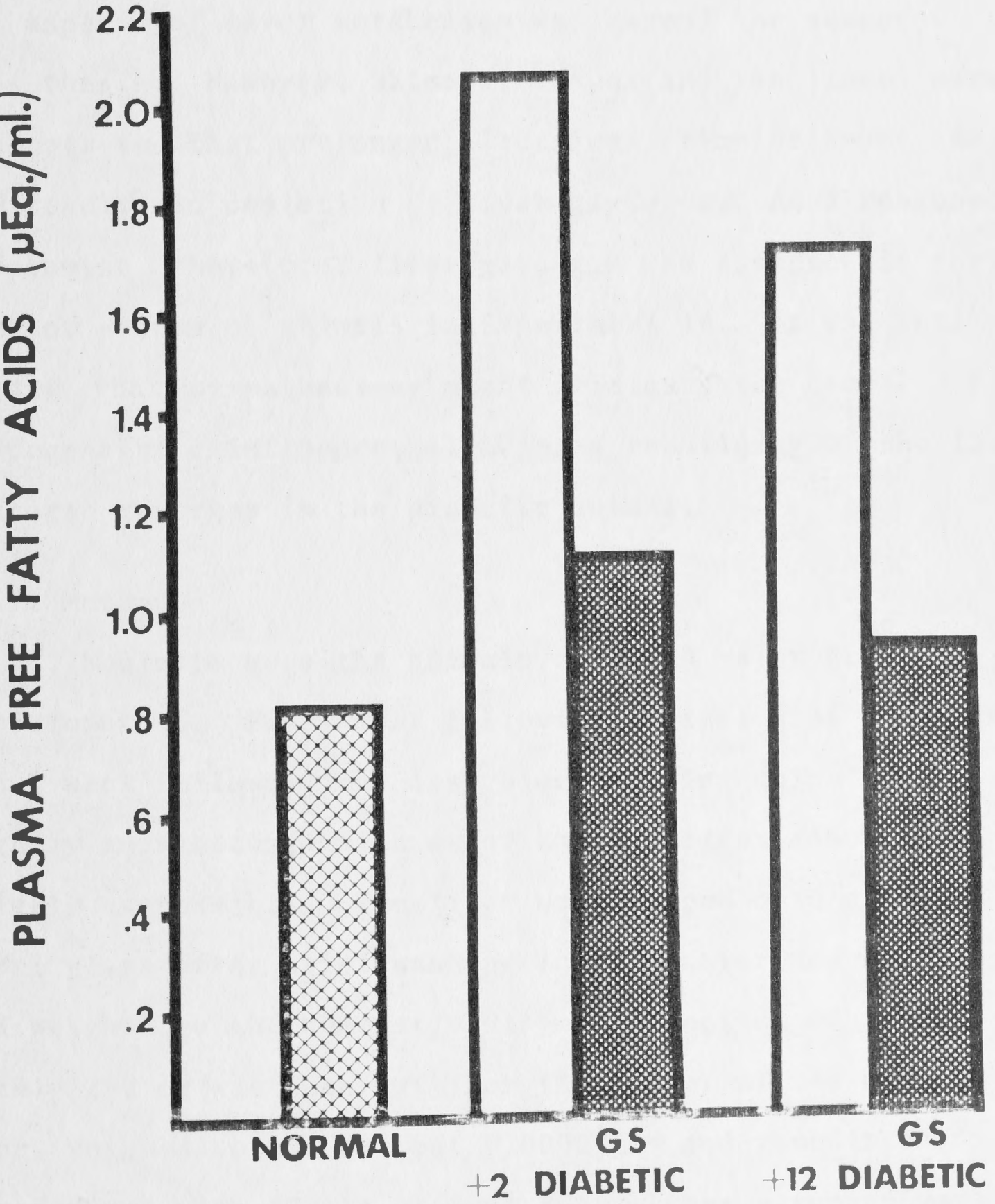
6.4.2 Results

The results are shown in Figure 18. Plasma FFAs were reduced both 10 hours following a single injection of GS ($t = 11.03$; $p < .01$ for a two-tailed test) and 24 hours following a 4-day series of GS injections ($t = 2.81$; $p < .02$ for a two-tailed test). This confirms, for GS, results obtained with dibenzyline.

