ANTERIOR HYPOTHALAMIC KNIFE-CUTS AND THE OVARIAN HYPERPHAGIA-OBESITY SYNDROME

by

STEVEN K. GALE

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July 22, 1977 — Anthony Sclafani
date — Chairman of Examining Committee

July 28, 1977 — Florence L. Denmark
date — Executive Officer

Dr. A. Sclafani

Dr. C. T. Lee

Dr. P. Bronstein

Supervisory Committee

The City University of New York

Abstract

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Adviser: Professor Anthony Sclafani

The hypothesis that the overeating and weight gain produced by anterior hypothalamic transections in adult female rats results indirectly from functional castration was examined. In Experiment 1 direct comparisons were made between ovariectomized rats and animals with coronal knife-cuts through the anterior hypothalamic area (AHA). It was found that while both physiological manipulations produced hyperphagia and obesity, the feeding and weight facilitory effects of the AHA knife-cuts exceeded those resulting from ovariectomy. Estrogen replacement (1-ug/day) completely reversed ovarian obesity but did not alter the expression of AHA knife-cut-induced obesity. Knife-cut rats were not insensitive to estrogen, however, since they reduced their body weight, as did neurally intact rats, when injected systemically with a higher dose (5-ug/day) of the hormone. The findings of Experiment 1 also revealed that significant differences exist between anterior knife-cut and ovariectomized rats in their water consumption, water/food intake ratio,

linear growth, and Lee Obesity Index values. Taken together, these results suggest that functional castration is not a causative factor in the anterior knife-cut-induced obesity syndrome.

In Experiment 2 ovariectomized rats in the static phase of their ovarian obesity were given anterior coronal knife-cuts. It was observed that these transections produced additional weight gains in animals with a pre-existing estrogen insufficiency. Further, the body weight effects produced by ovariectomy and by anterior knife-cuts were found to be near additive when both physiological manipulations were combined in the same animal. The feeding responses of the anterior cut and ovariectomized rats to abrupt changes in environmental temperature were also examined in this experiment. The results of these temperature tests indicate that knife-cut damage to the AHA disrupts thermoregulation but this is not likely to explain the excess feeding and weight gain seen following anterior coronal transections.

In Experiment 3 it was observed that adult male rats given anterior knife-cuts became hyperphagic and obese. This finding further strengthens the view that an estrogen insufficiency could not adequately explain AHA knife-cut-induced obesity. It was also found that medial parasagittal knife-cuts were effective in producing overeating and weight gain in adult male rats. Further, rats given either set of knife-cuts displayed an initial overconsumption when switched to a high fat diet from their standard laboratory chow.

Experiment 4 examined whether or not ovariectomized rats with anterior coronal cuts would respond like neurally intact rats to the feeding and weight suppressive effects of exogenous estrogen administration. It was observed that anterior cuts, as well as medial parasagittal transections, did not block estrogen anorexia. These findings suggest that neither the anterior or medial transections produced their feeding and weight facilitory effects by directly damaging estrogen-sensitive feeding neurons.

It was observed in Experiment 5 that ovariectomized rats given either anterior coronal or medial parasagittal knife-cuts exhibited severe and persisting activity decrements which were independent of changes in ovarian hormone levels. Estrogen replacement (5-ug/day) was found to completely restore the wheel running activity of medial, but not anterior, cut rats to preovariectomy levels. These findings are discussed in light of the evidence implicating estrogen-sensitive and estrogen-insensitive brain areas in the modulation of spontaneous locomotor activity.

In Experiment 6 it was found that ovariectomized rats given either anterior coronal or medial parasagittal knife cuts displayed an exaggerated underconsumption on a .2% quinine-adulterated diet, decreased latencies to eat a highly palatable food in a novel environment, and, when offered a high-fat diet, both cut groups showed excellerated weight gains. These findings suggest that anterior coronal and medial parasagittal knife-cut rats are finicky eaters.

In summary, the results of this study demonstrate that the hyperphagia-obesity syndrome produced by anterior coronal knife-cuts does not result from the hyposecretion of estrogen as was initially suggested. The present findings also indicate that anterior neural transections do not interfere with the estrogenic suppression of feeding and body weight following systemic hormone injections. These findings indicate, therefore, that the overeating and weight gain produced by anterior coronal transections is independent of the hyperphagia-obesity syndrome resulting from the chronic withdrawal of estrogen. The feeding and body weight effects produced by anterior transections are consistent with other recent findings indicating that such knife-cuts damage a longitudinal feeding system which projects through the hypothalamus.

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INTRODUCTION

OVARIAN HYPERPHAGIA-OBESITY SYNDROME IN THE ADULT FEMALE RAT

Estrogen-Induced Changes in Ad Libitum Food Intake

It has long been known that bilateral ovariectomy in adult female rats produces hyperphagia and rapid body weight gain. Holt, Keeton, and Vennesland (1936) were among the first to observe these effects which have since been confirmed by numerous other investigators (Hervey and Hervey, 1966; Kakolewski, Cox, and Valenstein, 1968; Tartellin and Gorski, 1971). The onset of the overeating occurs within a few days following ovariectomy and continues for several postoperative weeks. Food consumption gradually declines towards preovariectomy levels although the body weight of the ovariectomized animal remains permanently elevated. The increase in body weight following surgery results primarily from an elevation in food intake (Landau and Zucker, 1976) and is associated with a disproportional increase in fat stores relative to lean body mass (Leshner and Collier, 1973). Ovariectomy has also been reported to increase the water consumption of adult rats (Tartellin and Gorski, 1971) as well as to augment their linear growth (Nyda, deMajo, and Lewis, 1948).

Ovariectomy-induced hyperphagia and obesity do not develop in rats injected systemically with estrogen at the time of spaying (Wade

and Zucker, 1970) and non-treated ovariectomized rats allowed to become obese reduce their feeding and lose weight in a dose-dependent fashion following peripheral estrogen injections (Mook et. al, 1972; Tartellin and Gorski, 1973; Zucker, 1972). The estrogenic suppression of feeding and body weight has also been reported after subcutaneous (Dubuc, 1974), oral (Bull et. al, 1974) and intrahypothalamic estrogen administration in ovariectomized animals (Beatty, O'Briant & Vilberg, 1974; Jankowiak and Stern, 1974; Wade and Zucker, 1970).

In contrast to the effects of estrogen replacement on the feeding and body weight of ovariectomized rats, the other major ovarian hormone, namely progesterone, fails to either prevent or reverse the ovarian hyperphagia-obesity syndrome (Hervey and Hervey, 1964, 1966; Rodier, 1971). Progesterone injections in high doses have been reported to increase the food intake and body weight of endocrine intact rats (Hervey and Hervey, 1964; Roberts, Kenney and Mook, 1972; Rodier, 1971) however considerable evidence now indicates that the feeding and weight promoting effects of this hormone result indirectly from progestational influences on endogenous estrogen levels (Wade, 1975). Thus, it appears likely that estrogen withdrawal is responsible for the hyperphagia-obesity syndrome resulting from ovariectomy.

During the normal ovarian cycle of the rat food intake and body weight have been shown to vary predictably with changes in plasma estrogen titers. That is, female rats overeat and gain weight during the diestrous phase of the cycle (Brobeck, Wheatland and Strominger, 1947) when estrogen secretion is greatly diminished (Hori, Ide and Miyake, 1968; Yoshinaga, Hawkins and Stocker, 1969). There is also

a marked elevation in the feeding and body weight of intact rats in early pregnancy, pseudopregnancy, and lactation which, like the overeating seen normally at diestrus, has been attributed, in part, to a reduction in estrogen's feeding inhibitory effects (Ota and Yokoyama, 1967; Wade and Zucker, 1969). Conversely, the food intake and body weight of intact rats shows a marked decline during the estrus phase of the ovarian cycle (Brobeck, Wheatland, and Strominger, 1947) when plasma estrogen titers are normally elevated (Hori, Ide, and Miyake, 1968; Yoshinaga, Hawkins, and Stocker, 1969). It has recently been shown, in fact, that even after normal ovarian cycling has ceased the food intake and body weight of aged females in constant vaginal estrus is significantly less than that of same age rats in vaginal states characterized by low levels of estrogen secretion (Cooper and Linnoila, 1976). These findings, taken together, indicate that the feeding and body weight of intact rats are influenced by naturally occurring changes in estrogen availability and are also consistent with the evidence cited earlier that the hyperphagia-obesity syndrome following ovariectomy results from a chronic estrogen insufficiency.

Estrogen-Induced Changes in Food-Motivated Behavior

The food-motivational effects produced by changes in ovarian hormone levels have received much less attention compared to the ad libitum changes in feeding and body weight produced either by ovariectomy or by estrogen administration. The available evidence, however, relating changes in plasma estrogen titers to food motivation demonstrate that high levels of this hormone in the peripheral plasma cause

a decrease, and low levels an increase, in food rewarded behaviors. For example, Harris and Heistad (1970) trained food-deprived female rats to press a lever to obtain food rewards and observed that responding markedly decreased during the estrus stage of the ovarian cycle. Further, systemic injections of estradiol, but not the oil vehicle, produced a dose-related decrement in the food-reinforced lever pressing of ovariectomized rats. In a similar motivational analysis Jennings (1973) observed that female rats in estrus were less willing to work for food than were rats in the diestrus phase of the ovarian cycle and this inhibition of feeding at estrus was not overcome by offering the animals highly palatable food rewards.

These findings which indicate a decrease in hunger motivation during either natural estrus or following estrogen replacement in ovariectomized rats are consistent with the well documented hypophagia seen in free feeding rats when blood estrogen concentrations are endogenously or experimentally elevated (Brobeck, Wheatland, and Strominger, 1947; Wade and Zucker, 1970). An examination of the meal patterns of rats during estrus also reveals that this stage of the ovarian cycle is associated with a significant reduction in the size of naturally occurring meals. Moreover, a similar decrease in meal size has been produced experimentally by the systemic administration of estrogen into hyperphagic ovariectomized rats (Blaustein and Wade, 1976).

In contrast to the inhibitory effects on food motivation observed when plasma estrogen levels are elevated, the findings of several studies indicate that the chronic withdrawal of estrogen following ovariectomy

enhances the animal's food rewarded responding. For example, the results of a study by Kenny and Mook (1974) indicate that ovariectomized rats will increase their daily bar pressing above presurgical levels to obtain food rewards. This finding suggesting enhanced food-motivated behavior following estrogen withdrawal has recently been confirmed by Gale and Sclafani (1977b) who also observed that ovariectomized rats worked harder for food than did operated controls in both short- and long-term bar pressing tests. Further, when allowed to become obese ovariectomized rats did not display a reduction in their bar pressing behavior compared to sham ovariectomized rats working for similar food rewards on demanding reinforcement schedules (Gale and Sclafani, 1977c). Taken together these findings reveal that ovariectomy increases the rat's food motivation and this is consistent, therefore, with earlier evidence that rats given free access to food in their home cage will overeat and gain weight following either chronic estrogen withdrawal or the transient reduction in the circulating level of this hormone at diestrus in intact cycling rats.

The enhanced food motivation of ovariectomized rats is also consistent with other evidence demonstrating that even a very bitter tasting diet fails to prevent the overeating and weight gain which follows estrogen withdrawal and brain damaged rats who remain normophagic on such noxious diets exhibit an abrupt hyperphagia and body weight gain when subsequently ovariectomized (Gale and Sclafani, 1977b). Finally, analysis of meal pattern data reveal that ovariectomized rats take larger meals soon after estrogen withdrawal (Gale and Sclafani, 1977b; Kenny and Mook, 1974) and continue to do so even after their elevated

food intakes return to preovariectomy levels (Blaustein and Wade, 1976). An increase in meal size following chronic estrogen withdrawal is also characteristic of the feeding pattern of intact rats during the diestrus stage of the ovarian cycle (Drewett, 1974) when there is only a transient reduction in the plasma concentration of estrogen.

ESTROGEN AND BODY WEIGHT SETPOINT

Several investigators have suggested that estrogen alters a level or setpoint at which animals regulate their body weight rather than directly affecting feeding per se (Mook et al., 1972; Redick et al., 1973; Wade, 1972; Zucker, 1972). According to this hypothesis endogenous estrogen normally restrains the body weight of female rats by lowering the setpoint, and ovariectomy raises this setpoint by abolishing the inhibition. Ovariectomy hyperphagia is, therefore, seen as an indirect consequence of the animal's attempt to regulate its body weight at a level higher than normal. Conversely, estrogen-induced hypophagia in obese ovariectomized rats is viewed as an indirect consequence of the animal's efforts to bring its body weight down towards preovariectomy levels. These feeding changes following estrogen withdrawal or replacement are considered secondary to adjustments in body weight and thus they persist only until a new body weight plateau is achieved.

Consistent with the setpoint interpretation of estrogen-induced changes in body weight regulation are the findings which indicate that

obese ovariectomized rats reduce their eating and lose weight following estrogen replacement but only until they reach the level of endocrine intact rats (Mook et al., 1972; see Wade, 1976). Additional support for the setpoint interpretation are the findings which reveal that the estrogenic suppression of feeding is blocked in ovariectomized animals whose obesity has been prevented. For example, Redick et al., (1972) have shown that ovariectomized-adrenalectomized rats fail to overeat and gain weight on a standard chow diet and when these rats are subsequently injected with estrogen they are unresponsive to the hormone's feeding suppressive effects. On the other hand, when they are offered a high fat diet which encouraged their overeating and weight gain, subsequent estrogen injections effectively suppressed their food intake and completely reversed the high-fat-induced obesity. Thereafter, continued estrogen injections failed to further reduce feeding or lower the body weight of the ovariectomized-adrenalectomized animals. Similar findings with hypophysectomized rats have also been reported by Zucker (1972). He observed that lean ovariectomized-hypophysectomized rats maintained stable food intake and body weight levels when injected with estrogen at doses that suppressed the feeding and weight gain of overweight animals given only prior ovariectomy.

The above findings suggest, therefore, that when postovariectomy hyperphagia and obesity are blocked by surgical intervention, estrogen's normally anorexigenic effects are greatly attenuated or even eliminated. Surgical intervention to limit weight gain does not, however, appear to be the critical variable in these studies since it

has been observed that estrogen administration also fails to reduce the feeding or body weight level of lean ovariectomized rats prevented from becoming obese by food restriction. Further, estrogen does decrease these measures in ovariectomized rats allowed to become obese when they are given ad libitum access to the same diet (Landau and Zucker, 1976). It has also been reported (Zucker, 1972), that in mature rats of differing body weights resulting from the prior manipulation of litter size, estrogen is significantly more effective in suppressing feeding and lowering the body weight of heavy rather than lean intact animals. Finally, also consistent with the setpoint interpretation that estrogen alters food intake as a means of regulating body weight is the finding that obese ovariectomized animals will undereat and eventually stabilize their body weight at a reduced level which is in direct proportion to the dose of estrogen used in replacement therapy (Wade, 1975; Zucker, 1972).

POSSIBLE BRAIN SITES MEDIATING THE FEEDING EFFECT OF ESTROGEN

Evidence for the Existence of Estrogen-Sensitive Sites in the Ventromedial Hypothalamus (VMH)[1]

As early as 1947, Brobeck, Wheatland, and Strominger had theorized that cells within the hypothalamus mediated the effects of estrogen on food intake and body weight. Kennedy and Mitra (1963) later suggested that the ventromedial nucleus was the principal hypothalamic subarea where estrogen influenced feeding, based in part, on their finding that rats with lesions in this area continued to display normal

ovarian cycling but failed to either decrease their feeding or increase their activity at estrus. Wade and Zucker (1970) were the first to specifically examine this hypothesis by directly implanting estrogen into the VMH and found that the hormone markedly reduced the feeding of ovariectomized rats. The suppression of food intake occurred within 12 hours of application to the brain and the reduction was similar in magnitude to the feeding reduction seen following peripheral estradiol injections. These investigators also observed that cholesterol and testosterone, two known precursors of estrogen synthesis, failed to affect feeding when similarly implanted in the vicinity of the ventromedial nucleus.

Wade and Zucker (1970) also reported that estrogen-induced feeding suppression following central application terminated once the hormone was removed from the VMH and that estrogenic brain stimulation failed to reduce food intake at other adjacent neural sites implicated in the central regulation of feeding behavior. For example, estrogen implants into the lateral hypothalamus (LH) or into the anterior hypothalamic area (AHA) were without effect on the eating of ovariectomized rats. More recent observations (Beatty, O'Briant and Vilberg, 1974; Jankowiak and Stern, 1974) have generally confirmed the findings that intracranial estrogen implants into the VMH suppress feeding whereas similar implants into adjacent brain sites fail to alter the rat's food intake. The results of these hormone cannulation studies, therefore, strongly suggest that estrogen selectively acts on cells within the VMH to decrease feeding and this confirms the original speculation of Kennedy and Mitra (1963).

Since locomotor activity has long been known to be highly dependent on the circulating level of estrogen in the female rat (Young and Fish, 1945) it is not inconceivable that implants of this hormone into the VMH might have increased the rat's running behavior and thereby indirectly affected its food consumption. Arguing against this possibility, however, are the findings of Colvin and Sawyer (1969) who observed that VMH estrogen implants were totally ineffective in altering the rat's locomotor activity although estrogen implants into the nearby AHA were capable of increasing their running. Neural specificity with regard to eating and locomotor activity is also suggested by the finding that implants of the cytotoxic agent actinomycin-D into the AHA inhibited spontaneous activity without affecting the rat's food consumption (Stern and Jankowiak, 1972). A similar dissociation also exists between brain sites involved in feeding and sexual behavior, that is, at neural areas where estrogen implants influences sexual behavior food intake measures typically remain unaffected (see Wade, 1976).

Consistent with the hypothesis of the VMH mediation of estrogen's feeding effects, Nance and Gorski (Note 1) reported that electrolytic lesions of this brain area prevented the normal reduction in food intake following peripheral estrogen administration. More recently, Nance (1975) has confirmed these findings using similar lesion placements and different dietary conditions. Further, he has provided some additional data which suggests that destruction of the VMH-arcuate nucleus complex, rather than the VMH itself, is more likely to result in a loss of sensitivity to the feeding suppressive effects of estrogen.

Kaestner (1974) also observed that VMH lesions would abolish the feeding suppression normally produced by systemic injections of estrogen. She found that soon after hypothalamic surgery, when VMH lesioned animals were in the dynamic phase of their obesity, estrogen injections failed to reduce food consumption or lower the body weight of these brain damaged rats. However, when permitted time to reach the static phase of their obesity Kaestner observed that ovariectomized rats with VMH damage were heavier than were sham ovariectomized animals with similar neural damage. Beatty, O'Briant and Vilberg (1975) have reported that static obese VMH lesioned rats were less responsive than were neurally intact animals to the feeding and weight suppressive effects of peripheral estrogen administration. Further, they observed that the static obese VMH rat exhibited less of a feeding enhancement than did unlesioned controls to the chronic removal of estrogen by ovariectomy. Beatty et al. (1975) interpreted their findings as support for the hypothesis that estrogen-sensitive feeding neurons exist within the VMH and, possibly within the adjacent arcuate nucleus.

In contrast to the above lesion findings, other ablation studies have questioned whether estrogen exclusively acts on the VMH to alter food intake. For example, King and Cox (1973) reported that estrogen remains fully effective in suppressing feeding in VMH lesioned rats, and Reynolds and Bryson (1974) have obtained similar results. These findings suggesting that VMH damage does not always prevent the anorexigenic effects of estrogen injections in lesioned rats confirm the earlier results of Montemurro (1971) who found that subcutaneous

implants of diethylstilbestrol, a synthetic estrogen, were capable of inhibiting the feeding of VMH lesioned mice. Thus it appears likely that estrogen can act on sites outside the VMH to influence food consumption. This possibility also receives support from the numerous findings which demonstrate that the weight gains produced by ovariectomy and that produced by VMH damage are near additive when both surgeries are combined in the same animal (Gale and Sclafani, 1977a; Kaestner, 1974; King and Cox, 1973; Powley, Opsahl, Van den Pol, Note 2).

Thus the hormone cannulation studies indicate that the VMH is the principle site for the estrogenic modulation of feeding, whereas the brain ablation studies report that VMH damage either completely blocks, attenuates, or has no substantial effect on estrogen's normally anorexigenic effects. One conceivable explanation for the apparent differences among the lesion results may involve the estrogen dose employed and the duration of the injection sequence. For example, the data reported by Nance (1975) indicating a complete inhibition of estrogen-induced anorexia were based on a single high dose of estrogen whereas the findings of King and Cox (1973), that VMH damage did not block estrogen-induced anorexia, were based on a more physiological dose of the hormone which was given daily over 25 treatment-days.

Alternatively, the reported differences in the feeding effects of estrogen injections in VMH lesioned animals may result from the differences in the extent and locus of neural tissue damaged by the electrolytic lesions. That is, a careful reading of the neuroanatomical

descriptions of the VMH lesion histology reveals that structures other than the intended target site were often destroyed such as the arcuate, anterior hypothalamic and dorsomedial nuclei. The specificity of neural damage to this brain region has previously been shown to be critical to several aspects of food intake (Epstein and Teitelbaum, 1967; Grossman, 1975; Sclafani, Berner and Maul, 1973). Thus, the seemingly contradictory results concerning the effects of systemic estrogen injections on the feeding behavior of rats with VMN and adjacent tissue damage are not entirely surprising given the variability in the actual brain tissue destroyed. At the same time, however, the finding that most clearly emerges from the ablation studies is that sites other than the VMN must also be capable of mediating estrogen's feeding inhibitory effects. This does not preclude the possibility that the VMN is the primary neural site of estrogen-induced feeding changes in the rat, as suggested by the hormone cannulation studies (Beatty, O'Briant and Vilberg, 1974; Jankowiak and Stern, 1974; Wade & Zucker, 1970) but rather, that other brain areas may play a more prominant or perhaps compensatory role in estrogen mediation of feeding following destruction of this brain area.

Other Estrogen-Sensitive Sites Including Those In the Anterior Hypothalamic Area (AHA)

Little is known about which central nervous system sites besides the VMH also participate in the estrogenic mediation of feeding or of the potential relationship of these as yet unspecified sites to neural areas previously implicated in the central regulation of feeding. Since some evidence suggests that the VMN may alter food intake by inhibiting

lateral hypothalamic neural activity (see Oomura, 1976) it is possible that estrogen-sensitive feeding fibers project laterally from the VMN towards the lateral hypothalamus. Implants of estrogen directly into the lateral hypothalamus fail to reduce the feeding of ovariectomized rats (Wade and Zucker, 1970) and lesions of this brain area do not substantially block ovariectomy-induced hyperphagia and weight gain (Harrell and Balagura, 1975). Furthermore, rats with parasagittal knife-cuts between the lateral hypothalamus and the VMN overeat and gain weight like neurally intact animals following chronic estrogen withdrawal (Gale and Sclafani, 1977a). The available evidence, therefore, lends no support to the notion that estrogen-sensitive feeding fibers project laterally from the VMH towards the LH or that the LH itself is sensitive to the feeding inhibitory effects of estrogen.

In a specific attempt to identify steroid-sensitive feeding sites by means of localized ablation, Cox and King (1974) observed that rats with lesions of the corticomedial nuclear group of the amygdaloid complex, a neural area shown to be implicated in the central control of feedint (Montgomery and Singer, 1975; Sclafani, Belluzzi, Grossman, 1970; White and Fisher, 1969), remained normally responsive to the feeding suppressive effects of systemic estrogen administration. Following ovariectomy these brain damaged rats overate and gained weight as did neurally intact animals. Other evidence from serum hormone assays indicates that lesions of the amygadaloid nuclear complex do not alter estradiol levels (Spies et al., 1976).

Several indirect lines of evidence which are each discussed below suggest that the anterior hypothalamic area (AHA) may be

involved in the estrogen mediation of feeding although the only study which directly cannulated this hormone into the AHA of ovariectomized rats failed to reveal a reliable feeding suppression (Wade and Zucker, 1970).

One line of study implicating the anterior hypothalamus in the hormonal mediation of feeding links changes in estrogen levels to brain catecholamines. The anterior hypothalamus has been identified (Booth, 1967; Leibowitz, 1976) as one of the most sensitive neural sites from which feeding can be elicited by microinjections of norepinephrine (NE) and ovariectomy selectively increases NE synthesis and turnover in the anterior hypothalamus (Bapna, Neff and Costa, 1971). Additional evidence from other studies reveal that the increased levels of NE seen after the chronic withdrawal of estrogen are accompanied by reciprocal decreases in brain dopamine (DA) levels in the anterior hypothalamus and both of these catecholamine changes in ovariectomized rats are restored following hormone replacement therapy (Donoso and Stefano, 1965). A similar pattern of altered catecholamines is also seen in intact female rats at diestrus; that is, NE levels in the anterior hypothalamus are increased, and DA levels decreased, during the phase of the ovarian cycle when estrogen levels are reduced only transiently (Stefano and Donoso, 1967). Thus low levels of estrogen resulting from surgery or occurring naturally are associated with increased NE synthesis in the anterior hypothalamus where microinjections of this catecholamine induce vigorous eating. Moreover, autoradiographic findings (Pfaff and Keiner, 1973; Stumpf, 1968) indicate that a high density of neurons within the anterior hypothalamus concentrate labelled estradiol.

Based on the above systemic fluctuations in brain catecholamines associated with changes in estrogen levels, as well as the degree in overlap between the distribution on NE-elicited feeding sites and estrogen-sensitive neurons in the anterior hypothalamus, Simpson and DiCara (1973) hypothesized that this neural area was the principal region mediating estrogen's feeding effects. More specifically, these investigators theorized that estradiol directly interferred with the conversion of dopamine to norepinephrine by inhibiting the activity of the rate limiting enzyme dopamine-beta-hydroxylase (DBH). Evidence which they interpret as supporting their hypothesis are the findings that intracranial DA injections into the anterior hypothalamus elicit eating in intact rats only during diestrus when estrogen levels are low and thereby conversion to NE is permitted. On the other hand, NE injections elicited feeding during either diestrus or estrous since NE, unlike DA, is beyond estradiol-induced enzyme inhibition. In a more recent study Simpson, Cummins and DiCara (1975) have provided direct evidence that estrogen alters DBH activity. The results of this later study, however, are inconsistent in relation to their original feeding hypothesis since estrus, a time of elevated estrogen levels, was associated with increased rather than decreased DBH activity.

An interaction between estrogen and brain catecholamines with regard to feeding has also recently been advanced by Fishman (1976). He postulated that the estrogen metabolite, 2-hydroxyestrone, suppresses feeding by indirectly altering the availability of norepinephrine. Increases in 2-hydroxyestrone resulting from elevated estrogen levels, according to this hypothesis, increase the half-life of norepine-

phrine and this in turn would decrease the formation of newly synthesized NE and thereby lead to a decrease in NE-elicited feeding. The hypothalamus, including the anterior region which has a high density of estrogen-concentrating neurons, is especially capable of converting estrogen to this active metabolite (Fishman and Norton, 1975).

