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Vagal mediation of hypothalamic hyperphagia and obesity.

Paul E. Sawchenko
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VAGAL MEDIATION OF HYPOTHALAMIC HYPERPHAGIA
AND OBESITY

A Dissertation Presented
By
PAUL E. SAWCHENKO

Submitted to the Graduate School of the University of Massachusetts in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

September 1979

Department of Psychology
VAGAL MEDIATION OF HYPOTHALAMIC HYPERPHAGIA AND OBESITY

A Dissertation Presented
By
PAUL E. SAWCHENKO

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ABSTRACT

Vagal Mediation of Hypothalamic Hyperphagia and Obesity

September, 1979

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Directed by: Professor Richard M. Gold

In Part I, three experiments examined the role of the abdominal vagus nerves in mediating the hyperphagia and obesity which result from ventromedial hypothalamic (VMH) damage. Complete abdominal vagotomy totally reversed preexisting lesion-induced hyperphagia and obesity. Vagotomy also eliminated the increase in fasting gastric acid secretion produced by VMH lesions. Although these results suggest vagal participation in the VMH syndrome, because vagotomy reduced food and water intake, and body weight independent of lesion status, and produced evidence of gastrointestinal dysfunction, the specificity of the vagotomy effect may be questioned.

The issue of specificity was addressed by varying both the extent of vagotomy and the sequence of brain and gut surgeries. Complete vagotomy also reversed the overeating and obesity produced by hypothalamic knife cuts. In addition, vagotomy prevented the development of knife cut effects in rats which received knife cuts and vagotomies at the same time, as well as in rats which were permitted to recover from the acute effects of vagotomy before receiving knife cuts. Selective section of the gastric vagal branches (sparing the hepatic and
coeliac branches) had virtually no effect on knife cut-induced hyperphagia and obesity, but, like the more extensive vagotomies, reduced water/food ratios and abolished knife cut-induced increases in fasting gastric acid secretion. These results indicate that upper gastrointestinal dysfunction alone cannot account for the blockade of hypothalamic hyperphagia and obesity by complete vagotomy, and implicate the coeliac and hepatic vagal branches as important to the expression of the syndrome.

Finally, the contribution of individual vagal branches to the VMH syndrome was estimated. Selective vagotomies which included the coeliac branch significantly reduced knife cut effects. Coeliac vagotomy alone produced a 43% reduction in weight gain produced by knife cuts. More extensive vagotomies which included the coeliac branch were more effective in blocking knife cut effects. Neither gastric, nor gastric plus hepatic vagotomy significantly reduced knife cut-induced hyperphagia and obesity. Hepatic vagotomy even produced evidence of potentiating knife cut effects. All knife cut rats overate and became obese (or more obese) when offered a high-fat diet. These results indicate specific vagal involvement in the VMH syndrome and suggest an important, but non-exclusive role for coeliac vagal mechanisms in generating hypothalamic hyperphagia. However, because all knife cut rats overate a high-fat diet, the vagus nerves may not provide a complete accounting for the syndrome.

In Part II, three experiments examined the role of the vagus in mediating the eating and drinking seen after injection of norepinephrine
(NE) into the paraventricular nucleus. After recovery from complete vagotomy, the eating response of sated rats to 20 nM NE was abolished, and the drinking response was attenuated. Vagotomized rats retained some capacity to rapidly increase eating in response to food deprivation, suggesting that the effect of vagotomy on NE-induced eating is, at least in part, specific. Efferent vagal blockade of intact rats with systemic atropine methyl nitrate (0.4 mg/kg) prior to central NE infusions yielded similar results. Finally, selective section of the coeliac vagus produced a 48% reduction of NE-elicited eating, while selective section of the gastric plus hepatic vagal branches (i.e., sparing only the coeliac branch) did not affect the ingestive response. Both vagotomized groups displayed an unimpaired capacity to increase food intake in response to systemic insulin injections.

These results suggest the participation of efferent vagal mechanisms in both the adrenergic feeding and VMH hyperphagia and obesity syndromes, and are consistent with a special role for some function under coeliac vagal control (perhaps insulin secretion) in mediating the effects of hypothalamic manipulations on feeding behavior.
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INTRODUCTION

Since the demonstration that overeating and obesity can result from electrolytic lesions of the ventromedial hypothalamus (VMH) (Hetherington & Ranson, 1940), considerable effort has been directed toward determining the role of the hypothalamus in the maintenance of energy balance and toward elaborating the mechanism(s) through which hypothalamic hyperphagia and obesity are manifested. This "VMH syndrome" has attracted attention from a number of perspectives, as judged by its incorporation into models of food intake and body weight regulation, brain function, autonomic organization, motivation, and obesity. Indeed, many apparent physiological and behavioral similarities between VMH lesioned rats and obese humans (e.g., Nisbett, 1972; Schachter, 1971; Schachter & Rodin, 1974) have led to a widespread use of the hypothalamic obese rat as a model for the study and treatment of human obesity.

A more complete understanding of the means by which the hypothalamus influences food intake and adiposity is important for several reasons. First, hypothalamic obesity is not uncommon in man; the some 3,000 reported cases of obesity which have been linked directly to hypothalamic tumor, trauma or inflammation (Bray & Gallagher, 1975) surely underestimate the clinical incidence of the syndrome. Since portions of the basomedial hypothalamus are uniquely devoid of a blood brain barrier (e.g., Oldendorf, 1975), this region would be expected to be particularly prone to injury. Second, given the parallels between the VMH lesioned rat and obese man, an understanding of the
mechanisms underlying hypothalamic hyperphagia and obesity could foster the development of treatments useful in managing human overweight. Finally, clarification of the mechanisms of the VMH syndrome would have important implications for the aforementioned theoretical models which are built around it. For example, hypothalamic hyperphagia has been suggested as being due to elimination of a brain center responsible for behavioral satiety, or, alternatively, as secondary to the metabolic changes which accompany the increased food intake. A better understanding of the syndrome may help to clarify this issue and might provide a clue as to how the neural mechanisms governing feeding and metabolism interact.

Hypothalamic Control of Food Intake

The VMH became entrenched in a "dual center" model of appetite regulation when Anand and Brobeck (1951) produced the complementary syndrome, that is, a prolonged failure to eat, by lesioning the adjacent lateral hypothalamic area (LH). It was then posited, and is still widely held, that the basis for hypothalamic hyperphagia was the tonic release of an LH-based feeding center from the inhibition of a VMH satiety center (e.g., Anand & Brobeck, 1951; Grossman, 1975; Stellar, 1954). Support for this model derived from studies such as those showing that electrical stimulation of the VMH caused a cessation of eating in fasted animals, while LH stimulation induced eating in sated animals (e.g., Wyrwicka & Dobrzechka, 1960). The intimate association between these areas was further demonstrated by the histological identification of their connecting fiber tracts (Arees & Mayer, 1967; Sutin & Eager,
and by the finding that electrical stimulation of either of these two regions produces a decrease in the spontaneous electrical activity of neurons in the other (Oomura, Ooyama, Yammamoto & Naka, 1967).

Based on studies showing that systemic injections of the neurotoxic glucose analog, gold thioglucone, selectively destroyed a population of neurons in the VMH and produced obesity, most variations of the dual center model incorporated the notion that the VMH contained glucoreceptors. These receptors were thought to sample the arteriovenous difference in blood glucose concentration, thereby receiving information related to the general availability of nutrient stores (i.e., satiety) (e.g., Mayer, 1955, 1966).

Despite its internal consistency, the validity of the dual center model has been questioned. Recent evidence has severely challenged the notion that the VMH serves as a satiety center. For example, like its intact counterpart, the VMH lesioned animal will reduce its food intake in direct proportion to the caloric value of imposed nutrient loads (Rowland, Meile & Nicolaidis, 1975; Russek & Morgane, 1963). Moreover, the VMH rat will become obese, however modestly, even if overeating is prevented by food restriction or limited forced feedings (Brooks & Lambert, 1946; Han, 1967), suggesting a purely metabolic component to hypothalamic obesity. The shortcomings of the satiety center concept are treated in detail in two recent reviews (Friedman & Stricker, 1976; Powley, 1977).

Physiological Effects of VMH Lesions
Parallel to experiments which attempt to partition the hypothalamus into antagonistic systems controlling food intake are other studies suggesting that the central efferent control of visceral autonomic functions might similarly be segregated into medial (sympathetic) and lateral (parasympathetic) hypothalamic zones (Ban, 1967; 1975). Since the physiological profile of the VMH rat might generally be characterized as one of relative parasympathetic hyperactivity, attention has recently turned to the possibility that hypothalamic hyperphagia might be a consequence of peripheral autonomic imbalance.

Virtually all metabolic compartments are drastically affected by VMH lesions; in general, the direction of these shifts is toward increased anabolic (storage) processes. Many of the metabolic changes have been shown to occur in the absence of excessive food intake, suggesting that these are primary effects of the lesion and may be etiologically important to the syndrome. In other words, "... an animal with VMH lesions may not increase its food intake in order to gain weight but because it is gaining weight." (Friedman & Stricker, 1976, p. 415).

The principal sites at which metabolic changes relevant to food intake and obesity might be effected, the liver, pancreas, gastrointestinal tract and adipose tissue, are each abundantly innervated by autonomic nerves. Changes in the activity of these nerves can markedly influence metabolic, secretory and motor activity at these sites (liver: Sawchenko & Friedman, 1979; pancreas: Gerich & Lorenzi, 1978; Woods & Porte, 1974; GI tract: Novin, 1977; Powley, 1977; adipose tissue: Havel, 1965; Wertheimer & Shapiro, 1948). Thus, lesion induced changes
in visceral autonomic tone could produce metabolic changes which, in turn, promote hypothalamic hyperphagia and obesity.

Most prominently mentioned as a causative factor of hypothalamic hyperphagia and obesity is the increase in circulating insulin levels seen after VMH lesions or hypothalamic fiber transections (knife cuts) (e.g., Bernardis & Frohman, 1970; Hales & Kennedy, 1964; Martin, Koninjendijk & Bouman, 1974; Steffens, Mogenson & Stevenson, 1972; Tannenbaum, Paxinos & Bindra, 1974). This hyperinsulinemia is apparent within minutes to hours after surgery (Rohner, Dufour, Karakash, LeMarchand, Ruf & Jeanrenaud, 1977; Steffens et al., 1972), suggesting a primary neurogenic effect, and its magnitude is positively correlated with subsequent ad libitum food intake (Hustvedt & Løvå, 1972).

Pancreatic islet hypertrophy (Han, Yu & Chow, 1970) and hyperinsulinemia (Martin et al., 1974; Powley & Opsahl, 1976) are present even if lesioned animals are pair fed with intact controls so as to control for the possible confounding effects of the excessive food intake and increased meal size which follow VMH lesions (Balagura & Devenport, 1970).

It is well known that exogenous insulin injections promote increased food intake and adiposity (e.g., MacKay, Calloway & Barnes, 1940; Panksepp, Pollack, Krost, Meeker & Ritter, 1975), although the mechanisms by which it does so are unclear. Most probably, insulin produces increased food intake via its acknowledged capacity to clear utilizable metabolic substrates, principally glucose and fats, from the circulation (Friedman & Stricker, 1976). The liver (Friedman & Stricker, 1976) and the brain (Mayer, 1966; Smith & Epstein, 1969) have
been proposed as sites at which insulin induced deficits in available metabolic fuels might be appreciated.

The means by which VMH lesions effect changes in insulin secretion may involve peripheral autonomic nerves. The pancreatic islets are innervated by both parasympathetic (vagus) and sympathetic (splanchnic) nerves (Woods & Porte, 1974). Either electrical or cholinergic chemical stimulation of the vagus nerves promotes increased insulin secretion while, on the sympathetic side, insulin secretion is stimulated by β-adrenergic receptors and inhibited by α-adrenergic receptors (Gerich & Lorenzi, 1978; Smith & Porte, 1976). The possible relevance of these autonomic mechanisms to the VMH syndrome is most clearly shown by the findings that severing the abdominal vagus nerves (vagotony) abolishes the hyperinsulinemia produced by VMH lesions (Louis-Sylvestre, 1976; Powley & Opsahl, 1976) and, at least under some conditions, the hyperphagia and obesity as well (see below).

Several laboratories have sought to assess the role of hyperinsulinemia in hypothalamic hyperphagia and obesity by administering VMH lesions to rats with experimental diabetes mellitus. Such studies have clearly shown that intact pancreatic β cells are necessary for the full expression of VMH lesion-induced weight gains and adiposity (Friedman, 1972; Vilberg & Beatty, 1975; York & Bray, 1972; Young & Liu, 1965). Diabetic VMH lesioned rats do overeat, however, but reports of the magnitude of hyperphagia in such animals vary considerably. Factors such as the severity of diabetes, maintenance diet, and type, if any, of insulin replacement therapy might each contribute to this discrepancy. Thus, while insulin-secreting β cells
of the pancreas are apparently critical to some facets of hypothalamic obesity, the extent and nature of their involvement remains unclear.

Virtually all of the increased body weight of VMH lesioned rats is due to excessive accretion of fat in adipose tissue. This is due, in part, to the increases in circulating insulin levels which result from the lesion. However, since elimination of hyperinsulinemia does not totally prevent lesion induced changes in fat metabolism and fat deposition (Goldman, Schnatz, Bernardis & Frohman, 1972 a and b), adipose tissue metabolism may be directly and primarily affected. Indeed, LeMagnen and colleagues (LeMagnen, Devos, Gaudilliere, Louis-Sylvestre & Tallon, 1973) have shown that VMH lesions abolish the normal daytime mobilization of fats from adipose tissue in the rat, a nocturnal species which typically consumes very little food by day. The decreased ability to rely on adipose tissue as a source of metabolic fuels during the daylight hours may account for the fact that the bulk of the overeating seen in VMH lesioned rats can be attributed to increased daytime intakes (Balagura & Devenport, 1970; Kakolewski, Deaux, Christensen & Case, 1971).

Both humoral and neural factors have been proposed as mediating the lesion induced changes in fat metabolism. A number of hypothalamic hormones have been isolated which are capable of influencing adipose tissue metabolism in the appropriate direction (e.g., Grimes, Mok & Martin, 1978; Kastin, Redding, Hall, Besser & Schally, 1975). None of these, however, has yet been shown to be necessarily affected by VMH lesions. Alternatively, adipose tissue receives direct autonomic (primarily sympathetic) innervation (Havel, 1965; Wertheimer & Shapiro,
1948). Both VMH lesions and the excision of the nerves entering a given adipose tissue depot have been shown to impair the mobilization of fats during such homeostatic challenges as food deprivation and cold stress (e.g., Cantu & Goodman, 1967; Cottle & Cottle, 1970; Sidman & Fawcett, 1954). These findings, coupled with studies showing that electrical stimulation of areas in and around the VMH does affect plasma free fatty acid, triglyceride and cholesterol levels (Gutstein, Schneck & Appleton, 1968, 1969), have led several authors to speculate that some portion of VMH lesion induced adiposity might be due to direct neural influences on adipose tissue (e.g., Bernardis & Goldman, 1976; Bernardis & Schnatz, 1971).

The VMH via its autonomic connections, has also been shown to directly affect the activities of key hepatic enzymes involved in the metabolism of amino acids, glucose and glycogen (see Sawchenko & Friedman, in press, for a review). Alterations in the balance between the mechanisms favoring storage versus mobilization of metabolic fuels in the liver may well contribute to lesion induced changes in food intake (Friedman & Strieker, 1976).

Finally, in addition to their direct effects on metabolic functions, VMH lesions produce primary alterations in gastric acid secretion (Powley, Opsahl, 1974; Ridley & Brooks, 1965) and in gastrointestinal motility (Ralph & Sawchenko, 1978). Although such findings provide further evidence of altered autonomic tone in VMH lesioned animals, their relevance to the mechanisms underlying hypothalamic hyperphagia and obesity is unknown.

In summary, VMH lesions produce a myriad of effects on peripheral
metabolic, secretory and motor functions. Many of these may be due to alterations in hypothalamic autonomic outflow to the viscera. In general, these changes promote increased storage of metabolic substrates, so much so that gluconeogenesis (the de novo synthesis of glucose from bodily protein), a process normally seen only in the post-absorptive state, is apparent in hypothalamic hyperphagic rats despite excessive food intakes (Holm, Hustvedt & Løvø, 1973). That many of the metabolic and visceral effects enumerated above occur in the absence of excessive food intake suggest that these are primary effects of the lesion and that they may be causes rather than consequences of the observed hyperphagia. The question now arises as to whether autonomic mechanisms can account for the behavioral effects of VMH damage.

The Central Neural Basis of Hypothalamic Hyperphagia

The central neuroanatomical substrates of the VMH syndrome remain incompletely defined. While the bulk of the lesion work has concentrated on the ventromedial hypothalamic nucleus as the anatomical focus of the syndrome, more recent work has clearly demonstrated that the most effective discrete lesions are placed more rostrally, in the region of the paraventricular nucleus (PVN) of the hypothalamus (Gold, 1973, 1975). This more rostral focus has been confirmed with fiber-of-passage-sparing amino acid lesions (Simson, Gold, Standish & Pellett, 1977), and is supported by the finding that parasagittal hypothalamic knife cuts must cross the plane of the PVN in order to effectively produce the syndrome (Gold, Jones, Sawchenko & Kapatos, 1977; Sclafani
Even more obscure are the central efferent pathways through which the syndrome is expressed. Owing to the influence of the dual center model, the few available studies of neuronal degeneration following VMH lesions have been aimed at defining local (i.e., intrahypothalamic) connections (Arees & Mayer, 1967; Sutin & Eager, 1969). Mayer and Arees (1968) observed, however, degeneration of the dorsal longitudinal fasciculus, a primary route of hypothalamic autonomic outflow, following medial hypothalamic damage induced by gold thioglucose injections in mice.

With the charting of the central monoamine pathways emerged attempts to attribute hypothalamic hyperphagia to disruption of transmitter-specific systems. A mild hyperphagia and obesity can be elicited by lesioning an ascending noradrenergic projection to the forebrain (Ahlskog, 1974; Ahlskog & Hoebel, 1973). The contribution of disruption of this noradrenergic system to hypothalamic hyperphagia is probably not significant, however, since the expression of noradrenaline depletion induced obesity depends on an intact pituitary (Ahlskog, Hoebel & Breisch, 1974) while VMH lesion and knife cut induced obesities do not (Ieni & Gold, 1977; Kurtz, Rozin & Teitelbaum, 1972).

Chronic hyperphagia has also been observed following intra-cerebroventricular infusions of neurotoxins specific for serotonin (Breisch, Zemlan & Hoebel, 1976; Saller & Stricker, 1976), but in this case the excessive weight gain is due to increased linear growth rather than to increased adiposity. Excessive linear growth and obesity are seen in knife cut induced hypothalamic hyperphagia (Gold & Kapatos,
1975) while normal or stunted growth occurs with VMH lesion induced hyperphagia (Bernardis & Frohman, 1968; Frohman & Bernardis, 1968). Taken together, these findings would indicate that alterations in linear growth are not critical to the expression of the syndrome and that serotonin depletions account for only a small portion of the effects of VMH lesions on food intake and adiposity.

Gold and coworkers have used the techniques of asymmetrical lesions and asymmetrical knife cuts to more clearly define the longitudinal fiber system which must be damaged to produce hyperphagia and obesity (Gold et al., 1977; Gold, Quackenbush & Kapatos, 1972; Kapatos & Gold, 1973). By pairing a unilateral parasagittal cut (which if represented bilaterally would produce obesity) with a contralateral cut in the coronal plane, whose anterior-posterior coordinate was varied across subjects, the relevant system was traced from the rostral level of the PVN through the midbrain (Gold et al., 1977; see also Sclafani & Berner, 1977). More recently, overeating and obesity have been observed in rats with asymmetrical cuts whose coronal member lies just rostral to the dorsal motor nucleus (DMN) of the vagus nerve (Sawchenko & Gold, unpublished observations).

Support for a role for the PVN in the hypothalamic control of food intake has come from chemical stimulation studies in which minute quantities of transmitter agonists or antagonists are microinjected into discrete brain loci. Building upon the seminal work of Grossman (1962a), who was the first to suggest that feeding behavior might be chemically coded in the brain, Leibowitz (1978) has shown the PVN to be the site where norepinephrine (NE) and other α-adrenergic agonists
elicit eating at the lowest doses. Norepinephrine has been shown to be released from the hypothalamus during spontaneously initiated feeding (Martin & Myers, 1975), and has been suggested as playing a role in hunger and satiety (Leibowitz, 1976). On the other hand, paralleling the situation in VMH lesioned animals, microinjections of NE into the hypothalamus, but not necessarily the PVN proper, have been demonstrated to produce such autonomic effects as increased gastric acid secretion (Carmona & Slangen, 1973), hypothermia, brachycardia, vasodilation (Carmona & Slangen, 1976), and increased insulin secretion (DeJong & Steffens, Note 2). That the overeating induced by both NE infusions and VMH lesions apparently share a common anatomical focus and several autonomic effects may indicate that the two phenomena share a common mechanism.

The evidence reviewed so far is generally consistent with the notion that disruption of the autonomic outflow from the PVN, particularly that to the DMN, may be primarily responsible for hypothalamic hyperphagia and obesity. A direct PVN to DMN projection has recently been identified anatomically (Saper, Loewy, Swanson & Cowan, 1976; Swanson, 1977). It is noteworthy that collaterals of this projection system have been shown to terminate on cells in the intermediolateral column of the spinal cord, the region which contains the cell bodies of preganglionic visceral sympathetic neurons. Thus, the PVN has potentially been linked to both parasympathetic and sympathetic function.

Despite the fact that it is best known for the production of posterior pituitary hormones, the implication that the PVN might play
a role in energy balance and autonomic regulation is not new. Chronic hypoglycemia following poorly localized PVN lesions was noted by early researchers (Barris & Ingram, 1936; Ingram & Barris, 1936). Electrical stimulation of the PVN has been shown to produce hyperglycemia (Lewy & Gassman, 1935) and, more recently, rather specific stimulation-bound hyperphagia (Atrens & Vietinghoff-Riesch, 1972). Vonderhae (1937) frequently observed PVN lesions in post-mortem autopsies of diabetic humans and proposed that this region, via its autonomic connections, might serve as an insulin regulating center!

In summary, although precise information on the efferent pathways through which hypothalamic hyperphagia and obesity are expressed is lacking, certain evidence links the rostral anatomical focus of the syndrome to autonomic centers in the brainstem and spinal cord. Such a connection is consistent with the possibility that the VMH syndrome might be mediated via peripheral autonomic nerves.

**Autonomic Mediation of Hypothalamic Hyperphagia and Obesity?**

The changes in food intake brought about by stimulation or lesions of the hypothalamus can be qualitatively reproduced by manipulations of the abdominal vagus nerves. For example, electrical stimulation of the abdominal vagi has been shown to produce overeating in sated animals Penaloza-Rojas, Barrera-Mera & Kubli-Garfias, 1969), while direct current blockade, presumably of vagal afferents, yields anorexia in hungry animals (Penaloza-Rojas & Russek, 1963). Vagotomy results in a transient (2-4 weeks) period of undereating and in a more permanent reduction of body weight in rats (Shay, Komarov & Gruenstein, 1949;
Powley & Opsahl, 1974) and rabbits (Rezek, 1975) maintained on standard laboratory diets.

The possibility that hypothalamic hyperphagia and obesity might be mediated via the vagus nerves was first addressed (and rejected) in the informal experiments of Brooks and Lambert (1946). This question was reopened when Powley and Opsahl (1974) clearly showed that preexisting VMH lesion induced hyperphagia and obesity could be reversed by sub-diaphragmatic vagotomy. This finding has since been replicated (Inoue & Bray, 1977; King, Carpenter, Stamoutsos, Frohman & Grossman, 1978), although other laboratories, using somewhat different paradigms have failed to confirm this result (Chikamori, Masuda, Izumi, Isaka & Tezuka, 1977; King et al., 1978; Wampler, Note 9; Wampler & Snowdon, 1979). Thus, considerable question remains as to the reproducibility of the effects of vagotomy on lesion induced hyperphagia and obesity.

Even if vagotomy can be shown to abolish the effects of VMH lesions on food intake and body weight, more challenging questions of the specificity of this effect must be addressed. Arguing for specificity are the observations that vagotomy often raises the current threshold required to elicit overeating via electrical stimulation of the LH (Ball, 1974; Powley & Opsahl, 1976) while sparing the genetic obesity of the Zucker "fatty" rat (Opsahl & Powley, 1974) as well as the modest obesity which results from ovariectomy in female rats (Eng, Gold & Wade, 1979). It should be noted, however, that the latter two varieties of overeating and obesity are generally slower to develop and are of a lesser magnitude than is hypothalamic obesity, and would presumably place lesser demands on the parasympathetic nervous system for the
processing of additional foodstuffs.