6.4.3 Discussion

The reduction in plasma FFA's most likely reflects a decrease in efflux from fat stores. Such a decrease would obviously be important to the diabetic both as protection against depletion of body energy reserves and in slowing the rate of gluconeogenesis and hence reducing both blood glucose levels and tendency to ketosis.

Figure 18. Plasma free fatty acid levels of 9- to 10-hour deprived normal and diabetic animals. Blood sampling was done on the diabetic animals either 2 days following induction of diabetes with streptozotocin and having had either 100 mg/kg of guanethidine (dark cross-hatching) or physiological saline (open bars) injected at the beginning of the fast or 12 days following induction of diabetes and 24 hours following the last of a series of 4 daily injections of either guanethidine (100 mg/kg) or saline.


PLASMA FREE FATTY ACIDS (μEq./ml.)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
NORMAL
GS +2 DIABETIC
GS +12 DIABETIC

6.5 Experiment 19: Sympathectomy and Diabetic Liver Glycogen

A proper analysis of the effect of sympathectomy on all aspects of liver metabolism was beyond the scope of this thesis. However, Shimazu, Fukuda and Ban [1966] have demonstrated that prolonged electrical stimulation of the VMH results in depletion of liver glycogen. As a reasonable compromise, therefore, liver glycogen was assessed in the 4 major groups of animals in Experiment 16. It was anticipated that sympathectomy might eliminate the neural glycogenolytic influence, allowing a rebuilding of the liver glycogen reserves in the diabetic animal.

6.5.1 Method

Subjects were the animals of the 4 major groups of Experiment 16. Five weeks following cessation of treatment, and 1 week following the last blood sample, animals were lightly anesthetized with ether and the liver removed as quickly as possible. The liver was dropped onto a frozen petri glass after brief washing in cold water and blotting, and weighed to the nearest 0.01 gm. A section of approximately 200 mg was then cut from the center of the major lobe, weighed to the nearest 0.0005 gms and immediately homogenized with 20 mls of 5.0% TCA. Liver glycogen was analyzed according to the iodine method of Van der Vies [1954]. A one-way, fixed effects analysis of variance

was carried out on both liver weight and glycogen data followed by Scheffé individual comparisons.

6.5.2 Results

Liver weights and glycogen content are reported in Table 8 (see Appendix P for F ratios). Livers were significantly heavier in the diabetic groups compared to their

TABLE 8

Liver weight and glycogen content (mean ± SD) of sympathectomized diabetic and sympathectomized normal animals and their respective control groups.

Group	Liver wt. (gms)	Liver glycogen (% wet weight)
Diabetic-sympathectomized	10.08 ± 1.26	1.39 ± 0.83
Normal-sympathectomized	8.06 ± 0.58	3.13 ± 0.59
Diabetic-control injected	10.99 ± 0.95	1.81 ± 1.06
Normal-control injected	8.43 ± 0.73	3.38 ± 0.48

respective controls ($p < .01$ in both cases). There were no differences between diabetic groups or between control groups. Liver glycogen in both diabetic groups was considerably reduced compared to controls ($p < .02$ in both cases). However, contrary to prediction, sympathectomy not only failed to ameliorate the glycogen deficiency of

the streptozotocin diabetic, but the trend is in the opposite direction. It is interesting to note that sympathectomized control animals also have a non-significant ($p < .10$) tendency towards smaller livers and less glycogen per gm of liver.

6.5.3 Discussion

The results of this experiment demonstrate that sympathectomizing diabetic animals whose liver glycogen is already greatly reduced does not result in any repletion of the liver glycogen reserve. It may be more reasonable to expect that inducing diabetes in already sympathectomized animals or initiating GS treatment immediately upon induction of diabetes would limit the depletion of liver glycogen.

The results are, however, interesting from two other points of view. First, sympathectomy tends to reduce the excess liver weight which accumulates in the diabetic. As this enlargement is likely to reflect the excessive gluconeogenic demands made on the diabetic liver, the result supplements those showing decreased gluconeogenic substrate [Experiment 18] and apparent glucose output [Experiment 17] following sympathectomy. Second, while the results of Shimazu, Fukuda and Ban [1966, cited in the introdution to this experiment] suggest that sympathetic arousal has a glycogenolytic effect, the present results demonstrate that

basal levels of SNS activity do not function to limit glycogen reserves.