A second line of study implicating the anterior hypothalamus in the estrogenic mediation of feeding results from research principally designed to examine hypothalamic and pituitary involvement in endocrine functioning. For example, in a series of studies involving partial deafferentiation of the basomedial hypothalamus, Halasz (1969) reported that dome-shaped transections through the anterior hypothalamus inhibited the cyclic release of gonadotropins and altered circulating levels of ovarian steroids. The transections completely blocked ovulation and, depending on their exact position, interferred with the occurrence of ovarian compensatory hypertrophy following unilateral ovariectomy. These findings in general support the results of both lesion and electrical stimulation studies indicating that critical fiber pathways responsible for the neuroendocrine regulation of reproductive behavior and estrus rhythm course through the anterior hypothalamus (see Barraclough, 1973).

While the scope of these neuroendocrine studies often did not include an examination of the attendant feeding and body weight changes produced by brain damage, the results of several reports using similar dome-shaped transections which pass through the border of the VMH and the AHA revealed that such damage produced marked obesity and presumed overeating (Blake et al., 1972, 1973; Mitchell

et al., 1972; Palka, Coyer and Critchlow, 1969). These findings suggest, therefore, that a diffuse system of hormone-sensitive feeding fibers may exist in the anterior hypothalamus.

Alternatively, it is possible that these transections cause overeating and subsequent weight gain in female rats not by an interruption of feeding fibers but rather as an indirect consequence of gonadal failure. A chronic reduction in the circulating titer of estrogen is consistent with some observations that anterior transections cause highly irregular estrus rhythms which are often characterized by constant states of vaginal diestrous (Halasz and Gorski, 1967). Further, functional castration is also consistent with the evidence indicating a reduction in the ovarian and uterine weights of rats with anterior hypothalamic damage compared to similar measures in neurally intact rats (Halasz and Gorski, 1967; Halasz and Pupp, 1965). However, arguing against the likelihood that an estrogen insufficiency alone is responsible for the overeating and weight gain produced by anterior dome-shaped transections are other findings which show that similar neural damage is capable of enhancing the sexual receptivity of intact and recently ovariectomized rats and, more directly, of elevating serum estradiol levels (Rodgers and Schwartz, 1972; 1976). Since none of these studies of hypothalamic deafferentiation, however, were designed to examine specifically the relationship between the anterior hypothalamus and food intake, it is difficult to speculate as to whether direct damage to putative estrogen-sensitive feeding fibers in the AHA, endocrine dysfunction, or both are responsible for the overeating and weight gain produced by these transections.

A third line of study implicating the anterior hypothalamic area in the estrogenic mediation of feeding results from studies which have focused specifically on the anatomical localization of the neural fibers whose destruction is thought to be responsible for the VMH hyperphagia-obesity syndrome. Grossman (1971), for example, reported that knife-cuts which transect the mediolateral aspects of the anterior hypothalamus, just rostral to the ventromedial nucleus, produced excessive food intake and weight gain in female rats. Animals with appropriately placed anterior transections displayed a mild hyperphagia which persisted for approximately four to five weeks and became moderately obese, gaining about 15 to 20% in body weight. The duration of the overeating and the degree of weight gain following the anterior cuts closely resembled the food intake and body weight changes seen after chronic estrogen withdrawal and thus it is possible that these cuts caused a functional ovariectomy. Smaller cuts confined to the more medial aspects of the AHA did not result in any obvious feeding changes but did produce a disruption in estrous cycling (Sclafani, 1971).

Albert et al. (1971), employing knife-cuts similar to those of Grossman (1971), also reported a mild and transient hyperphagia in female rats after anterior hypothalamic cuts whereas similar transections in males failed to produce overeating. The failure of anterior knife-cuts in males to reliably alter feeding has also been reported by Paxinos and Bindra (1972) and more recently by Mabry and Campbell (1975). Based on their original finding of a sex difference following anterior transections Albert et al. (1971) theorized that the knife-cuts

produced feeding and weight facilitory effects in females by means of a chronic estrogen insufficiency. It is possible, however, that the failure of male rats to overeat and gain weight following anterior cuts may have resulted from the use of standard laboratory chow since some investigators have reported that hyperphagia and obesity following brain damage in males is more likely to occur when high fat diets are offered (Cox, Kakolewski, and Valenstein, 1969; Valenstein, Cox and Kakolewski, 1969). Therefore, the observed differential feeding effects produced by anterior knife-cuts in male and female animals, reported by Albert et al., (1971), may not be the simple result of brain damaged-induced castration in the female, but rather, may be related to the increased likelihood for observing overeating in females than males when less than optimally palatable diet are provided.

Thus, in summary, the studies which have examined: (a) estrogen and brain catecholamines interactions, (b) the reproductive and endocrine consequences of neural deafferentiation, and (c) the neural circuitry underlying the VMH hyperphagia-obesity syndrome each provide suggestive evidence that estrogen may influence feeding directly by acting on hormone-sensitive sites in the anterior hypothalamic area. Moreover, that estrogen may act on sites in the anterior hypothalamic area to alter feeding is also compatible with studies cited earlier which indicate that estrogen-sensitive feeding neurons exist in the VMH but not exclusively so. It is conceivable, therefore, that such neurons may comprise a hormone-sensitive pathway which directly interconnects the anterior hypothalamic area

with the VMH. Such a proposed neural pathway is consistent with the known location of hypothalamic estradiol-concentrating cells (Pfaff and Keiner, 1973) and with evidence indicating intrahypothalamic neural projections between the anterior hypothalamic area and the VMH (Chi, 1970). It is also conceivable, however, that the feeding changes following damage to the anterior hypothalamic area can be explained principally on the basis of functional castration since ovarian disturbance is typically observed after neural transections which invade this brain region. Finally, the possibility exists that knife-cuts which traverse the anterior hypothalamic area may alter food intake neither as a result of damage to an estrogen-sensitive feeding system nor by causing functional castration.

The main purpose of this study was to examine the role of the AHA in the estrogenic mediation of feeding by means of the encephalatome technique (Sclafani, 1971) which permits selective neural transections with a minimum of tissue damage. Knife-cuts were placed in the caudal portion of the AHA, just rostral to the VMH, since the hormone cannulation studies uniformly indicated that estrogen implants into the VMH suppressed feeding (Beatty, O'Briant and Vilberg, 1974; Jankowiak and Stern, 1974; Wade and Zucker, 1970) and other evidence demonstrated that parasagittal knife-cuts lateral to the VMH did not block the overeating following chronic estrogen withdrawal (Gale and Sclafani, 1977a). Peripheral estrogen injections were subsequently administered to knife-cut and neurally intact rats to determine to what extent, if any, such transections would interfere with estrogen's normal anorexigenic effects.

GENERAL PROCEDURES

SUBJECTS

One-hundred and sixty-three female and twenty-one male CFE rats were obtained from Charles River Breeding Laboratories (Wilimington, Mass.) at approximately 100 days of age and individually housed in Wahmann LC-75/A wire mesh animal cages. Except where indicated, subjects were housed in an air-conditioned colony room which was maintained on a 12:12 hr. light/dark cycle.

SURGERY AND HISTOLOGY

Subjects were anesthetized with Equi-Thesin (2.5 ml/kg of body weight) prior to receiving: (I) hypothalamic knife-cuts, (II) ovariectomy, or (III) sham surgery.

(I) Knife-cuts were made by the encephalatome technique of Sclafani (1971) in which an inner cutting wire was extended a predetermined distance through the guide shaft of a knife assembly mounted to a Kopf stereotaxic electrode carrier. The knife assembly was mounted with a set of compression springs and a trigger device, which when released, automatically elevated the knife assembly a fixed height (Hamilton, Worsham, and Capobianco, 1973). Knife-cuts could also be achieved by manually raising and lowering the knife assembly with the trigger device in a locked position. The guide shaft could be rotated within the knife assembly and securely positioned so that it was fixed either at a 90^{o} (i.e. coronal) or exactly parallel (i.e. parasagittal) to the midline. All coronal cuts were made with the trigger-release

method so as to transect the relatively dense fiber tracts in the anterior hypothalamus. The parasagittal cuts were all effected manually with the trigger device in the locked position. Burr holes were drilled through the skull of all knife-cut subjects for the insertion of the knife assembly and the mid-sagittal sinus was exposed to allow a precise medial to lateral alignment with the aid of a stereotaxically-mounted microscope (Berner and Sclafani, 1975).

(a) Anterior coronal knife-cuts (ANT) were made just rostral to the ventromedial nuclei using stereotaxic coordinates determined from preliminary brain surgery. The guide shaft was positioned in the brain using the coordinate system of DeGroot (1967) at: AP=6.8, H=-2.5 and L=2.1. A 2.2mm cutting wire was then extended so that the transection continued for approximately 0.1mm on the contralateral side of the brain. The trigger device of the knife assembly was then activated so that a 2.5mm vertical excursion of the cut was achieved. After the cutting wire was retracted inside the guide shaft, the knife assembly was removed from the brain and the procedure duplicated on the contralateral side to produce a bilateral cut approximately 4.0mm in length.

(b) Parasagittal knife-cuts through the medial hypothalamus (MED) were made with the guide shaft positioned in the brain at DeGroot coordinates: AP=8.0, H=-2.5 and L=0.8. A 3.0mm cutting wire was then extended in a posterior direction and the cut effected manually by raising the knife assembly 2.5mm. Following retraction of the cutting wire the knife assembly was removed from the brain and the procedure duplicated on the contralateral side.

(II) Ovariectomy involved the bilateral removal of the ovaries, periovarial sac, and ovarian fat through dorsolateral flank incisions. The wall of the peritoneal cavity was sutured with surgical thread and the outer incisions closed with 9mm stainless-steel wound clips which were removed approximately two weeks following endocrine surgery.

(III) Sham knife-cuts for both anterior and medial hypothalamic transections involved positioning the rat in the stereotax, drilling the skull, and exposing the dura. Sham ovariectomized animals were treated exactly as were rats given ovariectomy except that no tissue was excised.

HISTOLOGY

At the conclusion of each experiment the knife-cut rats were sacrificed by an overdose of anesthetic and perfused intracardially with isotonic saline followed by a 10% formol-saline solution. After each brain was removed from the calvarium and stored for a minimum of three weeks in the formol-saline solution, the location of the neural transection was determined from 50 tissue sections stained with cresyl violet.

STATISTICAL ANALYSIS:

The food intake and body weight data from each experiment of the study were analyzed by either a two- or three-factor analysis of variance, where appropriate, for the factors surgery and injection condition over experimental days. Following significant F ratios the difference between all pairs of means was evaluated using a Newman-

Keuls test (Winer, 1962). Body weight change, rather than the absolute level of body weight was employed for statistical analysis since this has been shown to be a more accurate measure of the effects of several physiological manipulations on the state of energy balance (Collier, 1969).

EXPERIMENT 1

Coronal transections which pass through the anterior hypothalamic area (AHA) of adult female rats produce a moderate hyperphagia and obesity (Albert, Storlien, Albert, and Mah, 1971; Grossman, 1971), although when such cuts are restricted to within the boundary of the fornices these knife-cut-induced eating and weight facilitory effects are not obtained (Sclafani, 1971). The duration of the overeating and the degree of obesity produced by effective AHA transections closely resemble the pattern of increased food consumption and excess weight gain seen after chronic removal of estrogen by ovariectomy (Kakolewski, Cox and Valenstein, 1969; Tartellin and Gorski, 1973). This suggests that AHA knife-cuts may indirectly produce hyperphagia and obesity by causing a functional ovariectomy, as has been suggested by Albert et al. (1971), and this is consistent with neuroendocrine studies which reveal that AHA damage produces severe ovarian disturbances (Halasz, 1969). Also in accord with the hypothesis that transections of the AHA produce their feeding and weight promoting effects by altering ovarian function is the finding that similar knife-cuts in males fail to reliably alter eating or body weight (Mabry and Campbell, 1975; Paxinos and Bindra, 1972).

It seems possible, therefore, that AHA knife-cut-induced hyperphagia and obesity result incidentally from functional castration although it is also possible that such neural transections produce their effects by directly damaging estrogen-sensitive feeding neurons which may exist in the AHA (Simpson and DiCara, 1973) without necessarily

affecting ovarian functioning. Finally, AHA knife-cut-induced overeating and weight gain may result from the combined effects of functional castration and damage to estrogen-sensitive feeding neurons. The purpose of the present study was to examine the first two alternative hypotheses by directly comparing the effects of anterior transections and ovariectomy on the feeding and weight regulation of adult female rats. Since the ovarian hyperphagia-obesity syndrome is fully reversed by estrogen replacement therapy (see Wade, 1976), it was reasoned that if the feeding and weight facilitory effects of knife-cuts results secondarily from brain damaged-induced estrogen hyposecretion then replacement of this hormone should also reverse the effects of AHA damage on food intake and body weight. If, on the other hand, exogenous estrogen administration in knife-cut rats fails to suppress feeding and body weight, then the hypothesis of functional castration is not supported.

METHOD

Subjects: Fifty-two female rats ranging in body weight from 234-278 g. served as subjects and data are presented for 45 subjects that completed the study. Seven rats were discarded from the study as a result of misplaced knife-cuts, failure to survive endocrine surgery, or postoperative illness.

Procedure: Food intake and body weight measures were obtained daily for 21 preoperative and 80 postoperative days. Daily measures of water intake were recorded for five days prior to surgery and during postsurgical days 1-10, 25-30, and 75-80. Subjects were divided into

three groups equated for body weight and received one of the following surgical treatments: (a) ovariectomy (OVX); (b) coronal knife-cuts in the anterior hypothalamus (ANT); or (c) sham ovariectomy (CON-OVX) or sham knife-cuts (CON-ANT). Since the feeding effects of both sham surgical procedures were identical, their data were pooled to form one control group (CON). On postoperative Day 31 each group was subdivided into two groups equated for food intake and body weight gain prior to receiving injections of either estradiol benzoate (EB) or the sesame oil-vehicls (OIL). Hormone treated rats (OVX-EB, ANT-EB, and CON-EB) were injected with 1-ug of EB daily for twenty days followed by ten days of 5-ug EB injections (Days 51-60). Vehicle treated rats (OVX-OIL, ANT-OIL, and CON-OIL) were injected daily with oil for the 30 treatment days. On Days 61-80 all animals were handled but not injected.

Daily vaginal smears were taken on the morning of each of 14 preoperative and for 30 postoperative days and were classified as estrus, if they contained predominantly cornified cells, or as diestrus if the smear consisted almost entirely of leukocytes. At the time of surgery and on postoperative Day 80 body length measures were determined under anesthesia by placing the subject in a supine position and measuring the naso-anal length to the nearest 1.0mm with calipers. At the conclusion of the experiment the brains were prepared for histology as described. The ovaries of the knife-cut and sham operated rats were dissected free and their wet weights to the nearest 1.0mg were determined.

RESULTS

Histology

A photomicrograph of the anterior transections is presented in Figure 1A and a schematic representation is provided in Figure 2. The anterior knife-cuts produced a continuous coronal transection through the caudal half of the anterior nucleus of the hypothalamus at the level of AP=6.8 to 7.0. The height of the dorsal aspect of the cut extended from just beneath the anterior commissure to .3 to .5mm from the base of the brain. Some cuts extended more deeply and reached the ventral surface of the brain; presumably damaging the dorsal supraoptic commissure. The cuts sectioned the inferior aspects of the paraventricular nuclei medially and the arcuate nuclei just below it. In almost all animals the columns of the fornix were severed at least partially and the anterior transections also interrupted fibers of the stria terminalis in their more medial descent into the VMH. In a few rats the knife-cuts were displaced rostrally and dorsally by the more massive fibers comprising the stria medularis which were otherwise left intact. Laterally, the cuts extended to the medial edges of the lateral hypothalamus although in a few animals this damage was only unilateral.

Food Intake and Body Weight

The body weight findings of this experiment are illustrated in Figure 4 and Table 1 summarizes the food intake results. The analysis of variance for both of these measures are contained in Table 2. Pre-

operatively there were no significant differences among any of the six groups in their food consumption of body weights. Following surgery the knife-cut and ovariectomized rats were hyperphagic and gained weight rapidly during the 30-day pretreatment period. By Day 15, rats with ovariectomy or knife-cuts had gained more weight than did the controls (ANT=37.9 or OVX=30.7 vs. CON=6.4 g.; $p<.01$ for both comparisons) and were eating significantly more ($p<.05$) chow than were the controls (19.4 or 17.6 vs. 14.0 g/day, respectively). The food intake and weight gain differences between the ANT and OVX animals were not statistically different during the first fifteen postoperative days. However, just prior to the injections on Day 30, the knife-cut rats were still hyperphagic compared to controls whereas the ovariectomized animals were eating at a level only slightly and non-significantly above that of the controls. Both the ANT and OVX rats continued to outweigh ($p<.01$) the CON animals at the end of the first thirty postoperative days and by this time the food intake and the body weight differences between the ovariectomized and knife-cut rats became statistically reliable (both $p<.05$).

Following the 1-ug EB injections, the ovariectomized rats reduced their feeding and rapidly lost weight compared to oil-injected ovariectomized rats who continued to gain weight at the same level as did the oil-injected controls. By Day 50 the OVX-EB rats had gained less weight (37.5 vs. 71.5 g.; $p<.01$) and were consuming less chow (12.7 vs. 14.9 g/day; $p<.05$) than were the OVX-OIL animals. In contrast, the same hormone treatment failed to suppress the food intake or body weight of the ANT-EB compared to that of the oil-injected knife-

cut animals. At the end of the 20-day treatment period with the low dose of estrogen the ANT-EB and ANT-OIL groups had gained approximately the same weight from preoperative levels (93.3 vs. 100.1 g; $p > .05$; respectively) and were eating similar amounts of the chow diet (ANT-EB=19.6 vs. ANT-OIL=18.8 g/day; $p > .05$). Like both knife-cut groups, the control rats injected with either the hormone or oil-vehicle also gained approximately the same weight (CON-EB=15.9 vs. CON-OIL=16.4 g; $p > .05$) during this 20-day treatment period and both of these sham operated groups were consuming similar amounts of the chow diet on Days 48-50 (14.4 vs. 15.1 g/day; $p > .05$; respectively).

Comparisons between the ANT-OIL and OVX-OIL groups on Day 50 revealed that the knife-cut rats had gained more weight (100.0 vs. 71.6 g; $p < .05$; respectively) and were eating more chow (18.8 vs. 14.9 g/day; $p < .05$) than were the ovariectomized animals, who, in turn, were heavier ($p < .05$) than were the CON-OIL subjects (30.6 g.). The oil-injected ovariectomized animals continued to consume similar amounts of chow on Days 48-50 as did the control rats given the same injections (14.9 vs. 15.1 g/day; $p > .05$; respectively). The ANT-EB rats remained overweight and hyperphagic compared to hormone-treated ovariectomized and control animals by the end of this 20-day injection period and no significant food intake or body weight differences emerged between the latter two groups as a consequence of the 1-ug. EB injections.

Following the 5-ug EB injections which began on Day 51, the OVX-EB animals continued to undereat and lose weight, however, by

Days 58-60 they were actually consuming more chow, but not significantly so, than during the final three days of the 1-ug EB injections (13.3 vs. 12.7 g/day; respectively). When injected with the high dose of EB, the knife-cut rats for the first time reduced their food intake and ate less than did oil-injected knife-cut animals, however, this effect was short-lived and by Days 58-60 the ANT-EB animals were consuming only slightly less chow than were the ANT-OIL rats (15.8 vs. 16.7 g/day; $p > .05$ respectively). The controls injected with the 5-ug EB dose initially reduced their food consumption, as did the ANT-EB rats, and by the end of this 10-day treatment period they were also eating at or near the same level as were the oil-injected control rats (CON-EB=14.9 vs. CON-OIL=15.5 g/day; $p > .05$; respectively). Comparisons of body weight revealed that all three hormone injected groups lost similar amounts of weight as a consequence of the high-dose estrogen injections (ANT EB=6.7, OVX EB=4.5 and CON EB=5.2 g.). Over this same 10-day treatment period the relative feeding and body weight differences between the three oil-injected groups were maintained.

After the hormone treatment was terminated, all EB injected groups displayed an initial hyperphagia with the most pronounced and persistent feeding enhancement seen in the OVX-EB rats. At the conclusion of the experiment the ANT-EB rats had gained less weight than did the ANT-OIL animals (102.6 vs. 112.5 g; $p < .05$; respectively), although the ANT-EB animals were eating more, but not significantly so, than were the ANT-OIL animals on Days 78-80 (ANT EB=16.3 vs. ANT OIL=15.8 g/day). Both of the knife-cut groups also weighed more ($p < .05$) than did the hormone- and oil-treated ovariectomized groups

on Day 80. The OVX-OIL rats were not reliably heavier than were the OVX-EB rats (86.7 vs. 72.8 g.; $p > .05$; respectively) on Day 80 but the EB-treated ovariectomized rats were eating significantly more ($p < .05$) chow than were same-surgery oil-injected rats on Days 78-80 (17.2 vs. 14.0 g/day; respectively). Finally, both ovariectomized groups weighed significantly more ($p < .01$) than did similar hormone- or oil-treated control groups on Day 80 and neither the slight food intake or body weight differences that existed between the CON-EB and CON-OIL groups were statistically different at the conclusion of the experiment.

Water Consumption and Water/Food Intake Ratio

The water consumption and water/food intake ratios are summarized in Table 3 and the analysis of variance for both of these measures is contained in Table 4. Preoperatively there were no significant differences among any of the groups on these two measures. Following surgery, the knife-cut rats exhibited a more than three-fold increase in their fluid consumption. By Days 29-30 they were drinking significantly more ($p < .01$) water (79.5 vs. 25.0 ml/day) and their water/food intake ratio was significantly greater ($p < .01$) than that of the controls (3.58 vs. 1.71). The ovariectomized rats also increased ($p < .05$) their water consumption initially (OVX=33.0 vs. CON=23.5; ml/day; Days 9-10) but by Days 29-30 they were drinking at control levels (24.4 vs. 25.0 ml/day, respectively; $p > .05$), and their water/food intake ratio was not significantly different from that of the control animals (1.53 vs. 1.71; $p > .05$; respectively). Comparisons between the

ANT and OVX rats further revealed that the knife-cut-induced hyperdipsia exceeded ($p < .05$) the transient overdrinking produced by ovariectomy (75.5 vs. 33.0 ml; Days 9-10).

The final water intake measures obtained on Days 79-80 indicated that the knife-cut rats continued to drink more than did either the ovariectomized or control animals (ANT=61.0 vs. OVX=25.0 and CON=23.5, ml/day; $p < .05$ for both comparisons) and there were no statistical differences between the water consumption of the latter two groups. The water/food intake ratio of the knife-cut rats remained above the level of the ovariectomized and control rats on Days 79-80 (ANT=3.78 vs. OVX=1.60 and CON=1.68 $p < .05$ for both comparisons). There were no statistically significant differences in either the water consumption or water/food intake ratio within the ANT and CON groups (EB or OIL) as a result of prior hormone or oil vehicle injections during treatment Days 30-60. The OVX rats given EB, however, were consuming more water ($p < .05$) than the OIL-injected ovariectomized rats on Days 79-80 but their water/food intake ratio was almost identical to that of the OVX-OIL animals.

Vaginal Cytology and Ovarian Weights

Postoperatively the sham operated animals exhibited an initial interruption in estrous rhythm but as early as Day 12 the rats in each sham operated group had all displayed a complete ovarian cycle and except for one rat, all maintained a normal 4- or 5-day estrous rhythm thereafter. In contrast, only 25% of the knife-cut animals exhibited an estrous cycle by Day 12 and two of these four subjects failed to display

a second or third estrous rhythm. Seven of the remaining twelve knife-cut rats showed no cyclic activity during the 30-day pretreatment period and were considered to be anestrous since their vaginal smears were exclusively leukocytic. Three rats with brain damage showed a pattern of constant vaginal estrus for 30 days and two additional knife-cut subjects displayed a prolonged estrous phase (12 to 21 days) followed by one or two days of diestrus before resuming a pattern of continued estrous. Thus, of the sixteen rats who received knife-cuts, only two subjects showed no substantial postoperative modification in estrus cycling while of the remaining fourteen brain damaged rats, seven had vaginal smears that were predominantly leukocytic and five had smears that were classified as estrus. Histological verification of the transections did not indicate any noticeable difference in the location of the cuts between those animals with different vaginal cytologies.

The final ovarian wet weights of the knife-cut (ANT-EB and ANT-OIL) and control subjects (CON-EB and CON-OIL) are summarized in Table 5 and the analysis of variance is presented in Table 6. The ovaries of both brain damaged groups weighed significantly less ($p < .001$) than did their respective hormone or oil treated controls and no significant ovarian weight differences emerged between either the ANT-EB or ANT-OIL groups, or between the CON-EB or CON-OIL groups.

Naso-Anal Measurements and Lee Obesity Index Values

The pre- and postoperative naso-anal lengths and Lee Obesity

Index* values for all subgroups are summarized in Table 5 and the analysis of variance for these measures is contained in Table 7. Prior to surgery there were no significant differences among any of the six subgroups on either of these two measures. Eighty days following surgery, there were no differences in naso-anal lengths between knife-cut rats or control animals given similar hormone- or oil-injections (ANT-OIL=21.9 vs. CON-OIL=22.0 cm; and ANT-EB=22.2 vs. CON-EB=21.8 cm; $p > .05$ for both comparisons) and no significant differences in linear growth emerged between either of the knife-cut or between either of the sham-operated groups as a consequence of the prior hormone- or oil-vehicle injections. In contrast to the knife-cut rats, the ovariectomized animals significantly increased their lengths compared to same-treatment sham-operated controls (OVX-OIL=22.7 vs. CON-OIL=22.0 cm; and OVX-EB=22.7 vs. CON-EB=21.8 cm; $p < .01$ for both comparisons) and there were no significant differences in linear growth between the ovariectomized groups at the conclusion of the experiment.

The terminal Lee Obesity Index values of the knife-cut and ovariectomized rats were both significantly elevated compared to their respective hormone- or oil-injected controls (ANT-OIL=331 or OVX-OIL=313 vs. CON-OIL=301; $p < .01$ for both comparisons; and ANT-EB=333 or OVX-EB=311 vs. CON-EB=303; $p < .01$ for both comparisons). There were, however, no reliable differences in the final obesity index

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* The Lee Index is a reliable estimate of carcass fat content based on measures of body weight and naso-anal length (Bernardis, 1970); values that exceed 300 indicate obesity in terms of an excess in body fat.

values between either of the knife-cut groups or between either of the ovariectomized groups. Comparisons between the two surgical groups independent of prior hormone or oil injections revealed that the knife-cut rats had more carcass fat ($p < .05$) than did the ovariectomized animals by Day 80.