Several lines of evidence argue against specificity. It has been suggested (Booth, 1976; Panksepp, 1975) that since vagotomy disrupts and delays gastric emptying (e.g., Opsahl & Powley, 1974; Ralph & Sawchenko, 1978; Sawchenko, Gold & Ferrazano, 1977) that the gastrointestinal tract of a vagotomized animal might not be able to accommodate sufficient food to generate or maintain an obese state. In addition, since vagotomy alone produces undereating and weight loss in otherwise normal animals, its antagonism of hypothalamic obesity might be nonspecific. In support of this view are the reports that lesions administered to rats which have recovered from the acute effects of vagotomy are effective in producing overeating and obesity in rats maintained on standard lab diets (Chikamori et al., 1977; King et al., 1978; Wampler, 1977).

Finally, if vagotomy can be shown to specifically abolish hypothalamic hyperphagia and obesity, the question remains as to whether the causes of the overeating and obesity can be further localized to lesion induced alterations in particular vagally mediated functions. Powley has argued for the involvement of the whole of the abdominal vagal system in suggesting that the syndrome is due to tonic increases in parasympathetic tone (Powley & Opsahl, 1976) or to phasic increases in the cephalic reflexes of digestion (Powley, 1977). On the other hand, given the weight of the evidence suggesting that hyperinsulinemia may contribute to the hyperphagia and obesity, the possibility that some vagally mediated functions might be more etiologically important than others cannot be ignored.
The issue of anatomical and functional specificity can be addressed by using selective vagal denervations, variants of procedures which grew out of the need to selectively denervate the acid-secreting parietal cells of the stomach, while minimizing complications, in human ulcer patients (see Kennedy, 1967 for a review). Such procedures have only recently been applied to biobehavioral problems (e.g., Adachi, Niijima & Jacobs, 1976; Sawchenko, et al., 1977). The abdominal vagal system of the rat is well-suited to such procedures in that it is a relatively simple system, consisting of two main (gastric) trunks which ride along the esophagus and innervate the stomach. The anterior trunk gives off a branch to the liver (hepatic branch), while the more posterior trunk gives rise to a branch which passes through the coeliac ganglion en route to the pancreas and other abdominal organs (Legros & Griffith, 1969). The anatomical specificity of these branches is supported by the demonstration that hepatic and pancreatic functions are spared following selective gastric vagotomy or gastrectomy in man (Ishigami, Fuchimoto, Wakabayashi & Toshimitsu, 1974; Lenninger, Magee & White, 1965; McKelvey, Toner, Connell & Kennedy, 1973; Wakabayashi, 1973).

Overview

In Part I of the dissertation, the role of the vagus nerves in mediating knife cut induced hypothalamic hyperphagia is assessed. By varying the sequence of brain and visceral surgery, and by employing the above-mentioned selective vagotomy procedures, the nature and extent of vagal involvement in the mediation of the syndrome is estimated. If
upper gastrointestinal dysfunctions are responsible for the blockade of hypothalamic hyperphagia and obesity by complete abdominal vagotomy, then selective section of the gastric branches should mimic the effect of the more extensive vagotomy. On the other hand, given the evidence implicating excessive insulin secretion as a key factor in the generation of hyperphagia and obesity, it might be predicted that selective section of the coeliac branch would be especially effective in blocking lesion or knife cut effects.

In Part II, parallel studies are carried out to assess the effects of vagotomy on the overeating elicited by medial hypothalamic infusions of norepinephrine. This method of producing overeating, which shares several key attributes with lesion-induced hyperphagia, might well be expected to operate via a similar mechanism.
PART I
VAGAL MEDIATION OF HYPOTHALAMIC HYPERPHAGIA

CHAPTER I
THE EFFECT OF SUBDIAPHRAGMATIC VAGOTOMY ON ELECTROLYTIC LESION INDUCED HYPOTHALAMIC HYPERPHAGIA AND OBESITY

This first experiment was an attempt to confirm and extend the Powley and Opsahl (1974) study which demonstrated that preexisting hypothalamic obesity could be reversed by subdiaphragmatic vagotomy. The procedure follows closely that of this earlier study except that female rather than male rats were employed, and two different lesioning currents were used. A group with 12 milicoulomb (mC) lesions repeated the Powley and Opsahl (1974) procedure, while a group with 24 mC lesions was added to determine whether the effects of larger lesions, which promote greater weight gains (Gold, 1975), would also be reduced by vagotomy.

Method

Subjects. Thirty-seven adult female Charles River CD albino rats were individually housed in hanging stainless steel cages in a colony room maintained at 21 ± 1°C and on an artificial 12/12 hr light/dark cycle. All animals had unlimited access to Purina Lab Chow pellets and tap water. A 14 – 18 day period of adaptation to these conditions preceded any surgery. At the end of this period body weights ranged from 240 –
Brain Surgery. Three groups were formed on the basis of approximately equal body weight. One group was to receive small (12 mC) lesions, the second larger (24 mC) lesions, and the third to be sham lesioned.

Surgery was carried out over four consecutive days with at least two members of each group undergoing surgery on each day. Animals were anesthetized with sodium pentobarbital (Nembutal\textsuperscript{R}) (40 mg/kg, IP). Supplemental injections of atropine sulfate (0.1 mg, SC) and penecillin (20,000 U, IM) were given at the time of surgery.

Electrolytic lesions were stereotaxically administered by passing either 1.0 mA anodal current for 12 sec (12 mC lesions) or 1.5 mA for 16 sec (24 mC lesions) through stainless steel electrodes insulated except for 0.5 mm at their conical tips. Stereotaxic coordinates for both lesioning currents were, with the incisor bar 3 mm below the interaural line, 6.5 mm rostral to the earbars, + 0.5 mm lateral to the superior sagittal sinus, and 8.3 mm below the dura (Gold, 1973; 1975). Sham lesioned rats were comparably treated, except that their brains were not invaded beyond the pricking of the dura.

After surgery, all animals were returned to their home cages, where, following an overnight period of food deprivation to prevent asphyxiation due to excessive gorging, they were allowed free access to food and water for 40 days. Body weights and food and water intakes (all to the nearest 1 g) were measured semi-weekly.

Gut Surgery. Forty days after brain surgery, each of the three groups was divided on the basis of post-lesion body weight change. One group
each of the 12 mC lesioned (7 rats), 24 mC lesioned (8 rats), and sham lesioned animals (6 rats) was then administered subdiaphragmatic vagotomies. The remaining three groups were sham vagotomized.

Under Nembutal anesthesia, an incision was made 1 cm to the rat's left of ventral midline, extending 2-4 cm from the level of the xiphisternum. The stomach was lifted from the abdomen, supported over a probe placed horizontally beneath the esophagus, and wrapped in gauze soaked in warm saline. With the aid of a dissecting microscope (Zeiss 20X), the sheath containing the gastric artery and vein were blunt-dissected free of the esophagus, and about a 1 cm length of each vagal trunk was dissected free of the esophagus and excised with iridectomy scissors. Nerve sections were performed as high on the esophagus as possible to ensure interruption of the hepatic and coeliac vagal innervation. The stomach was then gently reinserted into the peritoneal cavity and muscle and skin separately sutured. Tetracycline was topically applied to the sutured wound.

The sham vagotomy procedure was comparable to the point of isolating and sectioning the nerves. In lieu of this, the nerves were visualized and the esophagus manipulated.

Following gut surgery, the animals were returned to their home cages where they were offered, in addition to their normal diet, a high-fat diet composed, by weight, of 67% powdered Purina Lab Chow and 33% vegetable shortening (Corbit & Stellar, 1964; Gold, 1970) to help combat post-vagotomy undereating and weight loss. The high fat diet was available until weight loss ceased.

Body weight and intake measures were taken for an additional 60
days.

**Obesity.** Each rat's naso-anal length, an index of linear growth, was measured with calipers at the time of brain surgery, at the time of gut surgery, and at the completion of the experiment. From this, Lee Obesity Indices (Lee, 1929), a measure highly correlated with body fat content (Bernardis & Patterson, 1968), was calculated by the formula

\[
\text{Lee Index} = \left(10^4\right) \left(\frac{\text{Body Weight (g)}}{\text{Naso-Anal Length (mm)}}\right)
\]

**Test for Completeness of Vagotomy.** After all behavioral data had been collected, vagotomies were tested for completeness using a modification of the procedure described by Powley and Opsahl (1974).

The rats were food deprived for 24 hrs prior to testing. Then, under Nembutal anesthesia, the rats were adrenalectomized (dorsal approach) and laparotomized using a 2-3 cm ventral midline incision. A tygon catheter was inserted into the stomach via a small duodenal incision and tied tightly into place. The free end of the catheter was fitted with a luer-lock adaptor.

The cervical vagi were then exposed with a 2-3 cm upper thoracic ventral midline incision. The nerves were blunt-dissected away from the carotid arteries and a suture was loosely looped around each nerve.

The stomach was then repeatedly washed by infusing and recollecting 5 ml warm isotonic saline until the perfusate was free of particulate matter. The animal was then allowed to rest undisturbed for 15 min. At the end of this interval, gastric acid secretions were collected by infusing and recollecting 10 ml (2 X 5 ml) warm saline. Secretions were immediately titrated to neutrality with 0.005 M NaOH, using 2
drops of 1% phenol red as an indicator. This procedure was repeated at 15 min intervals until stable baseline secretion rates were achieved (generally after 4-6 samples).

One cervical vagal trunk was then gently placed over a stainless steel hook electrode bathed in a pool of mineral oil. Square wave pulses (50 Hz, 0.4 msec duration, 4-6 V potential) were passed through the electrode for 5 sec out of every 20 sec for a 15 min period. Gastric acid secretions were then collected and titrated as above for at least four trials (60 min). The entire procedure was then repeated while stimulating the remaining vagal trunk.

Vagotomies were considered complete if any increase in gastric acid secretion in response to electrical stimulation was at least 2 SEM below the mean increase of sham-lesioned, sham vagotomized rats.

**Histology.** Immediately after the test for completeness of vagotomy, the animals were sacrificed by an overdose of Nembutal and transcardially perfused with isotonic saline and 10% formalin. The brains were extracted from the skulls, and later frozen, sectioned (40 u thick), and stained with cresyl violet to allow histological assessment of lesion size and placement.

**Data analysis.** The effects of lesions (at day 40) were analyzed using a one-way analysis of variance (ANOVA). The effects of vagotomy on lesion-induced changes in physiology and behavior were analyzed (at day 100) with a two-way ANOVA. Since group sizes were not all equal, the method of unweighted means was employed. At both time points, individual between-group differences were assessed using the Newman-
Keuls procedure for all pairwise comparisons (Myers, 1972).

Naso-anal length, obesity indices and body weight change measures at days 40 and 100 were analyzed. Behavioral measures reflect group means of individual daily averages over the 10 day periods immediately preceding lesions (days -10 - 0), vagotomy (days 30 - 40), and the completion of the experiment (days 90 - 100). Gastric acid secretion measures were made over days 104 - 130.

Results

A total of 30 animals completed all phases of the experiment. Each group contained five rats except the 24 mC lesioned, vagotomized group (24 mC-Vx group, n = 4) and the 12 mC lesioned, sham vagotomized group (12 mC-Sham Vx group, n = 6). The various rationales for excluding the remaining animals are given below.

Effects of lesions on ingestive behaviors and body weight. Both of the lesioning currents employed were effective in producing hyperphagia and obesity. Figure 1 shows body weight curves for all groups over the course of the experiment. At day 40, a significant overall effect of lesions on body weight was obtained (\(F(5,24) = 88.1, p < .001\)). Both groups with 24 mC lesions weighed significantly more than each of the other groups (p's < .01), but no difference between the 24 mC/Sham Vx and the 24 mC/Vx group was noted. Twelve mC lesions produced more modest, yet still significant, increases in body weight, relative to both the Sham lesion/Sham Vx group (p's < .01) and the Sham lesion/Vx group (p's < .05).
Figure 1. Mean (± SEM) body weight change following 12 mC, 24 mC, or sham electrolytic lesions (given on day 0) followed by either complete subdiaphragmatic vagotomy or sham vagotomy (given on day 40).
Table 1 shows mean body weight change, naso-anal length, and Lee Obesity Index for each group at days 0, 40, and 100. The excessive weight gain by lesioned rats was generally not due to excessive linear growth. Although a significant overall effect of lesions on naso-anal length at day 40 was obtained ($F(5,24) = 2.9, p < .05$), only one comparison achieved significance, with the 24 mC/Sham Vx group being reliably longer than the Sham Lesion/Sham Vx group ($p < .05$). Instead, the excessive weight gain was largely due to increased adiposity. A highly significant overall effect of lesions on Lee Obesity Indices at day 40 was obtained ($F(5,24) = 29.1, p < .001$). Both 24 mC lesioned groups were significantly more obese than each of the other groups ($p's < .01$), and both 12 mC groups were more obese than each of the sham lesioned groups ($p's < .01$).

Parallel to the effects on body weight were those on food intake ($F(5,24) = 25.3, p < .001$). Table 2 shows mean food intake, water intake and water/food ratios over the 10 day periods prior to lesioning (day 0), vagotomy (day 40), and the completion of the experiment (day 100). Over days 30 - 40, all four lesioned groups consumed more food than both sham lesioned groups ($p's < .01$). Both 24 mC lesioned groups ate more than the 12 mC/Sham Vx group ($p's < .01$), but only marginally more than the 12 mC/Vx group ($p's < .05$).

Despite the lesion-induced elevations in food intake and body weight, water intakes were not significantly affected by the lesions ($F(5,24) = 2.3, p < .05$). This lack of change in overall water intake in the face of elevated food consumption was reflected in markedly altered water/food ratios ($F(5,24) = 30.8, p < .001$). Both sham
Table 1

Mean (+ SEM) Naso-Anal Lengths (NAL (cm)) and Lee Obesity Indices (OBI) \(^1\) at the Time of VMH Lesions or Sham Lesions (Day 0), at the Time of Complete Subdiaphragmatic or Sham Vagotomy (Day 40), and at the Time the Experiment was Terminated (Day 100)

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 40</th>
<th>Day 100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAL</td>
<td>OBI</td>
<td>NAL</td>
</tr>
<tr>
<td>Sham Lesion - Sham Vagotomy</td>
<td>20.7 ± 0.3</td>
<td>301 ± 2</td>
<td>21.4 ± 0.2</td>
</tr>
<tr>
<td>12 mC Lesion - Sham Vagotomy</td>
<td>20.8 ± 0.2</td>
<td>298 ± 3</td>
<td>21.9 ± 0.2</td>
</tr>
<tr>
<td>24 mC Lesion - Sham Vagotomy</td>
<td>20.5 ± 0.2</td>
<td>303 ± 1</td>
<td>22.1 ± 0.4*</td>
</tr>
<tr>
<td>Sham Lesion - Vagotomy</td>
<td>20.5 ± 0.2</td>
<td>303 ± 3</td>
<td>21.5 ± 0.3</td>
</tr>
<tr>
<td>12 mC Lesion - Vagotomy</td>
<td>20.7 ± 0.3</td>
<td>306 ± 3</td>
<td>21.6 ± 0.3</td>
</tr>
<tr>
<td>24 mC Lesion - Vagotomy</td>
<td>20.6 ± 0.3</td>
<td>296 ± 5</td>
<td>21.8 ± 0.3</td>
</tr>
</tbody>
</table>

\(^1\)Normal range is 300 - 310

* Differs significantly from Sham Lesion - Sham Vagotomy value, p < .05 (Newman-Keuls test for all pairwise comparisons).

** Differs significantly from Sham Lesion - Sham Vagotomy value, p < .01
Table 2
Mean (± SEM) Food Intake (g) and Water/Food Ratios (ml/g) Over Ten Day Periods
Prior to Lesion Surgery (Day 0), Prior to Vagotomy Surgery (Day 40),
and Prior to Completion of the Experiment (Day 100).

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 40</th>
<th>Day 100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Food Intake</td>
<td>Water/Food</td>
<td>Food Intake</td>
</tr>
<tr>
<td>Sham Lesion - Sham Vagotomy</td>
<td>20.9 ± 1.1</td>
<td>1.51 ± .11</td>
<td>19.8 ± 1.0</td>
</tr>
<tr>
<td>12 mC Lesion - Sham Vagotomy</td>
<td>20.8 ± 0.5</td>
<td>1.53 ± .14</td>
<td>28.0 ± 1.2**</td>
</tr>
<tr>
<td>24 mC Lesion - Sham Vagotomy</td>
<td>20.7 ± 0.8</td>
<td>1.75 ± .19</td>
<td>32.9 ± 1.5**</td>
</tr>
<tr>
<td>Sham Lesion - Vagotomy</td>
<td>19.9 ± 0.9</td>
<td>1.53 ± .14</td>
<td>20.0 ± 0.6</td>
</tr>
<tr>
<td>12 mC Lesion - Vagotomy</td>
<td>21.2 ± 1.2</td>
<td>1.63 ± .12</td>
<td>28.9 ± 2.0**</td>
</tr>
<tr>
<td>24 mC Lesion - Vagotomy</td>
<td>20.2 ± 0.8</td>
<td>1.69 ± .10</td>
<td>33.2 ± 2.4**</td>
</tr>
</tbody>
</table>

* Differs significantly from Sham Lesion - Sham Vagotomy value, p < .05.

** Differs significantly from Sham Lesion - Sham Vagotomy value, p < .01.
lesioned groups displayed water/food ratios at day 40 which were greater than those of each of the lesioned groups (p's < .01) except the 12 mC/Vx group, whose water/food ratio, although reduced, was not significantly lower than that of either sham lesioned group.

**Effects of vagotomy.** Complete abdominal vagotomy produced a marked reversal of lesion-induced changes in food intake, body weight and obesity. The duration of the anorexia seen after vagotomy tended to be positively related to the body weight at the time of vagotomy. Under-eating and weight loss in the 24 mC/Vx group was particularly protracted and severe. While four animals of this group eventually stabilized body weights and food intakes (of a pelleted diet), three did not. Despite the constant availability of a high fat diet to these animals, which has been shown to support overeating and weight gain in VMH lesioned rats which have recovered from the acute effects of vagotomoy (Powley & Opsahl, 1974), these three animals continued to lose weight until death.

Despite such prolonged reductions in food intake, vagotomized animals were rarely, if ever, completely aphagic for more than one or two days. By day 100, at which time significant main effects of lesions (F(2,24) = 9.5, p < .001), vagotomy (F(1,24) = 45.6, p < .001), and a lesion X vagotomy interaction (F(2,24) = 6.3, p < .001) were evident, the food intakes of all vagotomized groups had stabilized at comparable levels which were non-sigificantly below those of sham lesioned, sham vagotomized rats. The intakes of both lesioned, sham vagotomized groups remained significantly above those of all other
groups (p's < .01). Food intakes of all groups over days 90 - 100 are given in Table 2.

The effects of vagotomy on food intake were mirrored by changes in body weight (see Figure 3). By day 100, significant overall effects on body weight of lesions (F(2,24) = 129.3, p < .001), vagotomy (F(1,24) = 36.9, p < .001) and their interaction (F(2,24) = 82.6, p < .001) were found. The weights of all vagotomized animals were reduced to comparable levels significantly below that of sham lesioned-sham vagotomized controls (p's < .01). Sham vagotomy, which had little effect on the weights of sham lesioned animals, produced a transient reduction in the body weights of the lesioned groups. At day 100, however, the body weight change of both of the lesioned-sham vagotomized groups remained significantly above that of the Sham lesioned/Sham vagotomized group (all p's < .01).

The reduced intakes and body weights of vagotomized rats appeared largely, but not entirely, due to decreased adiposity. A significant overall effect of vagotomy on naso-anal length at day 100 was obtained (F(1,24) = 7.8, p < .005). In general, the vagotomized groups tended to be shorter than the sham vagotomized groups, suggesting that some stunting may have occurred as a result of prolonged post-vagotomy undereating and weight loss. Of the vagotomized groups, only the 24 mC/Vx animals failed to achieve significance when compared to Sham lesioned/Sham Vx controls with respect to body length. Similarly, both of the lesioned-sham vagotomized groups were significantly longer than each of the vagotomized groups, with the sole exception of the 12 mC/Sham Vx vs 24 mC/Vx comparison, which did not achieve significance.
More dramatic was the effect of vagotomy of lesion-induced changes in obesity. With respect to Lee Obesity Indices, at day 100, significant main effects of lesions ($F(2,24) = 15.3$, $p < .001$), vagotomy ($F(2,24) = 176.5$, $p < .001$), and a significant lesions X vagotomy interaction ($F(2,24) = 53.7$, $p < .001$) were found. Vagotomy abolished the lesion-induced changes in adiposity to the extent that by day 100 all vagotomized groups' obesity indices (as well as that of the Sham lesioned/Sham Vx group) were significantly below those of both lesioned-sham vagotomized groups ($p's < .01$). Both lesioned-vagotomized groups maintained obesity indices which were significantly below that of Sham lesioned/Sham VX controls ($p's < .01$), and which were also below the 300 -310 range which is characteristic of normal rats (Bernardis & Patterson, 1968). The obesity index of the Sham lesioned/Vx group was not significantly depressed. Naso-anal lengths and obesity indices for all groups at day 100 are shown in Table 1.

Water intakes were profoundly reduced by vagotomy, independent of lesion status. As a result of this reduction, significant effects of lesions ($F(2,24) = 3.8$, $p < .05$), vagotomy ($F(1,24) = 33.8$, $p < .001$), and their interaction ($F(2,24) = 3.8$, $p < .05$) on water/food ratios were found. As was the case at day 40, lesioned groups with sham vagotomies displayed water/food ratios significantly below control values ($p's < .01$). With the pronounced reduction in water intake by vagotomy, the water/food ratios of the vagotomized groups were even further reduced, with all vagotomized groups' ratios being significantly lower than those of all other groups ($p's < .01$, except for the 12 mC/Sham Vx vs 24 mC/Vx comparison, which did not achieve significance).
The reduction in water/food ratios was so severe that 11 of the 14 vagotomized animals drank less (in ml) over days 90 - 100 than they ate (in g), an unusual condition in rats, whose water/food ratios typically range from about 1.4 - 2.0.

Gastric acid secretion. Two animals, one from each of the 24 mC/Vx and Sham lesion/Vx groups, failed to meet the criterion for completeness of vagotomy in the acid secretion test and were excluded from the data analysis. Figure 2 shows fasting gastric acid secretion under non-stimulated (baseline) conditions and the response to electrical stimulation of the cervical vagi. Under baseline conditions, significant main effects of lesions (\( F(2,24) = 3.5, p < .05 \)), vagotomy (\( F(1,24) = 26.5, p < .001 \)), and a significant lesion X vagotomy interaction (\( F(2,24) = 7.9, p < .005 \)) were noted. Multiple comparison tests revealed that the secretion of both the 12 mC/Sham Vx and the 24 mC/Sham Vx groups were elevated relative to each of the other groups (p's < .01), but did not differ significantly from one another.

A clear effect of vagotomy on the response to electrical stimulation of the cervical vagi was found (\( F(2,24) = 140.9, p < .001 \)), with each sham vagotomized group responding more vigorously than each vagotomized group (all p's < .01). None of the other comparisons were found to be statistically significant.

It was noted during the gastric acid secretion phase of the experiment that, despite 24 hr food deprivation, the stomachs of the vagotomized rats contained considerably more food than those of the sham vagotomized animals, as indicated by the number of saline washings which were required to obtain a perfusate which was free of particulate...
Figure 2. Mean (+ SEM) fasting gastric acid secretion under non-stimulated (baseline) conditions, and in response to electrical stimulation of the cervical vagi (stimulated). Open bars represent values of sham vagotomized groups; cross-hatched bars represent values of vagotomized rats. Baseline values are those obtained during the 15 min collection period immediately prior to the onset of electrical stimulation. Stimulated values are maxima seen in response to electrical stimulation.
matter. In addition, at autopsy the esophagi of vagotomized rats were observed to be distended to roughly twice their normal diameter, and, in some cases, were also packed with food.