6.6 Discussion of Chapter Six

The intake and weight data along with the data on blood glucose and FFAs would appear to mean a shift away from glucose output and towards more efficient conservation of body tissue and utilization of incoming nutrients. As this work is of an exploratory nature, little can be said about where in the metabolic systems the advantageous changes are taking place. There are a number of candidates.

As pointed out in the introduction to this chapter, sympathetic nerve stimulation can cause the release of glucagon [Bloom, Edwards and Vaughan, 1973; Marliss, Girardier, Seydoux, Wolheim, Kanazawa, Orci, Renold and Porte, 1973] and, where there is a certain level of residual insulin secreting capacity, this potential may be under active inhibition [Frohman and Bernardis, 1971].

The stimuli for mobilization of body fat may be largely of neural origin. Reducing FFA efflux by sympathetic block would limit gluconeogeneis. The decreased plasma FFAs may then account for the reduction in blood glucose. It has been generally presumed that the osmotic changes produced in the blood by high levels of glucose, and the water necessarily excreted in order to eliminate this glucose, accounted for the diabetic polydipsia. The reduction in blood glucose (whether by reduction in rate of gluconeogenesis or by some other mechanism) would account

at least for the drop in water intake. Food intake is more difficult. The hyperphagia of the diabetic presumably reflects the influence of a long-term body reserve regulation [Experiment 8]. Until the nature of this long-term system is established it will be impossible to say which metabolic effects of GS are responsible for the decrease in food intake.

Liver enzyme activity may be directly altered from excessive glycogenolysis and gluconeogenesis. Certainly it has been demonstrated that glycogen breakdown [Shimazu, Fukuka and Ban, 1966] and critical enzymes in the gluconeogenic and glycolytic pathways in the liver [Shimazu and Ogasawara, 1975] can be significantly modified by changes in hypothalamic activity. The whole question of the effect of insulin on handling of glucose by the liver is a difficult one. Certainly *in vivo* and *in vitro* types of preparations give dramatically different results [see, for example, Haft, 1968; and Madison, 1969]. While a discussion of these differences and the reasons for them is completely beyond the scope of this thesis, the following points may be made. In general there seems little doubt that insulin has a large effect on a range of liver functions. *In vitro* those effects are often difficult to demonstrate. For example Mortimore [1972] states that it appears that "insulin has little or no effect on glucose uptake and glycogenesis. On

the other hand, evidence from the intact animal clearly indicates that insulin is capable of stimulating glycogen synthesis". He then goes on to say, "One clue to a possible explanation has come from a report that glycogen synthetase activity increases after vagal stimulation *in vivo* [Shimazu, 1967]. Following this observation, Mondon *et al*. [Mondon and Burton, 1971] noted that glycogen deposition in perfused livers of fasted rats is greatly increased by insulin in the presence of acetylcholine and approaches rates of deposition seen in the intact rat. The possibility of neural influences cannot be ignored, and, if Mondon's findings are confirmed, one must consider the action of insulin in relation to the autonomic function of liver." These comments by Mortimore represent one of the very few references to insulin effect on liver function in terms of neural input. That this approach seems largely ignored probably reflects the overwhelming complexity of assessing the effect of change in one hormone *in vivo*. A cross-perfusion system along the lines of that employed by Bergman and Miller [1973] may provide the basis for at least a partial solution to the problem. This system would allow the manipulation of autonomic activity on an organ *in situ* with normal neural connections but whose blood supply was independent.

Finally, the present findings focus attention on the

role in diabetes of that portion of the autonomic nervous system whose prime function has been classically seen as mobilizing the body's resources in face of both internal and external stressors. As such the SNS may play its most devastating role in diabetes during the onset period of the juvenile form of diabetes mellitus. This period can rougly be characterized as follows. The person destined to become a juvenile diabetic is usually first admitted to hospital in, or near to, an insulin deficient coma. Treatment with insulin is effective in relieving the acute problem and the patient is usually discharged regulating blood glucose "normally" without the necessity, at this time, for insulin treatment. The relapse generally occurs some weeks, or even months, later and this time a full-blown, chronic, insulin-dependent diabetes results. As well as generally occurring around the time of the hormonal changes of puberty, the onset episodes often occur at times of other overt mental or physical (injury or illness) stress.