DISCUSSION

The present findings indicate that the ovariectomy hyperphagia-obesity syndrome is dissimilar from the feeding and body weight disorder produced by anterior hypothalamic knife-cuts in spite of an early resemblence in the overeating and weight gain between the two syndromes. That is, during the first thirty postoperative days the hyperphagia of the ovariectomized and knife-cut rats was of a similar magnitude and both surgical groups rapidly gained weight at near parallel rates. Thereafter, the elevated food intakes of the ovariectomized rats declined towards control levels and the body weights of the ovariectomized reached asymptote. In contrast to the ovariectomized rats, the knife-cut animals continued to overeat and gain weight throughout the entire postoperative period showing little or no sign of body weight stasis.

The hyperphagia-obesity syndromes produced by ovariectomy and by anterior knife-cuts are further distinguished from one another by differences in the feeding and weight responses observed following estrogen administration. That is, daily subcutaneous estrogen injections completely reversed ovariectomy-induced hyperphagia and obesity at doses that failed to inhibit brain damaged-induced overeating and

weight gain. The hormone injected knife-cut rats, however, like the controls injected with estrogen, were not insensitive to the anorexigenic effects of estrogen since both groups underate and lost similar amounts of weight when injected with the higher dose of the hormones.

Analysis of the water intake data of the anterior cut rats revealed a pronounced hyperdipsia which showed only a modest decline as late as the eleventh postoperative week. It is unlikely that this elevated water intake was a primary consequence of the knife-cut-induced overeating since the water intake of the brain damaged group exceeded the levelthat would have been anticipated based upon their hyperphagia alone. Stated another way, the water/food intake ratio of the knife-cut animals, but not the ovariectomized rats, was significantly elevated following surgery and remained above preoperative levels during the final water intake period. The hyperdipsia and elevated water/food intake ratio of the brain damaged rats confirms observations after similarknife-cuts placed in the anterior and posterior hypothalamus (Grossman, 1971; Sclafani and Berner, 1977) as well as after midbrain coronal transections (Wirtshafter, Pociask, Kent, Note 3). This suggests the existence of a thirst inhibitory system which travels longitudinally in the medial aspects of the diencephalon and is consistent with previous lesions findings which also indicate that such a neural thirst system extends through the level of the ventromedial hypothalamus (Gale, 1974).

In contrast to the marked and persistent hyperdipsia of the knife-cut rats in the present experiment, the ovariectomized animals displayed only a slight increase in their water consumption following surgery. This rise in water intake, unlike the hyperdipsia of the

knife-cut rats, was entirely coincident with the increased feeding of the ovariectomized animals. That is, the water/food intake ratio of the ovariectomized rats was never substantially elevated above pre-ovariectomy levels. The present results demonstrating an initial rise in the water intake following ovariectomy are consistent with earlier findings in same age (Tartellin and Gorski, 1971) and less mature ovariectomized rats (Leshner and Collier, 1973).

Differences in linear growth produced by ovariectomy and anterior knife-cuts further suggests that the two syndromes are independent of one-another. That is, ovariectomy caused an increase in linear growth while anterior cut rats had naso-anal lengths similar to those of the sham operated controls. Several studies have previously reported that damage to the VMH augments linear growth (Mitchell, et al., 1972; Palka Leibelt and Critchlow, 1971) and increases plasma growth hormone levels (Mitchell et al., 1973; Rice, Kroning and Critchlow, 1976), and this suggests that the VMH may contain elements of a neural system which normally inhibits growth. The present finding that rats with anterior knife-cuts grew at identical rates compared to animals with sham cuts suggests that such a neural system does not extend into the anterior hypothalamic-preoptic area of the brain. In contrast to rats with anterior cuts, the ovariectomized animals increased their linear growth and this confirms earlier findings (Nyda, Demajo, and Lewis, 1948). The failure of anterior cuts to alter somatic growth also suggests that such cuts do not produce a functional ovariectomy. A further distinction between the ovariectomy and anterior knife-cut-induced hyperphagia-obesity syndromes

is based on the marked differences in body fat produced by these two different surgical manipulations as estimated by Lee Index values. That is, while rats with either ovariectomy or anterior knife-cuts exhibited increased adiposity relative to that of the sham operated controls, the anterior cut rats were substantially more obese than were the ovariectomized animals by the end of the experiment.

In summary, significant differences in the degree of hyperphagia, magnitude of weight gain, and obesity index values suggests that the hyperphagia-obesity syndromes produced by ovariectomy and by anterior knife-cuts are likely to represent different feeding and body weight disorders. Further, differences in the pattern of water intake and measures of linear growth lend additional support to the view that the two syndromes have different etiologies. The finding that estrogen replacement therapy completely reversed ovarian, but not knife-cut-induced hyperphagia and obesity, strongly suggests that the primary cause of the brain damaged-induced feeding effects is not an estrogen insufficiency. These findings, however, do not preclude the possibility that reduced estrogen secretion may have added to the feeding and body weight increases produced by AHA knife-cut damage since examination of the vaginal cytology indicated that anterior transections almost invariable disrupted estrus rhythm, with prolonged diestrus the most frequently observed vaginal irregularity, and the ovaries of the knife-cut rats were underweight compared to those of the sham cut animals.

EXPERIMENT 2

The findings of the preceding experiment indicate that anterior knife-cuts and ovariectomy produce dissimilar feeding and body weight disorders but they do not completely rule out the possibility that gonadal failure may have contributed, in part, to the overeating and obesity caused by anterior hypothalamic damage. That is, following anterior knife-cuts an ovarian imbalance was indicated on the basis of prolonged estrous cycle irregularities and the subnormal ovarian weights of the brain damaged animals. A similar hypothesis that gonadal failure contributes to brain damaged-induced overeating has also been advanced by some to explain the greater weight gain seen in females compared to males following VMH electrolytic lesions in rats (Cox, Kakolewski and Valenstein, 1969; Valenstein, Cox and Kakolewski, 1969), and after goldthioglucos-induced chemical lesions in mice (Sanders, Lakey and Singh, 1973; Wright and Turner, 1973).

The main purpose of this experiment, therefore, was to determine the extent to which the hyperphagia-obesity syndrome produced by anterior knife-cuts was independent of the feeding and body weight changes caused by ovariectomy. This was achieved by first ovariectomizing the subjects and permitting them to overeat and stabilize their body weight prior to receiving the knife-cut surgery. If anterior cuts produce their feeding and weight facilitory effects by means of a functional ovariectomy, then rats with an already existing estrogen insufficiency should display no further feeding or weight changes following knife-cut surgery. If, on the other hand, the overeating and

obesity produced by anterior cuts is completely independent of ovarian influence then prior ovariectomy should not alter the expression of the knife-cut-induced hyperphagia-obesity syndrome. Further, it was reasoned that if both syndromes have separate etiologies then combined surgical treatments in the same animals should produce additive feeding and body weight effects.

Considerable evidence indicates that the anterior hypothalamic area plays an important role in the relationship between food consumption and body temperature regulation (Hamilton, 1975). For example, lesions of the anterior hypothalamic area raise colonic temperatures and reduce eating in normal ambient temperatures (Hamilton and Brobeck, 1964) whereas the artificial heating of this brain area lowers colonic temperature and increases eating in normal ambient temperatures (Hamilton and Ciaccia, 1971). The second purpose of this experiment was to examine the possibility that knife-cuts which damage the anterior hypothalamic area disrupt thermoregulation and may interfere with the animal's normal tendency to increase its feeding in cold ambient temperatures and decrease it in warm environments. To test this, rats with anterior knife-cuts were subjected to a series of abrupt shifts in environmental temperatures for brief periods.

METHOD

Subjects: Thirty-three female rats ranging in body weight from 233 to 257 g at the time of the initial surgery served as subjects and data are reported for 24 animals that completed the study.

Procedure: Food intake and body weight measures were obtained

daily for 10 preoperative and 170 postoperative days. The subjects were initially ovariectomized (OVX) or sham ovariectomized (SHAM) and fifty days later were given either anterior knife-cuts (ANT) or sham knife-cuts (SHAM). On Days 80-91 the rats were removed from the main colony and housed in a temperature and humidity controlled environmental chamber (Sherer 0ER68), in which the following ambient temperatures were maintained:(1) 24°C on Days 81-83; (2) 5°C on Days 84-85; (3) 24°C on Days 86-88; and (4) 35°C on Days 89-90. Prior to the temperature tests the core temperature of each subject was obtained by inserting a Yellowsprings thermister 5cm beyond the anal orifice. The subjects were returned to the main colony room on Days 91-170.

RESULTS

The body weight findings of this experiment are illustrated in Figure 5 and the feeding results are contained in Table 8. The analyses of variance for both of these measures are summarized in Table 9. Preoperatively there were no significant food intake or body weight differences among the four groups. Following surgery the ovariectomized rats were hyperphagic and rapidly gained weight, compared to animals given sham ovariectomy. By Days 18-20 the ovariectomized rats were eating more ($p < .05$) chow than the sham operated animals (OVX=18.1 vs. SHAM=15.8 g/day) and had gained significantly more ($p < .05$) weight than did the sham operated animals (61.9 vs. 27.6 g ; respectively). The difference in body weight gain between the ovariectomized and sham ovariectomized rats was further

increased ($p < .01$) by Day 50 (OVX=94.4 vs. SHAM=52.3 g.), although both operated and sham operated animals were consuming similar amounts of chow on Days 48-50 (15.9 and 15.5 g/day; respectively). Further analysis revealed that during Days 40-50, the ovariectomized and sham ovariectomized rats were increasing their weight gains at near identical rates just prior to the hypothalamic knife-cut surgery (OVX=.72 and SHAM=.74 g/day).

Following the knife-cut or sham brain surgery on Day 50, the SHAM+ANT and OVX+ANT groups were hyperphagic and gained weight rapidly. Thirty days following the operation the SHAM+ANT rats had gained more weight ($p < .01$) than did the SHAM+SHAM animals, as well as ($p < .05$) the previously obese OVX+SHAM animals [190.8 vs. 59.0 and 115.6 g ; respectively]. The SHAM+ANT rats were also consuming more ($p < .05$) chow than were either of the sham knife-cut groups on Days 78-80 [SHAM-ANT=35.2 vs. (SHAM+SHAM=15.5 or OVX+SHAM=16.5 g/day)]. The SHAM+ANT rats however gained less weight, but not significantly so, than did the OVX+ANT rats on Day 80 (190.8 vs. 207.0 g; respectively) and while both of these groups were hyperphagic compared to their respective non-cut groups, the differences between the knife-cut groups in their degree of overeating on Days 78-80 was not statistically reliable (SHAM+ANT=34.9 and OVX+ANT=35.2 g/day). During the thirty days following the brain surgery the knife-cut groups both gained similar amounts of weight, relative to their stable body weight levels just prior to the second operation (OVX+ANT=115.2 and SHAM+ANT=136.0 g; $p > .05$). The OVX+SHAM rats had gained more weight ($p < .01$) than the SHAM+

SHAM rats at the end of this thirty day period and both of these non-cut groups consumed similar amounts of the chow diet on Days 78-80 (16.5 and 15.5 g/day).

The mean core body temperatures obtained in the environmental chamber on Day 80 revealed that the groups with brain damage were hyperthermic ($p < .05$) compared to their respective endocrine controls (OVX+ANT=37.3° vs. OVX+SHAM=36.3°; SHAM+ANT=37.6° vs. SHAM+SHAM=36.2°). There were, however, no reliable differences in the colonic temperatures between the two groups given knife-cuts or between those given sham brain surgery. The feeding results of the temperature tests are illustrated in Figure 6. During the initial thermoneutral (24°C) period, the knife-cut groups continued eating more ($p < .01$) than did their respective non-cut controls, and neither the food intake difference between the cut, or between the non-cut groups was significant. Lowering the ambient temperature to 5°C resulted in a significant increase ($p < .05$) in the feeding of the non-cut groups while this brief cold stress failed to enhance, and in fact slightly decreased, but not significantly so, the food consumption of the cut group.

When the neutral ambient temperature was restored following the 5°C test, the non-cut groups significantly ($p < .05$) reduced their eating to the pretest baseline level. The knife-cut groups, when returned to the thermoneutral condition, did not reliably alter their feeding and continued to eat at the level seen during the just prior cold stress. Raising the ambient temperature to 35°C resulted

in a significant ($p < .01$) feeding reduction in all four group relative to that seen previously during the second thermoneutral test period (OVX+ANT=62.1%, OVX+SHAM=60.5%, SHAM+ANT=61.1%, and SHAM+SHAM=59.0%), although the knife-cut groups continued to consume more ($p < .05$) chow during the heat stress test than did their respective non-cut controls.

The subjects were returned to the main animal colony room on Day 91 and over the next 20-day period all four groups resumed a rate of weight gain similar to that observed prior to the temperature tests. During the final 50 days of the experiment the weight gain curves of the two knife-cut groups slowly began to diverge and by Day 140 the OVX+ANT had gained more ($p < .05$) weight than the SHAM+ANT animals (341.6 vs. 309.3 g; respectively). Over the same final 50-day period the OVX+SHAM rats maintained a significant ($p < .01$) and stable weight gain advantage over the SHAM+SHAM animals. At the conclusion of the experiment, on Day 170, the OVX+ANT rats had gained more ($p < .05$) weight than the SHAM+ANT animals (391.4 vs. 325.6 g; respectively) and both cut groups were consuming similar amounts of chow (30.1 and 28.3 g/day; respectively) on Days 168-170. Both knife-cut groups gained more ($p < .001$) weight and were eating more ($p < .01$) chow than their respective non-cut controls on Days 168-170. The OVX+SHAM rats, in turn, had gained more ($p < .05$) weight than did the SHAM+SHAM animals (140.7 vs. 89.3 g ; respectively) and there were no reliable food intake differences between these two groups on Days 168-170 (15.6 and 16.0 g/day). Finally, the terminal differences in weight gain between both cut groups (65.8 g)

closely approximated the differences in weight gain between the non-cut groups on Day 170 (51.4 g).

Histological examination of the knife-cuts revealed that they were bilaterally situated in the caudal portion of the anterior hypothalamic area and the damage produced by these transections was very similar to that observed in Experiment 1. All animals sustained bilateral damage to the medial edges of the lateral hypothalamus and the cuts extended slightly more ventral than that depicted in Figure 1A.

DISCUSSION

The finding that static obese ovariectomized rats consuming food at control levels will subsequently overeat and become more obese after anterior knife-cuts demonstrates that such neural transections do not cause their primary feeding and weight promoting effects by altering endogenous levels of estrogen. Rather, knife-cut induced overeating and weight gain appear to result directly from damage to the anterior hypothalamus or to fibers of passage which course through this brain region. The independence of the hyperphagia-obesity syndromes produced by ovariectomy from that following anterior knife-cuts is further supported by the present finding that the surgical manipulations produced near additive effects on weight gain when combined in the same animal. That is, the final difference in the weight gain between the ovariectomized and sham ovariectomized animals (i.e., ovarian obesity) very closely approximated the difference in the weight gain between the two knife-cut groups given

prior ovariectomy or sham ovariectomy. This additivity effect did not appear until the fifth or sixth postoperative week after the sham-ovariectomized knife-cut rats had begun to stabilize their food intake and body weight. The delayed expression of additivity, however, is not an unprecedented finding when neural and endocrine surgeries are combined in the same animal (Gale and Sclafani, 1977a; Kaestner, 1974; King and Cox, 1973) and may result from an initial ceiling effect on extremely rapid weight gain similar to the deferred weight gain seen in lactating rats given ovariectomy prior to the termination of nursing (Ota and Yokoyama, 1967).

The comparison between the SHAM+ANT and OVX+SHAM groups indicates that brain damage by itself produced a greater magnitude of hyperphagia and duration of overeating than did ovariectomy by itself. For example, the rats given knife-cuts alone remained hyperphagic compared to the SHAM+SHAM animals for 120 days following brain surgery, whereas after only 50 postoperative days the rats given ovariectomy alone had reduced their elevated intakes to control levels and remained normophagic thereafter. These findings indicating that ovarian hyperphagia, unlike the overeating seen after anterior cuts, persists for only several weeks following surgery and that the weight gain of the ovariectomized rats remains chronically elevated above control levels but below that of the knife-cut animals, confirm the results obtained earlier in Experiment 1.

The independence of the anterior knife-cut and the ovarian hyperphagia-obesity syndromes is further suggested by the present finding that, while ovariectomy did not alter the animal's colonic

temperature, anterior knife-cuts produced a marked elevation in the rat's core body temperature. The finding of knife-cut-induced hyperthermia is consistent with the observation of Hamilton and Brobeck (1964), that rats with extensive electrolytic lesions of the anterior hypothalamic area have elevated colonic temperatures. Despite this similarity, however, there appears to be few, if any, commonalities between the feeding effects produced by rostral hypothalamic lesions and those produced by anterior knife-cuts. For example, whereas the knife-cut rats in the present study were hyperphagic at 24°C, rats with rostal hypothalamic lesions are reported to be hypophagic (Hamilton & Brobeck, 1964). Further, the knife-cut rats of the present study displayed a feeding reduction when heat-stressed, unlike rats with rostral lesions, and when cold-stressed the knife-cut rats did not further increase their feeding in contrast to animals with anterior hypothalamic lesion tested at a similar environmental temperature (Hamilton & Brobeck, 1964). These differences between the temperature-dependent feeding responses of the knife-cut rats in the present study and those previously reported in animals with anterior hypothalamic lesions, suggest that while anterior cuts and lesions both produce hyperthermia they differentially effect a neural system involved in the thermogenic control of feeding.

With regard to chronic estrogen withdrawal, Marrone, Gentry and Wade (1976) have recently observed that soon after ovariectomy there is a drop in colonic temperature, but data concerning more long-term temperature changes in the rat following castration have not been previously reported. The present findings suggest that chronic removal

of estrogen, while it may produce more immediate changes in colonic temperature, does not interfere with the maintenance of a stable core body temperature whereas anterior hypothalamic knife-cuts, in contrast, produce hyperthermia as do lesions of this area, but the brain damaged-induced temperature and feeding effects may represent separate disorders.

The different feeding responses to abrupt shifts in temperature displayed by the knife-cut rats compared to the ovariectomized animals suggest, although perhaps less clearly, that the ovarian and anterior knife-cut hyperphagia-obesity syndromes are distinct from one another. For example, ovariectomized rats eating at control levels enhanced their feeding when placed in the cold whereas the knife-cut groups, in contrast, failed to augment their eating and this may have resulted from direct damage to brain areas previously implicated in the thermogenic control of feeding (see Hamilton, 1975).

It is possible that some additional factor, other than brain damage, may have contributed to the apparent feeding deficit of the knife-cut rats when placed in the cold. Both knife-cut groups, for example, were more obese than were the non-cut groups and this extra body fat may have decreased their heat loss in the cold and thus the body temperature of the anterior cut rats may have not dropped to a level which is necessary to mobilize additional feeding. On the other hand, compared to the non-cut groups the added insulation of the anterior cut rats did not exaggerate their feeding decreases when heat stressed. That is, the percent reduction in the anterior cut rat's food consumption relative to thermoneutral baseline levels was

similar to that seen in the non-cut groups. While other less obvious factors, or brain damage itself, may be responsible for the failure of the knife-cut animals to exhibit a feeding increase when cold stressed, it appears that they respond to heat as do the ovariectomized and sham ovariectomized rats by consuming less food. The present findings suggest, therefore, that compared to fully intact rats the mechanism(s) responsible for initiating overeating in the cold and undereating in the heat remain unaltered in ovariectomized rats but less well so in animals given anterior hypothalamic knife-cuts.

EXPERIMENT 3

If anterior knife-cuts produce their feeding and weight facilitory effects not incidentally by means of a functional ovariectomy, as the results of Experiments 1 and 2 indicate, but rather, by directly damaging feeding inhibitory fibers that course through the anterior hypothalamic area, then similar cuts in male rats should also produce overeating and obesity. Such cuts, however, have been reported not to reliably alter the food intake or body weight of male rats (Albert et al., 1971; Mabry and Campbell, 1975; Paxinos and Bindra, 1972).

It has been suggested previously (Cox, Kakolewski and Valenstein, 1969), that the effects of VMH damage on food intake and body weight are more readily attained in female rather than male rats and this may also apply in regard to the feeding and body weight effects produced by anterior knife-cut transections. One of the difficulties in observing brain damaged-induced overeating and weight gain in males compared to females may result from the accelerated growth rate seen in neurally intact males relative to females, and more recent evidence comparing brain damaged-induced obesity in males and females is consistent with this view (Gold, 1970; Rehovsky and Wampler, 1972).

The present study was designed to investigate whether or not anterior hypothalamic knife-cuts, like those which produced hyperphagia and obesity in female rats (Experiments 1 and 2), would have similar effects in males. Since previous studies using male

subjects have shown that parasagittal knife-cuts placed just medial to the fornix produce overeating and body weight gain (Albert et al., 1971; Gold, 1970; Mabry and Campbell, 1975; Paxinos and Bindra, 1972), unlike anterior transections, the effects of these parasagittal cuts on food intake and body weight were directly compared to those following anterior transections. Finally, to increase the likelihood of obtaining obesity the subjects were offered a high fat diet (Cox, Kakolewski and Valenstein, 1969).

METHOD

Subjects: Twenty-one male rats weighing from 366 to 391 g served as subjects and data are presented for 16 rats that completed the study. Three rats in the anterior transected group were discarded from the study as a result of grossly misplaced knife-cuts and the data of two additional rats discarded who developed chronic respiratory disease.

Procedure: Food intake and body weight measures were obtained daily for 10 preoperative and 35 postoperative days. Male subjects received one of the following surgical procedures: (a) anterior coronal knife-cuts (ANT), (2) medial parasagittal knife-cuts (MED), or (3) sham knife-cuts (CON). Prior to surgery and for the first 15 postoperative days the rats were maintained on Purina Chow (pellets). During the final 20 days of the experiment the pellet diet was removed and replaced with a high-fat diet consisting of 67% Purina Meal (powder) mixed with 33% Crisco vegetable fat (w/w). Since diets differing in caloric density were employed, the food intake

measures are expressed in terms of kcal (pellet diet=4.25 kcal/g and high-fat diet=5.92 kcal/g).

RESULTS

A photomicrograph of the medial knife-cuts is presented in Figure 1B and a schematic representation of these transections is illustrated in Figure 3. Histological analysis revealed that the medial cuts were situated between the medial and lateral hypothalamus in the parasagittal plane ranging from L=0.8 to 1.2. The medial cut extended rostrocaudally for about 3mm from the posterior half of anterior hypothalamic area to the premamillary region of the posterior hypothalamus. The medial cuts extended dorsally from slightly above the base of the brain to a level just beneath the inferior-most aspect of the paraventricular nucleus. The anterior knife-cuts were bilaterally situated in the coronal plane, essentially identical to those observed in the preceding experiment.

The body weight findings of this experiment are illustrated in Figure 7 and the feeding results are summarized in Table 10. The analyses of variance for body weight gain and caloric consumption are contained in Table 11. Preoperatively there were no significant differences in either the food intake or body weight measures among the three groups. When offered the pellet diet following brain surgery, both of the knife-cut groups (MED and ANT) exhibited accelerated weight gains compared to the animals given sham brain surgery. By Day 15 the MED and ANT rats had gained 68.4 and 48.9 g, respectively, which exceeded ($p < .05$) the 24.8 g weight gain displayed by the CON animals. The difference

in Day 15 weight gains between the two knife-cut groups fell short of significance. Food intake comparisons revealed that the MED rats were consuming more calories, but not significantly so, than were the ANT animals on Days 13-15 (157.1 and 144.0 kcal/day; respectively) and both knife-cut groups were overeating ($p < .05$) the pellets, compared with the intake of the CON rats on the last three days of this diet period (110.9 kcal/day).

When switched to the high-fat diet on Days 16-18, all three groups increased their caloric consumption compared with their previous pellet intakes on Days 13-15, but this increase was significant ($p < .01$) only for the knife-cut groups. The MED rats exhibited a 32% increase in their caloric consumption when offered the high-fat diet and this was greater ($p < .05$) than the 20% caloric increase displayed by the ANT animals. The CON rats, in contrast, increased their consumption on the high-fat diet by only 6% compared with their prior intakes on the pellet diet. By Day 35 the MED rats had gained more ($p < .01$) weight than did the ANT animals (236.2 vs. 140.2 g.), who in turn, gained more ($p < .05$) weight than did the CON animals (73.6 g) at the end of the experiment. The MED rats were consuming significantly more ($p < .05$) of the high-fat diet on Days 33-35 than were the ANT animals (187.7 vs. 131.2 kcal/day respectively) who, in turn, were eating slightly, but not significantly, more of the high-fat diet than were the CON animals (124.8 kcal/day) during the final three days of the experiment. Analysis of the body weight changes over the final 5-day period revealed that the medial cut animals were gaining weight at a more rapid rate than were either the anterior or

sham-cut rats [MED=9.2 vs. (ANT=3.1 or CON=2.8 g/day); $p < .05$ for both comparisons] while the difference between the latter two groups was not significant.

DISCUSSION

The results of this study indicating in male rats that parasagittal knife-cuts through the medial hypothalamus produced hyperphagia and obesity confirms the findings of other studies which have made similar medial transections (Albert et al., 1971; Gold, 1970; Mabry and Campbell, 1975; Paxinos and Bindra, 1972). The present results further indicate that knife-cuts through the anterior hypothalamus of male rats produce hyperphagia and obesity, as do the medial cuts, although the anterior knife-cut-induced feeding and body weight effects are less pronounced than those seen following the medial cuts. The finding that anterior knife-cuts produced overeating and weight gain in males strengthens the argument that an estrogen insufficiency alone could not adequately explain the hyperphagia-obesity syndrome in female rats. Also consistent with this view that a reduction in estrogen secretion is not the cause of anterior knife-cut-induced hyperphagia and weight gain, is the finding of Experiment 2 that prior ovariectomy does not block the full expression of anterior hypothalamic obesity. Anterior knife-cuts in males may have disrupted the secretion of testosterone but unlike estrogen the removal of the male sex hormone results in a modest feeding reduction and body weight loss (Kakolewski, Cox & Valenstein, 1968; Leshner & Collier, 1973); both of which are reversed by hormone replacement therapy (Gentry & Wade, 1976).

The failure of other investigators (Albert et al., 1971; Mabry and Campbell, 1975; Paxinos and Bindra, 1972), to reliably obtain overeating and excess weight gain in males following anterior knife-cuts, in contrast to the present findings, is not entirely clear. It is likely, however, that the exact lateral extent of the coronal transection was an important factor. That is, the present anterior knife-cuts were not confined to the most medial aspects of the medial zone of the hypothalamus, as were the knife-cuts previously used, but rather, extended more laterally into the perifornical region of the lateral hypothalamic zone. Recent evidence (Grossman and Hennessy, 1976; Sclafani and Berner, 1977) suggests that the fibers whose destruction is responsible for the overeating and obesity induced by hypothalamic brain damage probably travel through this perifornical region. However, since the exact position of the anterior cuts was not systematically varied in the present study a conclusion that the lateral extent of the cut was critical awaits further confirmation.