**Histology.** Figure 3 shows reconstructions of representative lesions from each of the four lesioned groups, plus a reconstruction of one 12 mC lesion which was ineffective in inducing overeating and excessive weight gain. The effective 12 mC lesions were found to be centered in the rostral half of the ventromedial hypothalamic nucleus (VMN), at about 0.5 mm lateral to the midline. These lesions were not restricted to the VMN, but extended rostrally, just beyond the confines of the VMN, dorsally into the most ventral portion of the dorsomedial hypothalamic nucleus, and, less frequently, medially to involve portions of the periventricular area. Two animals with 12 mC lesions failed to overeat and fatten. In these instances, lesions, although symmetrical, were more caudally placed, and, in the case of the ineffective lesions shown in Figure 1 (C), considerably more dorsal than those of the animals which did become obese. The 24 mC lesions were considerably more extensive. These lesions were centered at approximately the same point as the effective 12 mC lesions, but typically punched a hole in the basomedial hypothalamus, in all cases involving ventral periventricular tissue, as well as the ventral half of the dorsomedial hypothalamic nucleus. The lateral extent of these lesions was about to the lateral plane of the fornices, about 1 mm right and left of the midline. All of the animals given 24 mC lesions developed pronounced hyperphagia and obesity.
Figure 3. Reconstructions of representative 12 mC (A–C) and 24 mC (D and E) lesions drawn on plates from the Konig and Klippel (1963) rat brain atlas. Average daily weight gain over days 0-40 is shown in the lower right portion of each brain section. A: 12 mc lesion of an animal which subsequently received a sham vagotomy; B: 12 mC lesion of an animal which was vagotomized on day 40; C: 12 mC lesion which was ineffective in inducing overeating and excessive weight gain; D: 24 mC lesion of an animal which was sham vagotomized on day 40; E: 24 mC lesion of an animal which was vagotomized on day 40.
Discussion

The results of this experiment confirm the basic findings of Powley and Opsahl (1974) and are generally consistent with the results of subsequent studies (Inoue & Bray, 1977; King et al., 1978) which have shown that pre-existing hypothalamic lesion-induced hyperphagia and obesity can be reversed by complete subdiaphragmatic vagotomy.

Before discussing this principal finding, several features of the lesions employed here and their effects are worthy of comment.

The lesions used in the present study, although effective in producing hyperphagia and obesity, were somewhat less than optimally placed to achieve these ends. In attempting to faithfully reproduce the 12 mC lesions of Powley and Opsahl (1974), the coordinates and histological descriptions of their lesions (in male rats) were transposed for use in the female rats employed here. The result was lesions which were somewhat caudal to both Powley and Opsahl's placements, as well as to the site described by Gold (1973, 1975) where small lesions yield the greatest behavioral effects. Despite the fact that the larger, 24 mC lesions of the present study were similarly centered, these, by virtue of their larger size, did involve Gold's (1973, 1975) optimal site and produced markedly obese animals.

Such anatomical considerations may help to explain two rather unusual features of the syndrome produced by the lesions. The longest of the lesioned groups of the present study was significantly longer than the shortest of the control groups. While excessive linear growth can occur in association with the obesity produced by hypothalamic
knife cuts (Gold et al., 1977; Gold & Kapatos, 1975), growth following VMH lesions has generally been found to range from normal to somewhat stunted (e.g., Bernardis & Frohman, 1970; Bernardis & Skelton, 1965/1966). The increased growth found in one of my lesioned groups could be explained by the supposition that my caudally placed lesions should not have severely damaged the cell bodies or fibers which contain somato-statin (growth hormone release inhibiting hormone) (Elde & Hokfelt, 1978), while more rostrally placed lesions would be expected to interrupt this pathway. Alternatively, the fact that excessive growth was not consistently produced by my lesions might lead one to suspect that this isolated result may have been due either to random variability or measurement error.

A second notable feature of the lesions, which was consistently found across groups, was their lack of effect on water intake, with resultant reductions in water/food ratios. While reduced water/food ratios have previously been reported in VMH-lesioned rats (see Stevenson, 1969), even in these studies absolute water intakes were elevated in lesioned animals, relative to controls. Increased drinking following VMH lesions may occur as a secondary consequence of water loss which results from incidental damage to the antidiuretic hormone pathways to the posterior pituitary (Stevenson, Welt, & Orloff, 1950), as secondary to the increased food intake (Strominger, 1947), or, as a primary result of damage to a neural system involved in the control of drinking behavior and body water economy (Stevenson, 1969). It cannot be known whether the relatively low water intakes may have limited the development of overeating and obesity of lesioned rats in the present study, but it is
clear that elevated water intakes are not essential to the qualitative expression of the syndrome.

**Gastric acid secretion.** Consistent with the findings of others (Inoue & Bray, 1977; Powley & Opsahl, 1974; Ridley & Brooks, 1965), fasting gastric acid secretion was elevated in both lesioned-sham vagotomized groups. This observation, in providing an independent measure of tonic vagal activity, provides support for the notion that a shift in autonomic tone towards parasympathetic (vagal) hyperactivity may be mechanistically involved in the generation of hyperphagia and obesity. Also consistent with this view are the findings that vagotomy, which normalized food intake and adiposity, also abolished the lesion-induced increase in fasting gastric acid secretion.

Baseline acid secretion rates did not differ significantly as a function of lesion size, although a slight trend in this direction was apparent, while changes in body weight, food intake and adiposity were considerably greater in the animals with larger lesions. If the physiological changes which give rise to hyperphagia and obesity are closely related to that which produced the elevation in fasting gastric acid secretion, it might have been expected that the larger lesions would have produced a proportionately greater basal rate of acid secretion. Two factors may explain this apparent inconsistency. First, at the time gastric acid secretion measures were taken, food intakes of the two lesioned-sham vagotomized groups were quite comparable (see Table 2). Second, the measuring instrument may not have been sufficiently sensitive to detect any differences which might have
existed. The preparation and protocol used here (i.e., samples from barbiturate-anesthetized rats taken over relatively short intervals), although adequate as a test for completeness of vagotomy, is a far cry from the 1 - 4 hr collection intervals in unanesthetized rats equipped with chronic gastric fistulae which have been used to study gastric acid secretion, *per se* (e.g., Ridley & Brooks, 1965). Such considerations may also help to explain the finding that vagotomy, which is well-recognized as reducing basal gastric acid secretion in humans (e.g., Kennedy, 1974) and experimental animals (e.g., Anita, Rosiere, Robertson & Grossman, 1951), produced no significant reduction in baseline acid secretion, either in the present experiment or in other studies which employed a similar method (Inoue & Bray, 1977; Powley & Opsahl, 1974). Note, however, that baseline gastric acid secretion of vagotomized rats in the present experiment did tend towards the lower end of the normal range.

The verification procedure employed here and successfully used by others (Burge & Vane, 1958; Inoue & Bray, 1977; Opsahl & Powley, 1974; Powley & Opsahl, 1974) has been criticized by workers (King et al., 1978) who have adopted, instead, a procedure whereby a vagotomy is considered to be complete if a piece of filter paper placed directly on the gastric mucosa fails to pick up the color of an intravenously injected acid-base indicator (Legros & Griffith, 1969). King et al (1978) have anecdotally reported false positive "verifications" using the electrical stimulation procedure in rats which were intentionally incompletely vagotomized.

In my hands, the "color change" technique has proven less satis-
factory, as I have, on more than one occasion, failed to see a color change response in unoperated rats (Sawchenko, Note 4). Even in the absence of such an observation, I would still be inclined to opt for a procedure which involves a quantitative assessment of gastric acid secretion under conditions which have a firm basis in physiology over a qualitative technique whose basis, to my knowledge, is incompletely understood.

Effects of vagotomy on hypothalamic hyperphagia and obesity. The complete reversal of VMH lesion-induced overeating and obesity by vagotomy confirms the results of similar investigations (Inoue & Bray, 1977; King et al., 1978; Powley & Opsahl, 1974), and provides justification for suspecting that vagal mechanisms may be etiologically involved in producing hyperphagia and obesity. The present results also provide some evidence for the generality of these effects of vagotomy, as they were found to reverse VMH obesity of two different magnitudes in female rats. Previous studies had all used a single lesioning current in male animals. It must be noted, however, that one laboratory has failed to reverse VMH obesity with subsequent vagotomy (Wampler, Note 7; Wampler & Snowdon, 1979). Possible explanations for this discrepancy will be treated in subsequent chapters.

Before the nature and extent of any vagal involvement in generating the physiological and behavioral changes characteristic of the VMH syndrome can be estimated, several key questions must be answered. Paramount among these is the general issue of specificity which was outlined in the Introduction.

Since vagotomy alone produces a period of undereating and weight
loss in otherwise intact animals (e.g., Mordes, Herrera, & Silen, 1977; Powley & Opsahl, 1974; Shay, et al., 1949), its antagonism of hypothalamic hyperphagia and obesity may be functionally unrelated to the mechanisms through which VMH lesions produce their effects. In support of such a view are the observations (Chikamori et al., 1977; King et al., 1978; Wampler & Snowdon, 1979) that rats allowed to recover from the acute effects of vagotomy do show a marked overeating when subsequently administered VMH lesions. In these studies, reports of the magnitude of VMH lesion induced hyperphagia and obesity have ranged from comparable to sham vagotomized controls to slightly, but significantly, below lesioned, sham vagotomized control levels. In contrast, Rowland and Engle (1979) find no apparent overeating and obesity following VMH lesions in rats which had been vagotomized at an earlier date. The importance of surgical sequence to the expression of hypothalamic hyperphagia and obesity in vagotomized rats will be directly addressed in Chapter II.

Most prominently mentioned as a possible non-specific means by which vagotomy may antagonize hypothalamic hyperphagia and obesity are the upper gastrointestinal dysfunctions produced by vagotomy (Booth, 1976; Inoue & Bray, 1977; King et al., 1978; Panksepp, 1975). As noted above, vagotomy is well-known to produce a flaccid stomach which empties solid food slowly and erratically (e.g., Anita et al., 1951; Opsahl & Powley, 1974; Sawchenko et al., 1977; Snowdon & Epstein, 1970). The anecdotal observations of esophageal distension and significant food retention after fasting in vagotomized rats are in line with the view that upper gastrointestinal abnormalities may contribute to the vagotomy effect. The strikingly similar final levels of body weight change seen
in the present study among all vagotomized groups independent of lesion status, might be interpreted as resulting from comparable limitations in food handling capacity imposed by the gastrointestinal dysfunctions produced by vagotomy.

A second argument against specificity has recently been advanced by King et al (1978) who speculate that the chronically reduced water intakes and water/food ratios seen in vagotomized rats may result in dehydration which could, in turn, limit the capacity of vagotomized animals to overeat. Inconsistent with such an interpretation is the present finding that over days 90 - 100 the highest water/food ratio seen in vagotomized rats (1.04 ± 0.05 in the 24 mC/Vx group) was not significantly lower than the lowest of the lesioned-sham vagotomized groups (1.18 ± 0.10 for the 12 mC/Sham Vx group) despite highly significant differences in food intake between these two groups over this same interval (see Table 2). Also at odds with the view that reduced water intakes may limit overeating in vagotomized VMH lesioned rats is the finding that adulteration of normal rats' drinking water with bitter-tasting quinine, a treatment which produced water/food ratios quite comparable to those seen among vagotomized rats of the present study, produces little, if any, alteration in plasma electrolyte balance (Rowland & Flamm, 1977). Furthermore, reduced water/food ratios produced by vagotomies which exclude the hepatic branch, have been shown to be accompanied by quite normal fasting hematocrit and plasma osmolarity (Kraly, 1978). Thus, despite markedly reduced water intakes and water/food ratios, both normal and vagotomized rats appear to maintain good water balance.

As noted previously, some evidence in favor of the specificity of
the vagotomy effect may be gleaned from the observations that other forms of overeating, namely that associated with genetic defect (Opsahl & Powley, 1974), ovariectomy (Eng et al., 1979), increased dietary palatability (Gold et al., in press), and chronic insulin treatment (Rowland & Engle, 1979), are not substantially affected by vagotomy. However, since these varieties of overeating rarely place such dramatic and sustained demands on the animal's ingestive and processing capacities as does that which results from large VMH lesions or knife cuts, the general issue of specificity remains unresolved.

In summary, complete abdominal vagotomy was found to normalize VMH lesion-induced overeating and obesity in female rats. The present results also suggest that the magnitude of the VMH lesion effects does not influence their susceptibility to reversal by vagotomy. While these results are consistent with the thesis that vagal hyperactivity may mediate the development of lesion-induced hyperphagia and obesity, factors related to gastrointestinal dysfunction and the severity of the acute effects of vagotomy on eating and body weight may non-specifically limit the capacity of the vagotomized rat to overeat and fatten. The following experiment addresses itself to these issues.
THE EFFECTS OF COMPLETE VS SELECTIVE GASTRIC VAGOTOMY ON KNIFE CUTOFF-INDUCED HYPOTHALAMIC HYPERPHAGIA AND OBESITY

The principal finding of Chapter I, that pre-existing hypothalamic hyperphagia and obesity can be reversed by subdiaphragmatic vagotomy (see also Brooks et al., 1946; Inoue & Bray, 1977; King et al., 1978; Powley & Opsahl, 1974), raises the possibility that the release of some vagally mediated function(s) might be critically involved in the genesis of hypothalamic hyperphagia and obesity. The question now arises as to whether the effects of vagotomy are specific to the cause of hypothalamic obesity.

As discussed previously, the gastrointestinal dysfunctions and acute anorexia and weight loss which accompany vagotomy may non-specifically limit the capacity of the vagotomized rat to overeat. Related to this is the question of whether the generally more substantial overeating and obesity produced by hypothalamic knife cuts would also be eliminated by vagotomy. Finally, if the vagus is mechanistically related to the production of hypothalamic obesity, then we might ask whether such involvement is general, or whether the release of vagally mediated functions at particular target organs is preferentially involved.

In this experiment, the issue of specificity is addressed by (1) assessing the effects of vagotomy on the hyperphagia and obesity induced by parasagittal hypothalamic knife cuts, (2) varying the sequence
of operations, i.e., by allowing some rats to recover from the acute effects of vagotony before being administered potentially obesity-producing knife cuts, and (3) by varying the extent of vagotony. If disruption of upper gastrointestinal motor functions is responsible for the antagonism of hypothalamic obesity by vagotony, then selective denervation of the upper gastrointestinal tract should mimic the effects of more complete vagal transections. Such selective gastric vagotomies have been shown to produce the gastric stasis, altered clearance functions, and reduced gastric acid secretory response to insulin hypoglycemia commonly seen following more extensive vagal transections (Sawchenko et al., 1977).

Methods

Subjects. Adult female albino rats weighing 250-300 g at the time of first surgery were housed and fed as described in Chapter I.

Hypothalamic knife cuts. General surgical procedures were as previously described. Under Nembutal anesthesia, bilateral parasagittal hypothalamic knife cuts were produced at coordinates previously shown to produce maximum weight gains (Gold, 1973; Gold et al., 1977) using a retracting wire knife fashioned from a Hamilton microliter syringe (Gold, Kapatos, & Carey, 1973). With the incisor bar 3 mm below the interaural line, the knife, with its cutting wire retracted, was lowered at 8.0 - 8.5 mm rostral to the earbars and ± 0.9 mm lateral to the superior sagittal sinus to a depth of 7.5 mm below the dura. The 3 mm long cutting wire was then extended, and the knife lowered until the base of the brain
was contacted. The knife was then raised 3 mm, the cutting wire retracted, and the knife assembly removed from the brain. This procedure was repeated on the contralateral side.

The sham knife cut procedure was identical to that described for sham electrolytic lesions (Chapter I).

**Vagotomies.** Vagotomies and sham vagotomies were generally performed as previously described, excepting that two types of vagotomies were employed. Independent section of each of the four subdiaphragmatic vagal branches constituted what will be referred to as full vagotomies. Note that this differs from the procedure of Chapter I in that the main vagal trunks were severed at a much lower level, just above the gastroesophageal junction. Such a procedure would presumably spare a considerable portion of the vagal innervation of the esophagus. Section of the two main (gastric) trunks below the level of, and thus sparing, the coeliac and hepatic vagal branches constituted what will be referred to as selective gastric vagotomies. Figure 4 is a diagrammatic representation of the abdominal vagal branches, and shows the approximate level at which each was sectioned in the vagotomy procedures described here.

**Procedure.**

**Knife cuts before vagotomies.** Following adaptation to laboratory conditions, 19 rats were given bilateral parasagittal knife cuts and 15 sham knife cuts. All animals were then allowed to feed without restriction for 30 days. On day 30, eight of the knife cut rats were given full vagotomies, six gastric vagotomies, and five were sham
Figure 4. Diagrammatic representation of the subdiaphragmatic vagal trunks and their branches. Dashed lines indicate approximate levels of section in the various selective vagotomy procedures employed in Chapters 2 and 3. Complete (full) vagotomies involved independent section of each of the four branches; Gastric vagotomies involved section of the two main vagal trunks below the level of the hepatic and coeliac branches.
vagotomized. Similarly, full, gastric, and sham vagotomies were administered to five of the previously sham knife cut rats. Groups were matched for body weight.

All animals were then allowed to feed ad libitum for 40 days prior to the test for completeness of vagotomy and sacrifice. As before, any animal which lost weight following abdominal surgery was offered a high-fat diet until weight loss ceased. Body weights, and food and water intakes were measured semi-weekly. Naso-anal lengths were measured, and Lee Obesity Indices calculated, at the time of each surgery and at sacrifice.

**Vagotomies with knife cuts.** In this sequence, knife cuts (or sham cuts) and vagotomies (full or gastric or sham) were given at the same time (i.e., under the same anesthetic dose). The sequence of operations was alternated among animals within each condition.

Animals were maintained, and data taken, as described above.

**Vagotomies before knife cuts.** In this sequence, gut surgery was performed 30 days prior to brain surgery. Animals were maintained, and data taken, as described above.

**Verification of vagotomies and histology.** Both types of vagotomies were tested for completeness and histology performed as in Chapter I. In those instances where behavioral verification of the effectiveness of the knife cuts was potentially confounded by the administration of prior or simultaneous vagotomies, the placements of the knife cuts were judged, without knowledge of the behavioral results, by two independent, experienced observers.
Based on previous work from this and other laboratories (see discussion in Gold et al., 1977), knife cut placements were deemed to be appropriate if they met each of the following three criteria: (1) were within 0.6 - 1.5 mm lateral to the midline, (2) crossed the coronal plane of the PVN, and (3) were at least 2 mm in height and extended to the base of the brain.

Data analysis. Data were analyzed as described in Chapter I. Exceptions are noted in the text.

Results

Knife cuts before vagotomies. A total of 29 animals survived this operation sequence, and, where applicable, sustained appropriate knife cuts and/or met the criterion for completeness of vagotomy. All groups contained five rats except the knife cut-full vagotomy group (KC-Full Vx group) which contained four animals.

The hypothalamic knife cuts employed here were very effective in producing hyperphagia and excessive weight gain. Preliminary analyses of food intake and body weight data at day 30 revealed no significant differences between the three groups of animals which received knife cuts, or between the three groups which received sham knife cuts. Data from the groups with comparable manipulations have therefore been pooled into a single knife cut group and a single sham knife cut group for presentation below.

Table 3 shows mean food intakes for all groups over the seven day periods prior to brain surgery (day 0), prior to gut surgery (day 30),
Table 3

Mean (+ SEM) Food intake (g/day) Over Seven Day Periods Prior to Hypothalamic Knife Cuts or Sham Knife Cuts (Day 0) Prior to Sham, Full or Gastric Vagotomy (Day 30) and Prior to the Completion of the Experiment (Day 70).

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 30</th>
<th>Day 70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Knife Cut - Sham Vagotomy</td>
<td>20.8 ± 0.9</td>
<td>21.8 ± 1.0</td>
<td>23.0 ± 0.8</td>
</tr>
<tr>
<td>Knife Cut - Sham Vagotomy</td>
<td>21.7 ± 1.2</td>
<td>41.9 ± 3.5**</td>
<td>36.0 ± 1.9**</td>
</tr>
<tr>
<td>Sham Knife Cut - Full Vagotomy</td>
<td>21.5 ± 0.9</td>
<td>21.2 ± 0.8</td>
<td>20.4 ± 1.9</td>
</tr>
<tr>
<td>Knife Cut - Full Vagotomy</td>
<td>22.3 ± 1.0</td>
<td>42.8 ± 3.8**</td>
<td>20.8 ± 1.6</td>
</tr>
<tr>
<td>Sham Knife Cut - Gastric Vagotomy</td>
<td>20.0 ± 1.1</td>
<td>20.2 ± 1.0</td>
<td>21.8 ± 1.2</td>
</tr>
<tr>
<td>Knife Cut - Gastric Vagotomy</td>
<td>22.4 ± 1.6</td>
<td>45.1 ± 4.2**</td>
<td>33.1 ± 0.9**</td>
</tr>
</tbody>
</table>

** Differs significantly from Sham Knife Cut - Sham Vagotomy group, p < .01.
and prior to the completion of the experiment (day 70). Over days 23-30, knife cut rats' average daily intake (43.2 ± 1.9 g) was more than double that of sham knife cut animals (21.2 ± 0.6) (t(27) = 11.7, p < .001). As a result, by day 30, enormous differences in body weight change between the two groups were also apparent (t(27) = 9.9, p < .001). Figure 5 shows mean body weight change for all groups over the course of the experiment.

The increased body weight produced by knife cuts was largely, but not entirely, attributable to increases in adiposity. Body lengths and obesity indices for all groups at days 0, 30, and 70 are given in Table 4. At day 30, the knife cut animals were considerably more obese than the sham operated rats (t(27) = 12.7, p < .001). In contrast to the lesioned groups of the previous experiment, knife cut animals grew consistently longer than sham knife cut animals (t(27) = 5.9, p < .001). Drinking data for this, as well as for the other operation sequences, will be discussed below.

As was found to be the case in lesioned animals, full vagotomy completely reversed knife cut-induced changes in body weight, food intake and adiposity. The gastric vagotomy procedure, in contrast, had notably little effect on any of these parameters in either knife cut or sham knife cut rats.

Immediately following full vagotony, knife cut animals became extremely anorexic and lost weight rapidly. This occurred despite the fact that a high-fat diet was constantly available until weight loss ceased (between days 47-58). Four animals in this group continued to lose weight and died prior to the completion of the experiment. The
Figure 5. Mean (± SEM) body weight change for all groups in the knife cuts before vagotomy sequence. Bilateral parasagittal hypothalamic knife cuts or sham knife cuts were given on day 0; sham, full, or gastric vagotomies were added on day 30.
Table 4

Mean (± SEM) Naso-Anal-Lengths (cm) and Lee Obesity Indices¹ at the Time of Hypothalamic Knife Cuts or Sham Knife Cuts (Day 0), at the Time of Sham, Full, or Gastric Vagotomy (Day 30), and at the Time the Experiment was Ended (Day 70).

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 30</th>
<th>Day 70</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAL (cm)</td>
<td>OBI (cm)</td>
<td>NAL (cm)</td>
</tr>
<tr>
<td>Sham Knife Cut - Sham Vagotomy</td>
<td>20.9 ± 0.3</td>
<td>306 ± 3</td>
<td>21.5 ± 0.2</td>
</tr>
<tr>
<td>Knife Cut - Sham Vagotomy</td>
<td>20.8 ± 0.2</td>
<td>305 ± 3</td>
<td>22.3 ± 0.3**</td>
</tr>
<tr>
<td>Sham Knife Cut - Full Vagotomy</td>
<td>20.6 ± 0.2</td>
<td>301 ± 3</td>
<td>21.3 ± 0.2</td>
</tr>
<tr>
<td>Knife Cut - Full Vagotomy</td>
<td>21.0 ± 0.2</td>
<td>299 ± 3</td>
<td>22.4 ± 0.4**</td>
</tr>
<tr>
<td>Sham Knife Cut - Gastric Vagotomy</td>
<td>20.5 ± 0.2</td>
<td>305 ± 3</td>
<td>21.2 ± 0.3</td>
</tr>
<tr>
<td>Knife Cut - Gastric Vagotomy</td>
<td>20.7 ± 0.3</td>
<td>302 ± 2</td>
<td>22.1 ± 0.2*</td>
</tr>
</tbody>
</table>

¹Normal range is 300 - 310.

*Differs significantly from Sham Knife Cut - Sham Vagotomy group, p < .05.

**Differs significantly from Sham Knife Cut - Sham Vagotomy group, p < .01.
remaining four animals did reachieve stable levels of body weight main-
tenance on the lab chow diet. Over the seven days prior to completion
of the experiment (day 70), significant overall effects on food intake
of knife cuts ($F(1,23) = 110.0, p < .001$), vagotomies ($F(2,23) = 46.7,$
$p < .001$), and a significant knife cut X vagotomy interaction ($F(2,23) =
27.9, p < .001$) were found. The intake of both the KC-Sham Vx and the
KC-Gastric Vx groups was significantly greater than those of each of
the other groups (p's < .01, see Table 3). The intake of the KC-Sham
Vx group was marginally greater than that of the KC-Gastric Vx group
(p < .05). Despite their reduced body weights, the intakes of the two
groups with full vagotomy were not significantly lower than those of
Sham KC-Sham Vx controls.