Unhappily, no animal model now exists which even approximates the onset symptoms of the juvenile diabetic. However, the rat made abruptly diabetic with streptozotocin does exhibit many of the metabolic features of the juvenile onset period, including hyperglycemia, some degree of ketosis and severe wastage of body tissue. It would be of interest to determine whether ameliorating these onset

symptoms by, for example, prior sympathectomy would have even more substantial long-term remedial effects.

Further, it has recently been demonstated that a predisposition to diabetes is apparently produced by neonatal administration of subdiabetic doses of streptozotocin [Portha, Levacher, Picon and Rosselin, 1974]. Stress, in these animals, may be sufficient to induce diabetes and thus yield a more appropriate model for juvenile onset. Studying the effects of controlling SNS activity in such a model may prove rewarding.

CHAPTER SEVEN

GENERAL DISCUSSION

The major argument presented herein is that the bulk of the data generated by various manipulations of the VMH can be accounted for by assuming a direct role for the VMH in glucoregulation. The postulated receptor system is consistent with a good deal of data and the model, in general, would seem both to be cohesively logical and of some predictive value. A basis for its application to diabetes is provided in Chapter Six. As pointed out in the introduction to that chapter, diabetes is one of three areas of practical importance to which the model could be applied. In addition, although beyond the scope of this thesis, its relevance to neurogenic control of weight regulation and to the practice of complete alimentation of patients by the intravenous route should be explored.

7.1 Neurogenic control of weight regulation

The present argument may provide an anatomical basis for the phenomenon described by Nisbett [1972], Schachter [1971] and Stunkard [1968]. These authors discuss data concerning "environmental" influences on food intake and suggest that obese people are more responsive to external cues than normals. Thus obese subjects were found to be

significantly more susceptible to the influences of the social, cognitive, situational, and gustatory-quality aspects of nutrient ingestion. The specific suggestion of these authors is that, however it occurs, the obese person is biased toward responding to the external, rather than internal, cues associated with food intake.

Using the model of VMH function generated in this thesis, this suggestion can be interpreted as follows. In the "normal", non-obese animal ingestion of a familiar food will provide a series of metabolic responses which, in previous experience, have been found to result in: (a) reasonable post-prandial nutrient supply, and (b) stable long-term weight maintenance. With each novel food the appropriate series of metabolic responses must be redetermined. While it seems likely that the gustatory qualities of the novel food will initially bias both the amount ingested and the metabolic responses, the caloric value of the new food will eventually be felt. Thus, in the case of a neutral-tasting, calorically-dense food, the initial response would be, for instance, an insufficient secretion of insulin and the aversive consequence of a relative hyperglycemia. The alternatives available for subsequent meals are then either to: (a) reduce intake of the calorically more dense food, or (b) increase the metabolic (e.g., insulin) response and consume the same amount, ignoring

the long-term consequences (i.e., obesity) of such a course of action. The VMH, by the model generated in Chapter Three, is seen as a major influence both in the evaluation of the sensory qualities of the food and in making the appropriate metabolic adjustments. The VMH-lesioned animal is then seen, from this view, to represent the most gross case where an oversecretion of insulin results from ingestion of food creating an aversive hypoglycemic result. Denied the alternative of reducing the physiological response, the animal is forced to consume larger and larger amounts of food to the point of exhausting insulin supply.

It may be, then, that the obese person, either through physiological abnormality or maladaptive learning, has depressed VMH responsivity to stimulus conditions associated with food ingestion. Thus the obese person, like the VMH-lesioned animal, may respond to a relative oversecretion of insulin (and the aversive aftereffects), not by reducing insulin output, but by increasing food intake. Booth and his co-workers have suggested that people may be trained to greater sensitivity for the carbohydrate content of their diet by giving them verbal feedback on what they have eaten and asking them to note carefully their internal sensations. Booth, Lee and McAleavey [1976] have demonstrated that man, like the rat, can learn to use a food's flavor to anticipate satiating aftereffects. If this acquired

sensory satiety involves adapting the insulin secretory response, then it may be possible to use more direct and powerful biofeedback. Although rapid monitoring of insulin levels is at present not technically feasible, it may be possible to use gastric motility and/or pH as manipulable biofeedback phenomena correlated with the insulin response.