That the medial and anterior hypothalamic knife-cuts were each effective in producing hyperphagia and obesity in male rats is consistent with the view that both neural transections may have damaged a common pathway. The existence of such a pathway which may course through the VMH and possibly through the caudal portion of the anterior hypothalamic area has recently been suggested (Sclafani and Berner, 1977). The present finding that the medial cut rats, when switched from the pellet to the high fat diet, exhibited an exaggerated hyperphagia confirms the well known observation that animals with VMH lesions or knife-cut damage are finicky to good tasting diets

(Graff and Stellar, 1962; Miller, Bailey and Stevenson, 1950; Sclafani, Berner and Maul, 1975; Teitelbaum, 1955). The additional finding that anterior cut rats, like the medial cut animals, displayed a similar overconsumption when the high fat diet was initially presented is also consistent with the hypothesis that both knife-cuts may have damaged a similar underlying pathway.

While recognizing the limitation of comparisons between experiments, the analysis of the weight gains displayed by the anterior cut males in the present study and those exhibited earlier by the female subjects in Experiments 1 and 2 reveals that males and females gained similar amounts of weight (48.9 and 55.3 g; respectively), when fed the same pellet diet for 15 days. However, the changes in the weight gain of control males and females during the same 15-day period indicated that neurally intact males gained nearly twice as much weight as did females (24.8 and 12.8 g; respectively). Thus, although the absolute weight gains of anterior knife-cut males and females were quite similar, comparisons with same-sex controls indicated that the cut females actually displayed a 332% increase in weight gain whereas the cut males exhibited a 97% increase in weight gain. This finding suggesting similar absolute weight gains in males and females following anterior knife-cuts is in accord with recent evidence showing that VMH lesions and knife-cuts produce comparable weight gains in both sexes (Gold, 1970; Rehovsky and Wampler, 1972; Sclafani and Sperber, 1977). Furthermore, this similarity in brain damaged-induced body weight gain between males and females has also been shown when obesity is evaluated not as body weight per

se, but rather in terms of carcass fat deposition (Bernardis and Bellinger, 1976). Thus, the greater likelihood of obtaining obesity following VMH damage in females than in males (Cox, Kakolewski and Valenstein, 1969) may be due, in a large part, to the less rapid rate of weight gain of intact females compared to males.

EXPERIMENT 4

The results of the previous experiments demonstrated that transections which pass through the caudal half of the rostral hypothalamus produced overeating and body weight gain in adult rats of either sex. Since exogenous injections of estrogen in females failed to alter knife-cut-induced obesity at doses that completely reversed ovarian obesity (Experiment 1), it appears unlikely that the hyperphagia and obesity syndrome resulting from anterior knife-cuts is caused by a chronic estrogen insufficiency. Also arguing against functional castration as a possible cause of anterior knife-cut obesity is the finding that males given anterior neural transections exhibit a body weight gain comparable to that seen following similar hypothalamic transections in females (Experiment 3). Moreover, it appears that the ovarian and anterior hypothalamic obesity syndromes are almost fully independent of one another since the combined body weight effects of the two physiological manipulations are near additive when the rat is subjected to both surgeries (Experiment 2).

It remains possible that anterior brain damage, while not producing a functional castration, results in hyperphagia and obesity by directly damaging estrogen-sensitive feeding neurons in the anterior hypothalamus. Such damage, in effect, could reduce the estrogenic restraint on feeding and body weight at the level of the central nervous system without necessarily altering blood estrogen levels. This hypothesis of direct neural damage is not incompatible with the finding that ovariectomy and anterior cuts together produce near

additive body weight effects since the transections are likely to damage only some of the putative estrogen-sensitive feeding neurons in the anterior hypothalamus.

The finding which is most directly concerned with the hypothesis that estrogen-sensitive feeding neurons exist in the rostral hypothalamus indicates that animals with anterior cuts respond like neurally intact rats to estrogen's feeding and weight suppressing effects (Experiment 1). However, the ovarian and estrus rhythm data from the same experiment revealed that plasma estrogen levels were probably altered as a result of the cut and therefore the interpretation of normal responsivity to peripheral estrogen administration is confounded by unknown, and possibly unsystematic, changes in the level of endogenous ovarian hormones. In view of this, the present study was designed to reexamine whether or not rats with anterior cuts would respond like neurally intact animals to the feeding and weight suppressive effects of exogenous estrogen administration. To achieve this, ovariectomized rats received anterior knife-cuts and were later injected peripherally with estrogen. To explore further the food intake and body weight similarities between rats with anterior and medial cuts which were observed in Experiment 3, an additional group of subjects in the present study received medial hypothalamic transections and, like the anterior cut rats, were subsequently administered a series of estrogen injections.

METHOD

Subjects: Forty-two female rats weighing from 231 to 268 g

served as subjects and data are presented for 34 rats that survived the combined surgical treatments.

Procedure: Food intake and body weight measures were obtained daily for 14 preoperative and 130 postoperative days. All subjects were ovariectomized (OVX) and two days later received one of the following surgical treatments: (1) anterior knife-cuts (ANT); (2) medial knife-cuts (MED); or (3) sham surgery (CON). On day 31, the subjects in each group were divided into sub-groups equated for food intake and body weight gain and given daily injections of estradiol benzoate (EB) or the oil-vehicle (OIL). Hormone treated groups (ANT-EB, MED-EB, and CON-EB) were injected daily with 1-ug. EB for 10 days, followed by daily 5-ug. EB injections for 50 days. Vehicle treatment groups (ANT-OIL, MED-OIL, and CON-OIL) were injected each day with oil for 60 treatment-days. On Days 91-130 all six groups were handled daily but not injected.

RESULTS

The body weight findings of this experiment are shown in Figure 8 and Table 12 summarizes the food intake measures. The analyses of variance for body weight gain and food intake are contained in Table 13. Prior to surgery there were no significant differences among any of the groups in their food intakes or body weights. Following knife-cut surgery the MED and ANT groups were hyperphagic and rapidly gained weight compared to the CON group which received sham brain surgery. The weight gain of the medial cut rats was significantly greater ($p < .05$) than that of the anterior cut animals by Day 30 (MED=

151.7 vs. ANT=98.2 g) and both cut groups had gained more weight ($p < .01$) than did the control subjects thirty days following surgery (CON=56.4 g.). The food intake data on Days 28-30 revealed that the medial cut rats were also eating more ($p < .05$) chow than were the anterior cut animals (MED=49.2 vs. ANT=44.9 g/day) and both brain damaged groups were hyperphagic ($p < .01$) compared to the sham operated controls just prior to the treatment period (CON=25.1 g/day).

During the initial ten days of estrogen injections (1-ug EB/day), which began on Day 30, the hormone-treatment groups (MED-EB, ANT-EB and CON-EB) each gained less ($p < .05$) weight than did their respective oil-treatment controls. By Day 40 all three hormone-injected groups had lost similar amounts of weight relative to the weight changes exhibited by their appropriate oil treatment controls [MED (OIL vs. EB)= 25.2]; [ANT (OIL vs. EB)= 27.1]; and [CON (OIL vs. EB)= 23.6 g]. Analysis of the food intake data revealed that each of the hormone-treatment groups was also eating less ($p < .05$) chow than were their respective oil-treatment controls on Days 38-40. However, both of the hormone-injected knife-cut groups were consuming more ($p < .05$) chow than were the EB-injected sham cut animals on the final three days of this treatment period. The medial and anterior cut groups treated with oil for this 10-day injection period continued to gain weight ($p < .05$) compared to controls injected with oil, and both of these brain damaged oil-treatment groups were hyperphagic ($p < .05$) relative to the oil-injected group given sham brain damage.

When injected with the high-dose of EB, the medial cut rats did not initially alter their rate of weight change compared with that seen

earlier during the low-dose EB injections but by Days 71-80 their rate of weight change had declined to a level below ($p < .05$) that observed on Days 31-40 and the MED-EB rats actually lost weight during the remaining 10 treatment days. The anterior cut rats, who had lost weight when injected with the low-dose of EB initially, exhibited a further weight reduction ($p < .05$) when injected with the high-dose of EB by Days 51-60 and continued to lose weight during the remaining 30 treatment days. The sham cut rats, who also lost weight during the prior low-dose of EB, lost additional weight ($p < .05$) when injected with the high-dose of EB by Days 41-50. Thereafter, the CON-EB rats showed only a slight additional weight loss, despite the continued EB injections. Analysis of the food intake data on Days 88-90 revealed that the MED-EB rats were eating less ($p < .01$) chow than were the MED-OIL animals and, while both the ANT-EB and CON-EB rats were also consuming less chow than were their respective oil-treated controls, neither of these two differences reached significance.

At the conclusion of the estrogen treatment on Day 90, the body weight changes of the MED-EB and ANT-EB groups were very similar compared to the weight changes displayed by their appropriate oil-treated knife-cut controls [MED (OIL vs. EB)= -116.1] and [ANT (OIL vs. EB)= -106.0 g.; $p > .05$]. Both of these weight losses were greater ($p < .05$) than the relative weight loss exhibited by the controls given OIL or EB over the same 60-day treatment period [CON (OIL vs. EB)= -69.0 g.)].

Comparisons among the oil groups revealed by Day 90 that the medial cut rats had gained more ($p < .01$) weight than

had similar oil treated anterior cut animals (319.3 vs. 159.4 g ; respectively) and were eating more ($p < .05$) chow than were the oil-treated anterior-cut rats on Days 88-90. The ANT-OIL rats, in turn, had gained more weight ($p < .05$) than did the CON-OIL animals on Day 90 (159.4 vs. 88.8 g ; respectively), however, both groups were consuming similar amounts of chow during the final three oil-treatment days.

Following the termination of the hormone injections, the three EB groups rapidly increased their feeding within the first few post-injection days and renewed their preinjection weight gains. By Days 108-110 the CON-EB animals were consuming only slightly more chow than were the CON-OIL rats (23.8 vs. 23.0 g/day; respectively) whereas both knife-cut groups injected with EB were hyperphagic ($p < .01$) compared to their respective OIL injected controls [MED (EB vs. OIL)= 42.1 vs. 36.4 g/day; respectively and ANT (EB vs. OIL)= 37.3 vs. 26.2 g/day; respectively]. By the conclusion of the experiment on Day 130 the MED-EB rats had gained less weight ($p < .05$) than did the MED-OIL animals (356.0 vs. 404.4 g; respectively) but the MED-EB animals were eating more ($p < .05$) chow than were the MED-OIL rats on Days 128-130. Both medial cut groups had also gained more weight ($p < .01$) and were eating more chow ($p < .05$) than were their respective hormone- or oil-treated anterior cut groups on Day 130. The ANT-EB rats gained less weight, but not significantly so, than did the ANT-OIL animals by Day 130 (164.7 vs. 190.2 g ; respectively), but they were eating more ($p < .05$) chow than were the oil-treated anterior cut rats on Days 128-130. Finally, both

anterior groups had gained more weight ($p < .05$) and were eating more chow ($p < .01$) than were their respective EB- or OIL-injected sham cut controls while neither the food intake or differences in body weight gain between either of the sham cut treatment groups were statistically significant at the conclusion of the experiment.

Histological examination of the transections revealed that the medial cuts were situated in the parasagittal plane, just medial or lateral to the fornix, as indicated in Figure 3 and extended from the anterior hypothalamic area to the posterior hypothalamus. The anterior cuts were very similar to those of the previous experiments and are represented schematically in Figure 2.

DISCUSSION

The present finding that anterior hypothalamic transections produced overeating and body weight gain in ovariectomized rats confirms the results obtained earlier in Experiment 2. Further, the finding that anterior knife-cut-induced obesity in ovariectomized rats exceeded the level of obesity following sham knife-cuts in ovariectomized animals supports the hypothesis that functional castration is not the primary cause of anterior hypothalamic obesity. The present finding that medial hypothalamic cuts also produced obesity confirms the results of numerous other studies (Gold, 1970; Paxinos and Bindra, 1972; Sclafani and Grossman, 1969) and that the obesity produced by the medial cuts was greater than that seen after anterior cuts confirms the findings obtained earlier in Experiment 3 with male rats.

Analysis of the hormone-induced effects on food intake and body weight measures revealed that neither the anterior nor medial cuts blocked estrogen's feeding and weight inhibitory effects. That is, both knife-cut groups injected with the low dose of estrogen for 10 days reduced their eating and lost approximately the same amount of weight relative to their respective oil-injected controls. More importantly, the relative weight losses sustained by both knife-cut groups during this 10 day hormone treatment period were nearly identical to the weight loss exhibited by the estrogen-injected sham cut rats relative to oil-treated controls. The more long-term observation with the higher dose of the hormone also revealed that neither transection prevented the weight reducing effects of estrogen and both knife-cut groups lost similar amounts of weight over the 60 days of EB treatment relative to appropriate oil-injected controls. In fact, the relative weight loss of the knife-cut groups injected with estrogen was greater than that lost by the sham cut rats injected with EB for a similar duration. Thus, it is unlikely that either neural transections damaged an estrogen-sensitive feeding pathway since the most probably outcome of such damage would have been to attenuate estrogen anorexia not to potentiate it.

The finding that the weight loss induced by the hormone injections in the ANT-EB rats was greater than that seen in the CON-EB animals, however, is not entirely consistent with the hypothesis that anterior knife-cuts and ovariectomy produce additive body weight effects when combined in the same animal. That is, since all subjects were ovariectomized in the present study and the earlier

results of Experiment 1 indicated that anterior transections did not substantially modify estrogen anorexia, then EB treatment would be expected to produce comparable body weight reductions in both groups. A similar question also arises in interpreting the present finding of a greater weight loss induced by hormone injections in MED-EB rats relative to CON-EB animals, since other evidence indicates that medial transections and ovariectomy also produce additive body weight effects (Gale and Sclafani, 1977a).

The greater weight loss of the hormone injected knife-cut rats compared to the sham cut animals may have resulted from factors other than neurological insult. For example, previous studies have shown that estrogen's weight reducing effects are more pronounced in heavy versus lean intact rats (Zucker, 1972), and animals made overweight by dietary means are more susceptible to estrogen anorexia than are animals given only a standard chow (Redick et al., 1973). Thus, the level of obesity itself may be an important factor in assessing estrogen's weight reducing effects. It has also been reported that there is a lower limit beneath which even high doses of estrogen will not produce further body weight losses (Mook et al., 1972) and since the CON-EB rats were only mildly obese compared to the hormone-treated knife-cut rats, it is likely that they reached this lower body weight limit sooner than did the knife-cut rats. Continued estrogen injections, according to this theory, would produce additional weight losses only in the still overweight knife-cut animals. Thus, differences in the body weight level at the start of the hormone injection between the knife-cut and sham cut rats combined with a lower limit restricting

continued estrogen-induced weight loss may account for the present finding of a greater weight loss in the EB-injected ovariectomized knife-cut compared to ovariectomized sham cut animals given similar hormone injections.

In summary, the present knife-cut findings reveal that neither anterior nor medial hypothalamic transections block the anorexigenic effects of systemic estradiol injections. The present finding with anterior cuts in ovariectomized animals confirms the results obtained earlier in Experiment 1 and the further disclosure that medial cut, like anterior transections, also fail to block the feeding and the body weight reducing effects of estrogen replacement complements other evidence that medial transections do not prevent the expression of the ovarian hyperphagia-obesity syndrome (Gale and Sclafani, 1977a).

Finally, the postinjection finding that the feeding and weight suppressive effects produced by estrogen are reversed following the termination of hormone injections indicates that the observed feeding and body weight suppression effects are hormone dependent. That is, the three estrogen-treated groups displayed an almost immediate postinjection hyperphagia and weight gain and, by the end of the experiment, they had gained as much (CON-EB) or only slightly less weight (ANT-EB and MED-EB) than did their respective oil-injected controls. Since both hormone-injected knife-cut groups were hyperphagic relative to their oil-injected controls, it seems likely that the hormone-treated knife-cut groups would have reached the levels of their appropriate oil-injected controls if a longer postoperative period were provided.

EXPERIMENT 5

The results of the previous experiment demonstrated that anterior hypothalamic knife-cuts did not block the feeding and weight suppressive effects of peripheral estrogen injections. It is well known that estrogen stimulates spontaneous locomotor activity in adult rats (Stern and Murphy, 1972; Young and Fish, 1945) and during the 4-day estrous cycle rats display an activity rhythm which correlates with plasma estrogen levels as revealed by vaginal cytology (Brobeck, Wheatland and Strominger, 1947; Slonaker, 1924; Wang, 1923). Other evidence indicates that implants of estrogen directly into the anterior hypothalamic area increases wheel running activity (Colvin and Sawyer, 1969; Wade and Zucker, 1970). Thus, it is possible that anterior knife-cuts, while sparing an estrogen-sensitive feeding system, interfere with estrogenic influences on spontaneous locomotor activity. Consistent with this hypothesis is the finding that anterior hypothalamic transections significantly depress wheel running activity and disrupt estrous cycling Sclafani, 1971).

The purpose of the present study, therefore, was to examine whether or not anterior hypothalamic knife-cuts would alter the effects of estrogen on the spontaneous locomotor activity of adult rats. Since anterior transections may disturb ovarian cycling, as is indicated in Experiment 1, and this might effect locomotor activity, all subjects were ovariectomized prior to brain surgery as in the preceding experiment. In view of the similarity observed earlier in Experiment 3 between the feeding and body weight effects produced by anterior and

medial knife-cuts, the present study also investigated the locomotor effects produced by parasagittal knife-cuts through the medial hypothalamus.

METHOD

Subjects: Thirty-six female rats ranging in body weight from 231-255 g served as subjects and data are reported for 30 rats that survived both the endocrine and hypothalamic surgeries.

Procedure: Subjects were housed on arrival in Wahmann LC-34 activity cages for a period of three weeks and given ad libitum access to Purina Chow (pellets) and water. Daily spontaneous activity measures (revolutions/24 hr) and body weight were recorded during the last two weeks of this preoperative baseline period and for fifty-nine days following brain surgery. All subjects were ovariectomized and subdivided into three groups equated for body weight and activity scores. Two days later each group received either: (1) anterior coronal knife-cut (ANT); (2) medial parasagittal knife-cuts (MED); or (3) sham surgery (CON). Five weeks following brain surgery, each of the three groups was subdivided into three subgroups equated for body weight and activity scores and injections daily with either estrogen (EB) or the oil vehicle (OIL). The hormone treatment groups (ANT-EB, MED-EB and CON-EB) were injected for 25 days with 5-ug EB/day whereas the oil treatment groups (ANT-OIL, MED-OIL, and CON-OIL) were injected daily with equal volumes of the vehicle over the same 25-day period. At the end of the treatment period

on Day 59 the hormone injected animals were overdosed with an anesthetic and the brains of the knife-cut animals prepared for histology. Due to intragroup variability in running scores following brain surgery, the activity measures were expressed as a percentage of the baseline wheel running.

RESULTS

The postoperative changes in wheel running are depicted in Figure 9 and the analysis of variance for this measure is contained in Table 14. The body weight gains of the ovariectomized rats following knife-cuts or sham surgery are shown in Table 15 and the analysis of variance for these data are summarized in Table 14. Preoperatively, the three groups were well matched on wheel running (ANT=3182, MED=3203, and CON=3239 rev/24 hr) and there were no significant differences in the preoperative body weights of the three groups (241.2, 242.1, and 238.5 g.; respectively). Following the combined endocrine and brain surgery, all groups reduced ($p < .01$) their activity. By Day 7 the medial rats were hypoactive ($p < .05$) compared to the anterior animals; the medial rats were running at 5.8% of baseline and the anterior rats were running at 19.9% of baseline. Both of the knife-cut groups were significantly hypoactive ($p < .05$) when compared to the control rats, who, after one week, were running at only 46.5% of baseline.

In the second postoperative week the controls showed a mild increase ($p < .05$) in their activity, however, during the remaining three non-injection weeks their wheel running gradually declined and

stabilized at or near the level observed at the end of the first post-surgical week (Day 35: CON=45.1% of baseline). Rats with anterior and medial knife-cuts exhibited only mild activity increases during the second postoperative week and during the remainder of the non-injection period the medial cut rats continued to run less ($p<.05$) than did the anterior cut animals. By Day 35 the medial cut rats were running at 10.7% of their baseline activity level which was significantly less ($p<.05$) than the 26.2% activity decrease displayed by the anterior cut rats, and compared to controls, both cut groups remained hypoactive ($p<.05$) five weeks following brain surgery.

Over the first 35 postoperative days the medial cut rats gained more weight than did the anterior cut animals (117.7 vs. 88.9g; respectively) although this difference failed to be significant. Both knife-cut groups, however, gained more ($p<.05$) weight than did the CON animals (53.6g).

During the 25 hormone treatment days, the ANT-EB, MED-EB, and CON-EB rats all increased ($p<.05$) their wheel running activity compared to the levels seen earlier during the last week of the non-injection period. The EB treated groups were also more ($p<.05$) active than were their respective OIL injected groups but this difference was significant only for the medial and sham cut groups. By the final injection day the MED-EB and CON-EB rats were running slightly above their preoperative activity levels (102.4% and 107.9% of baseline; respectively) and there were no reliable activity differences between these two groups. Both the MED-EB and CON-EB groups were more active ($p<.05$) than were the ANT-EB rats on Day 59 who were running

at only 57.2% of their baseline activity. The ANT-OIL, MED-OIL, and CON-OIL groups, on the other hand, displayed no substantial change in their wheel running activity over the 25 treatment days, compared to their activity levels during the final week of the non-injection period. By the final oil treatment day, the MED-OIL rats were running at 10.1% of their baseline level which was less ($p < .05$) than the 27.6% activity level of the ANT-OIL rats and both knife-cut groups remained significantly hypoactive ($p < .05$) compared to the CON-OIL rats on Day 59 who were running at 43.3% of their baseline activity.

The body weight findings for Days 35-59 reveal that the MED-EB rats lost slightly, but not significantly, more weight than did the ANT-EB animals (58.9 vs. 41.2 g ; respectively), and both knife-cut groups lost more ($p < .01$) weight than did the CON-EB rats (20.1 g). By the end of the hormone treatment period an analysis of the body weight gains from the preoperative baseline period revealed that the MED-EB and ANT-EB rats gained only slightly more weight than did the CON-EB animals (Table 15). During treatment days 35-59, the body weight gain of the MED-OIL rats exceeded ($p < .01$) that of the ANT-OIL rats (63.6 and 22.9 g ; respectively) and the ANT-OIL animals, in turn, gained more ($p < .05$) weight than did the CON-OIL rats (7.2 g). Analysis of the Day 59 weight gain from the preoperative baseline period revealed that the MED-OIL rats gained more weight ($p < .01$) than did the ANT-OIL animals, who in turn, outgained ($p < .01$) the CON-OIL rats (Table 15).

Histological examination revealed that the medial knife-cuts

were essentially identical to those seen in the previous experiments. The medial transections were positioned just medial or lateral to the fornix and extended from the anterior hypothalamic area to the posterior hypothalamus (Figure 3). The anterior knife-cuts were positioned in the caudal half of the anterior hypothalamic just rostral to the VMH, as described in Experiment 1, and extended bilaterally from the midline to the medial edge of the LH (Figure 2).

DISCUSSION

Ovariectomy has long been known to reduce, and estrogen replacement to restore, the spontaneous locomotor activity of adult female rats (Young and Fish, 1945). The present finding that sham knife-cut ovariectomized rats failed to reestablish locomotor activity at preovariectomy levels without estrogen replacement confirms the importance of this hormone in the maintenance of spontaneous activity. More importantly, the present finding demonstrating that ovariectomized rats given hypothalamic transections exhibited a greater reduction in wheel running than did animals given sham cuts demonstrates that neither the activity depressing effects of anterior or medial hypothalamic knife-cuts are dependent on changes in ovarian hormone levels.

A reduction in wheel running has previously been reported after lesions in the vicinity of the ventromedial nucleus of hypothalamus (Gladfelter and Brobeck, 1962; Hetherington and Ranson, 1940; Kennedy and Mitra, 1963) and the present finding that hypothalamic knife-cuts produce hypoactivity suggests that these cuts may alter

locomotor activity by transecting some of the same neural pathways destroyed by VMH lesions. The present finding of a greater reduction in wheel running after medial rather than anterior transections confirms previous knife-cut findings (Sclafani, 1971). Thus, it is possible that despite some quantitative differences between the wheel running reductions exhibited by the knife-cut groups both neural transections damaged a common activity pathway, such as that suggested by Kennedy (1964) or by Colvin and Sawyer (1969), which travels diffusely through the hypothalamus. On the other hand, the present finding that estrogen administration increases the activity of rats with medial knife-cuts to preovariectomy levels whereas, the same hormone treatment was much less effective in restoring the activity of rats with anterior knife-cuts suggests that both neural transections may have damaged, to some degree, separate activity systems.

Wade and Zucker (1970) have theorized that spontaneous locomotor activity is influenced by two brain areas, namely an estrogen sensitive region in the anterior hypothalamic-preoptic area (AHA-POA) and an estrogen insensitive region localized within the ventromedial-lateral hypothalamic area (VMH-LHA). The proposed involvement of these two brain areas in modulating locomotor activity was based, in part, on their cannulation findings and those of Colvin and Sawyer (1969) which indicated that implants of estrogen directly into the AHA-POA increased, whereas implants into the VMH-LHA did not alter the wheel running activity of rats. Other evidence suggesting that the AHA-POA contains estrogen-sensitive

activity neurons is the finding that damage to this area by the cellular disruption produced by implants of actinomycin-D selectively block estrogen-induced wheel running (Stern and Jankowiak, 1972). One exception to the finding that the VMH-LHA is insensitive to estrogen-mediated activity effects is the observation by Kennedy (1964) that lesions in the ventromedial region of the hypothalamus appear to abolish the locomotor increases produced by systemic injections of this hormone. However, several of the lesions that blocked estrogen-induced activity changes were also located in the AHA-POA and since the rostral border of the ventromedial nucleus was probably destroyed by the ventromedial area lesions, it is not unlikely that the failure of these brain damaged animals to increase their activity in response to estrogen injections may have resulted from overlapping neural damage which extended into the region just anterior to the ventromedial nucleus. Such an interpretation is consistent with Kennedy's own conclusion that the ventromedial nucleus is insensitive to estrogen and that rats with lesions of this nucleus fail to increase their running in response to estrogenic stimulation because of direct damage to activity neurons situated more rostally in the anterior hypothalamus.