Even more pronounced differences in body weight change at day 70
were apparent, with significant overall effects of knife cuts ($F(k,23)$
$= 88.5, p < .001$), vagotomies ($F(2,23) = 67.6, p < .001$), and their
interaction ($F(2,23) = 55.3, p < .001$) in evidence. Neither sham- nor
gastric vagotomy had any apparent effect on knife cut-induced increases
in body weight. The total weight change of both the KC-Sham Vx and the
KC-Gastric Vx groups was significantly greater than that of each of the
other groups (p's < .01). No significant difference between these two
groups with respect to body weight change was apparent. The weights
of both full vagotomy groups stabilized at levels significantly below
those of both the Sham KC-Sham Vx and the Sham KC-Gastric Vx groups (all
p's < .01, except for the Sham KC-Gastric Vx vs Sham KC-Full Vx com-
parison, $p < .05$). The total overall reduction in body weight by
vagotomy was most severe in the KC-Full Vx group, whose weight was
significantly lower than that of all other groups, including the Sham KC-Full Vx group ($p < .05$). Note that gastric vagotomy alone produced no significant reduction in body weight relative to control (Sham KC-Sham Vx) animals.

The weight reductions produced by full vagotomy in both knife cut and sham knife cut rats were almost completely attributable to changes in adiposity. At day 70 a significant overall effect of knife cuts on naso-anal length was apparent ($F(1,23) = 9.2, p < .005$; see Table 4). As was the case at day 30, all knife cut groups were significantly longer than all sham knife cut groups ($p$'s < .01). Despite the fact that they had experienced lengthy periods of undereating and weight loss, the lengths of rats with full vagotomies (with or without knife cuts) were only slightly suppressed, in no case achieving significance in comparison with their respective knife cut or sham knife cut controls. In contrast, Lee Obesity Indices at day 70 largely paralleled the body weight data. Significant main effects of knife cuts ($F(2,23) = 13.0, p < .001$), vagotomies ($F(2,23) = 9.1, p < .005$) and a significant knife cut X vagotomy interaction ($F(2,23) = 4.7, p < .025$) were found. As was the case with the body weight change data, both the KC-Sham Vx and the KC-Gastric Vx groups were more obese than each of the other groups ($p$'s < .01). While the obesity indices of both full vagotomy groups did come to lie below the 300 - 310 range considered representative of normal rats, only the Sham KC-Sham Vx vs KC-Full Vx comparison achieved significance ($p < .05$).

Knife cuts with vagotomies. Using this operation sequence, it was found
that full vagotomy could prevent the development of knife cut induced hyperphagia and obesity, while vagotomy had no apparent effect. A total of 30 animals (five per cell) met the criteria for appropriate knife cuts and/or vagotomies and are included in the data analysis.

Body weight curves for all groups over the course of the experiment are displayed in Figure 6. At day 35, at which time the experiment was terminated, significant overall effects on body weight change of knife cuts ($F(1,24) = 84.1, p < .001$), vagotomies ($F(2,24) = 27.6, p < .001$), and a significant knife cut X vagotomy interaction ($F(2,24) = 32.7, p < .001$) were found. After surgery, animals with gastric vagotomies and knife cuts gained weight fully as rapidly as did animals with sham vagotomies and knife cuts. Both the KC+Sham Vx and KC+Gastric Vx groups' final level of body weight change was significantly greater than that of each of the other groups (p's < .01); the difference between these two groups with respect to body weight change was not significant. Full vagotomy, when coupled with knife cuts, yielded a body weight function which initially closely resembled that of animals with full vagotomy alone. By day 35, however, the KC+Full Vx rats' overall weight change was not significantly different than that of Sham KC+Sham Vx rats. The result contrasts somewhat with the effects of Sham knife cuts plus full vagotomy, which produced total weight changes significantly lower than those of the Sham CK+Sham Vx group.

Food intake values over the last seven days of the experiment largely paralleled the body weight data. Table 5 shows food intakes for all groups averaged over the seven day periods prior to surgery and
Figure 6. Mean (± SEM) body weight change for all groups in the knife cuts with vagotomy sequence. Knife cuts or sham knife cuts were given under the same anesthetic dose as either sham, full, or gastric vagotomies.
Table 5

Mean (+ SEM) Food Intake (g/day) Over Seven Day Periods Prior to Combined Knife Cut and Vagotomy Surgeries (Day 0) and Prior to the Termination of the Experiment (Day 35).

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Knife Cut - Sham Vagotomy</td>
<td>21.0 ± 1.4</td>
<td>21.1 ± 0.7</td>
</tr>
<tr>
<td>Knife Cut - Sham Vagotomy</td>
<td>20.1 ± 0.8</td>
<td>43.9 ± 2.4**</td>
</tr>
<tr>
<td>Sham Knife Cut - Full Vagotomy</td>
<td>20.3 ± 1.2</td>
<td>20.6 ± 1.1</td>
</tr>
<tr>
<td>Knife Cut - Full Vagotomy</td>
<td>21.4 ± 0.8</td>
<td>21.3 ± 1.5</td>
</tr>
<tr>
<td>Sham Knife Cut - Gastric Vagotomy</td>
<td>20.1 ± 0.5</td>
<td>21.3 ± 0.8</td>
</tr>
<tr>
<td>Knife Cut - Gastric Vagotomy</td>
<td>21.5 ± 1.1</td>
<td>46.5 ± 4.5**</td>
</tr>
</tbody>
</table>

**Differs significantly from Sham Knife Cut - Sham Vagotomy group, p < .01.
prior to the completion of the experiment. The intakes of both the KC+Sham Vx and the KC+Gastric Vx groups were more than double that of each of the other groups (p's < .01). None of the other comparisons approached significance, although as in the previous experiments, the intakes of the groups with full vagotomies did tend toward the lower end of the normal range.

Effects on linear growth which resulted from this operation sequence were somewhat different than those which were seen when knife cuts were given 30 days prior to vagotomies. Table 6 shows naso-anal lengths and obesity indices for all groups at day 0 and at day 35. By the end of the experiment, significant main effects of knife cuts (F(1,24) = 14.7, p < .001) and vagotomies (F(2,24) = 6.8, p < .005) were found. Both the KC+Sham Vx and the KC+Gastric Vx groups grew significantly longer than each of the other groups (p's < .01, except the KC+Gastric Vx vs either the KC+Full Vx or the Sham KC+Sham Vx groups, p's < .05). The KC+Full Vx group, while longer than both the Sham KC+Full Vx (p < .01) and Sham KC+Gastric Vx groups (p < .05), failed to achieve significance in comparison to the Sham KC+Sham Vx controls, suggesting that full vagotomy attenuated, but did not abolish, the increased growth produced by knife cuts. Additional evidence of decreased growth rates following vagotomy was to be found in the observation that the Sham KC+Sham Vx group grew to be significantly longer than the Sham KC+Full Vx group.

While changes in growth may have, in part, accounted for the weight lost by rats with full vagotomies, differential effects of the various knife cut and vagotomy combinations on adiposity (obesity indices) were also apparent, and closely paralleled the body weight data. At day 35,
Table 6

Mean (+ SEM) Naso-Anal Length (cm) and Lee Obesity Indices\(^1\) at the Time Combined Knife Cut and Vagotomy Surgeries were Performed (Day 0), and at the Time the Experiment was Terminated (Day 35).

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 35</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAL</td>
<td>OBI</td>
</tr>
<tr>
<td>Sham Knife Cut - Sham Vagotomy</td>
<td>20.9 ± 0.2</td>
<td>305 ± 3</td>
</tr>
<tr>
<td>Knife Cut - Sham Vagotomy</td>
<td>21.3 ± 0.3</td>
<td>300 ± 4</td>
</tr>
<tr>
<td>Sham Knife Cut - Full Vagotomy</td>
<td>20.7 ± 0.2</td>
<td>303 ± 3</td>
</tr>
<tr>
<td>Knife Cut - Full Vagotomy</td>
<td>20.9 ± 0.2</td>
<td>300 ± 2</td>
</tr>
<tr>
<td>Sham Knife Cut - Gastric Vagotomy</td>
<td>20.7 ± 0.3</td>
<td>307 ± 3</td>
</tr>
<tr>
<td>Knife Cut - Gastric Vagotomy</td>
<td>20.9 ± 0.2</td>
<td>304 ± 4</td>
</tr>
</tbody>
</table>

\(^1\) Normal range is 300 - 310.

*Differs significantly from Sham Knife Cut - Sham Vagotomy value, \(p < .05\).

**Differs significantly from Sham Knife Cut - Sham Vagotomy value, \(p < .01\).
significant main effects of knife cuts ($F(1,24) = 48.8$, $p < .001$) vagotomies ($F(2,24) = 9.8$, $p < .001$), and a significant knife cut X vagotomy interaction ($F(2,24) = 8.9$, $p < .005$) were evident. Both the KC+Sham Vx and the KC+Gastric Vx groups became more obese than each of the other groups ($p$'s $< .01$). The reduced adiposity noted previously among rats with full vagotomies was again apparent, as the obesity index of the Sham KC+Full Vx group was significantly lower than that of the Sham KC+Sham Vx controls ($p < .01$).

**Knife cuts after vagotomies.** Thirty-two animals met the completeness of vagotomy and/or histological criteria and were included in the data analysis. On the basis of preliminary $t$-tests, no differences in food intake or body weight change at day 30 were found to exist between the two groups in each of the sham vagotomy, full vagotomy, and gastric vagotomy conditions. The data for all animals in each condition have therefore been pooled for presentation here. All groups contained five animals except the Full Vx-KC group and the Gastric Vx-KC group which contained six rats each.

The pattern of effects produced by sham, full and gastric vagotomies was quite consistent with that reported for the other operation sequences. Figure 7 shows body weight curves for all groups over the course of the experiment. At day 30 a significant overall effect of vagotomy on body weight change was found ($F(2,29) = 21.6$, $p < .001$). After showing the characteristic period of acute anorexia and weight loss, the rats with full vagotomies reattained stable levels of body weight maintenance, having gained less weight over the 30 day postoperative period than
Figure 7. Mean (+ SEM) body weight change for all groups in the knife cuts after vagotomy sequence. Sham, full, or gastric vagotomies were given on day 0; bilateral parasagittal hypothalamic knife cuts were added on day 30.
either the sham or gastric vagotomy groups (p's < .01). No effect of vagotomies on food intake was found (F(2,29) = 1.3, p < .10) (see Table 7), although the rats with full vagotomies again tended to eat less than both the sham and gastric vagotomy groups over days 23 - 30.

In this experiment, the overall change in linear growth did not achieve significance (F(2,29) = 1.5, p < .10), although the tendency for rats with full vagotomies to be shorter than either of the other groups was again apparent. Vagotomy did produce an overall effect on obesity indices (F(2,29) = 6.2, p < .01), with the full vagotomized animals being significantly less obese than either the sham or gastric vagotomy groups (p's < .01). Table 3 gives naso-anal lengths and obesity indices at the time of gut surgery (day 0), brain surgery (day 30), and at the completion of the experiment (day 60).

When knife cuts or sham knife cuts were administered on day 30, the differential effects of full vs gastric vagotomy on the overeating and obesity produced by knife cuts was again apparent. By the time the experiment was terminated (day 60), significant main effects of knife cuts (F(1,26) = 44.8, p < .001), vagotomies (F(2,26) = 24.2, p < .001), and a significant knife cut X vagotomy interaction (F(2,26) = 8.1, p < .005) were evident. While the rate of weight gain of the Gastric Vx-KC group did, for a time, lag behind that of the Sham Vx-KC group. by day 60, the total weight change of these two groups did not differ significantly, and both groups had gained far more weight than any of the other groups (p's < .01). In contrast, knife cuts administered to animals which had recovered from the acute effects of full vagotomy produced no discernably excessive increases in body weight, as the
Table 7

Mean (+ SEM) Food Intakes Over Seven Day Periods Prior to Vagotomy Surgery (Day 0), Knife Cut Surgery (Day 30), and the Completion of the Experiment (Day 60).

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 30</th>
<th>Day 60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Vagotomy - Sham Knife Cut</td>
<td>19.4 ± 0.7</td>
<td>21.4 ± 0.9</td>
<td>22.5 ± 0.9</td>
</tr>
<tr>
<td>Sham Vagotomy - Knife Cut</td>
<td>20.0 ± 0.6</td>
<td>20.9 ± 1.2</td>
<td>44.1 ± 2.9**</td>
</tr>
<tr>
<td>Full Vagotomy - Sham Knife Cut</td>
<td>20.2 ± 1.4</td>
<td>19.7 ± 1.6</td>
<td>19.9 ± 0.9*</td>
</tr>
<tr>
<td>Full Vagotomy - Knife Cut</td>
<td>19.8 ± 0.8</td>
<td>13.7 ± 0.9**</td>
<td>20.1 ± 1.2</td>
</tr>
<tr>
<td>Gastric Vagotomy - Sham Knife Cut</td>
<td>20.4 ± 0.6</td>
<td>20.8 ± 1.1.</td>
<td>21.5 ± 1.0</td>
</tr>
<tr>
<td>Gastric Vagotomy - Knife Cut</td>
<td>20.7 ± 1.0</td>
<td>21.6 ± 1.1</td>
<td>39.7 ± 2.1**</td>
</tr>
</tbody>
</table>

*Differs significantly from Sham Vagotomy - Sham Knife Cut value, p < .05.

**Differs significantly from Sham Vagotomy - Sham Knife Cut value, p < .01.
Table 8
Mean (+ SEH) Naso-Anal Lengths (NAL) (cm) and Lee Obesity Indices (OBI)\(^1\) at the Time
of Vagotomy Surgery (Day 0), at the Time of Knife Cut Surgery (Day 30),
and at the Time the Experiment was Terminated (Day 60).

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th></th>
<th>Day 30</th>
<th></th>
<th>Day 60</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAL</td>
<td>OBI</td>
<td>NAL</td>
<td>OBI</td>
<td>NAL</td>
<td>OBI</td>
</tr>
<tr>
<td>Sham Vagotomy - Sham Knife Cut</td>
<td>20.2 + 0.2</td>
<td>304 + 3</td>
<td>21.0 + 0.5</td>
<td>308 + 5</td>
<td>21.6 + 0.2</td>
<td>312 + 3</td>
</tr>
<tr>
<td>Sham Vagotomy - Knife Cut</td>
<td>20.3 + 0.3</td>
<td>305 + 6</td>
<td>21.3 + 0.3</td>
<td>303 + 6</td>
<td>22.4 + 0.3**</td>
<td>341 + 8**</td>
</tr>
<tr>
<td>Full Vagotomy - Sham Knife Cut</td>
<td>20.4 + 0.2</td>
<td>308 + 3</td>
<td>20.9 + 0.2</td>
<td>296 + 3**</td>
<td>21.3 + 0.2</td>
<td>293 + 5**</td>
</tr>
<tr>
<td>Full Vagotomy - Knife Cut</td>
<td>20.5 + 0.2</td>
<td>305 + 5</td>
<td>21.0 + 0.2</td>
<td>298 + 3**</td>
<td>22.2 + 0.3**</td>
<td>299 + 4*</td>
</tr>
<tr>
<td>Gastric Vagotomy - Sham Knife Cut</td>
<td>20.6 + 0.3</td>
<td>303 + 2</td>
<td>21.1 + 0.4</td>
<td>307 + 3</td>
<td>21.4 + 0.3</td>
<td>304 + 4</td>
</tr>
<tr>
<td>Gastric Vagotomy - Knife Cut</td>
<td>20.4 + 0.3</td>
<td>301 + 3</td>
<td>21.2 + 0.4</td>
<td>302 + 3</td>
<td>22.6 + 0.3**</td>
<td>336 + 5**</td>
</tr>
</tbody>
</table>

\(^{1}\)Normal range is 300 - 310.

*Differs significantly from Sham Vagotomy - Sham Knife Cut group, \(p < .05\).

**Differs significantly from Sham Vagotomy - Sham Knife Cut group, \(p < .01\).
weight change of this group remained comparable to that of the Full Vx-Sham KC animals, and significantly below that of both the Sham Vx-Sham KC and the Gastric Vx-Sham KC groups (all p's < .01 except for the Full Vx-KC vs Gastric Vx-Sham KC comparison, p < .05).

The general pattern of food intake data over days 53 - 60 was also congruent with the results obtained in the other operation sequences. Significant main effects of knife cuts ($F(1,26) = 57.2$, $p < .001$), vagotomies ($F(2,26) = 25.0$, $p < .001$), and a significant interaction term ($F(2,26) = 15.6$, $p < .001$) were again apparent. Both the Sham Vx-KC and the Gastric Vx-KC groups ate significantly more over this interval than any of the other groups (p's < .01), with the Sham Vx-KC group also eating somewhat more than the Gastric Vx-KC group ($p < .05$). Independent of the presence or absence of knife cuts, full vagotomy again resulted in food intakes which were numerically, but generally not significantly, below Sham Vx-Sham KC control levels. Only the Sham Vx-Sham KC vs Full-Vx Sham KC comparison achieved significance in this regard ($p < .05$, see Table 7). Gastric vagotomy alone again had no significant effect on food intake.

In this operation sequence, the changes in body weight appeared to be primarily attributable to fluctuations in adiposity. A significant effect of knife cuts on naso-anal length was obtained ($F(1,26) = 18.3$, $p < .001$), with all knife cut groups having grown significantly longer than all sham knife cut groups (p's < .01; see Table 8). While some evidence for stunting following full vagotomy was apparent in the previously described operation sequences, no such effect was apparent here, as none of the other comparisons achieved significance. Sig-
nificant overall effect of knife cuts ($F(1,26) = 60.7, p < .001$), vagotomies ($F(2,26) = 42.0, p < .001$), and a significant knife cut X vagotomy interaction ($F(2,26) = 8.4, p < .005$) on obesity indices were found, however, and paralleled the body weight data nicely. Both the Sham Vx-KC and the Gastric Vx-KC groups achieved obesity indices which were significantly greater than those of each of the other groups (p's < .01), but which did not differ significantly from one another. Both groups with full vagotomies, on the other hand, displayed obesity indices which were significantly below those of both the Sham Vx-Sham KC and the Gastric Vx-Sham KC groups (p's < .01).

Water/Food ratios. Preliminary analyses revealed that the water/food ratios obtained for animals within any given knife cut and vagotomy combination over the seven day period prior to the completion of the experiments did not differ as a function of operation sequence. I have therefore collapsed water/food ratio data across sequences for presentation here. Figure 8 presents water food ratios for each of the six possible brain and gut surgery combinations over the periods prior to any surgery (pre-op), and after intakes had stabilized under conditions when both brain and gut surgeries were in effect, that is, just prior to the completion of the experiments (post-op).

In contrast to the effect of VMH lesions described in Chapter I, water intakes following knife cuts were elevated, but the increases observed were roughly proportional to increases in food intake also produced by the knife cuts. As a result, no overall effect of knife cuts on water/food ratios was obtained although ratios following knife cuts were quite variable (mean = 1.66; range: 1.06 -2.70). Analysis
Figure 8. Mean (+ SEM) water/food ratios (ml/g) for each of
the six possible combinations of knife cuts or sham knife cuts
with sham, full, or gastric vagotomies. Data are collapsed across
operation sequences. Values indicated are averages over the seven
day period prior to any surgery (pre) and prior to the termination
of the experiment (post) when both knife cut and vagotomy surgeries
were in effect.
WATER/FOOD RATIO (ml/g)

SHAM VAGOTOMY

FULL VAGOTOMY

GASTRIC VAGOTOMY
of variance revealed only a significant main effect of vagotomies on water/food ratios \( F(2,85) = 14.1, p < .001 \). Consistent with the results of Chapter I, full vagotomy dramatically reduced water/food ratios, independent of knife cut status, relative to all other groups \( (p's < .01) \). Gastric vagotomy produced a less marked reduction in water/food ratios, again independent of whether or not knife cuts were present, relative to both sham vagotomy groups \( (p's < .01) \).

**Gastric acid secretion.** A total of nine rats were eliminated from the study after having failed to meet the criterion for completeness of vagotomy on the acid secretion test. Of these, four were rats with knife cuts and gastric vagotomies and two had knife cuts and full vagotomies. Each of the rats with knife cuts and incomplete gastric vagotomies showed overeating and weight gains which lay within the ranges of their respective sham vagotomy - knife cut control groups. The two rats with knife cuts and incomplete full vagotomies also overate and became obese, but notably less so than did their sham vagotomy-knife cut controls, reaching only 35 and 58% of the mean values of their respective sham vagotomy-knife cut control groups.

It was noted during the acid secretion tests that the stomachs of rats with either full or gastric vagotomies retained considerably more food following a 24 hr fast than did the sham vagotomized rats, as judged by the number of saline washings required to rid the stomach of retrievable solid matter. In Chapter I it was observed that the esophagi of vagotomized rats were oftentimes grossly distended and packed with food. It is especially noteworthy that this phenomenon was far less apparent, if not completely absent, in the rats which were
vagotomized using the somewhat different technique employed here (see Methods section).

Like the water/food ratio data, the gastric acid secretion results were collapsed across operation sequences for presentation below. Two sham vagotomized animals failed to survive the acid secretion phase of the experiment and whatever data was obtained from them during this phase was not considered in the analysis. The behavioral data from these animals was included, however, in the preceding analyses.

Gastric acid secretion data are presented in Figure 9. Under baseline conditions, significant main effects of knife cuts (F(1,33) = 36.0, p < .001), vagotomy (F(2,83) = 30.0, p < .001), and a significant interaction (F(2,83) = 26.7, p < .001) were obtained. The knife cut-sham vagotomy animals displayed fasting acid secretion rates which were significantly greater than, indeed more than double, that of any of the other groups (p's < .01), indicating that both the full and gastric vagotomy procedures were effective in blocking the increased gastric acid secretion produced by knife cuts. None of the other comparisons achieved significance.

Clear effects of knife cuts (F(1,33) = 39.1, p < .001), vagotomies (F(2,83) = 239.5, p < .001) and their interaction (F(2,83) = 35.0, p < .001) on the secretory response to electrical stimulation of the cervical vagi were noted. Both sham vagotomized groups' responses to stimulation were significantly greater than those of each of the other groups (p < .01). Thus, both full and gastric vagotomy abolished the rise in acid secretion produced by vagal stimulation. In addition, the knife cut-sham vagotomy animals, whose baseline secretion rates
Figure 9. Mean (+ SEM) fasting gastric acid secretion under non-stimulated (baseline) conditions, and in response to electrical stimulation of the cervical vagi. Values are collapsed across operation sequences. Open bars represent values of sham vagotomized groups; striped bars represent values of full vagotomy groups; cross-hatched bars represent values of gastric vagotomy groups. Baseline values are those obtained during the 15 min period immediately prior to the onset of electrical stimulation. Stimulated values are maxima seen in response to electrical stimulation.
were elevated, also displayed a greater secretory response to vagal stimulation than did sham knife cut - sham vagotomized controls (p < .01).

**Histology.** Figure 10 shows a reconstruction of a representative knife cut. The bilateral parasagittal cuts employed here were found to be a highly reliable means of inducing hyperphagia and obesity. Of 34 animals whose knife cut placements were subject to behavioral confirmation, uncomplicated by prior or simultaneous vagotomies, all but three became hyperphagic and obese. Data from the three animals which failed to fatten were not considered in the data analysis. Four knife cut placements which were not subject to unconfounded behavioral confirmation were judged by one or both observers not to conform to the criteria for effective cuts outlined in the methods section. Data from these animals were also discarded.

Effective cuts were generally found to be from 2.5 - 3.0 mm in height, ranging from about 0.7 - 1.3 mm lateral to the midline, typically lying just medial to the fornices. The coronal plane of the PVN was crossed by the rostral aspects of all knife cuts which were either proven or judged to be appropriately placed.

**Discussion**

The results show that full vagotomy eliminates overeating and obesity induced by hypothalamic knife cuts and provides some direct evidence that the antagonism of hypothalamic hyperphagia and obesity by vagotomy may indeed be specific.
Figure 10. Representative bilateral parasagittal knife cuts drawn on a plate from the Konig and Klippel (1963) rat brain atlas. The paraventricular nucleus is labelled (fm).
Effects of knife cuts. The knife cut placements employed in the present study were quite comparable to those which have been found in this laboratory and others (e.g., Gold, 1973; Gold et al., 1977; Sclafani & Berner, 1977) to cause maximal hyperphagia and weight gains. The magnitude of these knife cut-induced effects were even greater than those seen following the large (24 mC) electrolytic lesions employed in Chapter I.

As has repeatedly been shown to be the case following VMH lesions (Inoue & Bray, 1977; Powley & Opsahl, 1974; Ridley & Brooks, 1965), knife cuts also produced increased fasting gastric acid secretion, a finding which both underscores the commonality of the lesion and knife cut syndromes and provides physiological evidence suggestive of vagal hyperactivity in knife cut animals.

