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# HYPOTHALAMUS, ENDOCRINES, AND BEHAVIOR

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# METABOLIC AND ENDOCRINE ASPECTS OF THE VENTROMEDIAL HYPOTHALAMIC SYNDROME

Metabolic and endocrine factors were examined in relation to the behavioral syndrome (hyperphagia, hyperdipsia, and increased aggression) associated with ventromedial hypothalamic damage. Male rats were subjected to parasagittal knife cuts that separated the medial from the lateral hypothalamic areas; following surgery, some rats were given free access to food while others were restricted to normal quantities of food. Compared to control animals, the restricted-food rats exhibited hyperinsulinemia as early as 36 hr. after surgery, without any change in plasma glucose levels. Growth hormone secretion was not significantly altered. The blood samples of free-food rats, which had become obese, showed hyperinsulinemia, mild hyperglycemia, and elevated levels of free fatty acids. These results suggest that the interruption of the medial hypothalamic influence on the lateral hypothalamus produces hyperinsulinemia directly, and that further increases in insulin, glucose, and free fatty acid levels are caused by overeating. The surgical cuts produced an increase in aggressive behavior but no change in the circulating levels of testosterone. Possible neural pathways that mediate endocrine changes are discussed.

# METABOLIC AND ENDOCRINE ASPECTS OF THE VENTROMEDIAL HYPOTHALAMIC SYNDROME

by

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# INTRODUCTION

Destruction of the ventromedial region of the hypothalamus (VMH) produces several behavioral and bodily changes in the rat which include hyperphagia, hyperdipsia, obesity, and an increase in aggression (Hetherington & Ranson, 1940, 1942a, 1942b). These and other reported symptoms have been labelled as the "ventromedial hypothalamic syndrome" (for a review, see Stevenson, 1969; Hoebel, 1971). Generally, it has been assumed, explicitly or implicitly, that the primary effect of VMH destruction is on behavior; any metabolic and endocrine disturbances that have been shown to exist have been regarded as secondary consequences of the behavioral changes (Brobeck, Tepperman, & Long, 1942-43; Hales & Kennedy, 1964). However, it is now well established that the hypothalamus, in general, and the VMH, in particular, are intimately involved in the control of the secretion of several hormones (Ganong, 1966; Frohman & Bernardis, 1968). This suggests the possibility that some of the symptoms of the VMH syndrome may arise as a direct consequence of disturbances in neuroendocrine mechanisms. The present study is concerned with clarifying the role of metabolic and endocrine factors in relation to the behavioral syndrome observed following interruption of VMH function. The specific questions investigated are posed after a brief review of the major relevant findings.

## The Ventromedial Hypothalamic Syndrome

The early investigations of Hetherington & Ranson (1940, 1942a) showed that bilateral electrolytic lesions of the region of the ventromedial hypothalamic nucleus (VMN) resulted in extreme obesity, stunting of body growth and hyperirritability in the rat. Diabetes insipidus was noted in the

presence of the more anterior lesions (Hetherington & Ranson, 1942b). Brobeck, Tepperman, and Long (1942-43) emphasized the marked increase in food intake which occurs following the lesions and attributed the hypothalamic obesity to this ravenous eating or hyperphagia. The studies of Anand and Brobeck (1951) demonstrated that bilateral lesions in the lateral hypothalamus (LH), in the same rostrocaudal plane as the VMN, led to aphagia and to death of the animal from starvation. Because combined lesions of the VMN and LH resulted in aphagia, never hyperphagia, it was suggested that the LH was the more basic mechanism and that the VMN exerted an inhibitory influence on the LH, removal of which leads to overeating (Anand, Dua, & Schoenberg, 1955; Stellar, 1954). Electrical stimulation of the VMN and LH produces effects opposite to those obtained with lesions. Stimulation of the LH results in increased food intake whereas stimulation of the VMN decreases food intake (Anand & Dua, 1955; Hoebel & Teitelbaum, 1962). In general, these data led to a theory of the hypothalamic regulation of food intake in which the LH was designated the "feeding" center and the VMN the "satiety" center.

Recent experiments provide evidence of direct fiber connections from the medial to the lateral hypothalamic area, thus furnishing some anatomical basis for the interaction between the VMN and the LH (Arees & Mayer, 1967; Sutin & Eager, 1969). Using knife cuts to sever discrete neural pathways, several researchers have demonstrated that parasagittal knife cuts lateral to the ventromedial hypothalamic area produce hyperphagia, hyperdipsia, obesity, and irritability in the rat (Albert & Storlien, 1969; Sclafani & Grossman, 1969; Gold, 1970; Paxinos & Bindra, 1972). In a comparison of the effects of ventromedial hypothalamic lesions and parasagittal knife

cuts it was found that the degree of hyperphagia and weight gain was more marked in the lesioned animals (Albert, Storlien, Albert, & Mah, 1971; Sclafani, 1971). These findings suggest that factors other than the release of a lateral hypothalamic feeding center may be involved in the production of hyperphagia and obesity.