The present finding that the ovariectomized medial and anterior knife-cut rats were both hypoactive during the non-injection period, but to differing degrees, suggests the possibility that medial and anterior cuts may have damaged the same activity system, such as that in the VMH-LHA region, with the medial cut damage exceeding that produced by the anterior cuts. However, the finding that the

medial cut rats, unlike the anterior cut animals, increased their activity to baseline levels in response to estrogen, as did the sham cut rats, supports the view that only the anterior cuts damage an estrogen-dependent activity system such as that which may exist in the AHA-POA area. Thus, while an examination of activity levels during the non-injection period alone do not provide any suggestive evidence that the differential activity effects of the medial and anterior cuts are other than quantitative, activity comparisons between the cut groups following estrogen injections reveals a distinct qualitative difference between the cuts. The present findings indicating that the medial and anterior cuts damage an activity system which may be estrogen-independent but only the anterior cuts damage an estrogen-dependent activity system are readily compatible with the proposal of Wade and Zucker (1970) that two neural areas influence locomotor activity and these areas, based on direct cannulation studies, are estrogen sensitive (AHA-POA) and insensitive (VMH-LHA).

It is also possible that knife-cut-induced hypoactivity results not from damage to activity systems per se but indirectly from the hyperphagia which follows the brain injury. Kennedy and Mitra (1963), for example, have observed that inactivity follows several manipulations which cause excessive food intake and weight gain such as refeeding after a fast. While this possibility cannot be ruled out completely in the present study as the cause of hypoactivity, it appears unlikely. For example, both knife-cut subgroups subsequently given oil injections gained more weight, and presumably ate more, during the non-injection period following surgery than they did during the later

injection period, and this is most pronounced for the anterior rats. In spite of this, the reduced activity levels seen in both cut groups by the first postoperative week remain virtually unchanged throughout the entire experiment.

The body weight findings of the present experiment confirm those obtained earlier with anterior and medial cuts indicating that both transections produce excess weight gains with those of the medial cut rats exceeding the gains of the anterior cut animals. The weight gains of both cut groups, however, were generally less than those obtained in Experiment 4 in which the subjects were housed in conventional stationary cages. A similar attenuation in body weight gain has been reported in medial cut rats housed in activity cages (Sclafani and Rendel, 1977), as in the present experiment, and in normal (Collier, 1970) as well as dietary obese rats (Sclafani and Springer, 1976) given the opportunity for daily exercise. The body weight findings of the estrogen treated rats indicating that both ovariectomized knife-cut groups lost more weight than did the ovariectomized sham cut rats also confirms the findings obtained earlier (see Discussion, Experiment 4).

EXPERIMENT 6

It is possible that some of the feeding fibers that have been implicated in the VMH hyperphagia-obesity syndrome (Gold, 1973; Sclafani and Berner, 1977) may have been damaged by anterior knife-cuts which pass through the caudal portion of the anterior hypothalamic area, just rostral to the VMN. The findings of Experiments 3, 4, and 5 indicated that the excess weight gain produced by the coronal anterior knife-cuts was less pronounced than that produced by the parasagittal VMH knife-cuts and no qualitative differences between the feeding and body weight effects of the cuts emerged. Moreover, rats with either of the neural transections showed a similar exaggerated overconsumption when initially switched to a high-fat diet from a less palatable chow diet (Experiment 3) and it is well known that diet palatability plays an important role in the expression of the VMH hyperphagia-obesity syndrome (Graff and Stellar, 1962; Miller, Bailey and Stevenson, 1950; Sclafani, Berner and Maul, 1975; Teitelbaum, 1955). Based on these suggestive findings the present experiment explored further the similarities between coronal knife-cuts through the anterior hypothalamus and parasagittal knife-cuts which transect the medial hypothalamus.

METHOD

Subjects: The oil-injected rats of the preceding experiment served as subjects.

Procedure: Anterior (ANT; n=5), medial (MED; n=5) and sham cut (CON; n=5) ovariectomized rats were given free access to Purina Meal(powder)and water for a three-week period following their removal from the activity cages of Experiment 5. During this new baseline period, subjects were housed in standard stationary cages and food intake and body weight measures were obtained daily. Following this baseline period the subject's diet was mixed with quinine hydrochloride to achieve a .2% adulteration and this served as their only food source for the next two consecutive days. Subjects were then returned to their plain powdered chow diet for two weeks and their latency to eat a palatable food (Fruitloops, Kelloggs) in a novel environment was recorded on the last day of this period. Briefly, the rats were removed from their home cages, placed in a circular arena measuring 58.4 cm in diameter with white walls 45.7 cm in height. The latency in seconds was then recorded until the first bite was taken (see Sclafani, 1972). Following the latency-to-eat test, the powdered chow diet was replaced for two weeks by a high-fat diet (33% Crisco vegetable fat and 67% plain Purina Meal) and body weight measures only were recorded daily during this final test period. At the conclusion of the behavioral testing the knife-cut subjects were overdosed with anesthetic and their brains prepared for histology, as described.

RESULTS

The anterior cut, medial cut, and sham cut rats all gained weight on the powdered chow diet following their transfer from activity to standard stationary cages. At the end of this 3-week baseline

period the medial cut rats had gained more ($p < .05$) weight than did the anterior cut animals (48.4 vs. 34.2 g.) and both cut groups had outgained ($p < .01$) the controls (26.0 g.). The mean food intake during the final two days of this baseline period revealed that the medial cut rats were eating more ($p < .05$) food than were the anterior cut animals (29.1 vs. 25.0 g./day) and, compared to the intakes of the sham cut animals (22.8 g./day), both knife-cut groups were significantly hyperphagic ($p < .05$). Just prior to the 2-day quinine test, the medial rats were also heavier ($p < .05$) than were the anterior rats (430.3 vs. 377.8 g.) and while the anterior rats weighed more than did the controls (362.2 g.) this latter body weight difference did not reach statistically significance.

The results of the quinine feeding test, which are shown in Figure 10, indicate that all three groups reduced their consumption when given .2% quinine adulterated diet. The two cut groups ate significantly less ($p < .01$; for both comparisons) of this diet (MED= 9.0 and ANT=14.4 g.) than did the sham cut group (CON=17.1 g.). When expressed as a percentage of their plain powdered chow intakes, the controls reduced their intake by 25% which was less ($p < .05$) than either the 43% or 69% feeding reduction exhibited by the anterior and medial cut groups respectively. Comparisons between the two knife-cut groups revealed that the medial cut rats ate less ($p < .05$) of the .2% quinine diet, and displayed a greater ($p < .05$) relative reduction in quinine intake, than did the anterior cut animals.

Following the quinine test all subjects were given free access to the plain powdered chow diet for two weeks. During the last two

days of this period the medial cut rats were consuming more ($p < .05$) food than were the anterior animals (28.0 vs. 24.5 g /day) who, in turn, were eating slightly but not significantly more powdered chow than were the controls (23.4 g /day). Just prior to the latency-to-eat test, the medial rats also weighed more ($p < .05$) than did the anterior rats (446.6 vs. 390.4 g) who by now were significantly heavier ($p < .05$) than were the controls (363.6 g). The results of the latency-to-eat test, which are depicted in the left panel of Figure 11, indicate that the medial cut rats ate sooner ($p < .001$) than did the anterior cut animals (22.6 vs. 97.2 sec) and the latencies of the cut groups were of a shorter duration ($p < .01$) than were those of the sham cut controls (258.6 sec).

The three groups were given free access to a high-fat diet during the final two weeks of the experiment and after the first week on this diet, both cut groups had gained more ($p < .05$) weight than did the sham cut controls (MED=69.8, ANT=52.6, and CON=18.2) although the difference between the medial and anterior groups did not reach significance. By the end of the second week (see Figure 11, right panel), the medial cut rats had gained 125.0 g which was more ($p < .05$) than the 66.8 g weight gain exhibited by the anterior cut rats, and both groups had further outgained ($p < .01$) the controls (21.6 g). During the final five days on the high-fat diet the medial cut rats' rate of weight gain exceeded ($p < .01$) their earlier rate during the last 5-day period on the powdered chow diet (5.7 vs. 3.1 g/day; respectively) and the anterior cut rats also exhibited a significant ($p < .01$) increase in their rate of weight gain when similar high-fat

and powdered chow diet comparisons are made (1.7 vs. 0.9 g /day; respectively). The sham cut rats, in contrast to the knife-cut animals, were gaining weight no faster, and in fact slightly slower, on the final five high-fat diet days than they had earlier during the last 5-day period on the powdered chow diet (0.5 vs. 0.7 g /day; respectively). Analysis of the rate of body weight gain among the three groups over the last five days of the experiment revealed that the medial cut rats were gaining weight more ($p < .05$) rapidly than were the anterior animals (5.7 vs. 1.7 g /day) on the high-fat diet and the anterior group's rate of weight gain exceeded ($p < .05$) that of the controls (0.5 g /day) over the same final 5-day period.

DISCUSSION

The results of the quinine test indicating that both knife-cut groups ate significantly less of the adulterated chow diet than did the sham cut controls suggests that rats with either medial or anterior transections are finicky eaters when fed a poor tasting diet. This exaggerated underconsumption on the quinine diet confirms previous findings in rats with knife-cuts between the medial and lateral hypothalamus (Sclafani, Springer and Kluge, 1976) and with VMH lesions (Teitelbaum, 1955). The greater feeding reduction seen in the overweight medial cut rats compared to that seen in the slightly obese anterior cut animals suggests the possibility that the degree of excess weight gain *per se* may have influenced the undereating of the quinine diet. This view is consistent with other evidence which

indicates that non-obese rats with medial knife-cuts or lesions do not show an exaggerated underconsumption to quinine adulteration of the diet (Ferguson and Keesey, 1975; Franklin & Herberg, 1974; Sclafani, Springer & Kluge, 1976). At the same time, however, the present findings also suggests that obesity itself is not essential for quinine finickiness since anterior cut rats markedly underconsumed the quinine diet and yet they were not significantly heavier than were sham cut controls at the time of testing. Other evidence also reveals that finickiness to quinine adulteration of the diet can occur in the absence of obesity (Bevan, 1973; Gale and Sclafani, 1977a; Graff and Stellar, 1962).

The present results indicating that the high-fat diet further potentiated the obesity produced by both knife-cuts suggests that rats with either medial or anterior transections are also finicky eaters when offered good tasting diets. That is, the high fat diet magnified the knife-cut-induced obesities and this enhanced weight gain on a highly palatable diet was previously seen in Experiments 3 with medial cut males, but less so in males with anterior cuts. The finding with medial cuts confirms numerous studies which have shown that VMH knife-cuts produce exaggerated weight gains on high fat diets (Gale and Sclafani, 1977a; Sclafani, Berner and Maul, 1975; Sclafani, Springer, Kluge, 1976). These findings with the high-fat diet together with those observed with the quinine adulterated diet strongly suggest that dietary factors play a critical role in the expression of anterior, as well as medial, knife-cut-induced obesity.

The results of the latency-to-eat test indicating that non-

deprived rats with medial knife-cuts eat sooner than do sham cut rats in a novel environment confirms previous findings in rats with similar neural damage (Gale and Sclafani, 1977c; Sclafani, 1972). The results from this test reveal that anterior cuts, like medial cuts, also produce reduced latencies to consume a highly palatable food when non-deprived. Knife-cut rats do not exhibit these shorter latencies to eat when they are food deprived, or if a less palatable test diet is offered (Sclafani, 1972), and this suggests that both cuts may have increased the rat's appetite for good tasting foods but not its hunger drive (see Sclafani, 1976). However, since some studies have reported that rats with neural damage that does not result in overeating also display decreased latencies to consume palatable foods (Davenport and Balagura, 1971; Neill, Ross and Grossman, 1974), is it possible that brain damage itself may be responsible, in part, for the reduction in the feeding latencies observed in the present study.

The present finding that anterior knife-cut rats are finicky to quinine adulterated and high-fat diets further distinguishes this brain damaged-induced hyperphagia and obesity syndrome from that produced by ovariectomy. That is, the ovarian hyperphagia and obesity syndrome is virtually independent of diet palatability factors insofar as quinine adulteration of the diet does not block, nor does the use of a high fat diet potentiate, ovariectomy-induced body weight gain (Gale and Sclafani, 1977a). Further, in contrast to the present finding with anterior cut rats, ovariectomized animals do not display reduced latencies to eat palatable foods when non-deprived

(Gale and Sclafani, 1977c). Thus, in summary, the quinine, latency-to-eat, and high-fat diet tests reveal that rats with anterior or medial knife-cuts exhibit a very similar ingestive pattern which is characterized by a finickiness to both good and bad tasting diets as well as an enhanced readiness to eat a palatable food in a novel environment when non-deprived. The present results also suggest that ovarian obesity appears to differ from that produced by anterior cuts in that ovariectomized rats are not finicky eaters nor do they exhibit reduced latencies to consume highly palatable foods in a novel environment when food-deprived.

GENERAL DISCUSSION

The results of this study demonstrate that the hyperphagia-obesity syndrome produced by anterior coronal knife-cuts does not result from the hyposecretion of estrogen as was initially suggested (Albert et al., 1971). The present findings reveal also that anterior neural transections do not interfere with the estrogenic suppression of feeding and body weight following peripheral hormone injections. These results suggest, therefore, that the overeating and weight gain produced by anterior coronal transections is independent of the hyperphagia-obesity syndrome resulting from the chronic withdrawal of estrogen. The effects of anterior transections on food intake and body weight obtained in the present study, are consistent with other findings indicating that such cuts damage a longitudinal feeding system which projects through the hypothalamus whose destruction is presumed to be responsible for the classic VMH obesity syndrome.

Evidence against functional castration as the cause of anterior knife-cuts-induced obesity is provided by the finding that estrogen replacement completely reversed ovarian obesity at doses that did not alter the expression of the obesity produced by the anterior transections. While it is possible that such cuts could have produced a functional castration by rendering the rats insensitive

to subsequent estrogen treatment, this was not supported by the present results. That is, hormone injections at doses that were capable of reducing the body weight of rats with intact ovaries produced similar weight losses in animals given anterior coronal knife-cuts (Experiment 1). Equally as important, ovariectomized animals given anterior knife-cuts or sham knife-cuts in Experiments 4 and 5 lost weight when injected with estrogen. In fact, the results of these two experiments indicated that obese ovariectomized knife-cut rats were more susceptible to estrogen's weight reducing effects than were the ovariectomized sham cut animals. This finding is consistent with the results of earlier studies indicating that estrogen suppresses body weight more in heavy than lean rats (Redick, Nussbaum, and Mook, 1973; Zucker, 1972).

The results of the present study reveal also that medial parasagittal knife-cuts, like the anterior transections, did not impair the estrogenic suppression of feeding and body weight (Experiments 4 and 5). This finding is consistent with previous evidence that similar medial parasagittal knife-cuts do not block the expression of the ovarian hyperphagia-obesity syndrome (Gale and Sclafani, 1977a). Rats with medial parasagittal knife-cuts also lost similar amounts of weight compared to animals with anterior coronal knife-cuts when injected with estrogen.

Further evidence that estrogen hyposecretion was not a causative factor in anterior-knife-cut-induced obesity was the finding that these neural transections produced excess weight gain in male rats. Also arguing against estrogenic involvement in anterior

knife-cut-induced obesity is the present finding that these transections produced additional weight gain in previously ovariectomized rats in the static phase of their ovarian obesity. The present observation that anterior coronal knife-cuts and ovariectomy produced near additive body weight effects when combined in the same animal (Experiment 2) provides further supports for the view that the knife-cuts did not cause overeating and obesity by removing estrogen's inhibitory effects on feeding and body weight.

Several differences between the two syndromes emerged which indirectly suggest that the obesity produced by anterior coronal cuts was not the incidental result of functional castration. For example, the body weight gains of the anterior cut rats always exceeded those seen following ovariectomy and estimates of body fat revealed that anterior knife-cut rats accrued more fat than did ovariectomized animals. A further distinction between the two syndromes was that the ovariectomized rats, in contrast to the anterior cut animals, exhibited an increase in their linear growth. Ovariectomized and anterior knife-cut rats also differed from one another in their latency to eat measures, consumptions of the quinine-adulterated diet, and weight gains on the high-fat diet. That is, the anterior knife-cut animals ate the highly palatable test food significantly sooner than did the ovariectomized rats and they underconsumed the quinine-adulterated compared to the ovariectomized rats. When offered the high-fat diet the anterior cut rats increased their rate of weight gain in contrast to the ovariectomized animals. Finally, the anterior cut rats, unlike the ovariectomized

animals, were chronically hyperdipsic as indicated by the sustained elevations in their water/food intake ratios.

These numerous distinctions between the two syndromes do not completely rule out the possibility that the feeding and body weight effects produced by anterior knife-cuts and ovariectomy may share a common underlying basis. That is, some of these behavioral effects could have resulted from damage to separate systems which are not invariably linked to the feeding and body weight disorders produced by either the anterior knife-cuts or by ovariectomy. As indicated earlier, however, the possibility that ovariectomy and anterior knife-cuts produce similar hyperphagia-obesity syndromes appears unlikely since estrogen replacement completely reversed ovarian obesity but not that produced by anterior coronal transections. The independence of the two obesity syndromes, moreover, is indicated by the finding of near additivity in the body weight effects produced by both physiological manipulations in the same animal.

The present results demonstrate that anterior coronal transections do not interfere with the estrogenic mediation of feeding. At the same time, the activity findings obtained in Experiment 5 reveal that these transections, unlike the medial parasagittal knife-cuts, damage an estrogen-dependent activity system since replacement therapy with estrogen failed to restore the wheel running activity of the anterior cut rats to the level seen in neurally intact animal while similar hormone injections fully restored the activity of the medial cut animals. The present results also indicate that

both cuts may have damaged an estrogen-independent activity system but to differing degrees. These observations are compatible with the view that two separate neural areas influence locomotor activity and these areas differ from one another with respect to their sensitivity to the estrogenic stimulation of spontaneous activity (see Discussion, Experiment 5).

Based primarily on the hormone cannulation studies (Beatty, O'Briant and Vilberg, 1974; Jankowiak and Stern, 1974; Wade and Zucker, 1970) it has been suggested that estrogen influences feeding by acting on the VMH. Of these studies, however, only that of Wade and Zucker (1970) employed two of the controls requisite for a valid interpretation of the effects of estrogen exclusively on this brain tissue, namely, a control procedure for the non-specific responses of other chemically-related substances and a control procedure for the identification of a distinct neural area as the site of action by delivering the hormone under study to adjacent neural loci (see Myers, 1974).

Despite the use of these control procedures, considerable caution should still be exercised before the VMH or other neural areas are accepted as the site of action for estrogen in the brain. For example, Wade and Zucker (1970) suggested that estrogen probably does not enter the general blood supply following cannulation into the VMH since ovariectomized rats displayed concurrent anestrous vaginal smears. It had previously been reported that the minimum effective dose of estrogen required to activate sexual behavior was higher than that needed to produce cornification of the

vaginal epithelium (Davidson et al., 1968) so that leakage of the hormone into the systemic circulation was likely to be too low to affect the rat's sexual behavior. However, Drewett (1973) has more recently shown that feeding is significantly suppressed by blood levels of estrogen which do not alter vaginal cyctology and, further, that the feeding suppressive effects induced by estrogen precede those changes in sexual behavior facilitated by estrogen. Thus, it is entirely conceivable that estrogen could have entered the general blood supply following cannulation into the VMH and altered feeding at sites remote from the VMH without there being any observable changes in vaginal cytology or in sexual behavior. It appears necessary, therefore, that direct measurement of estrogen from the blood be obtained in order to exclude the possibility that the feeding effects of estrogen cannulated into the VMH are not being exerted elsewhere.

Evidence from some VMH ablation studies also suggests that estrogen may affect feeding by acting via the VMH, however, these findings are not easily reconciled with those from other ablation studies indicating that the destruction of the VMH fails to block the estrogenic suppression of feeding. For example, Beatty et al. (1975) reported an attentuation of the feeding suppressive effects of estrogen in VMH lesioned animals, although this effect was rather mild despite the fact that the lesions produced extensive bilateral damage to the VMH. On the other hand, Nance (1975) reported that VMH damage completely blocked the estrogenic suppression of feeding but this conclusion was reached on the basis of a single

hormone injection.

In contrast to the studies above, King and Cox (1973) and Reynolds and Bryson (1974) have reported that estrogen continued to suppress feeding in rats with comparable VMH lesion damage following a series of hormone injections. Further, estrogen has been shown to fully reduce the food intake of mice with gold thioglucose-induced lesions of the VMH (Blaustein, Gentry, Roy and Wade, 1976). Extensive lesion damage which destroys an area extending rostrocaudally from the anterior most aspects of the arcuate nucleus to the ventral premammillary nuclei also fails to prevent the estrogenic suppression of feeding (Gale and Sclafani, unpublished observations). Thus, the VMH ablation findings do not provide compelling evidence which would implicate this neural area as the major brain site mediating estrogen's effects on feeding.

Some doubts also exist concerning the importance of the anterior hypothalamic area (AHA) in the estrogenic mediation of feeding. For example, it was initially proposed that estrogen affected feeding in this brain region by diminishing norepinephrine (NE)-induced feeding (Simpson and DiCara, 1973). According to this hypothesis estrogen interferred with the conversion of dopamine to NE by specifically altering the activity of dopamine-beta-hydroxy-lase (DBH), the enzyme responsible for the conversion. More recent results, however, are inconsistent with the original hypothesis since at estrous, a time of reduced feeding, DBH activity was shown to be significantly increased, not lowered as the hypothesis predicted (Simpson, Cummins, and DiCara, 1975). Also

adding to the unlikelihood that estrogen alters feeding by acting on the AHA is the finding that cannulation of this hormone into the AHA fails to suppress the feeding of ovariectomized rats (Wade and Zucker, 1970). Further, implants of the cytotoxic agent actinomycin-D into the AHA fail to alter food intake but are capable of severely disrupting spontaneous locomotor activity (Stern and Jankowiak, 1972). Thus, as with the VMH, there appears to be sufficient evidence to question whether estrogen exerts its feeding effects via the AHA.

The present knife-cut findings do not lend any support to the view that estrogen sensitive feeding pathways exist in the ventromedial area of the hypothalamus. That is, anterior coronal transections just rostral to the VMN did not interrupt the estrogenic suppression of feeding and this suggests that an estrogen-sensitive pathway probably does not project rostrally from the VMN towards the AHA. The present finding that the medial parasagittal knife-cuts also did not block estrogenic suppression of feeding suggests that such a putative hormone-sensitive pathway does not project laterally from the VMN as well. Finally, the recent knife-cut findings of Hennessy and Grossman (1976) show that estrogen injections remained completely effective in suppressing feeding in rats with coronal transections placed just behind the VMN and this indicates that such an estrogen-sensitive feeding system also does not project caudally to the posterior hypothalamus. Taken together, these findings seriously question the existence of a well defined hormone-sensitive feeding pathway in the vicinity of the ventromedial area

of the hypothalamus.

Since the knife-cuts did not directly damage the ventromedial nucleus the present findings do not exclude the possibility that the VMN and its surrounding tissue are the locus for estrogen-sensitive feeding neurons as has been suggested by some investigators (Beatty, O'Briant, and Vilbery, 1975; Nance, 1975). However, questions raised by the hormone cannulation, regional ablation and knife-cut studies cited earlier do not strongly support such a view. With these considerations in mind it seems likely that estrogen may normally alter feeding by acting via extra hypothalamic sites or by its effects on non-neural tissues. For example, evidence suggests that estrogen is capable of affecting anterior pituitary hormone secretion (D'Angelo and Fisher, 1969) and it is possible, therefore, that estrogen could exert its primary feeding effects by this means or less directly so by affecting the secretion of pituitary target glands. However, an intact pituitary does not appear to be essential for estrogenic suppression of feeding since hypophysectomized weanling rats will reduce their food intake following peripheral injections of estrogen (Wade, 1974). Estrogenic effects on nutrient metabolism, utilization, and storage are numerous and widespread including changes in the hepatic control of blood sugar (Matute and Kalkhoff, 1973), plasma insulin levels (Costrini and Kalkhoff, 1971), gastric emptying (Crean, 1963), lipoprotein lipase activity (Wilson, Flowers, Carlile, and Udall, 1976) and regional adipose tissue cellularity (Krotkiewski, 1976) and it is possible that estrogen could alter feeding by acting directly

or indirectly to influence some of these functions.

The present results demonstrate that anterior coronal and medial parasagittal knife-cuts through the hypothalamus produce hyperphagia and obesity and it is possible that these neural transections may have damaged a common underlying feeding pathway. Support for this view is provided by the present findings which reveal that rats with either set of cuts exhibited several qualitatively similar feeding behaviors. That is, rats with anterior coronal and medial parasagittal knife-cuts displayed exaggerated overconsumptions on the high-fat diet and subnormal intakes on the quinine-adulterated diet. Further, animals with either set of knife-cuts exhibited decreased latencies to eat a highly palatable food in a novel environment when food deprived. The anterior and medial knife-cut findings obtained in this study also agree with current neuroanatomical observations of brain damaged-induced obesity indicating that the pathway severed by these transections is part of a longitudinal feeding system which travels through the hypothalamus.

The existence of such a pathway is supported by the findings of several studies which have employed knife-cut surgery and regional ablation (see below). Coronal knife-cuts placed rostral to the VMN have previously been reported to produce overeating and weight gain if such cuts are not restricted in their lateral extent (Albert et al., 1971; Grossman, 1971; Paxinos and Bindra, 1972; present study). More recent evidence indicates also that coronal knife-cuts placed posterior to the VMN in the perifornical region

also produce hyperphagia and weight gain (Grossman and Hennessy, 1976; Sclafani and Berner, 1977). These findings indicate, therefore, that excess food intake and subsequent weight gains can be produced by coronal knife-cuts ranging from an area just rostral or caudal to the VMN.

Further evidence for the existence of a longitudinal feeding pathway is provided by the results of asymmetrical knife-cut and lesion studies in which damage to one side of the brain is combined contralaterally with a unilateral brain insult. For example, Jones, D'Angelo, and Sawchanko (Note 4) have recently reported that a medial parasagittal knife-cut combined contralaterally with a unilateral anterior coronal knife-cut, similar to the bilateral coronal knife-cuts of the present study, produced overeating and obesity. Feeding and body weight increases were also produced with this combination of asymmetrical cuts by varying only the coronal knife-cut from the frontal level of the paraventricular nucleus to sites in the caudal midbrain. Using a similar asymmetrical technique Sclafani and Berner (1977) have shown that a unilateral medial parasagittal combined contralaterally with either a unilateral coronal knife-cut in the posterior hypothalamus, or with a unilateral coronal knife-cut in the midbrain tegmentum at the level of the red nucleus, produced overeating and weight gain. Finally, hyperphagia and obesity have been reported following a unilateral medial parasagittal knife-cut combined contralaterally with a unilateral electrolytic lesion in the mammillary region of the posterior hypothalamus (Gold, Quackenbush, and Kapatos, 1972). Thus evidence from both symmetrical and asymmetrical surgical techniques with anterior, medial, and posterior transections support the view for

a longitudinal feeding system.