The constellation of knife cut effects was not entirely identical to that produced by lesions. Consonant with previous studies (Gold et al., 1977; Gold & Kapatos, 1975) and in contrast to the effects of VMH lesions, knife cuts were consistently found to produce reliable increases in body length, which may, in part, account for the more substantial rates of weight gain typically seen in knife cut, relative to lesioned, animals. Knife cuts alone also resulted in increased water intakes, which was roughly proportional to the increased food intake, the end result being that water/food ratios remained about the same.

Effects of full vagotomy. As was found to be the case in lesioned animals, subsequent full subdiaphragmatic vagotomy completely reversed
knife cut-induced increases in body weight, food intake, and adiposity. The weights of the knife cut-full vagotomies group stabilized at a level below that of the sham knife cut-sham vagotomy group and even below that of the animals with full vagotomies alone. This latter result contrasts with the strikingly similar final levels of body weight maintenance seen in Chapter I among all full vagotomy groups, independent of lesion status, and would suggest that the level of weight maintenance following full vagotomy is subject to some variability. Perhaps some secondary consequence of prolonged undereating and weight loss, which has been shown capable of reducing chronic levels of body weight maintenance in otherwise normal animals (Sawchenko, Gold, & Bisson, in press), may have contributed to the final low level of body weight maintenance seen in the knife cut-full vagotomy group. In line with previous studies (e.g., King et al., 1978; Mordes et al., 1977; Powley & Opsahl, 1974), food intakes were reduced by full vagotomy in both knife cut and sham knife cut rats to a level somewhat, although not always significantly, lower that that of sham knife cut-sham vagotomized controls.

The changes in body weight seen following knife cuts and full vagotomies were largely mirrored by changes in obesity indices, indicating that the different levels of weight maintenance seen following the various knife cut and vagotomy combinations are largely attributable to alterations in the body fat compartment. Rates of linear growth were only marginally reduced by full vagotomies in this operation sequence.

These effects of full vagotomies were also generally apparent in the
other operation sequences employed. When full vagotomies and knife cuts were administered under the same anesthetic dose, a weight function similar to that seen in sham knife cut-sham vagotomized animals was initially apparent. The final levels of weight maintenance seen in these animals, although somewhat higher than that of rats with full vagotomies alone, and slightly below that of sham vagotomized-sham knife cut controls, did not achieve significance in either comparison. The food intakes and obesity indices of these knife cut - full vagotomy rats were within normal limits, buttressing the conclusion that full vagotomy again completely eliminated knife cut induced hyperphagia and obesity.

In contrast to the results found in the other operation sequences, full vagotomy in this sequence attenuated the increased growth produced by knife cuts and produced similar evidence of stunting in sham knife cut animals. The lack of excessive growth following knife cuts and full vagotomies was probably due to the fact that in the immediate post-operative period, these rats displayed the typical post-vagotomy anorexia and weight loss, a condition which would likely dictate that available foodstuffs be diverted toward meeting energy needs, at the expense of growth. In any case, the fact that stunting was inconsistently produced by full vagotomy would indicate that the resultant changes in body weight are due primarily to reductions in body fat content, as opposed to lean body mass.

In the final operation sequence, knife cuts judged potentially effective by two independent observers were completely ineffective in increasing body weight, food intake or adiposity in rats which had
recovered from the acute effects of full vagotomies.

Collectively, these results would indicate that the antagonism of hypothalamic hyperphagia and obesity by vagotomies, at least in part, is specific. The fact that animals recovered from the acute effects of full vagotomies failed to overeat and fatten when knife cuts were subsequently administered would suggest that the reversal of pre-existing hypothalamic hyperphagia and obesity by full vagotomies is not wholly a consequence of non-specific acute effects of vagotomies. This, however, does not preclude the possibility that some secondary consequence of having at some time experienced the acute anorexia and weight loss seen in all full vagotomy groups may contribute to the failure of hyperphagia and obesity to develop in rats whose vagotomies were administered prior to, or at the same time as, hypothalamic knife cuts. The results obtained with gastric vagotomies provide no insight into this issue, as gastric vagotomy alone yielded no remarkable post-operative undereating and weight loss.

Some laboratories using the full vagotomy before VMH lesion sequence have reported that hyperphagia and obesity develops in vagotomized animals almost to the extent that it does in sham vagotomized controls (Chikamori et al., 1976; King et al., 1973; Wampler, Note 9; Wampler & Snowdon, 1979). Others have found results compatible with those reported here, i.e., that prior full vagotomies prevents the development of overeating and obesity when lesions or knife cuts are added at a later date (Gold et al., in press; Rowland & Engle, 1979). A consideration of the effects of the gastric vagotomies employed here on the knife cut syndrome may provide a possible explanation for these
discrepancies.

Effects of gastric vagotomy. Independent of the sequence of operations, gastric vagotomy was found to produce no significant reduction of knife cut-induced increases in body weight, and only modest reductions in food intake (in two of three experiments), relative to animals with knife cuts and sham vagotomies. Similarly, final levels of linear growth and obesity indices were, in all sequences, not significantly below those seen in knife cut-sham vagotomized rats.

The gastric vagotomy procedure employed here has been shown to produce the alterations in gastric acid secretion and gastric emptying of both food and water characteristic of more extensive full vagotomies (Sawchenko et al., 1977). In the present experiment, both full and gastric vagotomies were found to abolish both the knife cut-induced increase in fasting gastric acid secretion, as well as the increase in acid secretion seen in sham vagotomized rats in response to electrical stimulation of the cervical vagi. Thus, both gastric vagotomy, which had virtually no impact on hypothalamic hyperphagia and obesity, and full vagotomy, which completely blocked or reversed knife cut induced increases in body weight food intake and adiposity, had similar effects on an independent physiological measure of gastric function. These results, then, do not provide a possible explanation for the observation (Chikamori et al., 1976; King et al., 1978; Wampler, Note 9; Wampler & Snowdon, 1979) that hypothalamic hyperphagia and obesity can become or remain fully developed in rats whose vagotomies rely on a gastric acid secretory response as an index of
completeness of vagotomy. The gastric secretion test appears to be one which is functionally irrelevant to the mechanisms underlying the syndrome.

Mechanisms of vagal blockade of hypothalamic obesity. Most of the studies which have failed to significantly attenuate lesion-induced hypothalamic hyperphagia and obesity by prior full vagotomy have used the vagotomy procedure described by Snowdon & Epstein (1970). In this procedure, the main vagal trunks are first severed at a level near the stomach and then stripped upwards off the esophagus and excised. If the initial section of the vagal trunks (see Figure 4) were at too low a level, this procedure could result in vagotomies in which the hepatic and/or coeliac vagal innervation remained intact. The differential effects of full gastric vagotomies described here strongly implicate these (hepatic and coeliac) branches, either alone or in conjunction with the gastric vagi as critical to the expression of the syndrome. This contention is consistent with the observation that two knife cut animals with full vagotomies which were judged to be incomplete on the gastric acid secretion test still gained substantially less weight than did their appropriate sham vagotomized, knife cut controls.

A recent report in which the level of vagal transection was varied in VMH-lesioned rats (Carpenter et al., 1978) has provided results which are, in some respects, compatible with the results and interpretation offered here. Carpenter and colleagues (1973) report that transection of the vagal trunks at a high level, just below the diaphragm (analogous
to the full vagotomy procedure of Chapter I) produced a high percentage of deaths which were attributed to gastrointestinal disorders and/or dehydration. Transections just above the stomach (presumably comparable to the gastric vagotomy procedure of Chapter II) produced only modest reduction of lesion-induced increases in food intake and body weight. Animals with mid-level transections displayed highly variable effects, ranging from severe weight loss to marked weight gain. Perhaps this variability may have been due to inconsistent involvement of the hepatic and/or coeliac vagal branches. The descriptions of surgical procedures provided by Carpenter et al (1978) are not sufficiently detailed to provide any additional insight into this issue.

The results of the present experiments have a direct bearing on the suggestion that the antagonism of hypothalamic hyperphagia and obesity by full vagotomy can largely be attributed to limitations in food-handling capacities imposed by gastrointestinal dysfunction (Booth, 1976; Carpenter et al., 1973; Inoue & Bray, 1977; King et al, 1978; Wampler & Snowdon, 1979; Panksepp, 1975). The full vagotomy procedure employed here involved independent section of each of the four principal abdominal vagal branches, as opposed to the more conventional and extensive vagotomy procedure which involves section of the main vagal trunks at a much higher level. Since the present full vagotomy procedure was fully effective in blocking the knife cut syndrome, and since no obvious evidence of esophageal distention and food retention was noted, it is highly unlikely that esophageal flaccidity is a principal contributor to the antagonism of hypothalamic
obesity by vagotomy. That the gastric vagotomy procedure produced evidence of gastric stasis and altered gastric secretory capacities similar to that seen following full vagotomy, would also indicate that upper gastrointestinal disorders are not a major factor mediating the blockade of knife cut induced obesity by full vagotomy.

Also consistent with the contention that upper gastrointestinal dysfunction cannot alone account for the blockade of hypothalamic obesity by vagotomy is the often overlooked report of Brooks et al (1946), who found that surgical removal of 80 - 90% of the stomach (gastrectomy) of obese-VMH lesioned rats had little long-term effect on hyperphagia and obesity. Such a radical gastrectomy procedure must surely have denervated much of the upper gastrointestinal tract, and placed enormous demands on the remaining components to process sufficient food to maintain an obese state. Brooks and colleague (1946) noted that the stomach remnant and upper duodenum in gastrectomized VMH-lesioned rats hypertrophied markedly to partially compensate for lost gastric storage functions.

While gastric and esophageal abnormalities do not appear to be capable of explaining the effects of full vagotomy on hypothalamic obesity, the possibility that dysfunction of gastrointestinal mechanisms not under gastric vagal control may contribute to the effects of full vagotomy cannot be discounted. The coeliac branch of the vagus, for example, is known to provide the innervation of a portion of the small intestines (e.g., Kennedy, 1974; Legros & Griffith, 1969; Ishigami et al, 1974). Perhaps some secretory or motor function under control of the coeliac vagus, which is severed by full vagotomies but spared by
gastric vagotomies, may account for the blockade of hypothalamic obesity. While such a contribution may exist, it is unlikely that small intestinal dysfunction could provide a complete explanation of the normalizing effects of vagotomy on hypothalamic obesity, particularly in light of the Brooks et al (1946) data which testifies to the adaptive capabilities of the gastrointestinal tract, and the absence of any reports documenting gross small intestinal dysfunction following VMH lesions or knife cuts.

Water/food ratios. A consideration of the water/food ratio data, while apart from the main point of these experiments, is important as these data lend credence to the results obtained with gastric vagotomies insofar as they demonstrate that gastric vagotomies were not without behavioral effects.

As we found to be the case in Chapter I, full vagotomies reduced water/food ratios independent of knife cut status. Gastric vagotomy also reduced water/food ratios relative to sham vagotomized rats, but not to the extent that full vagotomy did. This result would suggest that the reduced water/food ratios characteristic of vagotomized rats (e.g., King et al., 1973; Kraly, 1975) are largely attributable to alterations in upper gastrointestinal function. Both full (Kraly, 1978; Snowdon & Epstein, 1970) and gastric (Sawchenko et al., 1977) have been shown to increase the gastric emptying rate of water. It is therefore possible that some consequence of this effect, either discomfort produced by premature "dumping" of ingested fluids into the duodenum (see Deutsch, Molina, & Puerto, 1976), or perhaps the premature
activation of gastrointestinal receptors which serve to inhibit water intake, may account for the reduced water/food ratios seen following vagotomy.

The fact that gastric vagotomy qualitatively reproduced the effects of full vagotomy on drinking and water/food ratios should discourage the practice recently advocated by King et al (1978) of using reduced water/food ratios as an informal index of completeness of full vagotomy.
CHAPTER III

EFFECTS OF SELECTIVE SUBDIAPHRAGMATIC VAGOTOMIES ON KNIFE CUT-INDUCED HYPOTHALAMIC HYPERPHAGIA, OBESITY, AND FINICKINESS

On the basis of the results of the preceding experiments, it appears likely that hypothalamic hyperphagia and obesity may indeed be mediated through the subdiaphragmatic vagi. That selective gastric vagotomy alone does not limit overeating or weight gains implicates the remaining hepatic and/or coeliac branches as necessary for the expression of the syndrome.

In this experiment the effects of various types of selective vagotomies on knife cut-induced hypothalamic hyperphagia and obesity are assessed. Rather that the bilaterally symmetrical parasagittal cuts of Chapter II, asymmetrical cuts are employed here. Such cuts, described previously, have been shown to produce overeating and weight gains of a magnitude comparable to those seen following large electrolytic lesions, but avoid some of the potentially confounding ancillary effects of symmetrical ablations which are unrelated to the appearance of overeating and obesity (Gold et al., 1977).

Following examination of the effects of selective vagotomies on knife cut-induced overeating and obesity in rats fed a standard lab chow diet, a period of access to a high fat diet was allowed. Such diets are consumed especially avidly by animals with VMH lesions or knife cuts (e.g., Carlisle & Stellar, 1969; Corbit & Stellar; Gold, 1970). Powley and Opsahl (1974) have shown, albeit equivocally, that
vagotomized VMH lesioned rats, which are eating normal amounts of a standard laboratory diet, will overeat a high fat diet. Such a result, if confirmed, would indicate that the exaggerated preference for tasty foods ("finickiness") characteristic of the VMH animal is spared by vagotomy, and would also show that the vagotomized rat with knife cuts is capable of overeating.

**Method**

**Subjects.** Adult female rats weighing 250-300 g were used. Housing and feeding conditions were as described in Chapter I, except where otherwise noted.

**Surgery.** General surgical procedures for brain and gut surgery were as previously described. The asymmetrical knife cut procedure is detailed in Gold et al (1977). Each knife cut rat received a unilateral 3 mm long X 3 mm high parasagittal knife cut such as described in Chapter II. In addition, a 2 mm long X 3 mm high contralateral coronal cut was placed with a medially directed knife at 5.5 mm anterior to the earbars and 2.1 mm lateral to the midline.

Immediately before or after brain surgery animals were given either a selective vagotomy or sham vagotomy. Table 9 shows the number of animals initially operated in each group, the reasons for excluding some from the data analysis, and the final number of rats in each cell.

Vagotomies were generally performed as they were in Chapter II. The approximate levels at which the various vagal branches were sectioned also corresponded to those described previously (refer back to
Table 9
Composition of Groups with Various Knife Cut and Selective Vagotomy Combinations

<table>
<thead>
<tr>
<th>Knife Cuts</th>
<th>Vagotomy</th>
<th>Operated</th>
<th>Excluded because of:</th>
<th>Histology</th>
<th>Vagotomy</th>
<th>Sick/Dead</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Gastric</td>
<td>-</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Hepatic</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Coeliac</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Sham Knife Cuts</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Gast. + Hep.</td>
<td>-</td>
<td>7</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Gast. + Coel.</td>
<td>-</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Coel. + Hep.</td>
<td>-</td>
<td>7</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Full</td>
<td>-</td>
<td>7</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>5</td>
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<tr>
<td>Sham</td>
<td>-</td>
<td>12</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Gastric</td>
<td>-</td>
<td>7</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Hepatic</td>
<td>-</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Coeliac</td>
<td>-</td>
<td>11</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Asymmetrical Knife Cuts</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Gast. + Hep.</td>
<td>-</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Gast. + Coel.</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Coel. + Hep.</td>
<td>-</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Full</td>
<td>-</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Totals</td>
<td>127</td>
<td>4</td>
<td>16</td>
<td>11</td>
<td>96</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Procedure. Following surgery, animals were returned to their home cages where lab chow pallets and water were freely available for 30 days. To avoid a possible confound in the second phase of the experiment (see below), ten percent sucrose solutions, rather than the high fat diet, were given to anorexic animals, as needed.

Following the initial 30 day period, all animals were placed exclusively on the 33% high fat diet described in Chapter I for 30 days, and were then reinstated on lab chow for a final 30 day period. Naso-anal lengths, pursuant to calculation of Lee Obesity Indices, were measured at the time of surgery, and, under light ether anesthesia, at the time of each dietary change, and at the conclusion of the experiment. In contrast to the previous chapters, food intakes are reported as energy equivalents of the weight of food consumed (KCal/day). Since the caloric densities of the lab chow (3.61 KCal/g) and high fat (5.5 KCal/g) differ, reporting intakes as KCal facilitates comparisons across diets.

Verification of vagotomies. At the completion of the experiment, animals were anesthetized and laparotomized. The region of the esophagus and stomach was thoroughly scanned for vagal elements under 20 X magnification. In cases where rats were grossly obese, it was sometimes necessary to remove large fat deposits from the abdomen. Each animal individually screened for the integrity of each of the four vagal branches without knowledge of the surgical status of the animal. Only those rats whose vagal status at autopsy coincided with the originally intended
surgery were included in the data analysis.

**Histology.** Rats were perfused and their brains prepared as described previously, except that the brains were sectioned in the horizontal plane to allow visualization of both the parasagittal and coronal cuts in a single section. As in Chapter II, the placements of the cuts were judged, without knowledge of the behavioral data, by two independent observers. The criteria for placement of the parasagittal cut were the same as those used in the previous chapter. The coronal cut was considered to be appropriate if it extended to the base of the brain, was at least 2 mm in height, and extended at least 1.5 mm lateral to the midline.

**Data analysis.** The basic analyses were described in Chapter I. Exceptions are indicated in the text.

**Results**

**Effects of selective vagotomies on knife cut-induced hyperphagia and obesity - lab chow diet.** The various selective vagotomy procedures were found to produce quite different effects on the overeating and obesity which resulted from asymmetrical knife cuts. Over the initial 30 day postoperative period, when all animals were maintained on the standard lab chow diet, significant overall effects on body weight change of knife cuts ($F(1,80) = 147.5, p < .001$), vagotomies ($F(7,80) = 11.6, p < .001$) and a significant knife cut X vagotomy interaction ($F(7,80) = 7.9, p < .001$) were found. Figure 11a shows mean body weight changes for all knife cut groups (the sham knife cut- sham
Figure 11a. Mean body weight change of rats which received asymmetrical knife cuts and a selective vagotomy (or sham vagotomy) on day 0. Sham knife cut-sham vagotomized controls are included for comparisons. A high-fat diet was in effect over days 30-60. At all other times the animals ate lab chow pellets.
Figure 11b. Mean body weight change of rats which received sham knife cuts and a selective vagotomy on day 0. A high-fat diet was in effect over day 30-60. At all other times the animals ate lab chow pellets. Note that the scale on the ordinate differs from that in Figure 11a.
vagotomy group is included for comparisons) over this and subsequent phases of the experiment. For the sake of clarity, sham knife cut groups are plotted separately (in b).

In general, vagotomies in which the coeliac branch was left intact were ineffective in blocking the weight gains produced by knife cuts. The KC-Gastric Vx and KC-Gastric plus Hepatic Vx groups' overall weight change did not differ significantly from that of the KC-Sham Vx Group. Hepatic vagotomy even produced indications of potentiating the knife cut effect on body weight, as this group gained more weight over the initial 30 day postoperative interval than did KC-Sham Vx animals (p < .01).

In contrast, vagotomies which included the coeliac branch were variably effective in reducing knife cut-induced increases in body weight. The KC-Coeliac Vx group achieved a total body weight change which reached only 57% of that of the KC-Sham Vx group (p < .01), but which still exceeded the weight increase of the Sham KC-Sham Vx controls (p < .01). The KC-Coeliac plus Hepatic Vx procedure produced a comparable overall suppression in weight change relative to CK-Sham Vx rats (p < .01), which did not differ significantly from that seen following knife cuts coupled with coeliac vagotomy alone. Coeliac plus gastric vagotomies had more severe effects, yielding weight gains which did not differ significantly from those seen in Sham KC-Sham Vx controls. Full vagotomy (i.e., coeliac plus gastric plus hepatic vagotomy) again completely abolished knife cut induced increases in body weight, and yielded an overall weight change which was less than that of the Sham KC-Sham Vx group (p < .01).
Among the sham knife cut groups (Figure 11b) it is noteworthy that none of the selective vagotomies which involved only a single branch significantly reduced body weight gains, relative to Sham KC-Sham Vx rats. More extensive vagotomies generally produced more pronounced decrements in body weight change. Most severe of these were the gastric plus coeliac and full vagotomy procedures, which yielded weight gains significantly less than those seen in the Sham KC-Sham Vx groups (p's < .01).

Caloric intakes generally corresponded to these body weight effects. The intakes of the knife cut groups, expressed as kilocalories per day (KCal/day), for 10 day blocks over the course of the experiment are shown in Figure 12. Again data from sham knife cut-sham vagotomized controls are included for comparison. Average daily intakes for all groups over the initial 30 day postoperative period were analyzed. Significant main effects of knife cuts ($F(1,80) = 165.7, p < .001$), vagotomies ($F(7,80) = 18.4, p < .001$), and a significant knife cut X vagotomy interaction ($F(7,80) = 5.0, p < .001$) were evident. Of the knife cut groups, neither the KC-Gastric Vx nor the KC-Gastric plus Hepatic Vx groups' intakes were found to differ significantly from those of KC-Sham Vx rats. Consistent with the body weight data, the KC-Hepatic Vx animals' intakes were elevated, relative to KC Sham Vx controls ($p < .01$).

Each of the knife cut groups whose vagotomies included the coeliac branch showed intakes significantly lower than those of knife cut controls (p's < .01). Of these, the KC-Coeliac Vx ($p < .01$) and the KC-Coeliac plus Hepatic Vx group ($p < .05$) ate significantly more than did
Figure 12a. Mean caloric intake for knife cut rats with selective vagotomies or sham vagotomies averaged over ten day blocks. The intakes of sham knife cut-sham vagotomized rats are also plotted for comparisons. A high-fat diet was in effect over days 30-60. At all other times the rats ate lab chow pellets.
Figure 12b. Mean caloric intakes for sham knife cut rats with selective vagotomies or sham vagotomies averaged over ten day blocks. A high-fat diet was in effect over days 30-60. At all other times the rats ate lab chow pellets. Note that the scale on the ordinate differs from that in Figure 12a.
Sham KC-Sham Vx controls, indicating that hyperphagia was reduced, but not eliminated, in these animals. The KC-Coeliac plus Gastric Vx group did not eat significantly more over this 30 day interval than did Sham KC-Sham Vx rats.

Among the sham knife cut groups, those with either full (p < .01), gastric plus coeliac (p < .01), or gastric plus hepatic (p < .05) vagotomies all ate less than Sham KC-Sham Vx controls. The bulk of these decrements appeared to be attributable to greatly reduced intakes over the ten day period immediately following surgery.

The changes in body weight were paralleled by changes in obesity indices. At day 30, significant main effects of knife cuts (F(1,30) = 169.9, p < .001), vagotomies (F(7,80) = 19.5, p < .001), and a significant interaction (F(7,80) = 8.9, p < .001) were found. Obesity indices at this and subsequent time points are given in Table 10. The results of individual comparisons with both the KC-Sham Vx and the Sham KC-Sham Vx groups are indicated. Because the results closely parallel the body weight effects, they will not be reiterated here. Note that despite their elevated body weights, the KC-Hepatic Vx groups' obesity index was not significantly greater than that of KC-Sham Vx controls. In addition, the groups whose body weights were significantly lower than that of the normal controls (i.e., the KC-Full Vx, Sham KC-Full Vx, and Sham KC-Gastric plus Coeliac Vx groups) also displayed obesity indices which were significantly below Sham KC-Sham Vx values (p's < .01).

Responses to a high-fat diet. The changes in eating and body weight
Table 10

Mean (+ SEM) Lee Obesity Indices at Four Different Stages
for Various Knife Cut and Selective Vagotomy Combinations

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-op</th>
<th>Day 30</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham KC-Sham Vx</td>
<td>306 ± 2</td>
<td>306 ± 2*</td>
<td>314 ± 3*</td>
<td>310 ± 2*</td>
</tr>
<tr>
<td>KC-Sham Vx</td>
<td>302 ± 2</td>
<td>348 ± 5*</td>
<td>372 ± 7*</td>
<td>374 ± 5*</td>
</tr>
<tr>
<td>KC-Gastric Vx</td>
<td>307 ± 3</td>
<td>350 ± 8*</td>
<td>377 ± 9*</td>
<td>367 ± 7*</td>
</tr>
<tr>
<td>KC-Hepatic Vx</td>
<td>300 ± 2</td>
<td>355 ± 6*</td>
<td>365 ± 5*</td>
<td>365 ± 6*</td>
</tr>
<tr>
<td>KC-Gast. + Hep. Vx</td>
<td>305 ± 2</td>
<td>347 ± 6*</td>
<td>372 ± 7*</td>
<td>371 ± 8*</td>
</tr>
<tr>
<td>KC-Coeliac Vx</td>
<td>304 ± 4</td>
<td>325 ± 5*+</td>
<td>355 ± 4*+</td>
<td>353 ± 5*+</td>
</tr>
<tr>
<td>KC-Coel. + Gast. Vx</td>
<td>304 ± 3</td>
<td>312 ± 6*</td>
<td>345 ± 5*+</td>
<td>343 ± 5*+</td>
</tr>
<tr>
<td>KC-Coel. + Hep. Vx</td>
<td>303 ± 2</td>
<td>320 ± 9*+</td>
<td>349 ± 11*+</td>
<td>350 ± 12*+</td>
</tr>
<tr>
<td>KC-Full Vx</td>
<td>302 ± 2</td>
<td>295 ± 4*+</td>
<td>334 ± 7*+</td>
<td>324 ± 4*+</td>
</tr>
</tbody>
</table>

1 Normal range is 300-310.

*Differs significantly from KC-Sham Vx value, p < .01.