7.2 Intravenous feeding and caloric regulation

The science of parenteral alimentation (the feeding of the entire nutrient supply by routes outside the alimentary canal, generally intravenously) is still relatively primitive. Achievements of satisfactory infusion mixtures over long periods of time are rare. Apart from the obvious failure to infuse essential amino acids and fatty acids, the recent report of Oohktens, Marsh, Smith, Bergman and Yates [1974] provides dramatic evidence of the anti-regulatory effect of continuous, long-term infusion of carbohydrate (typically given in humans via a destrose-saline infusion mixture).

A wide range of diet compositions can be orally ingested without significant fluctuation in calories of intake or weight [see Harper and Boyle, 1976]. Within those bounds any addition (by gastric, duodenal, or intravenous infusion) of nutrient to total energy supply would seem, logically, to be capable of compensation. Thus, if the

addition of glucose to an animal either intravenously or intragastrically does not require a reduction in oral intake of a magnitude such that supply of protein or essential fatty acids is critically impaired, then there should be perfect compensation (by reduced oral intake) for the caloric value of that infused glucose.

The initial study which explored this question [Adair, Miller and Booth, 1968], using continuous infusion intravenously (i.v.) over a period of days, reported absolutely no compensation for the infusion of 27.5 mls of 30% D-glucose per 24 hours (a rate which they calculated should raise the plasma level of glucose some 125 mg%). These negative results were replicated by Scharrer, Thomas and Mayer [1974] again using continuous i.v. glucose infusions. Additionally, these authors reported no effect of meal-tied glucose infusions. The amounts they infused in this latter experiment, however, represented only the addition of some 0.2 to 0.3 kcal of energy to meals averaging 4 to 5 kcal. The infused amounts are less than the standard errors which they report for meal size. Under these conditions any significant effect would be unlikely.

More recently Rowland, Meile and Nicolaides [1973; 1975] have reported depression ratios (DR, calculated as depression in intake (kcal)/amount infused (kcal); no compensation then yields a DR = 0 and perfect compensation

DR = 1) of some 0.42 to 0.52 for glucose infused continuously. That represents a reduction in intake of approximately half the amount infused. With the addition of a parallel infusion of 10 I.U. of insulin per 24-hour period the DR to i.v. glucose rose to precise compensation at 1.00. In a further extension of these results [Rowland, Meile and Nicolaides, 1975] the DR to i.v. glucose in VMH lesioned rats was shown also to be 1.00. They interpret these findings as meaning that the imperfect compensation in the normal animal is due to insufficient secretion of insulin to a glucose load which bypasses the orogastrointestinal system. They suggest this is not a problem in the VMH lesioned animal who is already markedly hyperinsulinemic and whose insulin secretory apparatus has presumably undergone sufficient hypertrophy to allow an adequate insulin output to the added glucose load.

An alternative explanation follows from the role of the VMH in glucoregulation as developed in the discussion of Chapter Three and hinges upon the study of Oohktens, Marsh, Smith, Bergman and Yates [1974] showing that marked oscillations in plasma insulin and glucose occur as a result of continuous i.v. glucose infusion. In the normal animal I have hypothesized that the VMH acts to stabilize energy (particularly glucose) supply to the CNS. It is seen to do so by: (1) augmenting pancreatic insulin secretion

(and, most reasonably, reducing both hepatic glucose production and efflux of FFAs from adipose) to the anticipation of a meal, thus creating a situation minimizing destruction of endogenous energy reserve and maximizing efficient disposition of the incoming nutrient, and (2) suppressing the reactive phase of insulin secretion which is driven first by the gastrointestinal vago-vagal reflexes and secondly by the actual perfusion of the pancreas by nutrient enriched blood. The suppression of these insulin-secreting phases (combined, most likely, with augmented hepatic glucose production) avoids any hypoglycemic overshoot.