Adding to the likelihood that the present bilateral anterior coronal and medial parasagittal knife-cuts severed the same longitudinal feeding pathway through the hypothalamus is the finding that bilateral posterior coronal knife-cuts also produced an exaggeraged overconsumption on a high-fat diet and subnormal intakes on a quinine-adultered diet (Sclafani and Berner, 1977). A further similarity between rats with different hypothalamic transections is that bilateral posterior coronal knife-cuts and medial parasagittal knife-cuts do not attenuate amphetamine anorexia (Sclafani and Berner, 1977) and bilateral anterior coronal knife-cuts also do not diminish the feeding suppressive response induced by amphetamine administration (Gale and Sclafani, unpublished observations). Thus, rats with either anterior coronal knife-cuts or medial parasagittal knife-cuts (present study) exhibit several qualitatively similar feeding behaviors compared to rats with coronal knife-cuts through the posterior hypothalamus (Sclafani and Berner, 1977). This indicates further that a longitudinal feeding system either ascends or descends through the hypothalamus and it is likely that this pathway is the neural substrate for the classic ventromedial hyperphagia-obesity syndrome.

The destruction of the ventral noradrenergic bundle (VNAB), which as initially described by Ungerstedt (1971) travels near the ventromedial area of the hypothalamus, also produces excess overeating and weight gain (Ahlskog, 1974; Ahlskog and Hoebel, 1973). Unlike the ventromedial syndrome, however, VNAB mediated hyperphagia is not associated with diet finickiness (Ahlskog,

1976). The two syndromes are further distinguished from one another behaviorally in that rats with ventromedial lesions, unlike animals with VNAB damage, do not display an attenuation of amphetamine anorexia and they overeat during both the light and dark portion of the day-night cycle (Ahlskog, Randall, and Hoebel, 1975). Since this pattern of diet finickiness, non-diminished amphetamine anorexia, and daytime and nighttime overeating has also been observed in rats with posterior coronal and medial parasagittal knife-cuts, it appears unlikely that either the knife-cut or VMH lesion syndrome is mediated by the VNAB as has been suggested (Gold, 1973; Kapatos and Gold, 1973).

In contrast to the knife-cut syndrome, however, it remains possible that the effects of ovariectomy on food intake and body weight may be mediated, in part, by the VNAB. For example, unlike rats with knife-cuts, ovariectomized animals (Gale and Sclafani, 1977a) and animals with VNAB damage (Ahlskog, Randall, Hoebel, 1975) are not finicky eaters. Further, the extent of obesity produced by ovariectomy and by VNAB damage is rarely as pronounced as that typically seen following hypothalamic knife-cuts. The vagus nerve, which has been implicated in the mediation of some of the autonomic dysfunctions characterizing the ventromedial obesity syndrome (Powley, 1977; Powley and Opsahl, 1976), does not appear to be essential for estrogenic mediation of feeding since ovarian obesity, in contrast to VMH obesity, is spared by vagotomy (Powley, Opsahl, and VanDenPol, Note 3) and perhaps vagotomy would also spare VNAB mediated obesity. On the other hand, some clear feeding differences exist between ovariectomized rats and animals with VNAB damage. For example, ovarian

hyperphagia is associated with increased day and nighttime feeding (Blaustein and Wade, 1976) unlike VNAB mediated hyperphagia which occurs primarily during the nighttime (Ahlskog, Randall, and Hoebel, 1975). Thus, while the involvement of the VNAB in the ovarian hyperphagia-obesity syndrome remains speculative, direct comparisons between the two syndromes may provide a clue for the identity of the estrogenic system mediating feeding.

In summary, the present findings reveal that the hyperphagia-obesity syndrome produced by anterior coronal knife-cuts does not result from the hyposecretion of estrogen as was initially suggested (Albert et al., 1971). Estrogen replacement completely reversed ovarian obesity but did not alter the expression of anterior knife-cut-induced obesity. That an estrogen insufficiency is not the cause of the obesity produced by anterior transections is also suggested by the finding that female rats with a pre-existing estrogen insufficiency, as well as male rats, became obese following anterior cuts. Evidence indicating that the two obesity syndromes have separate etiologies is also suggested by the finding that the effects of the combined surgeries was additive. The results of this study also disclosed that anterior coronal and medial parasagittal transections do not reduce the anoretic potency of exogenously administered estrogen. These findings, taken together, indicate that the estrogenic modulation of feeding is not mediated by the same fibers whose destruction is responsible for the overeating and excess weight gain resulting from hypothalamic knife-cuts. Finally, the results obtained in the present study support the view that multiple systems exist which function to restrain eating and at least two of these systems are independent of one another.

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FOOTNOTE

[1] The ventromedial area of the hypothalamus (VMH) is used throughout the text to denote the ventromedial nuclei (VMN) and their adjacent neural tissue. The ventromedial area includes the arcuate nucleus and the regions of the VMN which border on the fornices laterally, the pre mammillary nuclei posteriorly, the inferior aspects of the dorsomedial nucleus dorsally, the surface of the brain ventrally, and the area surrounding the rostral pole of the VMN anteriorly. In reference to the studies cited in this text, the terms VMH and VMN were used on the basis of the histological analysis and/or neuroanatomical description provided by the study. In cases where the exact distinction between the VMH and VMN was less than clear the more inclusive of the two terms (i.e., the VMH) was adopted.

MEAN FOOD INTAKE (g/day)

		Preoperative	Postoperative			
Group	N	(3-1)	(28-30)	(48-50)	(58-60)	(78-80)
OVX-OIL	7	14.1	15.7	14.9	14.3	14.0
ANT-OIL	8	13.3	21.8	18.8	16.7	15.8
CON-OIL	8	14.0	14.3	15.1	15.5	13.7
OVX-EB	6	14.6	16.0	12.7	13.3	17.2
ANT-EB	8	14.3	22.5	19.6	15.8	16.3
CON-EB	8	13.7	14.8	14.4	14.9	14.3

TABLE 1

SOURCE	df	FOOD INTAKE		df	BODY WEIGHT GAIN	
		MS	F		MS	F
Surgery (A)	2	61,378.1	21.6***	2	17,421.3	61.4***
Treatment (B)	1	17,622.3	11.6***	1	9,136.7	22.3*
AXB	2	12,774.9	7.8**	2	15,467.1	17.4*
Error	39	4,490.3		39	2,130.5	
Days (C)	4	19,351.3	35.1***	3	5,972.0	27.5***
AXC	8	41,241.6	16.1**	6	18,633.8	81.6**
BXC	4	21,348.5	7.8*	3	8,541.9	23.2***
AXBXC	8	14,462.6	12.4*	6	10,756.4	8.3**
Error	156	7,130.2		117	3,250.7	

$p < .05$*
$p < .01$**
$p < .001$***

TABLE 2

		MEAN WATER CONSUMPTION (ml/day)				WATER/FOOD INTAKE RATIO		
		Preoperative	Postoperative			Preoperative	Postoperative	
Group	N	(2-1)	(9-10)	(29-30)	(79-80)	(2-1)	(29-30)	(79-80)
OVX	13	23.0	33.0	24.4	25.0	1.62	1.53	1.60
ANT	16	22.5	75.5	79.5	61.0	1.63	3.58	3.78
CON	16	22.0	23.5	25.0	23.5	1.59	1.71	1.68

TABLE 3

SOURCE	df	WATER CONSUMPTION MS	F	df	WATER/FOOD INTAKE RATIO MS	F
Surgery (A)	2	51,075.0	33.9***	2	32.4	55.8***
Treatment (B)	1	1,829.6	1.5	1	4.3	1.3
AXB	2	1,520.6	0.8	2	2.5	.4
Error	39	376.9		39	.9	
Days (C)	2	17,778.4	39.4***	2	9.5	39.3***
AXC	4	15,488.3	34.3***	4	8.4	25.7**
BXC	2	451.7	3.2*	2	12.3	0.9
AXBXC	4	313.6	1.1	4	5.7	1.3
Error	78	151.7		78	3.2	

$p < .05$*
$p < .01$**
$p < .001$***

TABLE 4

		Ovarian Weight (mg)	Naso-Anal (N-A) Length (cm)		Lee Obesity Index*	
Group	N	Postoperative	Preoperative	Postoperative	Preoperative	Postoperative
OVX-OIL	8	--	21.2	22.7	301	313
ANT-OIL	7	48.4	21.3	21.9	298	331
CON-OIL	8	74.5	21.4	22.0	299	301
OVX-EB	8	--	21.4	22.6	300	311
ANT-EB	6	45.2	21.6	22.2	298	333
CON-EB	8	69.6	21.3	21.9	298	303

* Lee Obesity Index $= \sqrt{\frac{\text{body weight, g}}{\text{N-A length, cm}}} \times (10^3)$

TABLE 5

SOURCE	df	OVARIAN WEIGHT MS	F
Surgery (A)	1	5,108.7	160.3*
Treatment (B)	1	126.0	1.0
AXB	1	31.7	0.6
Error	28	4.7	

$p < .001$*

TABLE 6

SOURCE	df	NASO-ANAL (N-A) LENGTH MS	F
Surgery (A)	2	.81	4.59*
Treatment (B)	1	.31	0.27
AXB	2	1.26	0.43
Error	39	.22	

	df	LEE OBESITY INDEX MS	F
Surgery (A)	2	168.3	32.7**
Treatment (B)	1	34.9	1.0
AXB	2	111.5	0.6
Error	39	21.6	

$p < .01$*
$p < .001$**

TABLE 7

		MEAN FOOD INTAKE (g/day)				
		Endocrine Surgery		Brain Surgery		
		Preoperative	Postoperative	Preoperative	Postoperative	
Group	N	(3-1)	(18-20)	(48-50)	(78-80)	(168-170)
OVX +ANT	5	15.2	17.8	15.8	34.9	30.1
SHAM+ANT	5	16.1	15.7	15.3	35.2	28.3
OVX +SHAM	6	15.0	18.4	16.1	16.5	15.6
SHAM+SHAM	8	15.5	15.9	15.6	15.5	16.0

TABLE 8

SOURCE	df	FOOD INTAKE		df	BODY WEIGHT GAIN	
		MS	F		MS	F
Endocrine						
Surgery (A)	1	8,532.1	17.3**	1	13,273.8	43.9***
Knife-cuts (B)	1	12,411.5	9.4**	1	20,417.6	14.8***
AXB	1	14,306.1	4.6*	1	8,237.0	7.1*
Error	20	4,779.0		20	2,271.9	
Days (C)	1	4,787.1	8.4**	1	5,862.0	27.5**
AXC	1	3,841.0	13.6*	1	15,289.3	7.9*
BXC	1	12,455.1	2.4*	1	4,423.7	11.2**
AXBXC	1	6,862.4	2.8*	1	12,002.1	4.2*
Error	20	1,167.4		20	975.6	

$p < .05$*
$p < .01$**
$p < .001$***

TABLE 9

		MEAN FOOD INTAKE [kcal/day and (g/day)]			
		Preoperative	Postoperative		
Group	N	Pellets (3-1)	Pellets (13-15)	High-fat (16-18)	High-fat (33-35)
ANT	5	105.1 (24.7)	144.0 (33.9)	172.3 (29.1)	131.2 (22.2)
MED	7	103.6 (24.4)	157.1 (37.0)	206.7 (34.9)	187.7 (31.7)
CON	5	104.9 (24.7)	110.9 (26.1)	117.0 (19.7)	124.8 (21.1)

TABLE 10

SOURCE	CALORIC INTAKE df	MS	F	BODY WEIGHT GAIN df	MS	F
Surgery (A)	2	16,009.7	26.5***	2	45,363.0	20,6***
Error	14	604.1		14	2,206.1	
Days (B)	3	12,798.1	55.4***	2	39,627.0	90.9***
AXB	6	1,693.8	7.3*	4	4,984.2	11.4**
	42	231.1		28	463.1	

$p < .05$*

$p < .01$**

$p < .001$***

TABLE 11

		MEAN FOOD INTAKE (g/day)				
		Preoperative	Postoperative			
Group	N	(3-1)	(28-30)	(38-40)	(88-90)	(128-130)
ANT-OIL	5	20.0	44.3	37.4	25.2	27.7
MED-OIL	7	21.2	50.5	49.1	36.2	36.5
CON-OIL	5	21.5	24.9	25.9	23.6	21.4
ANT-EB	6	20.8	45.5	34.4	23.8	37.8
MED-EB	5	21.6	47.8	35.6	26.4	45.6
CON-EB	6	20.3	25.3	20.4	21.7	22.1

TABLE 12

SOURCE	df	FOOD INTAKE		df	BODY WEIGHT GAIN	
		MS	F		MS	F
Group (A)	2	2,183.4	72.3***	2	16,214.4	46.7***
Treatment (B)	1	1,342.1	64.5***	1	5,360.2	10.3***
AXB	2	854.3	17.4*	2	894.2	8.5***
Error	28	175.7	--	28	155.4	--
Days (C)	4	3,836.9	153.7***	3	48,763.0	103.7***
AXC	8	3,263.0	56.8***	6	16,433.2	26.2***
BXC	4	1,677.3	21.5*	3	1,083.6	15.4***
AXBXC	8	847.3	13.2*	6	961.9	2.3*
Error	112	129.1		84	98.2	

$p < .05$*
$p < .01$**
$p < .001$***

TABLE 13

SOURCE	df	CALORIC INTAKE MS	F	df	BODY WEIGHT GAIN MS	F
Surgery (A)	2	8,640.2	68.5***	2	3,661.6	8.0**
Treatment (B)	1	10,291.3	81.6***	1	13,480.0	40.4***
AXB	2	1,268.6	10.1***	2	954.6	2.4*
Error	24	126.2	--	24	457.4	
Days (C)	2	10,357.5	173.2***	1	2,548.2	18.0***
AXC	4	701.1	11.7***	2	1,414.8	12.9**
BXC	2	9,606.0	160.6***	1	18,691.3	132.4***
AXBXC	4	748.0	12.5***	2	478.0	3.4*
Error	48	59.8	--	24		

$p < .05$*

$p < .01$**

$p < .001$***

TABLE 14

		MEAN BODY WEIGHT GAIN (g)	
Group	N	Day 35	Day 59
ANT-OIL	5	96.4	119.3
MED-OIL	5	123.6	187.2
CON-OIL	5	49.5	56.7
ANT-EB	5	81.3	40.1
MED-EB	5	111.8	52.9
CON-EB	5	57.6	37.5

TABLE 15

FIGURE CAPTIONS

Figure 1. Photomicrograph of anterior coronal knife-cut (upper) and medial parasagittal knife-cut (lower).

Figure 2. Schematic representation from the DeGroot (1967) atlas of anterior coronal knife-cut with the lateral (L) coordinate of each parasagittal plane indicated at the right.

Figure 3. Schematic representation from the DeGroot (1967) atlas of the medial parasagittal knife-cut with the anterior-posterior (A-P) coordinate of each coronal plane indicated at the left. (The shaded area represents the range of the cut.)

Figure 4. Mean postoperative body weight gain of female rats given anterior coronal cut (ANT), ovariectomy (OVX), or sham (CON) surgery. (During Days 31-60 subjects in each group were injected daily with either estradiol benzoate [EB] at the doses indicated or with the oil vehicle [OIL].)

Figure 5. Mean postoperative body weight gain of female rats given ovariectomy (OVX) or sham ovariectomy (SHAM). Subjects were subsequently given either anterior knife-cut (OVX+ANT or SHAM+ANT) or sham cut (OVX+SHAM or SHAM+SHAM) surgery at times indicated by the heavy-set arrows. (During Days 81-90 a series of temperature tests were administered.)

Figure 6. Mean (+S. E. M.) food intake of rats sequentially placed in an environmental chamber at 24°C. (Days 81-83), 5°C. (Days 84-85), 24°C. (Days 86-88), and 35°C. (Days 89-90). (Abbreviations for group identification are provided in Fig. 5.)

Figure 7. Mean postoperative body weight gain of male rats given either anterior coronal knife-cut (ANT), medial parasagittal knife-cut (MED) or sham cut (CON) surgery. (Subjects were maintained preoperatively and for the first 15 postoperative days on Purina pellets and then given a high-fat diet from Days 16-35.)

Figure 8. Mean postoperative body weight gain of ovariectomized rats given either anterior coronal knife-cut (ANT), medial parasagittal knife-cut (MED) or sham cut (CON) surgery. (During Days 31-90 subjects in each group were injected daily with either estradiol benzoate [EB] at the doses indicated or with the oil vehicle [OIL].)

Figure 9. Mean postoperative wheel running activity of ovariectomized rats given anterior coronal knife-cut (ANT), medial parasagittal knife-cut (MED), or sham cut (CON) surgery. Activity is expressed as a percentage of baseline levels. (During Days 35-59 subjects in each group were injected daily with 5-ug of estradiol benzoate [EB] or with the oil vehicle [OIL].)

Figure 10. Consecutive two-day mean (+S. E. M.) intake of Purina chow (meal) and .2% quinine-adulterated diet by ovariectomized rats given anterior coronal knife-cut (ANT), medial parasagittal knife-cut (MED) or sham cut (CON) surgery.

Figure 11. Mean latencies to eat a highly palatable food in a novel environment by non-deprived knife-cut (ANT or MED) or sham cut (CON) ovariectomized rats (lt. panel). Mean body weight gain of knife-cut or sham cut ovariectomized rats given access to a high-fat diet for a two week period (rt. panel).

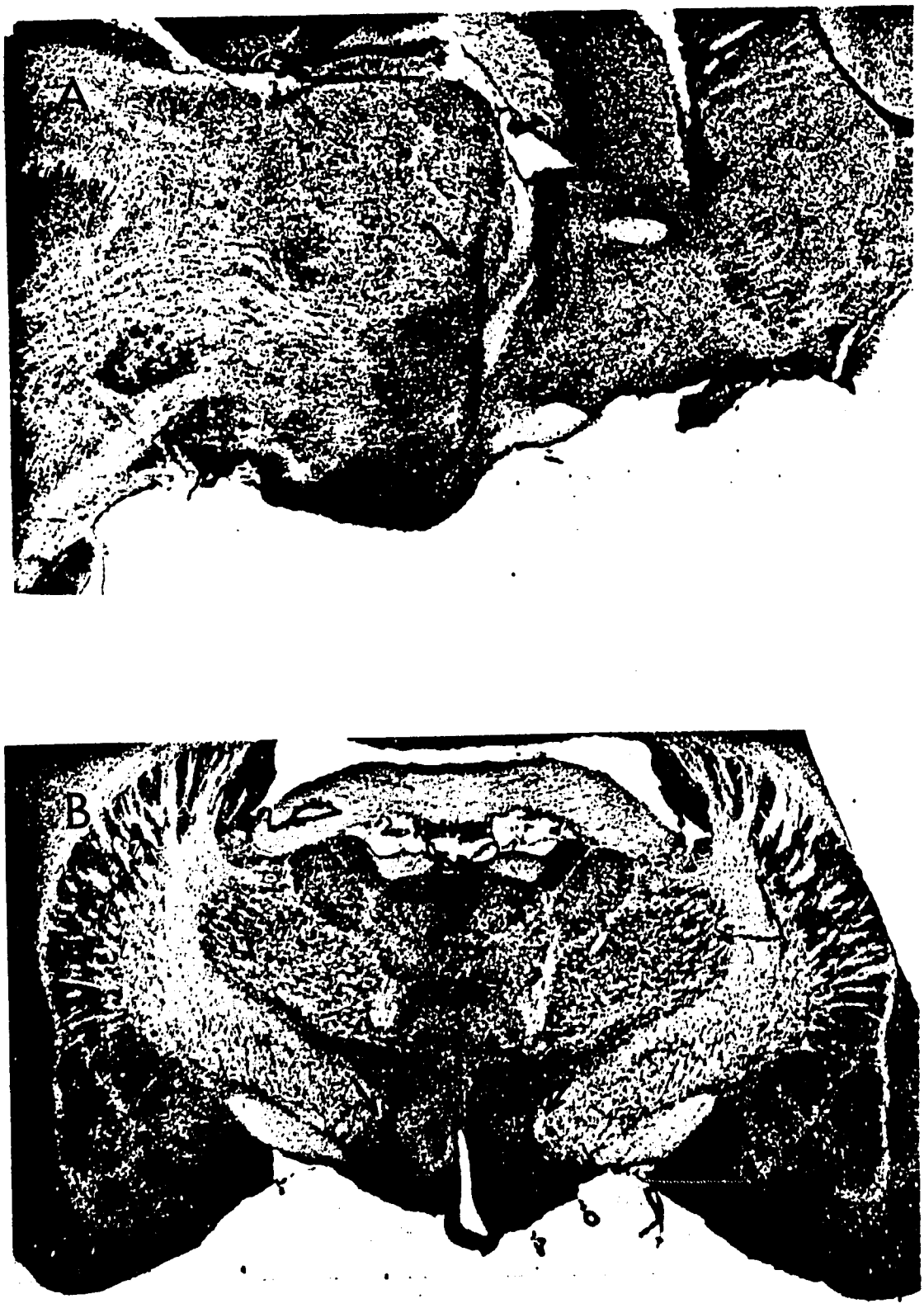


Figure 1

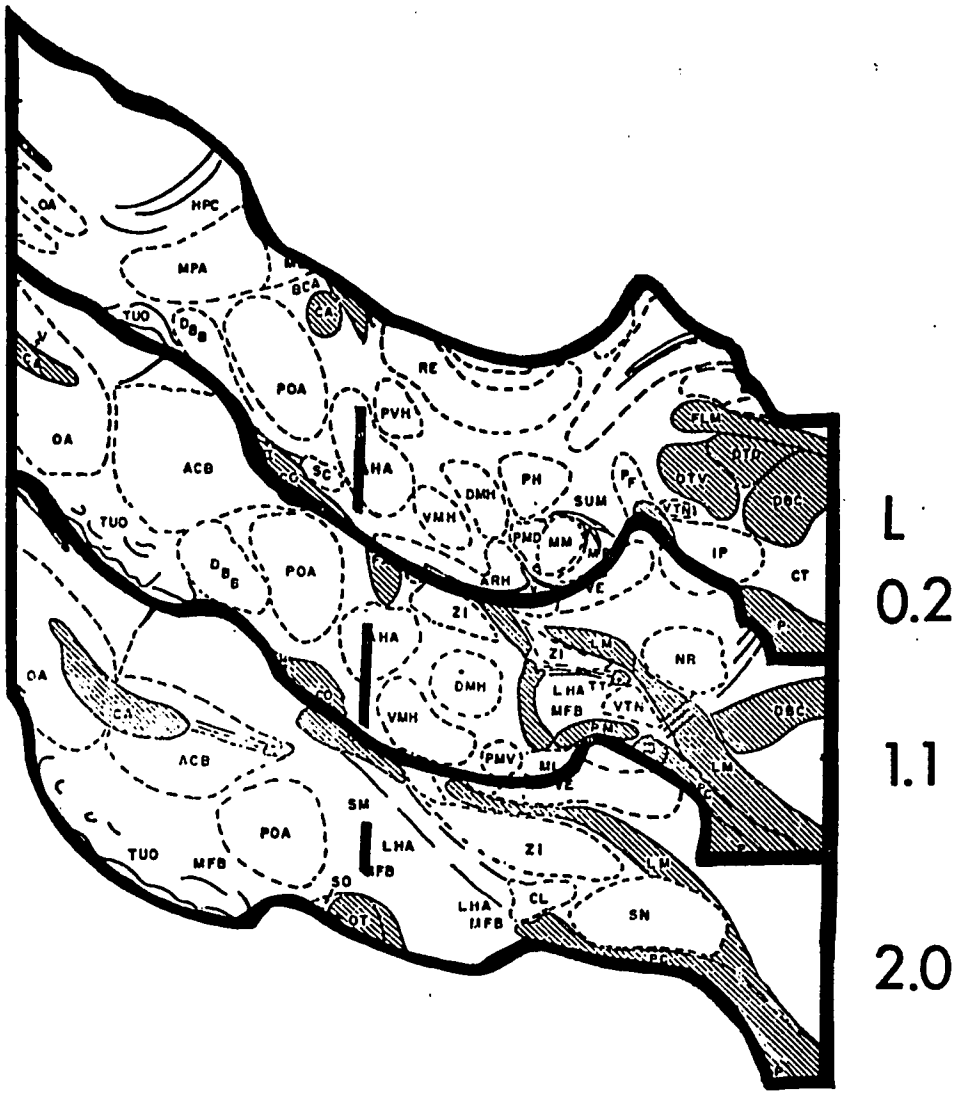

OA
HPC
MPA
BCA
TUO
RE
POA
PVH
ACB
SC
LHA
PH
DMH
SUM
VMH
PMD
MM
ARH
ZI
IP
CT
NR
LHA
MFB
VTN
PMV
SM
SO
OT
CL
SN
L
0.2
1.1
2.0