+Differs significantly from Sham KC-Sham value, p < .01.
seen over days 30-60, when a high-fat diet was in force, were similar in all groups. The responses were biphasic in nature and were of a far greater magnitude in knife cut groups. Following and initial burst of overeating and rapid weight gains over the first five to ten days on which the diet was offered, rates of body weight change stabilized at levels which were usually, but not always, comparable to those previously seen on the lab chow diet.

Because the knife cut groups had reached different weights over the first 30 days of the experiment, analysis was carried out on the absolute weight change which occurred over the period in which the high-fat diet was available (i.e., weight on day 60 minus weight on day 30). Non-readjusted body weight curves may be seen in Figure 11. All knife cut groups gained more weight on the high fat diet than did each of the sham knife cut groups (all p's < .01), although some differences in the magnitude of the responses among the knife groups were noted. Both the KC-Hepatic and KC-Full Vx groups gained significantly less weight over this interval than did KC-Sham Vx rats (p's < .01). All other knife cut groups' weight gains were comparable to those of KC-Sham Vx controls. While the KC-Hepatic Vx rats tended, as a group, to gain somewhat less weight than the other knife cut groups, their weight curve over days 30-60 is largely attributable to the aberrant behavior of one animal who gained only 17 g over this interval. The remaining KC-Hepatic Vx rats gained from 131-151 g. As for the KC-Full vagotomy group, these animals showed an initially less dramatic, but more sustained, pattern of overeating and weight gain, relative to the other knife cut groups. Note that by the end of the period of access
to the high-fat diet, the total weight change of the KC-Full Vx group had come to exceed that of Sham KC-Vx controls.

Caloric intakes over the interval in which the high-fat diet was offered tended to be greatest during the initial ten day period of access, following which intakes declined despite ongoing weight gains. When the diet was first introduced into the animals' cages, it was noted that many of the knife cut rats approached the food bowl and began eating almost immediately. This initial eating bout was generally sustained for several minutes. While introduction of the new diet typically provoked sham knife cut rats to sniff the food and explore the bowl, actual ingestion of the diet was rare, and when it did occur, was of a short duration.

Analysis of average caloric intakes over the high-fat diet phase revealed significant main effects of knife cuts \( (F(1,80) = 334.4, p < .001) \) and of vagotomy \( (F(7,80) = 4.5, p < .001) \). All knife cut groups ate more of the diet than did the sham knife cut groups \( (p's < .01) \). Among the knife cut groups, the KC-Hepatic Vx \( (p < .01) \), KC-Full Vx \( (p < .01) \), and KC-Coeliac Vx \( (p < .05) \) groups ate less than did KC-Sham Vx controls.

Among the sham vagotomized groups, only one comparison achieved significance, with the KC-Full Vx rats eating marginally less than Sham KC-Sham Vx controls \( (p < .05) \).

By the end of the period of access to the high-fat food, the changes in food intake and body weight had expressed themselves in generally increased obesity indices (refer back to Table 10). At day 60, significant overall effects of knife cuts \( (F(1,80) = 118.7, p < \)
.001), vagotomies \((F(7,80) = 8.5, p < .001)\), and a significant knife cut \(\times\) vagotomy interaction \((F(7,80) = 5.5, p < .001)\) were found with respect to obesity indices. While the obesity indices of all groups tended to be elevated, compared to day 30 values, most of the relative differences seen at day 30 had been maintained. Apart from the comparisons indicated in Table 10, note that the obesity indices of all knife cut groups with vagotomies which included the coeliac branch were significantly lower than that of the KC-Sham Vx group (\(p's < .01\)). Also note that the indices of the KC-Full Vx and KC-Coeliac plus Gastric Vx groups, which were at or below control levels at day 30, had by day 60 come to significantly exceed the index of the Sham KC-Sham Vx group.

At day 60, obesity indices of the sham knife cut groups generally tended to slightly exceed the 300-310 range characteristic of normal rats.

Return to lab chow diet. When the animals were reinstated on the lab chow diet, all groups tended to lose weight slowly over about the first half of the final 30 day interval, following which rates of body weight change tended to level off. Analysis of variance was performed on the total postoperative body weight change (i.e., over days 0-90). Significant overall effects of knife cuts \((F(1,80) = 262.0, p < .001)\), vagotomies \((F(7,80) = 6.7, p < .001)\), and a significant knife cut \(\times\) vagotomy interaction \((F(7,80) = 4.0, p < .001)\) were found. The principal results revealed by subsequent multiple comparisons were that all knife cut groups had gained more weight than all sham knife cut
groups (all p's < .01). All knife cut groups with vagotomies which included the coeliac branch gained less weight overall than did knife cut animals with vagotomies which excluded the coeliac branch (all p's < .01, except for the KC-Gastric Vx vs KC-Coeliac plus Hepatic Vx comparison, p < .05).

Obesity indices at the termination of the experiment (day 90) were generally quite comparable to those seen at day 60 (see Table 10). Despite the perturbations in food intake and body weight seen following a period of access to a high-fat diet, and the subsequent return to a lab chow diet, most of the relative differences in obesity indices which were apparent at day 30 had been maintained.

Water/food ratios. In contrast to the drinking produced by electrolytic lesions (Chapter I) or bilaterally symmetrical knife cuts (Chapter II), the asymmetrical knife cuts employed here produced quite variable effects on water/food ratios. As will be discussed below (see Histology) some of the knife cuts produced excessive drinking and urination characteristic of partial damage to antidiuretic hormone pathways to the pituitary. Twelve of 50 knife cut rats showed some evidence of this and displayed water/food ratios which ranged from 2.5 - 10.0 (mean = 3.79). The rats which displayed this effect were not evenly distributed across groups, the end result being that mean water/food ratios among the knife cut groups were quite variable. For this reason the drinking data were not analyzed.

This excessive drinking displayed by some knife cut rats apparently did not affect food intake and body weight changes, as the intakes and
body weights of these animals did not differ in a consistent fashion from those of the other members of their respective groups which did not show evidence of polydipsia.

**Anatomical verification of vagotomies.** While the number of animals whose vagotomies were judged at autopsy to be incomplete was indicated in Table 9, several observations made in the course of this phase of the experiment are worthy of comment. The most frequent reason for excluding animals from the study was the apparent regeneration of components of the main vagal (gastric) trunks. Evidence for this type of apparent regeneration was found in ten of the 16 animals whose vagal status of autopsy did not conform to the originally intended surgery (see also Kraly, 1978).

Six rats were judged to have sustained incomplete coeliac and/or hepatic vagotomies. The apparently regenerated branches in these animals were found to come off the main vagal trunks at approximately the same level that they do normally. Nevertheless, the esophagi of all animals were scanned up to the level of the diaphragm in case some regrowth might have occurred via an aberrant route. No evidence of any such sprouting was found.

The behavior of animals whose vagotomies were judged to be incomplete was not always consistent with what might have been predicted on the basis of the behavior of rats with verified vagotomies. For example, the total weight gain over days 0-90 of one KC-Coelaic plus Hepatic Vx rat which was excluded on the basis of a presumably regenerated coeliac branch, was somewhat less than the mean weight
gain of the KC-Coeliac plus Hepatic Vx group. Thus, it is not clear than any regeneration which may have occurred was necessarily functional.

**Histology.** Four animals were excluded from the study after having been judged by both observers to have sustained inappropriate knife cuts.

Drinking data were not reported here since some of the asymmetrical knife cuts produced evidence of excessive drinking and urination. In these animals, the coronal cuts were found to lie in or near the region of the median eminence, making it likely that the excessive drinking was a consequence of water loss due to disruption of antidiuretic hormone pathways to the pituitary. Some other cuts which did not produce polydipsia were also found to lie in this region, but these tended to produce less damage to the base of the brain near the midline that did cuts which did produce polydipsia.

Figure 13 shows one pair of knife cuts chosen randomly from each of the asymmetrical knife cut groups. The cuts are superimposed on a section of a plate from a horizontal rat brain atlas (Simson, Jones, & Gold, Note 7) which extends through the plane of the PVN. The parasagittal cuts were, in all respects, quite comparable to those described in Chapter II. The coronal cuts were typically 2.5-3.0 mm in height and 1.7-2.2 mm in maximum medial-lateral extent. The location of these cuts did vary somewhat (over about a 1 mm range) in the anterior-posterior plane, ranging from about the midextent of the ventromedial hypothalamic nucleus to the rostral tip of the premamillary nuclei.

**Discussion**

The results are again consistent with the contention that vagal
Figure 13. Reconstructions of asymmetrical knife cuts chosen randomly from each of eight experimental groups. Cuts are superimposed upon a section of a plate from a horizontal rat brain atlas (Simson et al., Note 7) which extends through the plane of the paraventricular nucleus. B–I represent, respectively, cuts from groups which also received sham vagotomy (B), gastric vagotomy (C), hepatic vagotomy (D), coeliac vagotomy (E), gastric plus hepatic vagotomy (F), coeliac plus hepatic vagotomy (G), coeliac plus gastric vagotomy (H), and full vagotomy (I). A: key; pom, medial preoptic nucleus; ha, anterior hypothalamic nucleus; fm, paraventricular nucleus; FMP, medial forebrain bundle; ZI, zona incerta; CAI, internal capsule; LM, medial lemniscus.
mechanisms are involved in mediating hypothalamic hyperphagia and obesity, although the extent of their specific involvement may not be as great as the results of the preceding experiments might have indicated. In addition the results suggest a special role for some function under coeliac vagal control as underlying the changes in eating, body weight, and adiposity characteristic of the VMH syndrome.

Over the first 30 days postoperatively, a clear pattern of changes in eating and body weight were produced by the various knife cut and selective vagotomy combinations. Vagotomies which included the coeliac branch significantly reduced the increases in food intake, body weight, and adiposity produced by knife cuts, as judged by comparisons with knife cut-sham vagotomized controls. This ranged from a 43% reduction in average weight gain, seen in the knife cut-coeliac vagotomy group, to a total blockade of the knife cut effects, seen in the knife cut groups with gastric plus coeliac or full vagotomies. The results obtained from the knife cut-coeliac vagotomy group are especially noteworthy, considering the fact that coeliac vagotomy in sham knife cut animals produced no significant decrements in eating or body weight. Any additional suppression of knife cut-induced overeating and obesity seen in animals with more extensive vagotomies which included the coeliac branch tended to occur as a function of the magnitude of the acute effects of these vagotomies on eating and body weight in sham knife cut rats (see Figure 11a and b). Therefore, while it may be argued that the greater effectiveness of the more extensive vagotomies in antagonizing knife cut effects occurred by virtue of the fact that these vagotomies reduced food intake and body weight in otherwise
normal animals, such a consideration would not seem pertinent to the interpretation of the effects of coeliac vagotomy alone.

In contrast, neither gastric nor gastric plus hepatic vagotomy (the latter being a procedure in which only the coeliac branch is spared) had any significant impact on knife cut-induced overeating or weight gains. Hepatic vagotomy even provided consistent evidence of potentiating the knife cut syndrome on all measures except obesity indices.

When a high-fat diet was offered, all knife cut rats, irrespective of vagotomy status, showed increased caloric intake and weight gains relative to sham knife cut rats. Even the knife cut-full vagotomy group overate and became significantly more obese than did sham knife cut controls, indicating that the exaggerated preference for high-fat diets (finickiness) which is characteristic of the VMH syndrome (Carlisle & Stellar, 1969; Corbit & Stellar, 1964; Gold, 1970; Kramer & Gold, in press; see also review by Powley, 1977), is spared by vagotomy. A finer-grained analysis of the responses of the knife cut groups in confounded by the fact that these groups entered the high-fat diet phase at very different starting weights. Therefore, the data do not allow quantitative estimations of the extent to which the finicky behavior might have been differentially affected by the various selective vagotomy procedures. The observation that all knife cut groups gained excessive weight on the novel diet would indicate that the differential effects of the selective vagotomies on the knife cut syndrome as seen over days 0-30 were almost certainly not a consequence of systematic between-group differences in knife cut effectiveness. The results seen on the high-fat diet extend the observations of
Powley and Opsahl (1974) who noted that VMH-lesioned rats which had recovered from the effects of full vagotomy could overeat and gain weight rapidly when granted brief periods of access to such food. In this study, however, the diet was not in effect long enough for the lesioned-vagotomized animals' body weights to come to exceed those of controls.

When, in the present study, the animals were reinstated on the lab chow diet, all groups tended to lose weight slowly over about the first half of the final 30 day interval, following which the rates of weight change had reached stable, if uniformly low levels. It is important to note that over the total 90 day postoperative period that the relative differences in body weight and adiposity seen between the knife cut groups at day 30 were quite well-maintained.

**Role of the coeliac vagus in hypothalamic hyperphagia and obesity.**

Because the coeliac vagus was, by itself, capable of supporting an undiminished hyperphagic syndrome (i.e., after gastric plus hepatic vagotomy), and because sectioning the coeliac vagus alone attenuated knife cut effects, this branch would appear to be most directly involved in mediating the effects of complete vagal denervation on hypothalamic hyperphagia and obesity.

In the rat, the coeliac branch of the abdominal vagus projects to the coeliac ganglion from which it provides the parasympathetic innervation of the pancreas and a portion of the small intestines. In other mammalian species, the coeliac branch has been shown to innervate other abdominal organs as well, including the spleen, kidney, and adrenal
gland. Such projections have not yet been described in the rat (Griffith & Legros, 1969; Ishigami et al., 1974; Honjin, 1956; Teitelbaum, 1932; Woods & Porte, 1974). The coeliac branch is the most distal point at which a relatively specific parasympathetic denervation of these organs can be achieved, since from the coeliac ganglion, vagal and splanchnic fibers merge to form mixed (parasympathetic and sympathetic) nerves (see Honjin, 1956).

Given the weight of the evidence which has implicated excessive insulin secretion as a principal mechanism underlying hypothalamic hyperphagia and obesity (see Introduction), one possible means by which coeliac vagotomy could antagonize the overeating syndrome is by preventing the hypersecretion of insulin which has been shown to occur as a primary consequence of VMH lesions or knife cuts (e.g., Hustvedt & Løvø, 1972; Tannenbaum, et al., 1974). Several lines of evidence are consistent with this view.

Electrical or chemical stimulation of vagal efferent mechanisms is well-known to elicit insulin secretion (see Gerich & Lorenzi, 1978; Smith & Porte, 1976; Woods & Porte, 1974 for reviews). In addition, both vagal stimulation (Penaloza-Rojas, 1969) and exogenous insulin injections (MacKay et al., 1940; Panksepp et al., 1975) have been shown to promote excessive food intake. Finally, in addition to reversing VMH lesion-induced hyperphagia and obesity, full vagotomy also abolishes hyperinsulinemia (Inoue & Bray, 1977; Louis-Sylvestre, 1976; Powley & Opsahl, 1976.

It is important to recognize that even full vagotomy has no gross
effect on fasting insulin levels, and only slightly reduces oral glucose tolerance (Louis-Sylvestre, 1976; Woods & Porte, 1974). Apparently only the capacity to release insulin in response to neurogenic stimuli is severely impaired. Therefore, it is unlikely that the effects of any form of vagotomy may be due to malaise or metabolic disturbances attributable to some semi-diabetic state.

Hyperinsulinemia does not appear to play an exclusive role in mediating the syndrome. Studies have shown that VMH lesions induce hyperphagia of varying degrees in corrected and uncorrected diabetic rats (Friedman, 1972; Vilberg & Beatty, 1975; Young & Liu, 1965; York & Bray, 1972). Additional insight into the role of pancreatic neural mechanisms in the VMH syndrome has recently been provided by Bray, Inoue, and Mullen (Note 1), who transplanted fetal pancreases into diabetic rats prior to making VMH lesions. This procedure results in a remission of diabetic symptoms and produces rats with normal insulin levels and secretory capacities (see also Louis-Sylvestre, 1978), but whose pancreases are presumably not innervated. When VMH lesions were given, hyperphagia and weight gains of diabetic rats with transplants were reduced, relative to that seen in VMH lesioned controls, but were not eliminated. These results are compatible with the findings and suggestion offered here, that is, that a neurogenic hyperinsulinemia is an important, but apparently not the only, mechanism underlying the VMH syndrome.

While the results are consistent with the large body of evidence implicating hyperinsulinemia as a contributory mechanism in hypothalamic hyperphagia, other mechanisms are possible because other abdominal
organs may receive projections from the coeliac vagus. One feasible mechanism might involve changes in secretory or motor functions of the small intestines, which also receive coeliac vagal input. It is conceivable that a disruption of intestinal motility by coeliac vagotomy could limit overeating and weight gains in some nonspecific fashion. This possibility is not considered likely, since intestinal motility appears to be primarily under myogenic control (Bortoff, 1976) and removal of presumably all neural influences has no apparent effect on motor function (Ahlman, 1976).

Gastrointestinal hormones, some of which are subject to vagal control, may provide more likely candidates, particularly in view of the fact that these can mobilize a variety of digestive processes, including insulin secretion (Rayford, Miller, & Thompson, 1976). However, in the absence of any indication that gut hormones could drive an effect of the magnitude of hypothalamic hyperphagia, the possibility that such hormones could play a mediating role must be considered tentative.

Other abdominal organs such as the spleen, kidneys and adrenal glands may receive input from the coeliac vagus. There is little reason to suspect, however, that neural influences on the spleen or kidneys might contribute to the syndrome in any specific way. As for the adrenals, whose innervation has long been a controversial topic (Teitelbaum, 1932), hyperphagia and obesity have been demonstrated in adrenalectomized rats with VMH lesions, and replacement doses of corticosterone do not promote any additional weight gain (York & Bray, 1972). In contrast, others have found that prior adrenalectomy, but
not adrenal demedullation, significantly attenuates lesion-induced overeating and weight gains (Mook, Fisher, & Durr, 1975). While this discrepancy is yet to be adequately explained, to hold that the coeliac vagus mediates hypothalamic hyperphagia via the adrenals would require that coeliac vagotomy produce a functional adrenalectomy, which is clearly not the case.

To summarize, the coeliac vagus appears to play a specific, but non-exclusive, mediating role in hypothalamic hyperphagia. Because the coeliac branch provides the parasympathetic innervation of the pancreas, this result is consistent with a variety of evidence which has implicated excessive insulin secretion as an important mechanism of hypothalamic hyperphagia. However, because the coeliac branch innervates other abdominal sites, possible mediating influences from other sources cannot be discounted.

**Hepatic vagotomy-knife cut interaction.** In some respects, as interesting a finding as the selective blockade by coeliac vagotomy of the knife cut syndrome, was the totally unexpected observation that hepatic vagotomy appeared to potentiate knife cut effects on eating and body weight. Several admittedly speculative explanations for such a result are possible, each of which, because of the direction of the effect, seems unrelated to the mechanisms underlying hypothalamic hyperphagia.

Although modest overeating and weight gains have been observed following hepatic vagotomy in otherwise intact male rats, such has not been found to be the case for female rats in the present study or in others (Sawchenko, Friedman, & Gold, Note 5; Mordes et al., 1977).
While it is conceivable that a disruption of gonadal function, such as is sometimes seen after VMH lesions (Valenstein, Cox, Kakolewski, 1969), may have shifted the knife cut-hepatic vagotomized female rats of the present study to a male pattern, this possibility is not considered likely, since normal estrous cyclicity has been observed in knife cut rats with or without full vagotomies (Eng, Gold, & Sawchenko, 1978).

Evidence that vagal afferent nerves from the liver play a role in hunger and satiety is based largely on experiments showing that the delivery of various metabolic fuels to the liver can preferentially inhibit food intake under a variety of conditions (see Friedman & Stricker, 1976; Novin and VanderWeele, 1977; Sawchenko & Friedman, 1979 for reviews). Acceptance of such a role for hepatic vagal mechanisms has been slow in coming, in part, because various forms of liver denervation have no gross effects on food intake (e.g., Bellinger, Trietly, & Bernardis, 1976). While hepatic vagotomy also does not affect overall food intake in female rats, it does alter their pattern of intake, such that the animals eat more during the day and less at night than they did prior to nerve section (Sawchenko et al., Note 5). In the normal rat hepatic vagotomy may not have gross effects on food intake because any lack of satiety input from the liver may be compensated by other receptor systems controlling food intake (Friedman & Stricker, 1976). In the hypothalamic hyperphagic rat, however, the elevated food intake may somehow provide conditions against which the absence of information from the liver pertaining to satiety may have a greater impact. Because the overeating of the VMH rat may largely be
attributed to increased daytime eating (LeMagnen et al., 1973), and because hepatic vagotomy disrupts eating rhythms, perhaps the potentiation of hypothalamic hyperphagia by hepatic vagotomy is a result of additional removal of any restraining influences on daytime food intake.

In contrast to their enhanced overeating and weight gains on a lab chow diet, knife cut rats with hepatic vagotomies tended to eat less and gain less weight on a high-fat diet than knife cut-sham vagotomized animals. It is difficult to attribute any special significance to this finding in view of the possibility that a ceiling effect may have been encountered.

**Specificity of more extensive vagotomies.** At this point, the contributions of the three principal divisions of the abdominal vagus nerves to the mediation of hypothalamic hyperphagia and obesity have been estimated. Because none of the vagotomies which involved only a single branch significantly affected eating or body weight in sham knife cut animals, the interpretation of these effects if relatively uncomplicated. While the coeliac branch would appear to play a mediating role in the syndrome, it seems clear that neither the hepatic nor the gastric branches are, by themselves, indispensably involved (see also Chapter II; Brooks et al., 1946; Carpenter et al., 1979). In light of these findings, the interpretation of the effects of more extensive vagotomies in general, and full vagotomies in particular, is not at all clear-cut.

The fact that the combined reductions in knife cut effects seen after coeliac vagotomy and that seen after gastric plus hepatic vagotomy
did not approach the complete blockade produced by full vagotomy might suggest that vagal mechanisms cannot provide a complete accounting for the syndrome. This would imply that the effectiveness of full vagotomy in blocking the knife cut syndrome is attributable, in part, to non-specific factors. In other words, the knife cut rat with full vagotomies may not overeat, in part, because it cannot or will not overeat.

More extensive vagotomies may stifle the development of hyperphagia and obesity by limiting the capacity of the animal to efficiently process large quantities of food. While the coeliac vagus may be the only branch which plays an important role in the expression of the syndrome, it is probably safe to assume that all branches show enhanced neural activity following VMH lesions or knife cuts. Indeed, the increased gastric acid secretion documented here and elsewhere (Inoue & Bray, 1977; Powley & Opsahl, 1974; Ridley & Brooks, 1965) provide evidence of this. While hyperactivity of extra-coeliac vagal mechanisms may not be important to the generation of the behavioral changes which follow medial hypothalamic damage, it would be expected to facilitate the digestion of excessive post-ablation food intakes by enhancing such processes as biliary secretion, gastric acid and enzyme secretion, pancreatic exocrine secretion, etc. Thus, more extensive vagotomies may limit the development of hyperphagia and obesity, in part, by imposing such non-specific limitations in food-handling capacity. Such an explanation is consistent with the finding that the degree to which more extensive vagotomies were effective in blocking the knife cut syndrome tended to occur as a function of the acute post-operative effects of these
vagotomies on eating and body weight in sham knife cut rats (the results obtained with gastric plus hepatic vagotomies provide a notable exception to this trend).

In a similar vein, an anthropomorphic argument can be invoked to suggest that discomfort or malaise may contribute to the complete blockade of the syndrome produced by full vagotomy. A major impetus to the development of highly selective gastric vagotomy procedures for the treatment of human ulcers was the fact that full vagotomy produced a great many adverse side effects, including nausea, vomiting, diarrhea, and weight loss (see Kennedy, 1974; Passaro & Stabile, 1978 for reviews). However, several lines of evidence are at odds with the view that non-specific factors can account for the efficacy of complete vagotomy in blocking hypothalamic hyperphagia. For one, it has been shown that even after recovery from the effects of full vagotomy, lesions or knife cuts remain ineffective in inducing overeating or obesity (see Chapter II; Gold et al., in press, Rowland & Engle, 1979). More importantly, rats with full vagotomies can overeat and fatten on a lab chow diet in response to such treatments as ovariectomy (Eng et al., 1979), and VMH-lesioned rats with vagotomies can overeat in response to chronic insulin injections (Rowland & Engle, 1979) or when offered a high-fat diet. These results would tend to indicate that the abolition of the knife cut syndrome by vagotomy is a specific, although here, too, it may be difficult to draw comparisons across treatment conditions.