Hetherington and Ranson's (1942a) original observation that VMH-lesioned animals "respond with exaggerated violence to slight tactile stimuli [p. 613]" has been subsequently confirmed by several lesion studies (Wheatley, 1944; Anand & Brobeck, 1951; Sclafani, 1971). The finding that rats subjected to parasagittal knife cuts separating the medial from the lateral hypothalamus also become extremely vicious suggests the importance of medio-lateral hypothalamic connections in the control of irritability or affective attack (Paxinos & Bindra, 1972). The VMH has also been implicated in the regulation of predatory attack; lesions in and around the VMN induce mouse killing in about 40% of non-killers (Echlancher & Karli, 1971; Malick, 1970), although the effect may be a transient one (Karli, Vergnes, & Didiergeorges, 1969), whereas lesions in the LH abolish mouse-killing (Karli & Vergnes, 1964; Panksepp, 1971). Rats with parasagittal knife cuts between the VMH and the LH did not exhibit any changes in mouse-killing behavior (Paxinos & Bindra, 1972). In general, then, the behavioral effects of parasagittal knife cuts that separate the VMH from the LH appear to be the same, except perhaps in degree, as the effects of VMH lesions. One of the objectives of this investigation was to determine whether parasagittal knife cuts have the same effects on metabolic and endocrine factors as have been reported for VMH lesions (see below).

Hypothalamic-Endocrine Relations

The best known of the neuroendocrine mechanisms are those controlling pituitary secretion, although the central nervous system also affects the secretion of the adrenal medulla, the pancreas and the gastro-intestinal tract (Ganong, 1966). Fisher, Ingram, and Ranson (1938) first presented evidence for hypothalamic control of posterior pituitary secretion. Vasopressin (antidiuretic hormone) is synthesized in the supraoptic and paraventricular nuclei of the hypothalamus and is transported along neurosecretory axons of the supraoptico-hypophyseal tract down through the median eminence into the posterior pituitary. Verney (1947) demonstrated that increased plasma osmolarity stimulates vasopressin release and postulated the presence of osmoreceptors in the hypothalamus. The most important function of vasopressin is to inhibit water diuresis, and damage to the synthesizing cells or their axons leads to rapid depletion of body fluids (Sawyer & Mills, 1966).

Within the last 10-15 years a whole new family of neurohormones, termed releasing and inhibiting factors, have been discovered in the hypothalamus which act directly on the pituitary to modulate the secretion of each of the anterior pituitary tropic hormones (McCann & Porter, 1969). In contrast to the posterior pituitary which is controlled by neural connections, control of the anterior pituitary is mediated through a humoral mechanism. Numerous portal-hypophyseal vessels carry blood from the capillaries of the ventral hypothalamus to the anterior pituitary and the hypothalamic neurohormones are released directly into the hypophyseal portal vessels for transport to the anterior pituitary. At least six of these factors have been identified and include corticotropin-releasing factor

(CRF), thyrotropin-releasing factor (TRF), growth hormone-releasing factor (GHRF), follicle-stimulating hormone-releasing factor (FSHRF), luteinizing hormone-releasing factor (LHRF), and prolactin inhibitory factor (PIF). Experiments involving interruption of all neural afferents to the medial basal hypothalamus have revealed that this area, termed the hypophysiotrophic area, is able to maintain nearly normal anterior pituitary function in the absence of neural connections from higher centers (Halász & Pupp, 1965). The importance of the hypothalamus in neuroendocrine regulation is shown by the fact that this area of the brain contains the final elements of several neural inputs which converge to influence endocrine secretion. In the light of these new findings, a reexamination of the role of neuroendocrine mechanisms in the VMH syndrome is warranted.

Neuroendocrine Mechanisms and the VMH Syndrome

The etiology of the obesity characteristic of the VMH syndrome has been attributed to the hyperphagia which occurs following the experimental lesion. Any metabolic disturbances that have been shown to exist have been assumed to be the secondary consequences of the overeating behavior (Brobeck, Tepperman, & Long, 1942-43). Hyperinsulinemia and elevated concentrations of plasma-free fatty acids have been found in adult rats with VMH lesions but the influence of the hypothalamus was assumed to be an indirect one mediated through the regulation of food intake (Hales & Kennedy, 1964). Recent studies showing that VMH lesions cause metabolic and endocrine disturbances in the absence of overeating suggest that these effects may be the direct result of the lesions (Frohman & Bernardis, 1968; Han & Frohman, 1970; Martin & Bouman, 1971; Hustvedt & Løvø, 1972).