It seems reasonable, then, to hypothesize that the failure of Nicolaides and Rowland [1975], Adair, Miller and Booth [1968] and Scharrer, Thomas and Mayer [1974] to observe perfect, or near perfect, compensation for the glucose continuously infused resulted, not from a failure to secrete adequate insulin to dispose of the glucose efficiently, but from the generation of energetically wasteful futile cycles of glycogen and fat synthesis followed by degradation. These cycles are seen to be generated by the VMH as a result of an inappropriate sequelae of events (or, more appropriately, lack of events) surrounding the occurrence of increased blood glucose levels. In the case of the VMH lesioned animals, these oscillations would be absent and the only abnormality would be the supranormal

insulin response to the infused glucose, a situation which should result in its efficient utilization. It would seem then that in the normal animal perfect compensation for the caloric value of glucose infused i.v. might be achieved either by some form of autonomic nervous block (sympathetic or parasympathetic depending on the nature of the VMH output pathways) or by tying the infusions to naturally occurring meals in such a way as to mimic the normal time course of blood glucose rise following such meals.

Three studies, using intragastric or intraduodenal infusions, are relevant to the hypothesis that perfect compensation for infused nutrient might depend heavily upon the temporal pattern of the infusion. Quartermain, Kissileff, Shapiro and Miller [1971] report relatively good caloric compensation with complete diet infusion which were designed either to mimic the natural meal pattern of the rat or given 10 minutes before each meal of a forced meal pattern (15 minutes access to food each 3 hours). Their intragastric infusions were administered through a nasopharyngeal tube. They may, therefore, have maximized compensation by signalling the onset of an infused "meal" by a change in the characteristics of the tube (i.e., volume or temperature). The studies of Thomas and Mayer [1968] and Snowdon [1975] come closest to the proposed periprandial i.v. infusions. Both used the nasopharyngeal tube and

infused liquid diet or glucose each time the animal pressed a bar for a portion of the oral diet. The overall results appear to provide poor support for the above hypothesis but the data may be misleading. Thomas and Mayer, for example, give a single figure representing the mean caloric intake over the first 3 days of periprandial infusion. The caloric compensation is little better using periprandial rather than continuous infusion over the total of these 3 days (DR = .75 vs. .73). However, the individual data for a single animal presented in their Figure 5 show a progressive decline in excessive caloric intake over the 4 days shown to a point of perfect compensation by day 4 of periprandial infusion. This is in contrast to the results of Nicolaides and Rowland [1975] who found no change in the degree of compensation after the first day of infusion. The basis for the relatively long delay to compensation in the Thomas and Mayer [1968] study may lie in the absence of a discriminable change in the characteristics of the ingested food versus its metabolic consequences. In other words, it may be more difficult for an animal to alter the set of its metabolic responses to a diet with which it is already familiar (and to which it has metabolic responses of a particular magnitude which have been reinforced previously by pleasant aftereffects than to a diet which has, at least, novel sensory characteristics. This could easily be tested. However, even if perfect caloric compensation could be

demonstrated for intragastric infusions by tying the infusions to normal meal patterns and by maximizing signal value, then the question would still remain as to whether the gastrointestinal reflexes are necessary for complete utilization of incoming nutrient.

In summary, speaking to the point of caloric regulation, it has still to be conclusively demonstrated that i.v. infusion of glucose does not result in a reduction of intake equal to the caloric value of that infused glucose. Rather, the negative results thus far reported can be questioned on the grounds that such infusions generate metabolically wasteful synthetic-degradation cycles and/or require reductions in oral intake below that necessary to obtain sufficient protein and essential FFAs.

Secondly, and of far broader import, millions of hospital patients are routinely maintained on continuous i.v. infusions of dextrose-saline for extended periods of time. If the findings of Oohktens, Marsh, Smith, Bergman and Yates [1974] are found to apply equally to humans, then it is apparent that these patients are being subjected to conditions of alternating hyperglycemia and hypoglycemia with the obvious potential for severe nervous tissue damage. The model for VMH function proposed in this thesis may provide both the means of understanding the phenomenon and a direction for research aimed at alleviating the problem.