A simple reconciliation of these opposing viewpoints does not present itself. The failure of the present study to reveal a precise
additivity of vagal contributions to hypothalamic hyperphagia may have resulted from several factors including limitations in the resolving power of the present paradigm, or perhaps, as suggested by the anomalous results seen in the knife cut-hepatic vagotomy group, that various selective vagotomy combinations may interact with knife cuts in a manner which is functionally unrelated to the mechanisms which give rise to overeating and obesity. Finally, given the results seen during the high-fat diet phase, it seems likely that extra-vagal mechanisms may indeed play a role in mediating at least some aspects of the syndrome.

Responses to a high-fat diet. The finding that all knife cut groups overate and became obese (or more obese) when offered a high-fat diet would indicate that vagal mechanisms cannot provide a complete accounting for all facets of the syndrome. The so-called "finickiness" of the hypothalamic hyperphagic rat appears to be spared by any form of vagotomy. While there are suggestions in the literature that the mechanisms which cause finickiness may be anatomically dissociable from those which produce hyperphagia (Bevan, cited in Hoebel, 1977; Graff & Stellar, 1962), these are inconclusive and it will be assumed that finickiness is an inherent aspect of the VMH syndrome (see also Kramer & Gold, in press).

The mechanisms underlying the overresponding by VMH rats to the sensory qualities of diets have been debated for some time and are, as yet, poorly understood (see review by Powley, 1977). It must be emphasized that the present experiment was not designed to investigate these mechanisms. The purpose here was only to test the generality of
the vagotomy effects observed over days 0-30 by determining whether or not knife cut-vagotomized animals were capable of overeating in a situation which normally commands such behavior in VMH animals. Nevertheless, several observations made in the course of the present study are worthy of comment.

One of the factors which has been implicated in the overconsumption of certain foods by VMH rats is the taste or texture of the diet. Addition of non-nutritive greasy material (e.g., vaseline or mineral oil) promotes excessive caloric intake in VMH rats (Carlisle & Stellar, 1969; Corbit & Stellar, 1964). Taste may be involved as well, since sweetened diets are also consumed more avidly by VMH rats than by controls (e.g., Teitelbaum, 1955). Consistent with the contention that orosensory factors may be involved are the informal observations made in the course of the present study that knife cut rats with or without vagotomies began to consume the high-fat diet almost immediately upon first exposure, as well as the finding that intakes of the high-fat diet were greatest during the ten day period immediately following its introduction. If taste and textural cues do underlie the exaggerated preference for high-fat diets, it would not be surprising that this aspect of the syndrome would be spared by vagotomy, whose known efferent effects operate strictly post-ingestionally.

The second principal mechanism by which the excessive consumption of fat-rich foods may be mediated may involve differential metabolic fates of high-fat, as opposed to high-carbohydrate diets (the standard lab chow diet may be considered to be a low-fat high-carbohydrate diet). As noted in the introduction, VMH lesions produce a marked shift in
metabolism towards the deposition and sequestration of nutrients in adipose tissue (lipogenesis) (see Friedman & Stricker, 1976). While excessive insulin secretion may be a prime mover in this process, alterations in fat metabolism are still seen if hyperinsulinemia is prevented by diabetes (Goldman et al., 1972a, 1972b). If the shift in fat metabolism towards lipogenesis is also spared by vagotomy, as presumably it would be, then the present results are not incompatible with a metabolic account of "finickiness".

Finally, there is some reason to suspect that vagotomy may interfere more with carbohydrate than with fat metabolism. The reduction in overall intake seen among vagotomized rats in a cafeteria feeding situation can be attributed to a reduction in calories consumed as carbohydrates (Fox, Kipp, & VanderWeele, 1976).

Thus, the vagus nerves are clearly not the exclusive mediators of the VMH syndrome. Lesion or knife cut-induced alterations in orosensory reactivity and/or fat metabolism which may lead to the overconsumption of fat-rich diets are not substantially affected by any form of vagotomy.

**Summary of Part I.** It has been shown that hypothalamic hyperphagia and obesity can be prevented or reversed by complete subdiaphragmatic vagotomy. A finer analysis of the roles of various vagal components has revealed that neither the gastric nor the hepatic vagal branches appear to play major roles in generating the obesity syndrome, although both branches appear to interact with knife cut effects. Hepatic vagotomy potentiates the syndrome perhaps by interfering with afferent output from the liver pertaining to satiety. Gastric vagotomy has no major impact on knife
cut induced overeating and weight gains, making it unlikely that gastro-
intestinal dysfunctions alone can account for the normalizing effects
of complete vagotomies. Gastric vagotomy does, however, reduce water
intake, and may play a permissive role in allowing excessive drinking
to occur. Only the coeliac vagus appears to play a major mediating
role, as this branch alone can support the full magnitude of knife cut
induced overeating and obesity, and coeliac vagotomy attenuates these
knife cut effects. The results obtained with coeliac vagotomy are
consistent with a large body of evidence which has implicated excessive
insulin secretion as a primary mediator of the VMH syndrome.

It is unclear whether the complete blockade of the syndrome by full
vagotomy can be attributed to the abolition of specific mediating vagal
influences. Non-specific factors such as malaise or impaired digestive
capacities may contribute to the more pronounced effects of more ex-
tensive vagotomies. Vagal mechanisms cannot account for all facets of
the syndrome as rats with any manner of vagotomy overeat and become
rapidly obese when offered a high fat diet.
PART II

VAGAL MEDIATION OF NOREPINEPHRINE-STIMULATED EATING

CHAPTER IV

EFFECT OF VAGOTOMY ON THE EATING ELICITED BY HYPOTHALAMIC INFUSIONS OF NOREPINEPHRINE

Microinjection of norepinephrine (NE) and other α-adrenergic agonists into various medial hypothalamic loci produces a robust eating response in sated animals (e.g., Booth, 1967; Grossman, 1962a; Herberg & Franklin, 1972; Leibowitz, 1970, 1975a, 1975b, 1978; Slangen & Miller, 1969). While the mechanisms underlying this effect remain unknown, evidence gathered within this NE feeding paradigm has been cited as supporting a role for hypothalamic adrenoceptors in the short-term control of ingestive behaviors (Grossman, 1975; Leibowitz, 1970, 1976; Slangen & Miller, 1969). For example, NE-elicited eating is typically preceded by a drinking response (Leibowitz, 1975a, 1975b), a sequence which parallels the normal ingestion pattern in this species. In addition, hypothalamic NE infusions have been shown to influence feeding at doses within the physiological range. Although the doses used to elicit eating from an animal at rest typically exceed the NE content of the entire hypothalamus, doses within the physiological range have been proven capable of increasing the size of a spontaneously initiated meal in rats (Ritter & Epstein, 1975). Perhaps the most compelling evidence for a role for hypothalamic NE in the normal control of food intake is
the finding that NE is released in the hypothalamus during spontaneous eating (Martin & Myers, 1975; Van Der Gugten, De Kloet, Versteeg, & Slangen, 1977). Such findings, as well as her own tests with a great variety of drugs which either directly or indirectly affect hypothalamic NE, have led Leibowitz (1970, 1975a, 1975b, 1976, 1978) to propose an updated version of the dual center hypothesis which holds that medial hypothalamic α-adrenergic receptors mediate normal satiety, while more laterally based β-receptors subserve spontaneous hunger.

While the data discussed above are consistent with the notion that exogenous NE stimulates eating by activating a central adrenergic "feeding system", such involvement has by no means been proven. It remains possible that the stimulation of eating by NE may be secondary to a physiological or metabolic change produced by the infusion. Part I of this dissertation and other studies have shown that other forms of overeating elicited by hypothalamic manipulations are attenuated or abolished by sectioning the abdominal vagus nerves. As discussed earlier, such findings might suggest that these overeating syndromes are mediated via autonomic influences on the viscera. The observations that hypothalamic NE infusions produce such evidence of vagal activation as increased gastric acid secretion (Carmona & Slangen, 1973), brachycardia (Borkowski & Finch, 1978; Carmona & Slangen, 1976), vasodilation (Carmona & Slangen, 1976) and increased insulin secretion (De Jong & Steffens, Note 2), give cause to suspect that this form of hypothalamically elicited overeating may also be mediated by the vagus nerves.

Part II addresses the possibility of vagal mediation of the eating
elicited by adrenergic stimulation of the paraventricular nucleus (PVN) of the hypothalamus. As noted previously, the PVN has recently been identified as the diecephalic site which supports the greatest eating responses to small infusions of α-adrenergic agonists. In the first experiment, the effectiveness of NE in eliciting eating and drinking responses before and after recovery from complete subdiaphragmatic (full) vagotony is tested.

**Methods**

**Animals.** Adult male Charles River CD rats weighing 280-340 g at the time of surgery were used here, and in all experiments in Part II. Animals were individually housed in hanging stainless steel cages in a colony room maintained at 21 ± 1°C and on a 12/12 hr light/dark cycle. Purina lab chow pellets and water were available without restriction.

**Surgery.** Under pentobarbitol anesthesia, all animals were stereotaxically implanted with unilateral guide cannulae, fashioned from the hubs of 27 ga stainless steel needles, which were aimed to terminate just above the PVN. Coordinates for this placement, with the skull flat between Bregma and Lambda, were 0.3 - 0.5 mm lateral to the midline, 7.0 - 7.5 mm rostral to the interaural line, and 6.0 - 6.5 mm below the dura. Cannulae were affixed to the skull with cranioplastic cement, which adhered to three machine screws partially driven into the frontal and parietal bones. To maintain patency, each cannula was equipped with a stainless steel obdurator cut to terminate flush with the cannula tip.

Before testing commenced, rats were allowed a minimum of seven
days to recover from surgery, during which time they were frequently handled and mock-injected (obdurator removed, cannula manipulated, and rat held for a time approximating that required to make an injection).

**Test procedure.** The test procedure was patterned after that of Leibowitz (1975a). On test days, fresh food and water were given 1 hr prior to any manipulation to ensure maximal staiation at the time of testing. At the end of this interval, the obdurator was removed and a 30 ga stainless steel injector was inserted through the guide cannula. Injectors were prepared so as to extend 1 mm beyond the tip of the guide. The injector was connected via polyethylene tubing (PE 10, Clay Adams) to a Hamilton mecroliter syringe which was prefilled with the fluid to be injected. Injections were carried out over a 15 sec period, and the injector was permitted to remain in place for an additional 15 sec before removal. Tests, except where otherwise noted, were of 1 hr duration and occurred during the lights-on phase of the day/night cycle. Food intakes, corrected for spillage, were weighed to the nearest 0.1 g at the end of the hour. Water intakes, measured to the nearest 0.5 ml with calibrated drinking tubes, were taken at 5 min and 60 min post infusion. At least one day was allowed between any two consecutive tests.

The vehicle for intracerebrally injected NE was 0.5 ul sterile isotonic saline. Solutions were made fresh within 12 hr prior to use and were twice passed through a micropore filter.

**Procedure.** Twenty-three cannulated rats were given, in counterbalanced sequence, two tests each with 20 nanomoles (nM) norepinephrine bitar-
trate, saline and mock injections. Those rats which reliably increased food intake on both NE tests were then divided into two groups and received either full subdiaphragmatic vagotomy or sham vagotomy.

After surgery, all animals were given dietary supplements of 10% sucrose solutions until weight loss ceased. When body weights stabilized, testing resumed. Three additional NE, saline, and mock injection trials were given to each animal. On one postoperative NE and saline test, intakes were monitored over longer periods to determine whether any interference by vagotomy might merely reflect a delayed response. On these occasions, food and water intakes were recorded at 1, 2, 3, and 24 hr post infusion.

An additional test was carried out to assess the capacity of the vagotomized rat to rapidly increase food intake. After the completion of all testing involving central infusions, food intakes were measured on consecutive days for 1 hr following no treatment (day 1) and following 24 hr food deprivation (day 2).

**Verification of vagotomies.** At the end of the experiment, vagotomies were tested for completeness using a variant of the procedure described in Chapter I (Sawchenko et al., 1977). This differs from the earlier procedure only in that insulin hypoglycemia, rather than vagal stimulation, is used to provoke gastric acid secretion.

Under nembutal anesthesia, rats were adrenalectomized and received stomach catheters as described earlier. After stable baseline secretion rates were observed, 1.0 U/kg regular insulin was injected intravenously (femoral vein) and the collection procedure repeated at 15 min intervals
for at least 90 min. Criteria for completeness of vagotomy were as detailed in Chapter I.

**Histology.** Animals were perfused with injectors in place. Their brains were then extracted and processed as in Part I. Cannula placements were mapped by projecting stained brain sections onto plates from the Konig and Klippel (1963) rat brain atlas.

**Data analysis.** Comparisons within groups were made using a correlated samples \( t \) test. Comparisons between groups involved the independent samples (Student's) \( t \).

**Results**

**Anatomical and general observations.** The findings reported in this section pertain to all three experiments in Part II.

Figure 14 shows the anatomical placements of the cannula tips for all rats employed in the three studies of Part II; the effectiveness of each in supporting an eating response to 20 nM NE prior to any other manipulation is indicated. Some ineffective placements are shown for contrast. No systematic differences in placements were noted between experiments.

The effective cannula placements, arbitrarily defined as those which supported an eating response to 20 nM NE which was at least 1 g greater than that seen in response to saline infusions, were found to lie in a band extending from the periventricular hypothalamus at the level of the optic chiasm, through the entire longitudinal extent of
Figure 14. Anatomical placements of tips of cannulae which were effective in supporting a reliable eating response to infusions of 20 nM NE for all animals included in the three experiments reported in Part II. Some ineffective placements are shown for contrast. Plate numbers are those of the König and Klippel (1963) rat brain atlas. Different symbols represent approximate magnitude of eating response (in 1 hr) to 20 nM NE. Key: • = 0 - 0.5 g; ▼ = 0.6 - 1.5 g; ▲ = 1.6 - 2.5 g; ◆ = 2.6 - 3.5 g; ▼▼ = 3.6 - 4.5 g.
the PVN and up to, and including, the medial aspect of the rostral pole of the dorsomedial hypothalamic nucleus. Along this longitudinal band, effective placements were generally found to lie medial to the fornices and, in the dorsal-ventral plane, ranged from the dorsal margin of the hypothalamus to just ventral to the level of the fornices. With one exception, the most effective cannula placements were found to lie within, or immediately adjacent to, the more lateral magnocellular portion of the PVN.

The average eating response to 20 nM NE of the 52 animals with effective cannula placements was 2.3 g in 1 hr. Eating was usually, but not always, preceded by a drinking response (average = 1.5 ml in 1 hr). Five animals which did eat in response to NE never showed a measurable drinking response. Most, but not all, of the elicited drinking took place during the first 5 min post infusion. Over all pre-manipulation NE tests, the eating and drinking responses were significantly correlated ($r = .45$, $p < .01$).

**Effects of vagotomy on ingestive behaviors elicited by NE.** Discounting attrition due to a lack of responsiveness to NE preoperatively (4 rats), failure to recover from the acute effects of vagotomy (1 rat), and failure to satisfactorily complete the verification procedure (1 rat), a total of 8 vagotomized and 9 sham-operated animals completed the experiment, and, in the case of the vagotomized rats, met the criterion for completeness of vagotomy. While vagotomized rats failed to increase acid secretion in response to insulin injections ($t(7) = 0.4$, $p > .10$), sham operated rats showed a robust response ($t(8) = 6.5$, $p < .001$). These results are depicted in Figure 15.
Figure 15. Mean (+ SEM) fasting gastric acid secretion before and after intravenous injection of 1.0 U/kg regular insulin in vagotomized and sham vagotomized rats. Baseline values are those taken during the 15 min collection period immediately preceding insulin injections. Insulin-stimulated values are maxima seen following insulin injections.
GASTRIC ACID SECRETION (uEq H⁺/15min)

SHAM VAGOTOMY
BASELINE

SHAM VAGOTOMY
INSULIN-STIMULATED
Under no condition did saline infusions produce a behavioral response which was significantly different than that seen following mock injections. All statistical comparisons involving behavioral responses to NE, therefore, are vs saline injection controls.

Figure 16 shows 1 hr food and water intakes elicited by NE before and after vagotomy or sham vagotomy. Prior to abdominal surgery, the mean food intake (± SEM) in response to 20 nM NE was 2.8 ± 0.3 g, as opposed to 0.3 ± 0.1 g in response to saline injections (\( t(16) = 14.2, p < .001 \)). Overall preoperative water intakes were 1.9 ± 0.3 ml under the NE condition and 0.3 ± 0.2 ml in response to saline (\( t(16) = 7.5, p < .001 \)).

Following surgery, vagotomized rats recovered normal rates of body weight change after 16 - 25 days, while sham-operated rats were considerably less affected, taking only 1 - 3 days to recover. Thirty days after surgery, when 1 hr testing resumed, the average body weight of the vagotomized group was 14% lower than that of the sham operated rats.

Postoperative testing revealed that NE was far less effective in stimulating food intake in vagotomized rats relative to either preoperative measures in the same animals (\( t(7) = 8.3, p < .001 \)) or postoperative measures in sham operated animals (\( t(15) = 6.7, p < .001 \)). Sham vagotomy produced a numerically small, and non-significant, reduction in eating elicited by NE (\( t(8) = 1.4, p > .10 \)).

Although NE injections in vagotomized rats were no more effective than saline in stimulating food intake (\( t(7) = 1.3, p > .10 \)), it is noteworthy that vagotomized rats' intakes in response to saline rose
Figure 16. Mean (± SEM) 1 hr eating and drinking responses before (pre-op) and after (post-op) recovery from complete sub-diaphragmatic vagotomy or sham vagotomy.
relative to peroperative measures, albeit nonsignificantly, despite smaller overall daily food intake on the part of vagotomized rats.

Water intakes were found to be reduced in vagotomized rats relative to the animals' own peroperative measures \((t(7) = 3.0, p < .05)\), but not relative to postoperative measures in sham vagotomized rats \((t(15) = 1.9, p > .05)\). Despite this reduction in the elicited drinking response, water intake in response to NE remained significantly greater than that seen in response to saline infusions \((t(7) = 5.2, p < .01)\).

Table 11 shows the results of the extended (24 hr) NE and saline tests in vagotomized and sham vagotomized rats. No clear evidence of delayed feeding or drinking in response to NE was noted among vagotomized rats. Neither feeding nor drinking was significantly elevated by NE at the 24 hr measurement in either vagotomized or sham operated rats.

Note, in addition, that the vagotomized rats showed the slight reduction in 24 hr food intake and the marked reduction in 24 hr water intake characteristic of the daily intakes of vagotomized rats as described in Part I.

In the test of the capacity of the vagotomized rats to rapidly increase food intake in response to a challenge other than that provided by NE, vagotomized rats consumed 3.5 ± 0.6 g of food in 1 hr following 24 hr food deprivation \((t(7) = 5.9, p < .001, \text{ vs non-deprived control values in the same animals})\), while sham operated rats ate 9.1 ± 1.0 g \((t(8) = 9.9, p < .001, \text{ relative to non-deprived conditions})\). The response of the sham operated rats was significantly greater than that of the vagotomized rats \((t(15) = 4.0, p < .01)\).
Table 11
Cumulative Food and Water Intake (Mean ± SEM) Following
Central Infusions in Vagotomized and Sham-Operated Rats

<table>
<thead>
<tr>
<th>Operation</th>
<th>Infusion</th>
<th>Food Intake at:</th>
<th>5 min.</th>
<th>60 min.</th>
<th>120 min.</th>
<th>180 min.</th>
<th>24 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vagotomy (8)</td>
<td>NE</td>
<td>--</td>
<td>0.6 ± 0.2</td>
<td>0.8 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>18.0 ± 1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>--</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.8 ± 0.2</td>
<td>17.8 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Sham (9)</td>
<td>NE</td>
<td>--</td>
<td>2.6 ± 0.4**</td>
<td>2.7 ± 0.4**</td>
<td>2.9 ± 0.4**</td>
<td>23.1 ± 0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>--</td>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>21.8 ± 1.1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Water Intake at:</th>
<th>5 min.</th>
<th>60 min.</th>
<th>120 min.</th>
<th>180 min.</th>
<th>24 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vagotomy (8)</td>
<td>NE</td>
<td>0.9 ± 0.3**</td>
<td>1.2 ± 0.4*</td>
<td>1.3 ± 0.3</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>0.1 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>Sham (9)</td>
<td>NE</td>
<td>1.6 ± 0.4**</td>
<td>1.9 ± 0.4**</td>
<td>2.1 ± 0.4**</td>
<td>2.3 ± 0.5**</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>0.0 ± 0.0</td>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.6 ± 0.2</td>
</tr>
</tbody>
</table>

* Differs significantly from saline control condition, p < .05 (Correlated samples t-test).

** Differs significantly from saline control condition, p < .10.
Discussion

The present results are generally consistent with a role for abdominal vagal mechanisms in mediating the eating induced by intracranial NE infusions. Complete subdiaphragmatic vagotomy virtually eliminated NE-induced eating, bringing the elicited response to a level comparable to that seen following saline control infusions. Eating in response to saline infusions was somewhat (although non-significantly) elevated in vagotomized rats, a finding which may be related to the recent observation (Louis-Sylvestre, 1978) of increased daytime food intakes following abdominal vagotomy in rats. In light of this result it is problematic as to whether the NE effect was eliminated, as opposed to being severely attenuated, by vagotomy.

Since vagotomy produces a rather protracted period of undereating and weight loss (e.g., Gold et al., in press; Mordes et al., 1977; Powley & Opsahl, 1974; Shay et al., 1949), questions must again be raised as to the competence of vagotomized animals to perform in brief (1 hr) tests. Some evidence for specificity was obtained in the present study. Water intake in response to NE was reduced, but not eliminated, indicating at least some residual capacity to ingest rapidly. In addition, vagotomized rats retained the ability to increase food intake in response to a food deprivation challenge. Although their response was less vigorous during the first hour of refeeding than that of sham operated controls, it was far greater than that seen under non-deprived conditions. Finally, no clear evidence of a delayed eating response to NE was observed. Collectively, these
results would indicate that the antagonism by vagotomy of NE-induced eating is, at least in part, a specific one. These data do not, however, rule out the possibility that non-specific impairments may play a role in the attenuation of NE-elicited eating. Indeed, the diminished capacity of the vagotomized rats to respond to a food deprivation challenge might suggest that non-specific impairments are in fact involved.

In retrospect, the choice of 24 hr food deprivation as an independent means by which to assess the capacity of vagotomized rats to rapidly increase food intake was somewhat ill-founded. Since such a procedure places considerably greater demand on the food-handling capacity of the animal than does central infusions of 20 nM NE, it is difficult to draw comparisons between intakes in response to the two challenges. In addition, since the time this experiment was carried out, we (Gold et al., in press) have obtained evidence that the diminished capacity of vagotomized rats to respond with alacrity to a challenge to ingestive behavior (in this case the addition of highly palatable "supermarket" foods to the animal's diet) disappears with repeated testing. Given these considerations, the role of non-specific factors as possible contributors to the diminished eating in response to NE by vagotomized rats remains at issue.

The present results may also have a bearing on the mechanisms by which NE stimulates water intake. Drinking induced by central NE infusions is not merely an adjunct of the elicited eating, since the onset precedes that of the eating, and the magnitude of the drinking response is not diminished when no food is available during testing.
Leibowitz has further shown that the drinking response involves both α- and β- receptors, while the feeding response is mediated by α- receptors alone (Leibowitz, 1975b). The partial sparing of the elicited drinking following complete abdominal vagotomy is consistent with the view that two drinking mechanisms are operative, one of which may be intimately associated with the mechanism that stimulates eating. Vagotomy does, however, drastically reduce ad lib (24 hr) water intake (Kraly et al., 1974; see also Table 11), as well as short-term intake in response to a variety of thirst-provoking stimuli (Kraly, 1978; Kraly et al., 1975). Therefore, it is possible that the reduced drinking by vagotomized rats in response to NE infusions might reflect a general limitation in the capacity of rapidly increase water intake, rather than a specific interference with the mechanisms underlying adrenergic drinking.