Figure 2

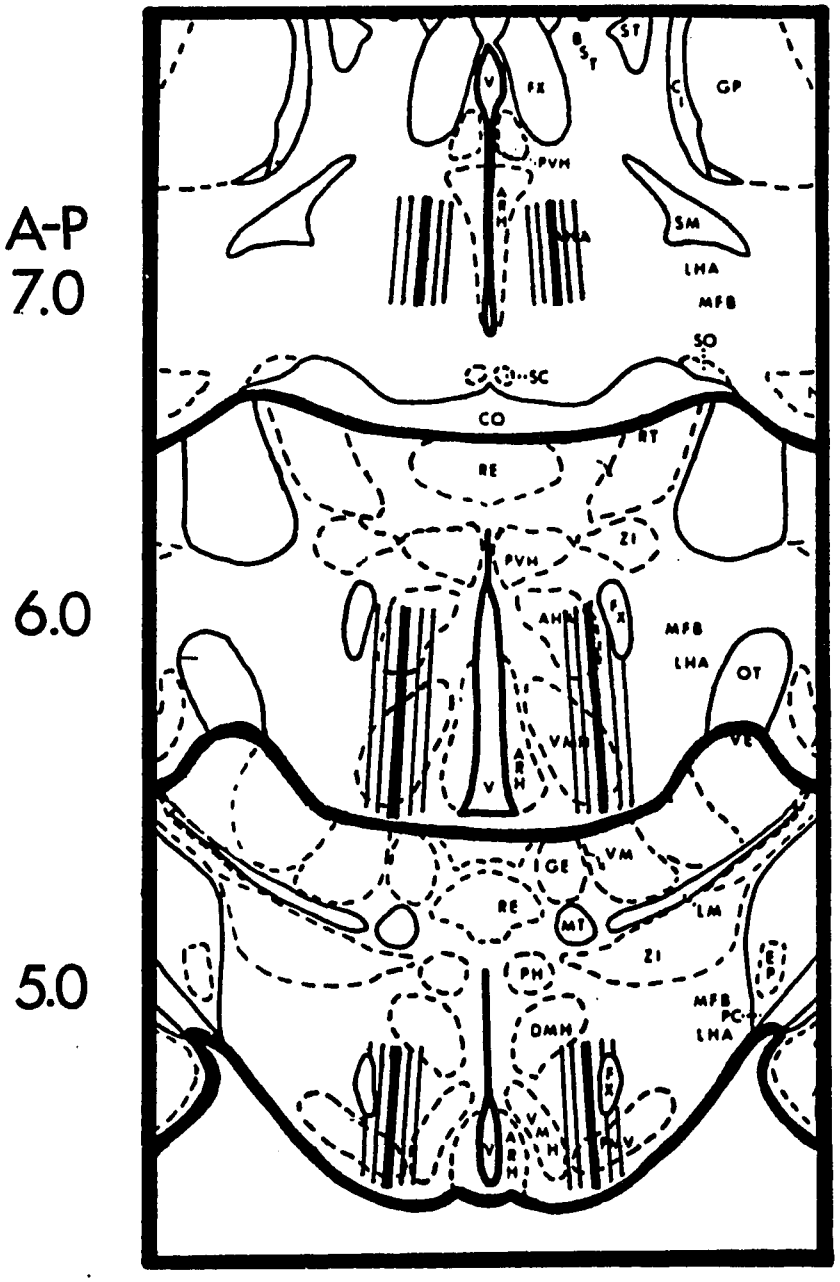
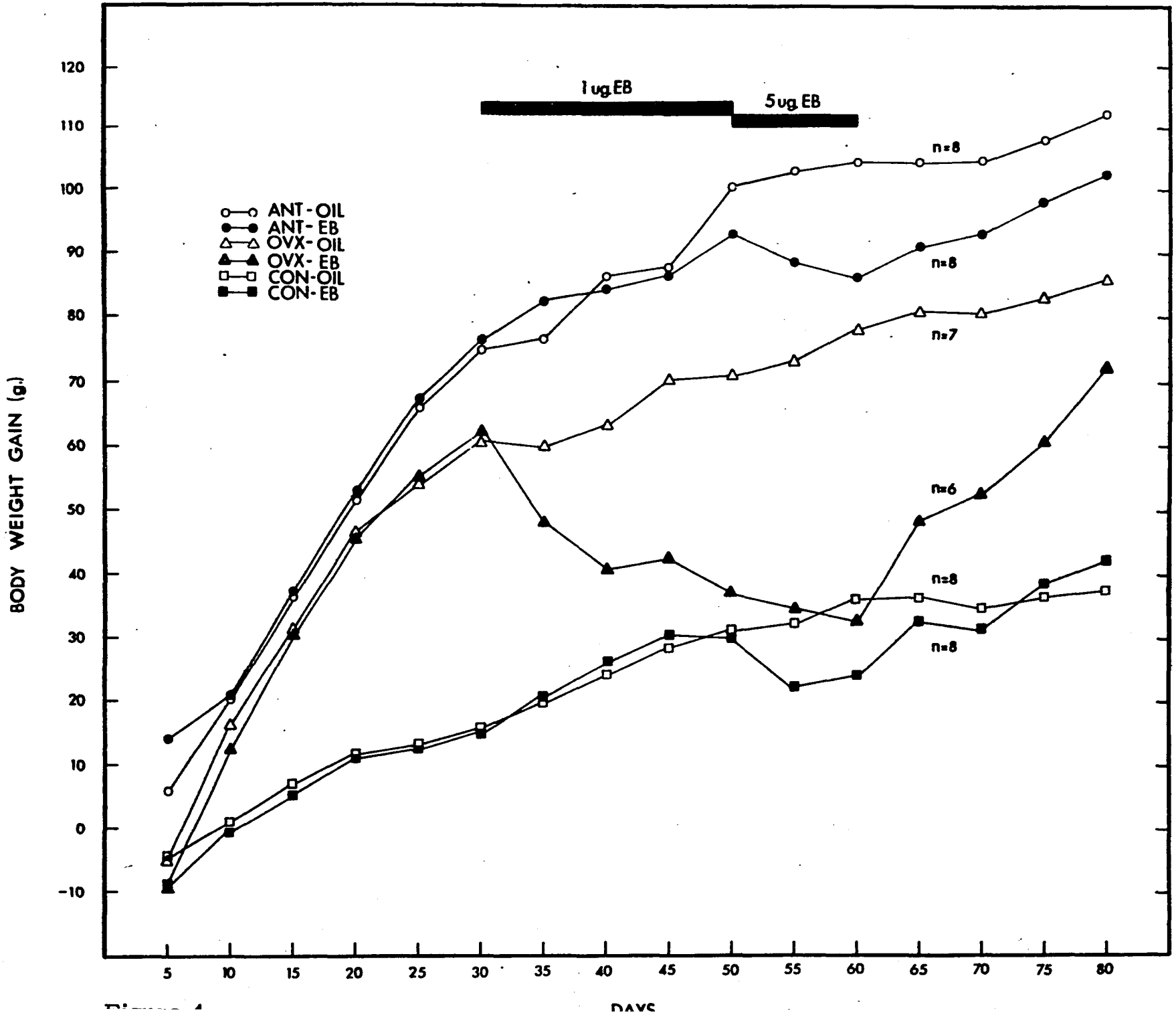

A-P
7.0
6.0
5.0
V
FX
ST
GP
PVH
SM
LHA
MFB
SO
SC
CO
RT
RE
ZI
AHN
OT
VMH
GE
VM
LM
MT
PH
DMH
PC

Figure 3


1 ug EB
5 ug EB
ANT - OIL
ANT - EB
OVX - OIL
OVX - EB
CON - OIL
CON - EB
n=8
n=8
n=7
n=6
n=8
n=8
BODY WEIGHT GAIN (g)
DAYS
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80

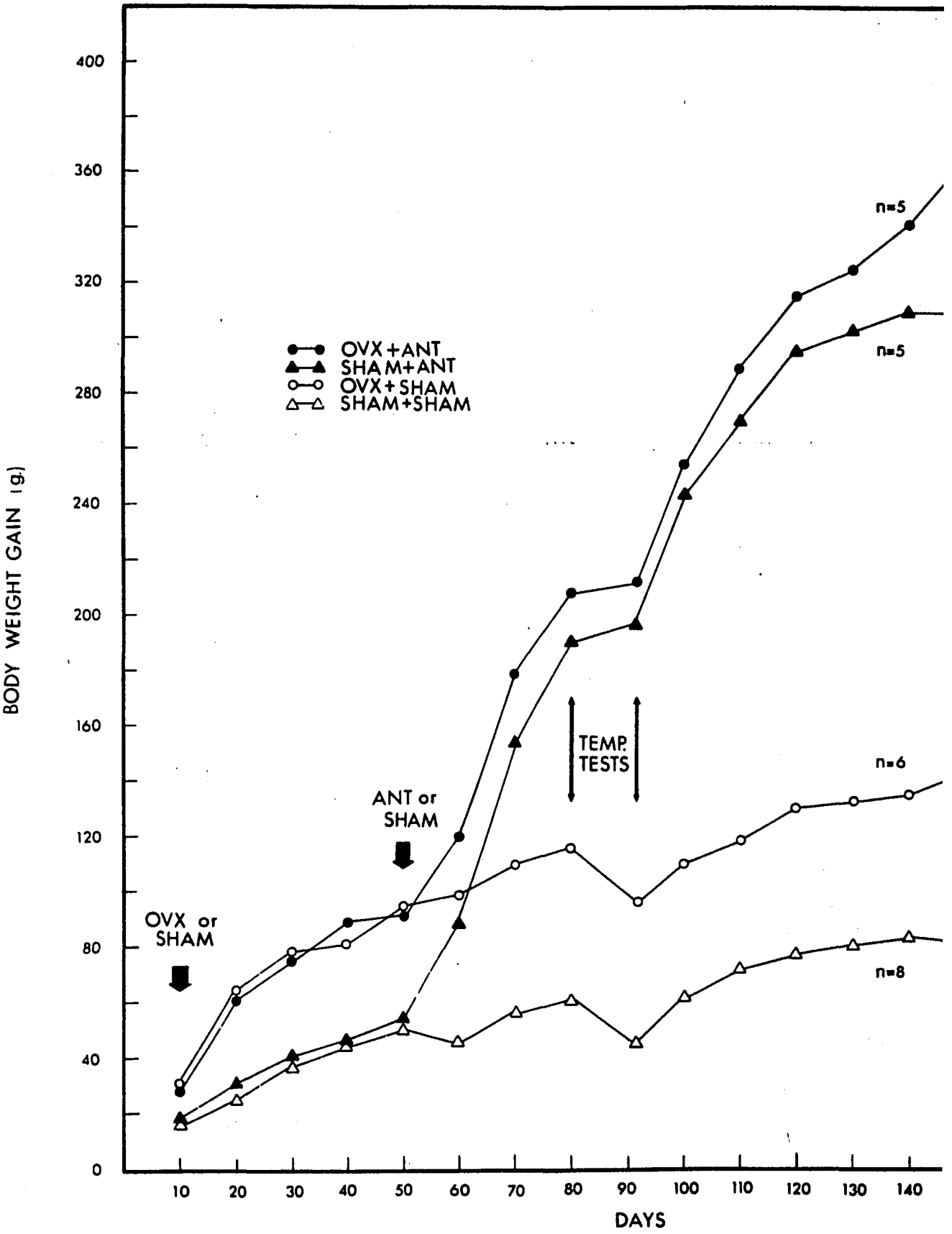
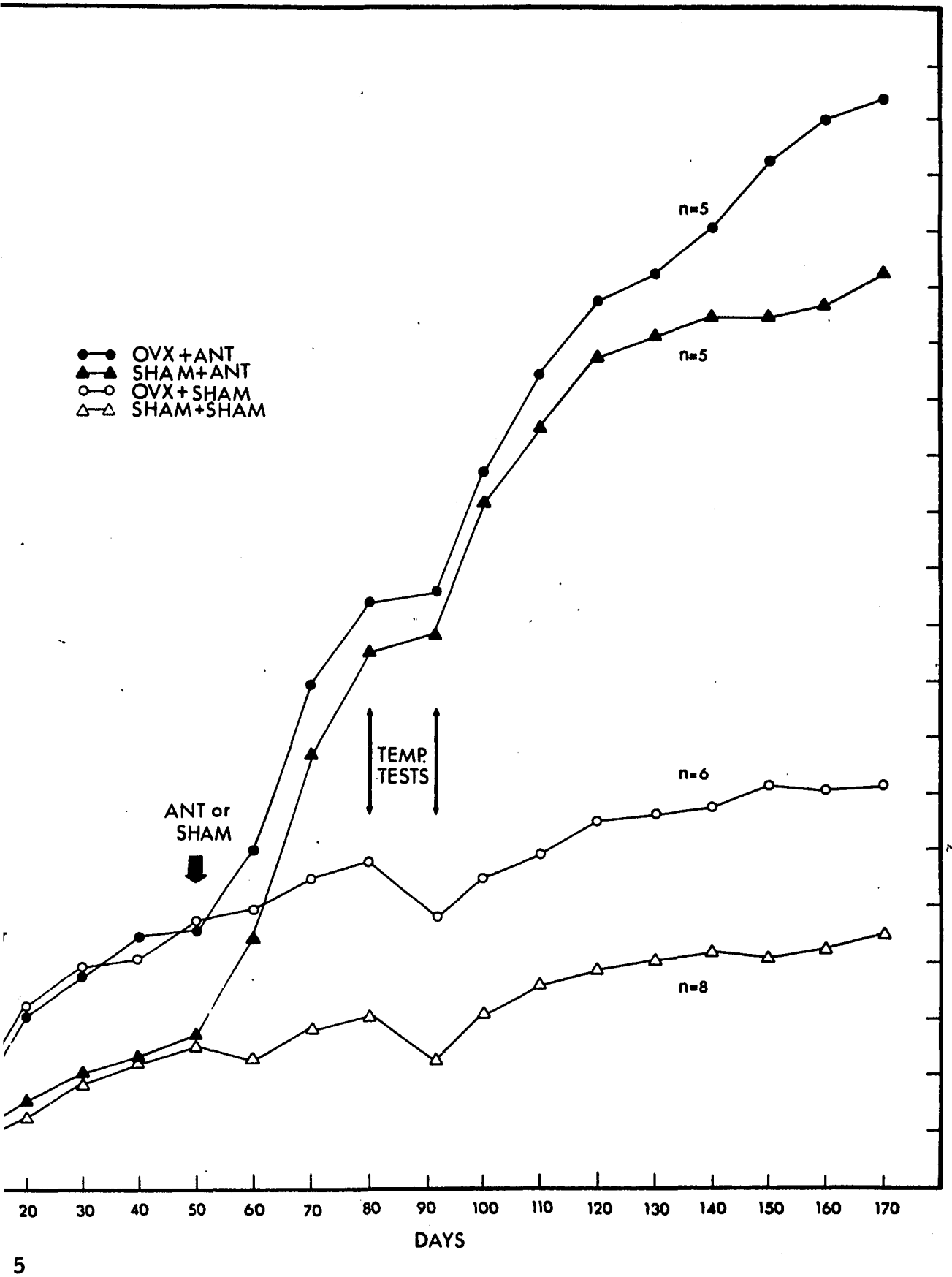

BODY WEIGHT GAIN (g)
400
360
320
280
240
200
160
120
80
40
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
DAYS
OVX+ANT
SHAM+ANT
OVX+SHAM
SHAM+SHAM
n=5
n=5
n=6
n=8
TEMP. TESTS
ANT or SHAM
OVX or SHAM

Figure 5


OVX+ANT
SHAM+ANT
OVX+SHAM
SHAM+SHAM
n=5
n=5
n=6
n=8
TEMP. TESTS
ANT or SHAM
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
DAYS

5

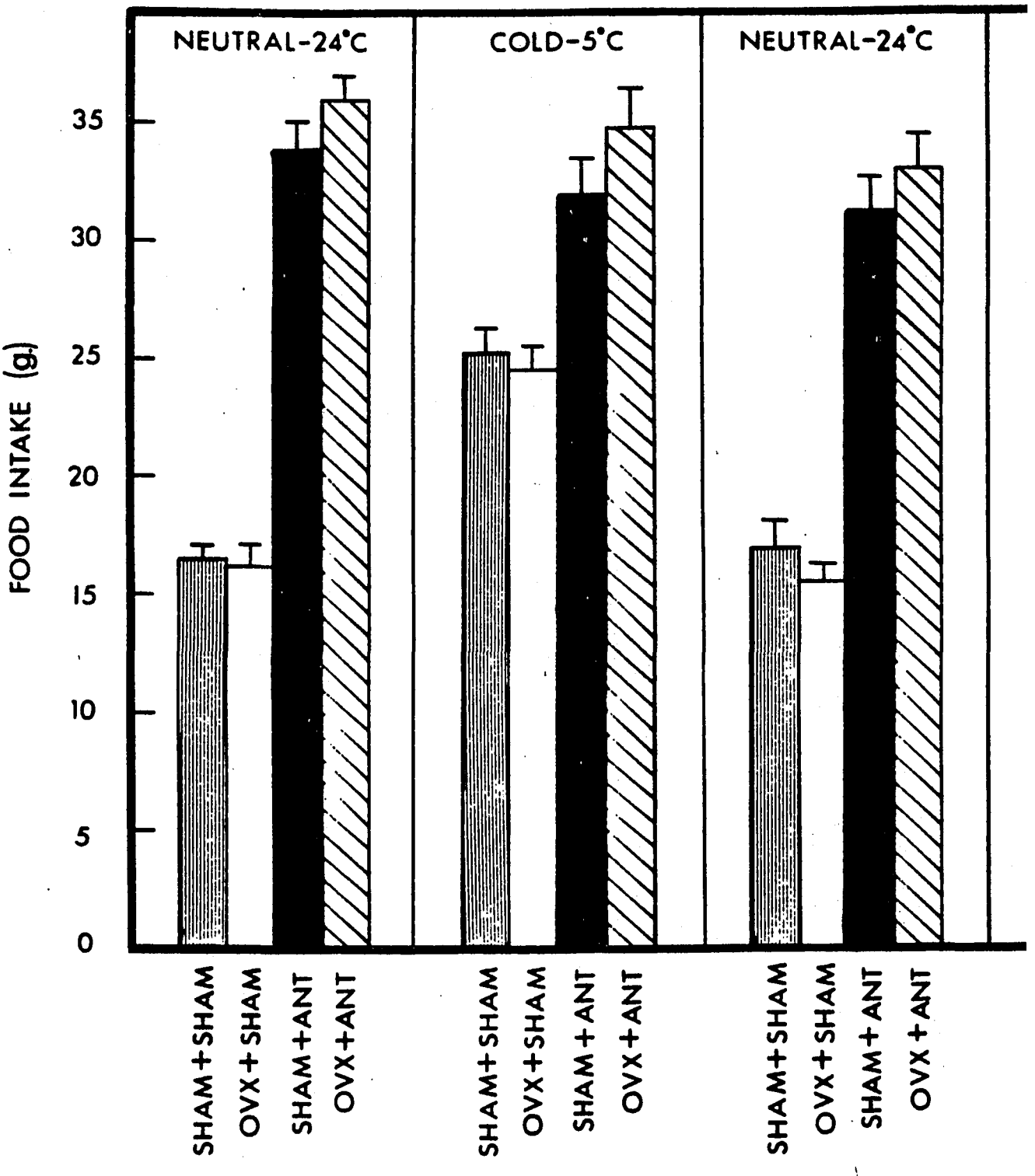
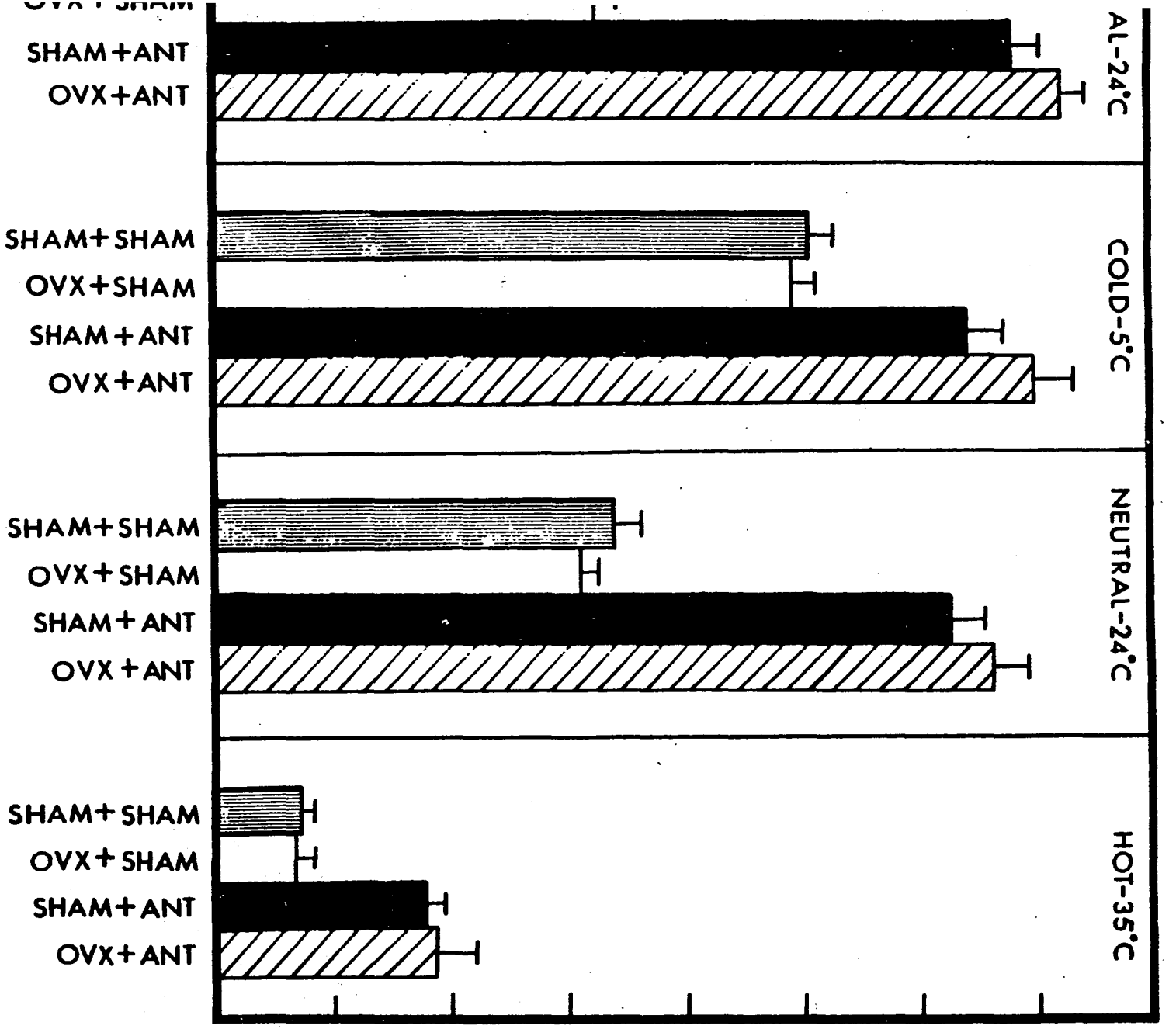

NEUTRAL-24°C
COLD-5°C
NEUTRAL-24°C
FOOD INTAKE (g)
0
5
10
15
20
25
30
35
SHAM+SHAM
OVX+SHAM
SHAM+ANT
OVX+ANT
SHAM+SHAM
OVX+SHAM
SHAM+ANT
OVX+ANT
SHAM+SHAM
OVX+SHAM
SHAM+ANT
OVX+ANT

Figure 6


SHAM+ANT
OVX+ANT
AL-24°C
SHAM+SHAM
OVX+SHAM
SHAM+ANT
OVX+ANT
COLD-5°C
SHAM+SHAM
OVX+SHAM
SHAM+ANT
OVX+ANT
NEUTRAL-24°C
SHAM+SHAM
OVX+SHAM
SHAM+ANT
OVX+ANT
HOT-35°C