7.3 Summary

Major points concerning the reinterpretation of the VMH role in energy balance can be reiterated as follows. The VMH is seen to stabilize glucose supply to the brain. It does so by initiating a series of metabolic actions (at least including the augmenting of insulin production, decreasing hepatic glucose output and reducing free fatty acid efflux) at the onset of a meal which prepare the animal for nutrient disposal and thus minimize the surplus of circulating nutrients attendant upon a meal. During, and immediately after, the meal, even while blood glucose is high, the VMH steadily reverses this metabolic pattern to anticipate the blood glucose fall and avoid a hypoglycemic overshoot. The VMH accomplishes these functions primarily via vagal output from receptors responsive to meal-related sensory events and blood-borne insulin and glucose concentrations.

The VMH is seen to have a role in the control of food intake only in so far as food intake (increase or decrease) is an important, but likely to be an emergency, glucoregulatory mechanism. It seems reasonable to presume that in an animal with ad libitum access to food, intake would anticipate deficit and therefore not normally occur as a glucoregulatory device.

There would appear to be no good evidence that the

VMH is involved in long-term body weight regulation. The results of the investigation into diabetic hyperphagia [Experiment 8] are interpreted as separating the effects of the VMH insulin-glucoreceptor from the systems monitoring body energy reserves. The observation that VMH-lesioned animals plateau at, and actively maintain, a particular body weight is reinterpreted as the outcome of a failure to suppress insulin secretion which occurs following the blood nutrient rise attendant upon a meal. To the degree that this inhibition is impaired, the oversecretion of insulin will drive incoming nutrients into fat, depleting readily available nutrient supply and entraining further intake. The particular body weight arrived at by any given VMH-lesioned animal is then seen as a balance between the degree of insulin oversecretion (to the point of effective depletion of readily available stores) and the insulin insensitivity engendered by deposition of fat in increasingly enlarged cells.

It is noted that, using this reasoning, any manipulation which decreases food intake (or probably even slows the rate of its ingestion) will also reduce the insulin oversecretion problem. This point is critical to the VMH "finickiness" question. Here it is argued that an animal's tendency initially to consume larger amounts of sweet foods and less of bitter would be sufficient to

entrain the most severe insulin hypersecretion (leading to the hypoglycemia-hyperphagia-hypoglycemia vicious circle) in the case of the former and break off this maladaptive sequence in the case of bitter substances. For purely textural changes the efficacy of any particular diet is seen to be related to the ease (i.e., rate) of its ingestion. Given this argument, it becomes of some importance to analyze closely the short-term responses (meal size and frequency of the initial meals) to changes of palatability and texture.

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APPENDIX A

F ratios from each of 12 one-way analyses of variance performed on the data (see Figure 1) of Experiment 1: VMH-Vagotomy

Dependent Variable	Weeks following VMH or sham lesion		Weeks following vagotomy or sham operation	
	1	2	1	2
Insulin df = 2, 20	26.47**	52.03**	39.97**	52.88**
Food Intake df = 2, 20	49.31**	20.47**	38.11**	10.30**
Body Weight df = 2, 20	86.34**	111.91**	63.92**	77.14**

**$p < .01$

APPENDIX B

F ratios from each of 12 one-way analyses of variance performed on the data (see Figure 2) of Experiment 2: LHA-Vagotomy.

Dependent Variable	Weeks following LHA or sham lesion				Weeks following vagotomy or sham operation	
	1	2	3	4	2	4
Insulin df = 2, 20		0.27			1.01	0.64
Food Intake df = 2, 20		7.10**			6.30**	4.51*
Body Weight df = 2, 20	8.67**	9.33**	10.04**	9.01**	14.53**	8.22**

* $p < .05$

** $p < .01$

APPENDIX C

F ratios from each of the 33 one-way analyses of variance performed on the data (see Figures 3 and 4) of Experiment 3: LHA-Sympathectomy.

Dependent Variable	Weeks after LHA lesions or sham		Weeks of GS or saline administration			Weeks following cessation of treatment					
	2	4	2	4	6	1	2	3	4	5	6
Food Intake df = 3, 32	11.14**	12.76**	9.37**	7.93**	10.00**	7.18**	11.28**	4.61**	9.28**	4.54**	3.41*
Water Intake df = 3, 32	14.81**	19.78**	14.91**	9.41**	11.88**	16.09**	10.64**	11.70**	11.63**	9.26**	10.96**
Body Weight df = 3, 32	8.97**	4.67**	5.00**	7.37**	10.12**	9.93**	12.10**	7.99**	10.18**	9.77**	8.08**

* $p < .05$
** $p < .01$

APPENDIX D

F ratios from each of the 6 one-way analyses of variance performed on the data (see Figure 7) of Experiment 4: VMH Insulin Injections.