The array of cannula placements found in the three experiments which comprise Part II (see Figure 14) is consistent with the extensive mapping of Leibowitz (1978) and confirmed by others (e.g., Booth, 1967; Matthews et al., 1978; Slangen & Miller, 1969) which identified the PVN as the diencephalic site which supports the greatest eating in response to small NE infusions. Five of the six most effective cannula placements of the present study were found to lie within or immediately adjacent to the magnocellular portion of the PVN. The issue of whether the PVN is the site of action of NE in stimulating ingestive behaviors, or whether it represents a focus of a more diffuse system of medial hypothalamic sites, is not critical to the present analysis and is discussed elsewhere (Leibowitz, 1978).
The parallels between the lesion and knife cut experiments presented in Part I and the results of the present experiment are obvious. Both the ablation (lesion or knife cut) and chemical stimulation phenomena appear to share the PVN as a common central neuroanatomical focus and are clearly susceptible to blockade by vagotomy. Before discussing the possible implications of these commonalities, a more detailed analysis of the nature of the vagal involvement in the adrenergic feeding syndrome is warranted.
CHAPTER V

EFFECTS OF METHYL ATROPINE ON INGESTIVE BEHAVIORS ELICITED BY NOREPINEPHRINE

The results of the preceding experiment gave cause to further examine the possibility that NE elicited eating might be vagally mediated. While the discussion thus far has emphasized vagal efferent hyperactivity as a probable mediator of hypothalamically elicited overeating, little direct evidence in support of such a view is available. A somewhat selective means of producing peripheral vagal efferent blockade is via injection of a muscarinic cholinergic blocking agent. The optimal drug for this purpose is methyl atropine, a quaternary amine which does not cross the blood brain barrier in substantial quantities, and which therefore may be assumed to have predominantly peripheral effects. The present experiment examines the effects of such pharmacological vagal blockade on NE induced eating and drinking.

Some information of the effects of atropine on hypothalamically elicited overeating is already available. Grossman (1962b) was unable to abolish NE induced eating even with massive (10 - 50 mg/kg) doses of atropine sulfate, a tertiary amine which does cross the blood brain barrier and which therefore has both central and peripheral effects. More recently Powley and his colleagues (Powley, MacFarlane, Markell & Opsahl, 1978) have reported no effect of 3 mg/kg doses of atropine sulfate on eating produced via electrical stimulation of the lateral
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Method

Subjects. Twenty-two cannulated adult male rats were housed and main-
tained as previously described.

Surgery and testing. Implantation and testing procedures generally
followed that described in Chapter IV. Injections were made in a some-
what different manner, however, and followed the procedure of Swanson,
Perez, and Sharpe (1972). A 1 mL glass syringe, mounted on an infusion
pump (Harvard Apparatus), was connected via PE 10 polyethylene tubing
to a 30 ga stainless steel injector. The volume of fluid contained per
unit length of tubing was determined prior to use. By introducing a
small air bubble into the tubing, and measuring the distance traversed
by that bubble during infusions, the volume of fluid injected could be
accurately measured. Infusion speed was 1 μl/min and infusion volume
was 0.5 μl.

Procedure. Rats were screened for NE induced eating and drinking during
two 1 hr tests with 20 nM NE. Those which reliably increased food intake
on both NE trials were then subjected, in counterbalanced sequence, to
the following regimen. Thirty min prior to central infusions, animals
received either 0.4 mg/kg intraperitoneal injections of atropine methyl
nitrate, or an equivalent volume of sterile isotonic saline. These were
followed by central infusions of either 20 nM norepinephrine bitartrate
or an equivalent volume of the saline vehicle.

After each animal had experienced all four possible combinations
of central and peripheral injections, an NE post-test was administered to determine whether the animal was still responsive to NE. Only rats which reliably increased eating on both pre- and post-tests were included in the data analysis. Histology was performed as detailed in Chapter I.

**Data analysis.** Comparisons between pre- and post-test data were made using the correlated samples t test. The remainder of the data were evaluated with a two-way analysis of variance with repeated measures on both factors. Individual between-group comparisons were made using the Newman-Keuls procedure.

**Results**

A total of 17 animals reliably increased food intake in response to NE pre-tests and retained this capacity at the time of the post-test. Figure 17 shows pre- and post-test eating and drinking responses to intracranial NE. On the post-test, the animals were still responsive to NE, but the magnitude of the eating response was significantly reduced relative to pre-test values ($t(16) = 2.4, p < .05$).

Figure 17 also shows the feeding and drinking responses to combined central (NE or saline) and peripheral (methyl atropine or saline) injections. With respect to the elicited eating response, analysis of variance revealed significant overall effects of central infusions ($F(1,16) = 34.1, p < .001$), peripheral injections ($F(1,16) = 45.2, p < .001$), and a significant interaction between the two ($F(1,16) = 27.5, p < .001$). Multiple comparisons between the individual group
Figure 17. Center panel: Mean (± SEM) eating (right bar of each pair) and drinking (left bar of each pair) responses to intrahypothalamic infusions of 20 nM NE (or an equivalent volume of saline) following pretreatment with systemic injections of 0.4 mg/kg atropine methyl nitrate (or an equivalent volume of saline). Right and left panels: Pre- and post-test values are ingestive responses to 20 nM NE which were used to identify animals with effective cannula placements. See text for details.
means indicated that under the NE/saline condition, the animals ate more than under any of the other conditions (all p's < .01). None of the other comparisons were significant, indicating a complete abolition by methyl atropine of the elicited eating response.

With respect to the drinking data, significant main effects of central infusions ($F(1,16) = 14.3$, $p < .005$), peripheral injections ($F(1,16) = 8.7$, $p < .01$) and their interaction ($F(1,16) = 14.8$, $p < .005$) were again found. Multiple comparisons indicated that the animals drank significantly more under the NE/saline condition than any other (p's < .01), indicating a reduction in the drinking response as a result of a pretreatment with atropine. The reduction was not complete, however, since drinking in the NE/atropine condition was greater than that seen in response to either control treatment (p's < .01).

**Discussion**

The present experiment provides some insight as to the nature of vagal involvement in the adrenergic stimulation of ingestive behaviors. The eating provoked by intracranial NE was eliminated by systemic pre-treatment with methyl atropine, a quaternary amine which does not cross the blood brain barrier, and which therefore may be assumed to have exclusively peripheral anti-muscarinic effects. As was the case following complete abdominal vagotomy (Chapter IV), the elicited drinking response was reduced, but not abolished. These observations are consistent with, but do not prove, the notion that the NE feeding effect is mediated through vagal efferent nerves. Although the muscarinic blocking effects of atropine are well established, and susceptibility
to atropine blockade is a widely accepted criterion for identifying an efferent vagal effect, certain evidence (reviewed by Paintal, 1971) has implicated acetylcholine as playing a role in the generation of afferent discharge in some peripheral sensory systems. Therefore, the implication that the NE effect is mediated through vagal efferent mechanisms is not unequivocal.

The present results stand in contrast to those of Grossman (1962b), who observed no impairment of NE-elicited eating with 10 or 20 mg/kg, and only a moderate reduction with 50 mg/kg doses of atropine sulfate. In a similar vein, Powley et al (1978) have reported a failure of 8 mg/kg doses of atropine sulfate to block eating elicited by electrical stimulation of the lateral hypothalamus. Possible explanations for these apparently discrepant results may involve differences in dosage, an interaction with some central effect of atropine sulfate, or, in the case of the electrically induced eating, basic differences in underlying mechanisms. Some evidence in support of the contention that hypothalamically elicited overeating is mediated through vagal efferent nerves derives from the observation that the hyperphagia produced by hypothalamic knife cuts is blocked by selective lesions of the dorsal motor nucleus of the vagus, the site from which the vagal efferent innervation of the viscera originates (Simson, Gold, Eng, & Sawchenko, Note 6).

The reduction of NE induced eating by methyl atropine is not likely to have been a result of non-specific impairments or malaise attributable to atropine injections. Doses of atropine several orders of magnitude greater than those reported here have been shown to produce
no significant reduction of deprivation induced feeding (Burks & Fisher, 1970; Khavari & Russell, 1969). Even chronic daily treatment of freely feeding rats with 50 mg/kg atropine sulfate produces only modest (12 %) reductions in food intake (Soulairac, 1969).

Less clear is the specificity of the attenuation of NE-elicited drinking by methyl atropine. Doses of methyl atropine comparable to those employed here have been reported to have no effect (Khavari & Russell, 1969), or to enhance (Chapman & Epstein, 1970) deprivation-induced drinking, apparently depending, respectively, on whether or not food was available during testing. Thus, based on the atropine data alone, the specificity of the peripheral cholinergic involvement in NE-induced drinking is questionable. When viewed in conjunction with the surgical data of the preceding experiment, these results would suggest that vagal efferent mechanisms do partially mediate, or are at least necessary for the expression of, the drinking stimulated by intra-cranial NE.
CHAPTER VI

THE EFFECTS OF SELECTIVE COELIAC VAGOTOMY ON THE EATING ELICITED BY INTRACRANIAL NOREPINEPHRINE

The obvious parallels between the effects of full vagotomy on the overeating induced by chemical stimulation and lesions or knife cuts of the basomedial hypothalamus justifies examination of the effects of selective coeliac vagotomy, which specifically attenuated knife cut-induced hyperphagia, on the eating elicited by intracranial infusions of NE. If coeliac vagotomy was found to be especially effective in antagonizing NE-induced eating, additional support would be provided for the hypothesis that the two overeating syndromes operate via a common mechanism.

Method

Subjects. Twenty-five cannulated rats were used. Housing and maintenance conditions were as described in Chapter IV.

Procedure. Using the injection and testing procedure of Chapter III, baseline food and water intakes on two 1 hr trials each with 20 nM NE, saline and mock injections were taken. The rats were then divided into three groups on the basis of baseline feeding responses to NE to receive either (1) section of the coeliac branch of the vagus nerve alone, (2) section of the remaining three abdominal vagal branches (anterior and posterior gastric, and hepatic), sparing only the coeliac
branch, or (3) sham vagotomies. General surgical procedures were as described in Chapters I and III and elsewhere (Sawchenko et al., 1977).

Following recovery from surgery, testing resumed, with each rat receiving at least two additional NE, saline, and mock injection trials.

After completion of the above phase of the experiment, a test was carried out to independently assess the ability of the vagotomized rats to respond to a challenge to food intake. In this instance, following one day on which 1 hr intakes were measured in the absence of any treatment, the animals were subcutaneously injected with 4 U/kg regular insulin. Food intakes were measured 1 hr after injection.

At the conclusion of the experiment, the animals were laparotomized and examined, with the aid of a 20X operating microscope, for the integrity of each of the four abdominal vagal branches without knowledge of their surgical status. Only those animals whose vagal status at autopsy coincided with the originally intended surgery are included in the data analysis.

Histology and data analysis. Histology and data analysis were performed as detailed in Chapter IV.

Results

Four animals were unresponsive to NE pre-operatively, one failed to recover from gastric plus hepatic vagotomy, and one was eliminated from each vagotomized group after having been judged at autopsy to have sustained inappropriate vagotomies.

Figure 18 shows 1 hr food and water intakes in response to 20 nM
Figure 18. Mean (+ SEM) 1 hr eating and drinking responses to 20 nM NE infusions before and after recovery from selective coeliac vagotomy, combined gastric plus hepatic vagotomy, or sham vagotomy.
2 - NE

PRE-OP (18)
POST-COEILIAC VX (7)
POST-GASTRIC + HEPATIC VX (6)
POST-SHAM VX (5)

WATER INTAKE (ml/hr)

FOOD INTAKE (g/hr)
NE infusions for all animals preoperatively, and following recovery from sham, coeliac, or combined gastric plus hepatic vagotomies. Prior to vagotony surgery, the animals consumed an average of 2.2 g of food 
\( t(16) = 8.5, p < .001 \), relative to saline control conditions), and drank 1.1 ml of water \( t(16) = 5.2, p < .01 \) in response to NE. For reasons which are not clear, these values were somewhat lower than those seen under similar conditions in the two preceding experiments. After surgery, sham operated rats reattained stable rates of body weight increase after an average of 1.4 days, while coeliac vagotomized animals required 2.8 days, and the combined gastric plus hepatic vagotomized rats required 10.3 days.

When testing resumed, 20 days after vagotony surgery, it was found that sham vagotomy did not significantly reduce the eating elicited by NE \( t(4) = 0.3, p > .10 \), relative to preoperative values). Similarly, combined gastric plus hepatic vagotomy had no significant effect on the eating response \( t(5) = 1.3, p > .10 \). Coeliac vagotomy, in contrast, produced a 48% decrease in the eating response to NE, from \( 2.3 \pm 0.2 \) g (preoperatively) to \( 1.1 \pm 0.3 \) g (postoperatively) \( t(6) = 2.6, p < .025 \). The postoperative eating by coeliac vagotomized rats in response to NE, was however, still greater than that seen in response to saline infusions \( t(6) = 4.1, p < .01 \), indicating that the elicited eating was reduced but not eliminated.

With respect to water intake, although drinking in response to NE was reduced in both vagotomized groups, neither reduction was statistically significant. However, neither was the elicited drinking in either vagotomized group significantly greater than that seen in
response to saline infusions (p's > .10). NE remained effective, relative to saline infusions, in stimulating excessive water intake sham vagotomy (t(4) = 5.6, p < .01).

The capacity of each group to rapidly increase food intake in response to a second, independent challenge was assessed in an insulin test. Each group did show increased food intake in the first hour following insulin injections, with the coeliac vagotomized animals eating 1.5 ± 0.3 g (t(6) = 5.7, p < .01, relative to the non-injected control condition), while the gastric plus hepatic vagotomized rats ate 1.3 ± .04 g (t(5) = 2.6, p < .05), and the sham-operated rats ate 1.8 ± 0.4 g (t(4) = 3.6, p < .05). Neither vagotomized group ate significantly less than sham operated rats in response to insulin injections (p's > .10).

Discussion

Selective section of the coeliac vagus attenuated, but did not eliminate, eating in response to NE infusions in the PVN. This reduction appears to have been quite specific. Coeliac vagotomy produced only very transient reductions in body weight, making it unlikely that its effect on the NE response was secondary to some untoward consequence of this type of vagotomy. In contrast, gastric plus hepatic vagotomy, which produced more extended periods of undereating and weight loss, failed to appreciably reduce NE-elicited eating. In addition, neither of the vagotomy procedures limited the ability of the animals to overeat in response to subcutaneous insulin injections. Finally, water intake in response to NE was not significantly reduced by any of the three
surgical procedures, although in the case of the vagotomized groups, this result was equivocal since the elicited drinking was not statistically greater than that seen in response to saline infusions.

As noted above, a variety of autonomic effects have been observed to occur as a result of intrahypothalamic, but not necessarily intra-PVN, NE infusions (DeJong & Steffens, Note 2; Borkowski & Finch, 1978; Carmona & Slangen, 1973, 1976). Recent anatomical work has identified direct projections from cell bodies in and around the region of the PVN to the vagal motor nuclei in the hindbrain, as well as to the perikarya of sympathetic preganglionic neurons in the spinal cord (Saper et al., 1976; Swanson, 1977). Thus, the PVN, previously known primarily for its neurosecretory functions, has been physiologically and anatomically linked to autonomic, and specifically vagal, function.

Of the autonomic effects known to result from intrahypothalamic NE infusions, that which has been most clearly established as being capable of stimulating ingestive behaviors is the release of insulin (DeJong & Steffens, Note 2). The relationships among the vagus nerves, insulin secretion and food intake have already been discussed. Thus, it is possible that one mechanism by which NE may stimulate eating is via a neurogenic release of insulin mediated by the vagus nerves. Given the problems in interpreting the effects of full vagotomy on NE-induced eating, whether other vagal mechanisms may, via interactions with the coeliac vagus, entirely account for the eating elicited by NE remains an open question.

Coeliac vagotomy produced an equivocal reduction in the drinking response to NE which, in light of the mechanism suggested for the food
intake response, may be related to the observation that exogenous insulins can stimulate drinking independent of any effect on food intake (Booth & Brookover, 1968; Booth & Pitt, 1968; Novin, 1964). However, because vagotomy appears to produce quite generalized drinking impairments (Kraly, 1978; Kraly et al., 1975), a more precise estimation of the contribution of vagal mechanisms to NE-induced drinking must await analysis of the effects of vagotomy on the response to NE in the absence of food.

While the evidence linking adrenergic mechanisms in the PVN to the control of insulin secretion is limited, the striking similarities between the lesion or knife cut syndromes and the adrenergic stimulation of eating, as regards both their central and peripheral neuroanatomical substrates, would lead one to suspect that the two varieties of overeating share a common mechanism. Additional evidence in support of this view may be found in the observation that posterior hypothalamic lesions, which produce hyperphagia and obesity, can eliminate eating induced by NE infusions into a more anterior hypothalamic site (Herberg & Franklin, 1972). However, that some hyperphagic animals continued to eat in response to NE might suggest that the mechanisms subserving the two overeating syndromes are not entirely coincident.

In conclusion, the results of Part II provide support for the notion that the eating, and, to a lesser extent the drinking elicited by adrenergic stimulation of the PVN are mediated through vagal efferent influences on the viscera. Whether vagal mechanisms can entirely account for the adrenergic feeding phenomenon remains at issue, but the attenuation of NE-elicited eating by vagotomy appears
to be, at least in part, specific. Some function under coeliac vagal control, perhaps insulin secretion, appears to play an important, but not an exclusive, role in mediating the effects of NE infusions on ingestive behaviors.
GENERAL DISCUSSION

To briefly summarize, in Part I it was found that full sub-diaphragmatic vagotomy reversed the increases in food intake, body weight, and adiposity seen after VMH lesions, and prevented or reversed the increases in these parameters seen after hypothalamic knife cuts. It is unlikely that the upper gastrointestinal dysfunctions produced by vagotomy can account for these results, since selective gastric vagotomy had no pronounced effect on any measure except water intake. By coupling various selective vagotomy procedures with knife cuts, it was found that nerve sections which included the coeliac branch were variably effective in antagonizing knife cut-induced hyperphagia and weight gains. Since knife cut rats with nerve sections in which only the coeliac branch was left intact showed unabated overeating and weight gains on a lab chow diet, the extent to which extra-coeliac vagal mechanisms may play a causative role in hypothalamic hyperphagia and obesity is questionable. When granted access to a high-fat diet, all knife cut groups overate, relative to sham knife cut rats indicating that the exaggerated preference for fat-rich foods which is characteristic of the VMH syndrome is spared by vagotomy. The vagus nerves, therefore, are not the exclusive mediators of hypothalamic obesity.

In Part II, parallel studies on NE-stimulated eating revealed that this phenomenon, too, was blocked by full vagotomy and reduced by coeliac vagotomy. The nature of the vagal mediation of NE-elicited eating appears to be efferent, since pretreatment with a peripherally
acting anti-muscarinic drug mimicked the effects of full vagotomy. These results provide fairly clear evidence that the vagus is necessary for the full expression of both hypothalamic hyperphagia and NE-induced eating. When coupled with other evidence in the literature, they further suggest that these two varieties of overeating share a common mechanism. As outlined above, the paraventricular nucleus (PVN) has been identified as being the principal anatomical focus of both the knife cut and adrenergic feeding syndromes (Gold et al., 1977; Leibowitz, 1978). Confirming a suggestion in the older literature (Heinbecker, White, & Rolf, 1944), it has recently been demonstrated that discrete electrolytic lesions of the PVN also produce a robust overeating and obesity (Eng, Gold, & Nunez, Note 3). The present results suggest that the parallel in the central nervous system extends to the periphery.

One possible model which would account for these findings would have an inhibitory projection from the PVN to the vagal motor nuclei in the medulla. The direct PVN-to-dorsal motor nucleus connections described previously (Saper et al., 1976; Swanson, 1977) could provide such a pathway, although other, polysynaptic routes have also been described (e.g., Ban, 1975; Mayer & Arees, 1968). The apparent paradox that both stimulation (via NE) and ablation (via lesions or knife cuts) techniques could both produce the same effect can be resolved if it is assumed that NE is an inhibitory transmitter at the PVN. Thus both local application of NE, and lesions or knife cuts would operate via disinhibition of descending autonomic projections from the PVN. Disinhibition of vagal neural activity would, by this scheme, give rise
to physiological and metabolic changes which promote the storage of metabolic substrates in adipose tissue. Excessive neurogenic stimulation of insulin secretion is likely to prove to be an important driving mechanism in this process. The resulting reduction in circulating metabolic fuels then stimulates eating by mechanisms which are still incompletely understood (see Friedman & Stricker, 1976).

The fact that both syndromes appear to produce their effects in comparable manners has a number of implications. In discussing the failure of vagotomy in knife cut rats to block overeating of a high-fat diet, it was noted that there were suggestions in the literature that hyperphagia and finickiness might be anatomically dissociable (Bevan, cited in Hoebel, 1977; Graff & Stellar, 1962). A recent report (Torris, Note 8) has indicated that, in contrast to the lesion or knife cut syndromes, NE-induced eating is not enhanced on a mineral oil - high-fat diet. This finding, coupled with an earlier suggestion that that overeating and finickiness might be pharmacologically distinct phenomena (Margules, 1970a, 1970b) might warrant a closer examination of whether finickiness and hyperphagia are anatomically separable in the VMH rat.

As has already been discussed, several authors have argued for a role for hypothalamic NE receptors in the day-to-day control of food intake (e.g., Grossman, 1975; Leibowitz, 1970, 1976; Slangen & Miller, 1969; Van Der Gugten et al., 1977). The most convincing evidence for such an assertion has come from studies which have shown that NE is released in the hypothalamus during spontaneous feeding (Martin & Myers, 1975; Van Der Gugten et al., 1977). To the extent that NE's
effects are attributable to vagal efferent influences on the viscera, the present results argue against the notion that hypothalamic NE subserves normal hunger and satiety. Perhaps the increased hypothalamic NE turnover seen during spontaneous eating is indicative of an activation of vagal mechanisms preparatory to the processing of ingested food, rather than to direct stimulation of ingestion via a hypothetical adrenergic "feeding system".

Similarly, the present results are consistent with the mounting evidence (see Friedman & Stricker, 1976; Powley, 1977) that neither lesion nor knife cut hyperphagia is a result of damage to neural systems which mediate normal hunger and satiety. To the extent that hypothalamic hyperphagia is attributable to vagal, as well as extravagal influences on metabolism, hypothalamic obesity may not provide a very useful animal model for the study of the etiology of simple human obesity (cf Mayer, 1966). However, by virtue of its obesity, per se, the VMH-lesioned or knife-cut rat clearly does share interesting attributes common to many forms of obesity (e.g., Schachter, 1971; Schachter & Rodin, 1974; Nisbett, 1972). Of greater intuitive appeal as a model of simple human overweight is the "supermarket" variety of dietary obesity (Sclafani, 1976). In this paradigm, rats offered such foods as marshmallows, cheese, peanut butter, etc., overeat and become rapidly obese. It is interesting to note that this form of obesity is not blocked by vagotomy (Gold et al., in press).

That neither the NE nor the knife cut syndromes may be completely blocked by vagotomy would indicate that other mechanisms will have to be invoked to provide a complete understanding of these phenomena.
One potentially profitable area of research might involve possible contributions from the sympathetic nervous system. The possible involvement of sympathetics is consistent with the finding that collaterals of the previously described direct connections between the PVN and the vagal motor nuclei reach sympathetic preganglionic neurons in the spinal cord (Saper et al., 1976; Swanson, 1977). Sympathetic nerves have been proven capable of stimulating the mobilization of fats from adipose tissue (e.g., Cantu & Goodman, 1967; Sigman & Fawcett, 1954), as well as insulin release via β-adrenergic synapses (Smith & Porte, 1976).

One key issue left unresolved by the present experiments is whether both knife cut- and NE-induced overeating of standard laboratory diets can be completely attributed to the specific involvement of vagal mechanisms. The fact that fully vagotomized animals can overeat in certain situations (e.g., Eng, et al., 1979; Opsahl & Powley, 1974; Rowland & Engle, 1970) would suggest that the vagus nerves may be exclusively involved, but in no instance has hyperphagia comparable in degree and duration to that of the VMH animal been demonstrated in vagotomized rats fed a lab chow diet. Perhaps the untoward side effects of full vagotomy are too widespread and severe to permit a definitive resolution of this issue.

In this regard, one final implication of this dissertation may relate to the use of selective vagotomies as investigative tools. The abdominal vagus nerves have traditionally been viewed as being a unitary structure. While various functions under vagal control certainly do interact and synergize to meet a common end (i.e., the
processing and storage of ingested foodstuffs), the results presented here might indicate that extensive vagal denervation may be of little utility in the pursuit of mechanisms underlying biobehavioral phenomena. Problems in interpreting the effects of full vagotomy may derive from gross impairments in ingestive or digestive capacities, an inability to even vaguely specify the loci at which the treatment is exerting its effect, and the fact, underscroed here, that within a given context, various selective vagotomies may have effects which are opposite in direction, and which therefore may be obscured by more extensive vagal denervations.
REFERENCE NOTES


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