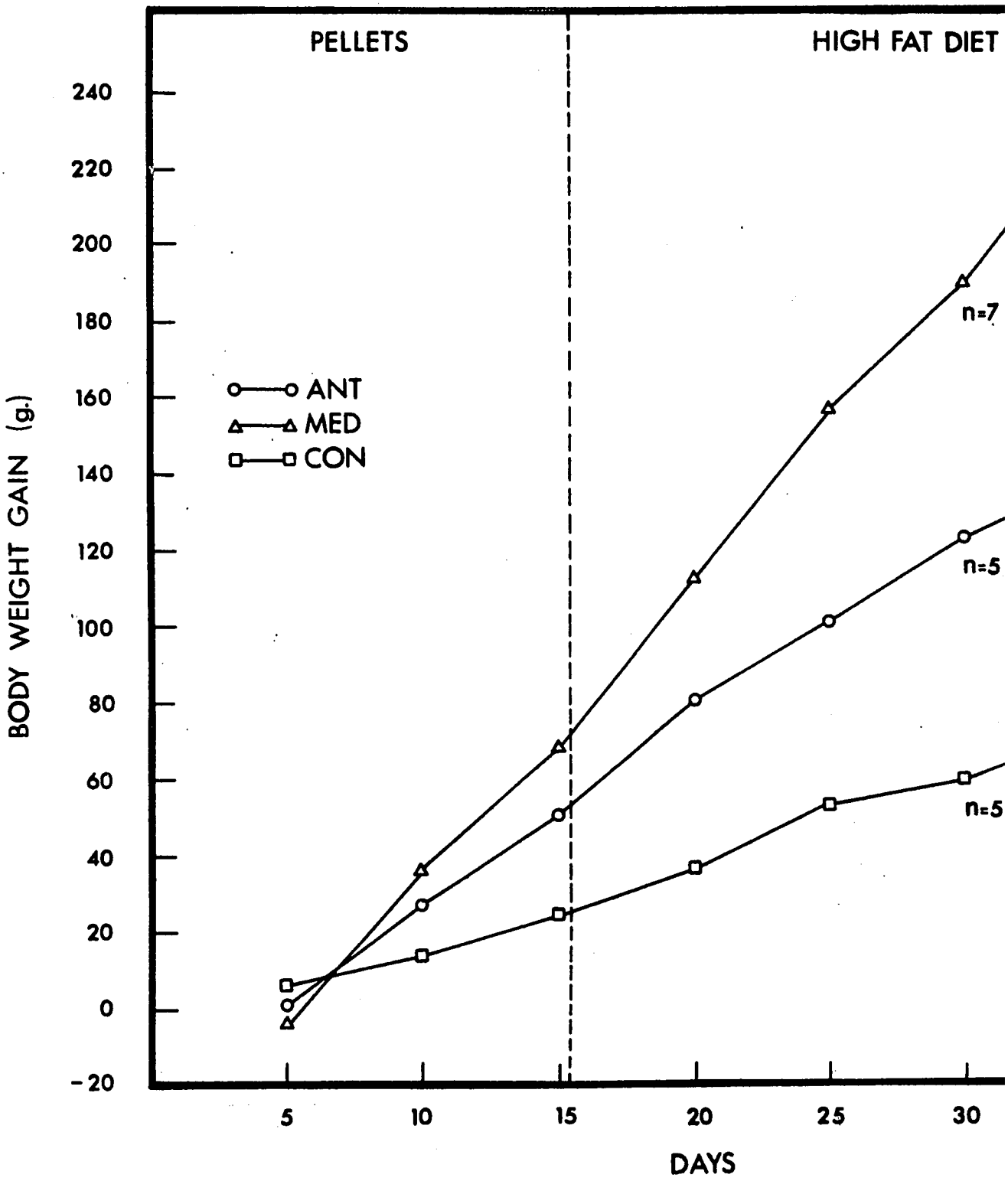
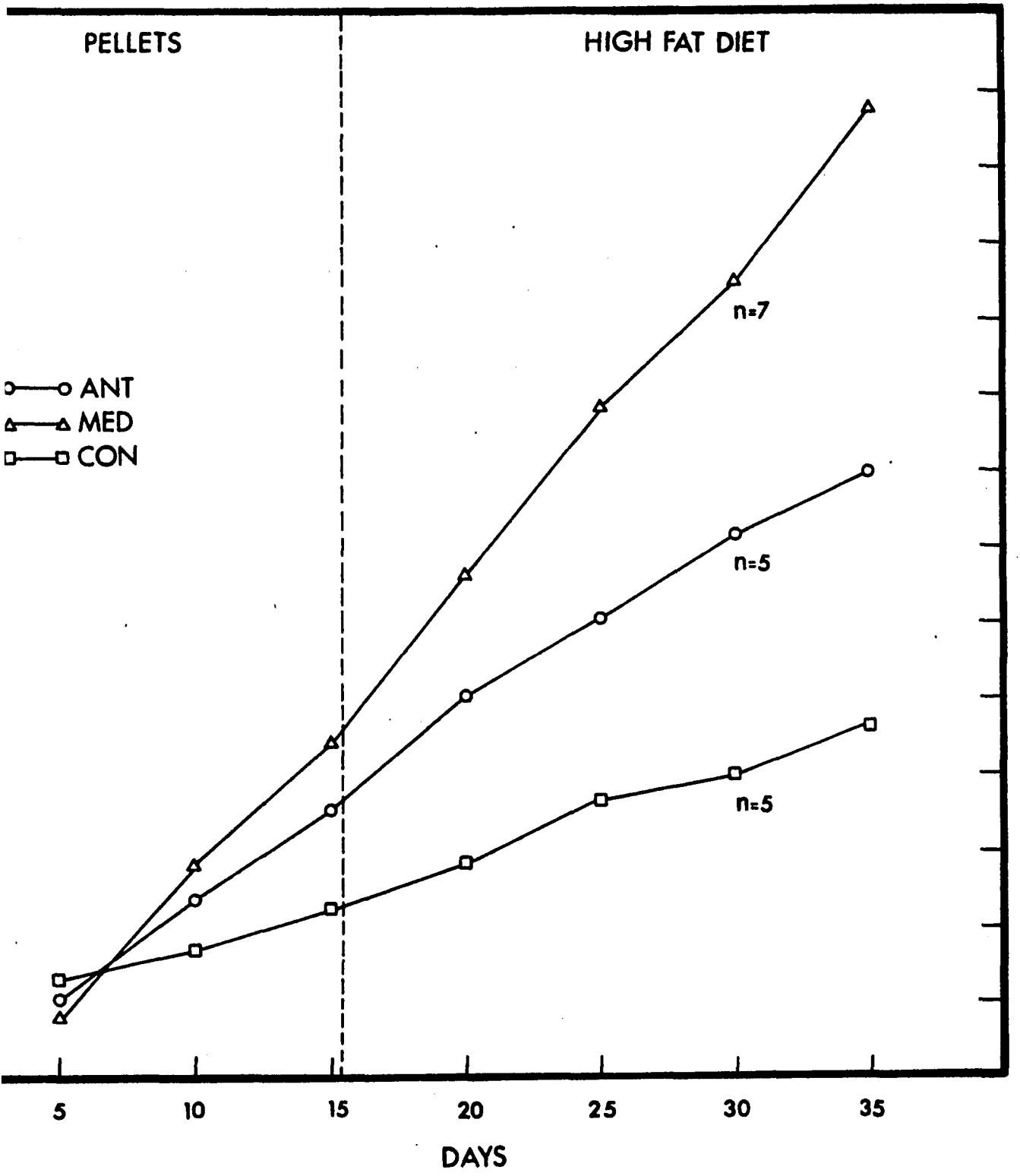
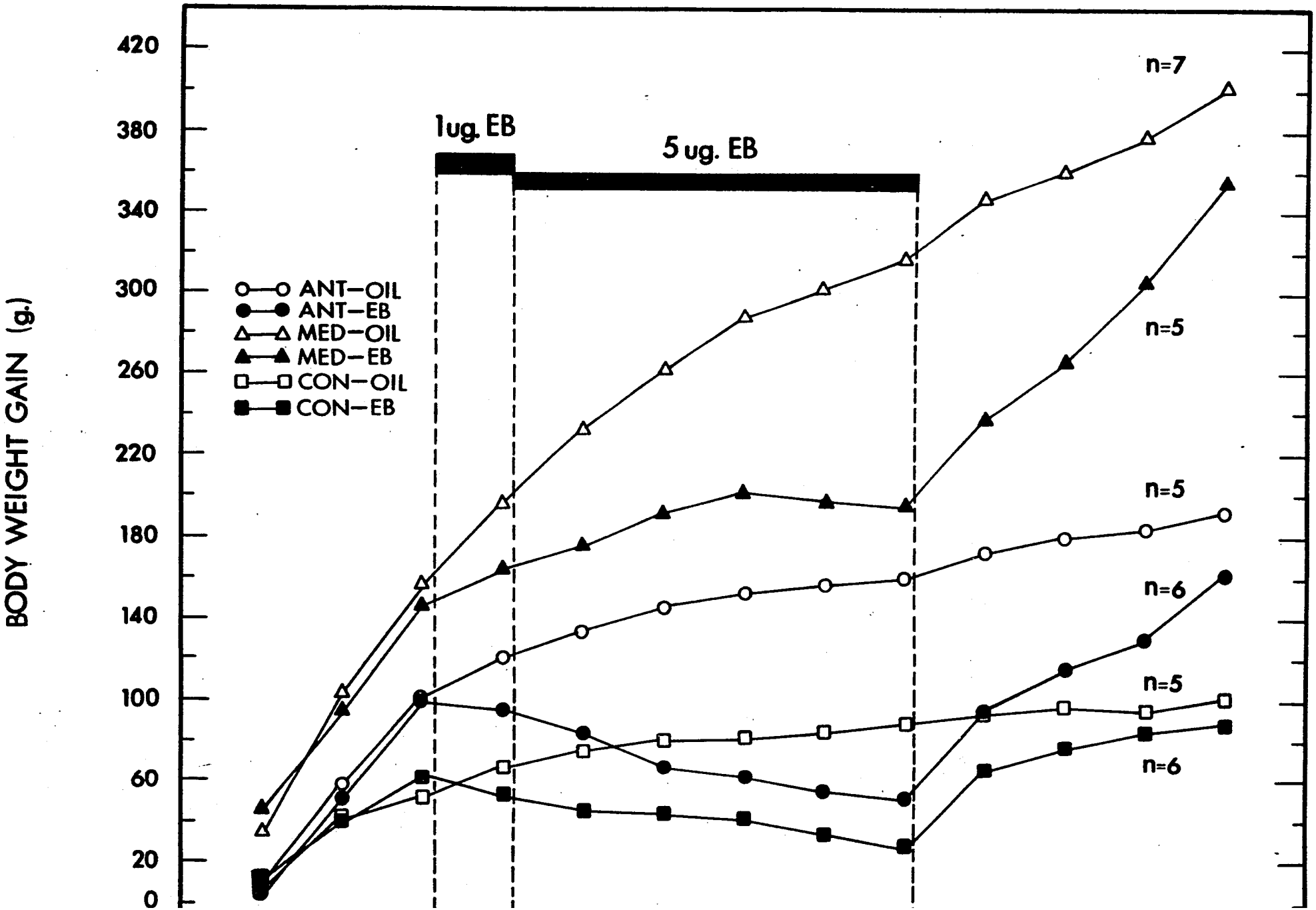

PELLETS
HIGH FAT DIET
240
220
200
180
160
140
120
100
80
60
40
20
0
-20
BODY WEIGHT GAIN (g.)
ANT
MED
CON
n=7
n=5
n=5
5
10
15
20
25
30
DAYS

Figure 7


PELLETS
HIGH FAT DIET
ANT
MED
CON
n=7
n=5
n=5
5
10
15
20
25
30
35
DAYS

e 7


BODY WEIGHT GAIN (g.)
420
380
340
300
260
220
180
140
100
60
20
0
1ug. EB
5 ug. EB
ANT–OIL
ANT–EB
MED–OIL
MED–EB
CON–OIL
CON–EB
n=7
n=5
n=5
n=6
n=5
n=6

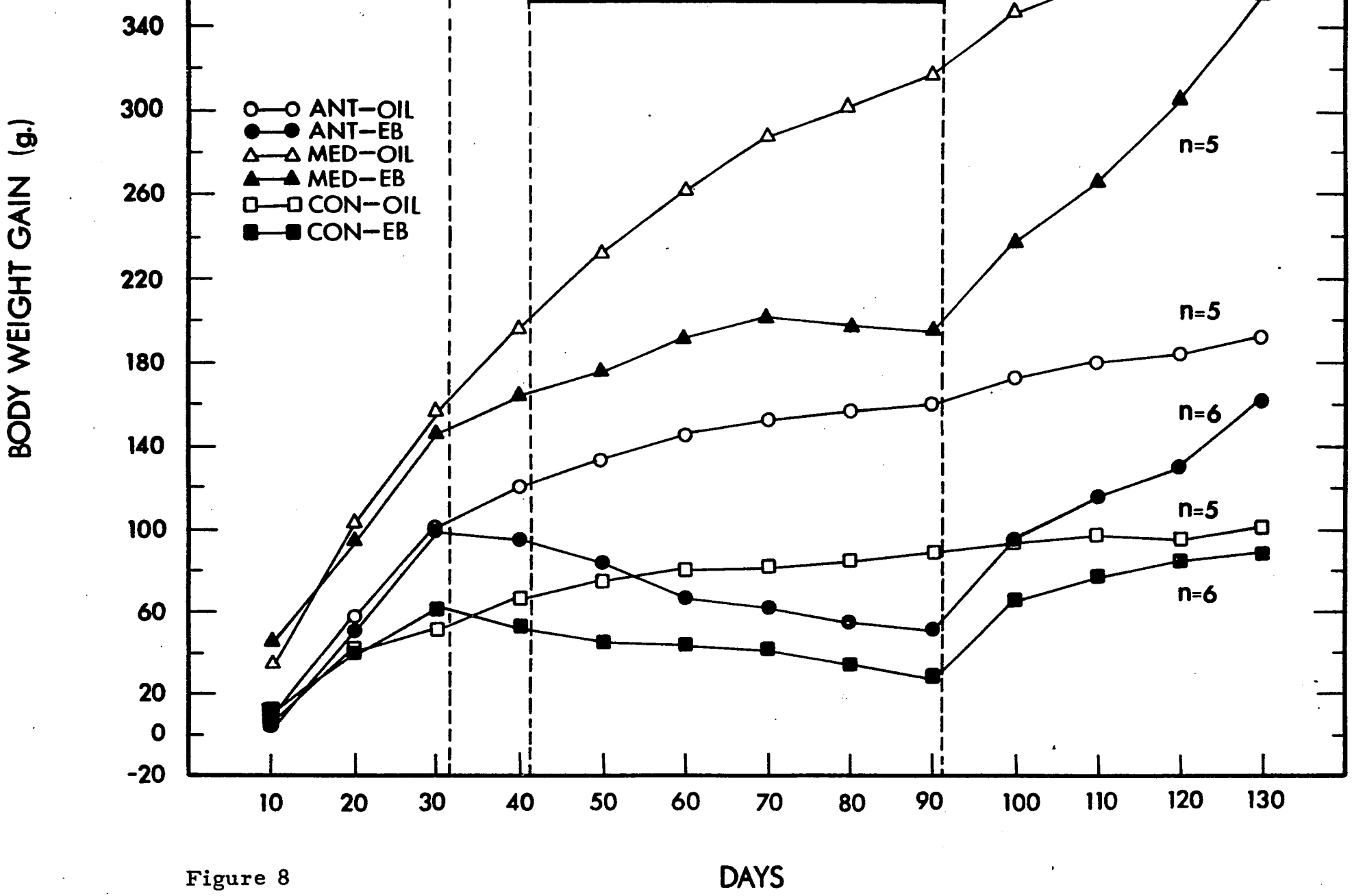
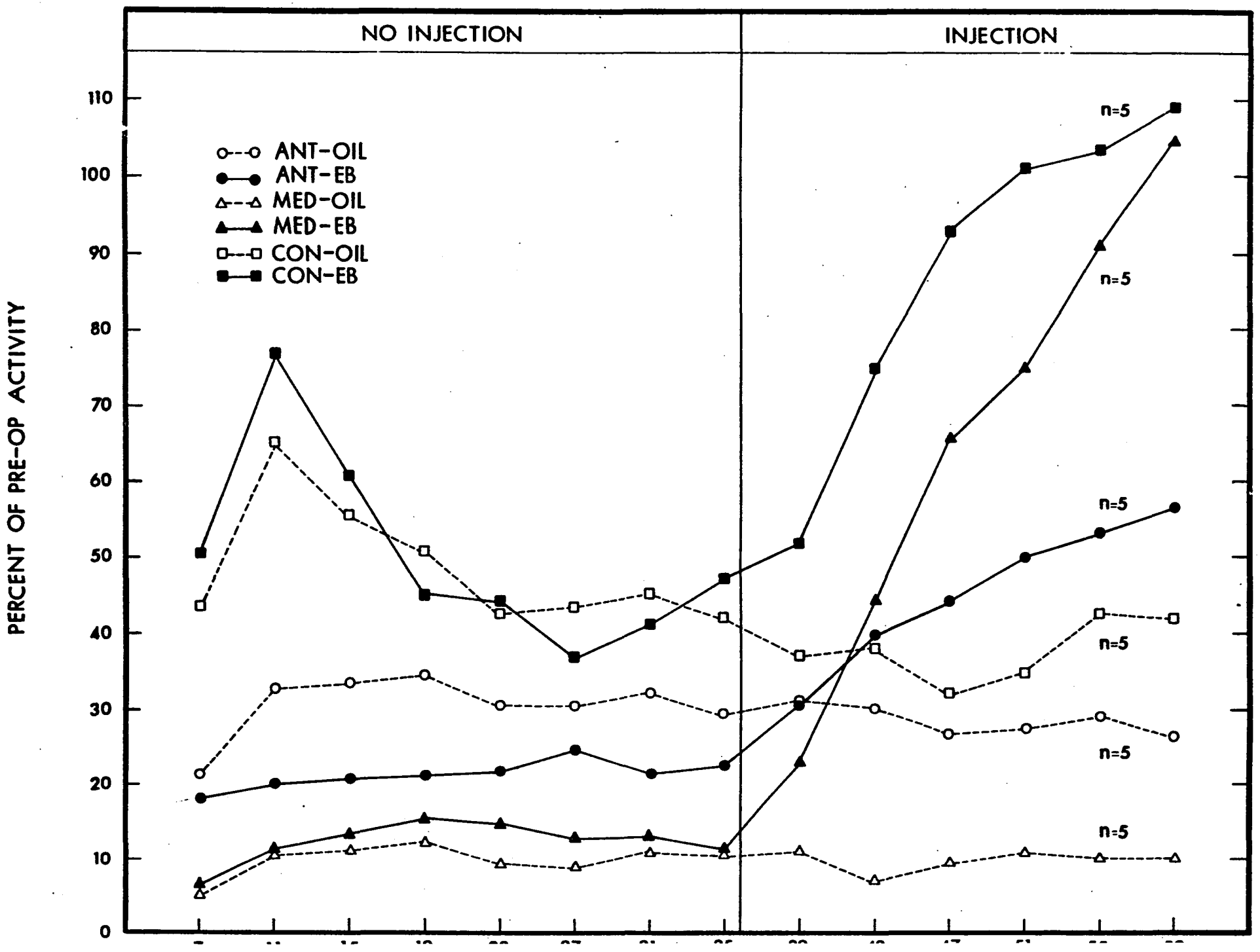

BODY WEIGHT GAIN (g.)
340
300
260
220
180
140
100
60
20
0
-20
10
20
30
40
50
60
70
80
90
100
110
120
130
DAYS
ANT–OIL
ANT–EB
MED–OIL
MED–EB
CON–OIL
CON–EB
n=5
n=5
n=6
n=5
n=6

Figure 8


NO INJECTION
INJECTION
ANT-OIL
ANT-EB
MED-OIL
MED-EB
CON-OIL
CON-EB
n=5
n=5
n=5
n=5
n=5
n=5
PERCENT OF PRE-OP ACTIVITY
0
10
20
30
40
50
60
70
80
90
100
110

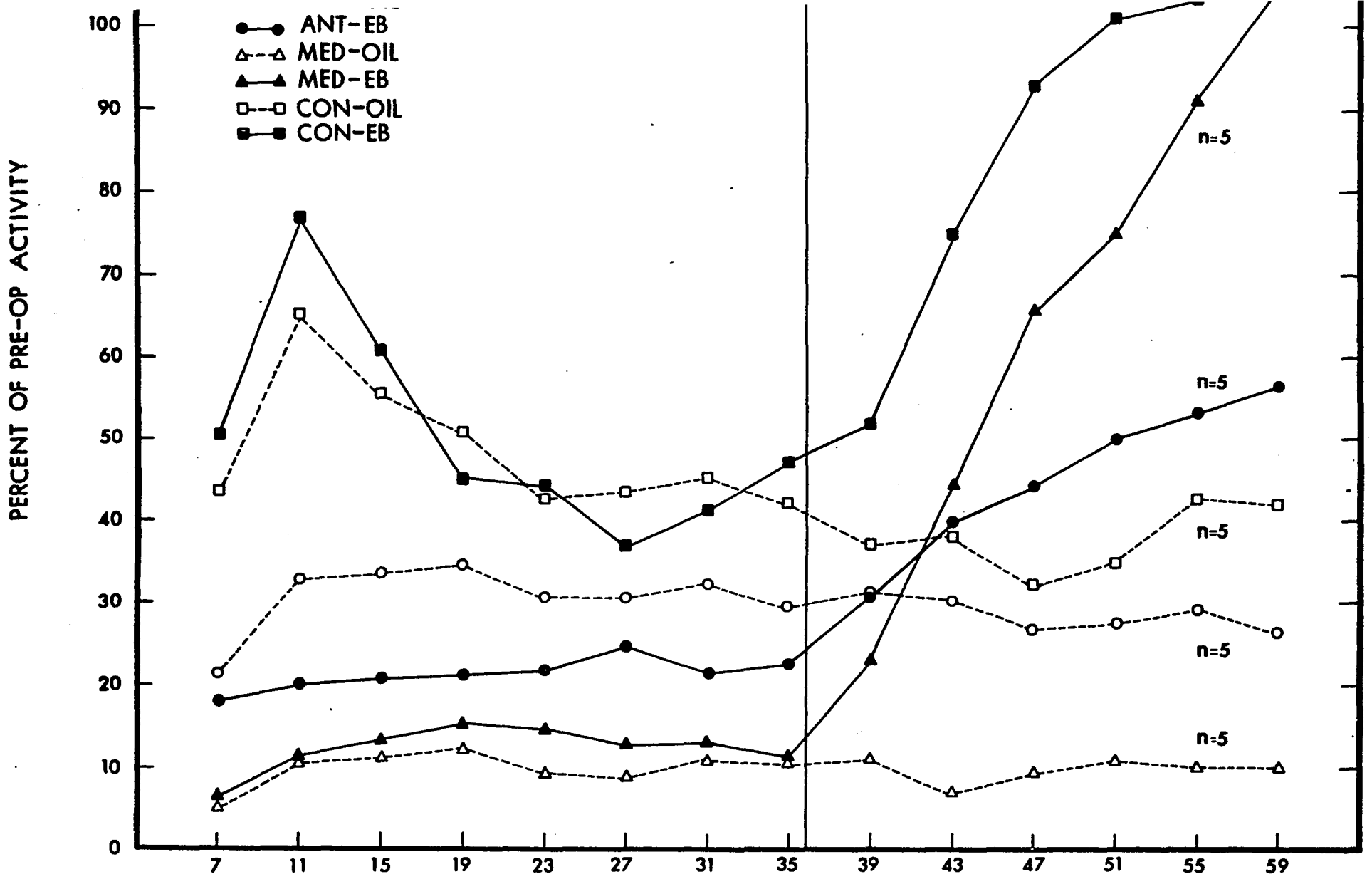

ANT-EB
MED-OIL
MED-EB
CON-OIL
CON-EB
PERCENT OF PRE-OP ACTIVITY
100
90
80
70
60
50
40
30
20
10
0
7
11
15
19
23
27
31
35
39
43
47
51
55
59
DAYS
n=5
n=5
n=5
n=5
n=5

Figure 9

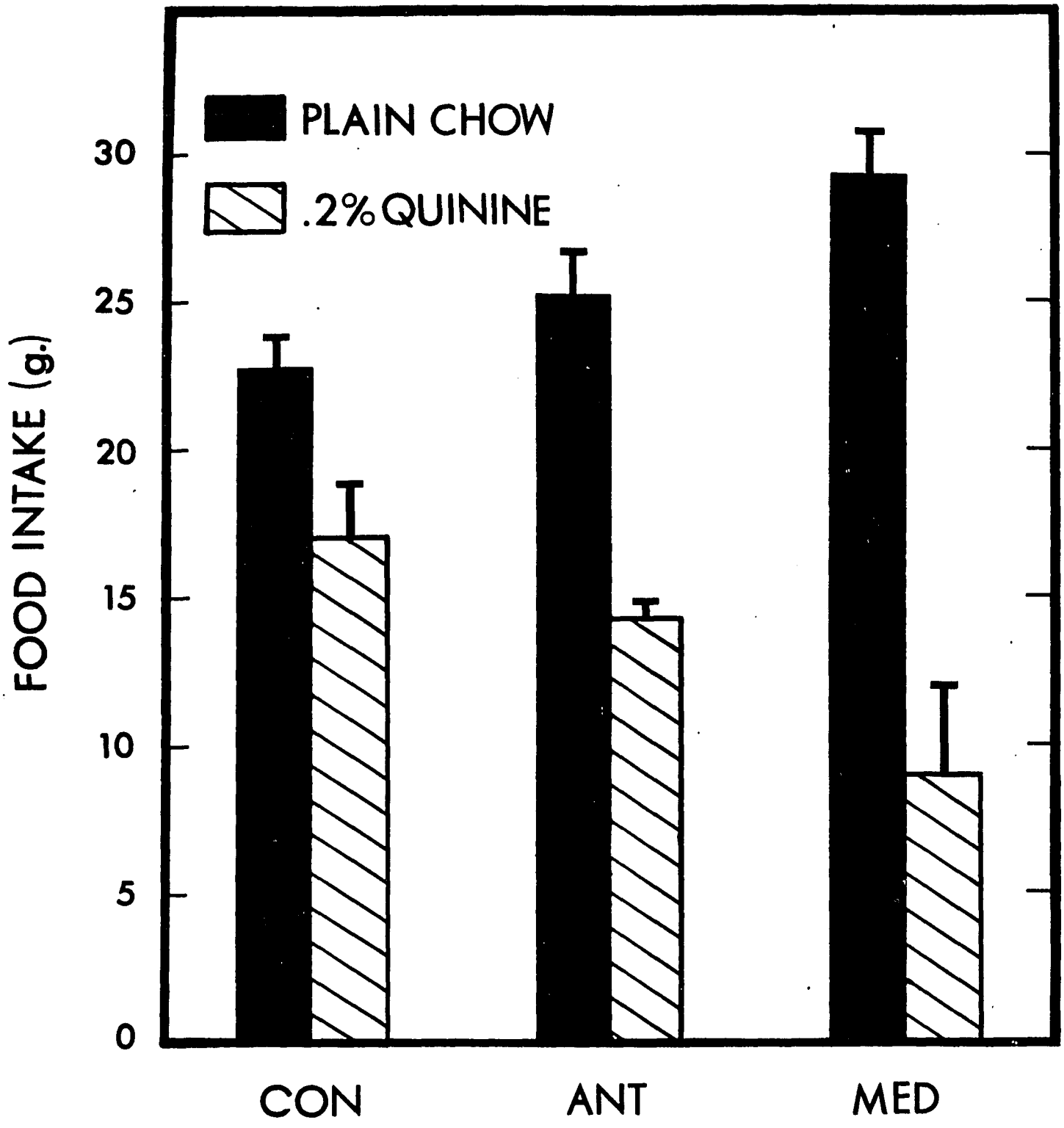


Figure 10

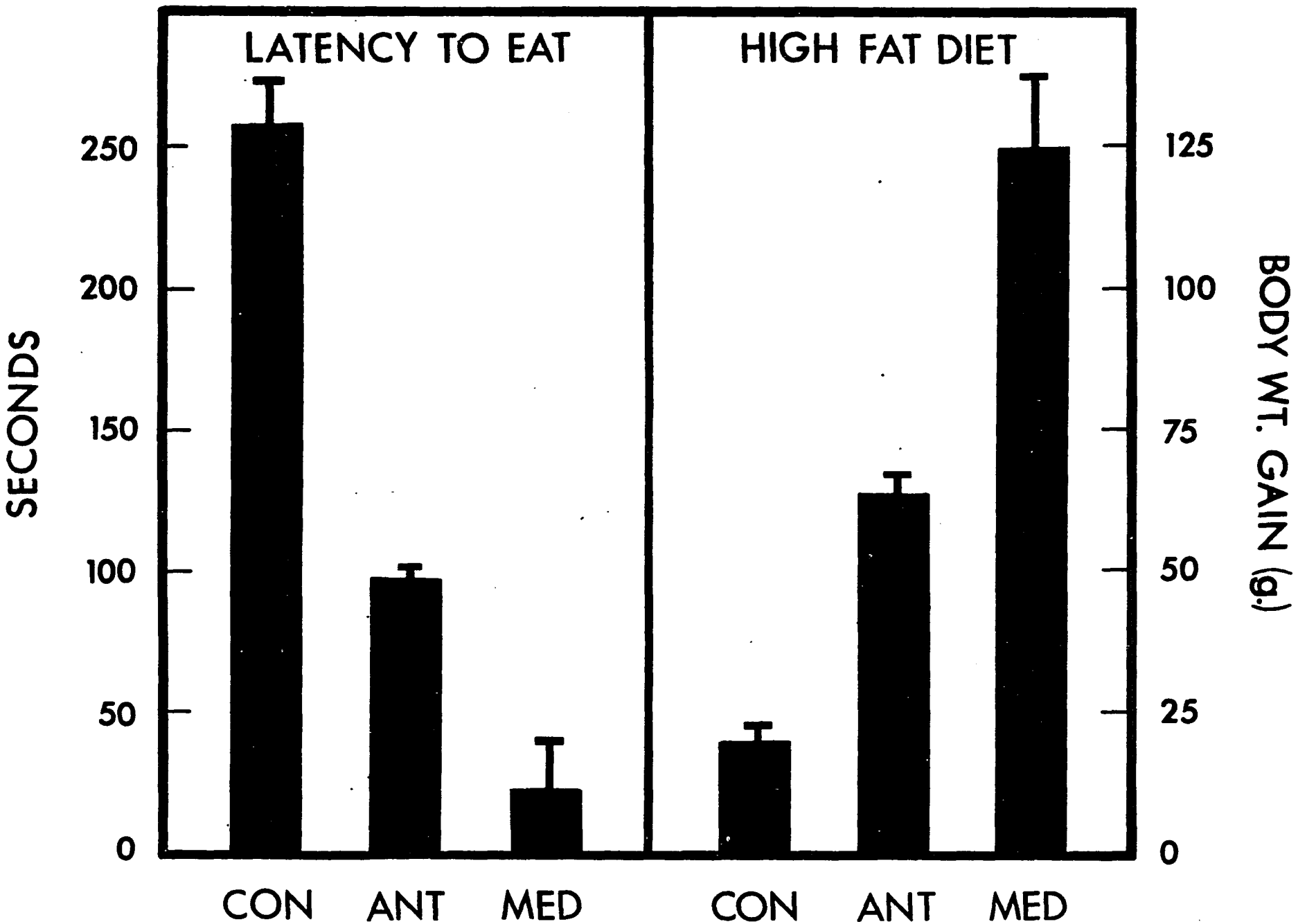

LATENCY TO EAT
HIGH FAT DIET
SECONDS
250
200
150
100
50
0
125
100
75
50
25
0
BODY WT. GAIN (g.)
CON
ANT
MED
CON
ANT
MED

Figure 11