Dependent Variable	Time in Minutes Following Injections of Insulin					
	0	5	10	20	30	60
Blood Glucose df = 3, 17	0.18	18.30**	4.01*	3.70	1.88	2.79

* $p < .05$

** $p < .01$

APPENDIX E

F ratios from each of the 6 one-way analyses of variance performed on the data (see Figure 8) of Experiment 5: Ventricular Insulin Injections.

Dependent Variable	Time in Minutes Following Injection of Insulin					
	0	5	10	20	30	60
Blood Glucose df = 2, 13	0.08	14.21**	6.84**	4.09*	2.88	1.54

* $p < .05$

** $p < .01$

APPENDIX F

F ratios from each of the 8 one-way analyses of variance performed on the data (see Figure 13) of Experiment 8: Diabetic Hyperphagia.

Dependent Variable	Time in Days Following Induction of Diabetes							
	1	2	3	4	6	9	12	15
Food Intake df = 4, 32	81.99**	63.65**	48.46**	46.22**	26.79**	17.91**	40.01**	33.82**

**p < .01

APPENDIX G

F ratios from the two-way analysis of variance performed on the one-half hour food intake data (see Table 1) of Experiment 9: Adrenaline Anorexia and Diabetes.

Source	df	F
Group	2, 24	17.92**
Treatment	2, 48	4.76*
Group X Treatment	4, 48	14.41**

* $p < .05$

** $p < .01$

APPENDIX H

F ratios from the two-way analysis of variance performed on the one-half hour food intake data (see Table 2) of Experiment 10: Adrenaline Anorexia and Vagotomy.

Source	df	F
Group	1, 12	3.33
Treatment	2, 24	47.94**
Group X Treatment	2, 24	1.98

** $p < .01$

APPENDIX J

F ratios from the two-way analysis of variance performed on the one-half hour food intake data (see Table 3) of Experiment 11: Adrenaline Anorexia

Source	df	F
Group	1, 10	1.11
Treatment	2, 20	38.47**
Group X Treatment	2, 20	0.90

**p < .01

APPENDIX K

F ratios from each of the 2 one-way analyses of variance performed on the data (see Table 4) of Experiment 12: Estrus Cycle and Insulin.

Dependent Variable	Groups
Insulin df = 2, 37	3.88*
Glucose df = 2, 37	0.06

*p < .05

APPENDIX L

F ratios from the one-way analysis of variance performed on the data (see Table 5) of Experiment 13: Estrus Cycle and Gastric Acid.

Dependent Variable	Groups
Acid secretion df = 2, 19	11.80**

**p < .01

APPENDIX M

F ratios from each of the 13 one-way analyses of variance performed on the data (see Figures 15 and 16) of Experiment 16: Sympathectomy and Diabetic Intake Utilization.

Dependent Variable	Pre-treatment	Days of Treatment with GS or saline				Days Post-treatment		
		3	14-15	21	42	13	14-15	21
Food Intake df = 3, 26	193.28**		56.83**	115.40**	90.00**		40.97**	76.66**
Water Intake df = 3, 26	104.73**	93.96**	82.88**	66.74**	119.00**		90.93**	84.43**

**p < .01

APPENDIX N

F ratios from each of the 4 one-way analyses of variance performed on the data (see Figure 17B) of Experiment 17: Sympathectomy, Ganglionic Block and Blood Glucose.

Dependent Variable	Pre-injection	Time Following GS or Saline Injection (Hours) 6	24	72
Blood Glucose df = 2, 13	0.44	33.19**	4.81*	1.03

* $p < .05$

** $p < .01$

APPENDIX P

F ratios from each of the 2 one-way analyses of variance performed on the data (see Table 8) of Experiment 19: Sympathectomy and Diabetic Liver Glycogen.

Dependent Variable	Groups
Liver Weight df = 3, 28	12.72**
Liver Glycogen df = 3, 28	17.94**

** $p < .01$