Weanling rats have been employed in several of these studies because they do not become hyperphagic after VMH lesions, and because their rate of somatic growth is faster and consequently a more prominant effect of the lesions on growth can be observed (Kennedy, 1957; Bernardis & Skelton, 1965). Weanling rats subjected to VMH lesions grow linearly at a slower rate than the controls and still become obese (defined as an increase in carcass fat) in the absence of overeating (Han, Lin, Chu, Mu, & Liu, 1965). With the development of sensitive radioimmunoassay techniques for the measurement of plasma growth hormone, Frohman and Bernardis (1968) showed that the growth retardation resulting from electrolytic destruction of the VMN in weanling rats is due to impairment of growth hormone secretion. The growth hormone content in the pituitary gland and in the plasma was significantly reduced in rats with VMN lesions, and fat deposition was enhanced in the absence of hyperphagia. Studies involving electrical stimulation of the VMN support the hypothesis that the VMN may be the site of origin of growth hormone-releasing factor. Stimulation only in the ventromedial nuclei and their border zones resulted in a consistent rise in plasma growth hormone whereas stimulation of other loci including anterior hypothalamus, preoptic area, lateral hypothalamus, and fornix was ineffective (Frohman, Bernardis, & Kant, 1968; Bernardis & Frohman, 1971a; Martin, 1972). The role of growth hormone in the VMH syndrome has been investigated using rats subjected to hypophysectomy and/or growth hormone therapy. Han (1968) observed that hypophysectomized rats with VMH lesions accumulated more body fat than their hypophysectomized controls when both groups were force-fed equal amounts of food. Growth hormone treatment produced equal linear growth in both the sham operated and lesioned rats but did not alter

the increase in fat accumulation. These findings demonstrate that obesity due to VMH lesions can develop in the absence of both overeating and pituitary involvement and indicate that other factor(s) must have contributed to the development of the obesity.

One such factor is insulin and the endocrine pancreas. The effects of VMH lesions on parameters of carbohydrate metabolism were investigated and it was found that increased levels of insulin were present in fasted weanling rats with VMH lesions, despite minimal changes in plasma glucose levels (Frohman, Bernardis, Schnatz, & Burek, 1969). They note that plasma insulin levels were significantly elevated by the fourth postoperative day in spite of a decreased food intake. A direct relationship between the VMH and the endocrine pancreas has been demonstrated, since the pancreatic islet size of tube-fed hypophysectomized adult rats bearing VMH lesions was found to be significantly larger than that of their controls (Han, Yu, & Chow, 1970). It has recently been established that hyperinsulinemia can develop in adult rats following VMH lesions, in the absence of hyperphagia. Han and Frohman (1970) report elevated plasma insulin levels by the fifth postoperative day in VMH-lesioned hypophysectomized adult rats as compared to controls when both groups were tube fed equal amounts of food. Hustvedt and Løvø (1972) found considerable elevation in plasma insulin levels with a concomitant small increase in blood glucose, two days after VMH destruction in adult rats subjected to restricted food intake. Firm knowledge of the plasma insulin and glucose changes during the first postoperative days is essential for a further understanding of the relation between the VMH and insulin secretion.

In general, the above findings indicate that the VMN is an important control site for the regulation of growth hormone and insulin secretion.

In an experiment in which the size of the lesion of the VMN was varied, no correlation was found between the parameters of growth and those of obesity (Bernardis & Frohman, 1970). This suggests that these functions are controlled by two different neuronal groups whose cell bodies lie within the VMN but whose axons project in different directions. Utilization of the more precise knife cut technique would perhaps delineate the specific neural pathways involved in this neuroendocrine control and make it possible to dissociate the linear growth and obesity effects.

Observations of human subjects show that obesity in man is accompanied by a number of metabolic and endocrine changes. These include hyperinsulinemia, blunted growth hormone responsiveness, and elevated serum-free fatty acids (Sims & Horton, 1968). The question as to whether these abnormalities in man have a primary role in the etiology of obesity or are a secondary consequence of overeating behavior has not as yet been resolved (Rabinowitz, 1968). The VMH animal provides an excellent preparation for studying the constellation of abnormalities occurring in experimentally-produced obesity.

Considerable evidence indicates that the secretion of gonadotropins (follicle-stimulating hormone and luteinizing hormone) is under hypothalamic control (Davidson, 1966). The finding that only anterior pituitary grafts situated in the medial basal part of the hypothalamus release sufficient amounts of follicle-stimulating hormone and luteinizing hormone to maintain the normal function of the gonads, supports the view that hypophysiotrophic factors influence both the synthesis and release of gonadotropins (Halász, Pupp, & Uhlarick, 1962-63; Halász & Pupp, 1965). The preoptic area has been implicated in the release of luteinizing hormone-releasing factor

(Halász, 1969) whereas control of follicle-stimulating hormone secretion appears to require an intact anterior hypothalamic area (Halász & Gorski, 1967).

Gonadal hormones, especially testosterone, have been shown to influence aggressive behavior in a number of species. Aggressive behavior in adult male hamsters decreases after castration and is restored by injections of testosterone propionate (Vandenbergh, 1971). Castrated male mice which had failed to exhibit isolation-induced aggression did so after the administration of testosterone (Sigg, Day, & Colombo, 1966). Testosterone-propionate implanted in the anterior hypothalamic preoptic area of the male ring dove brain activated aggressive behavior (Barfield, 1971). The results of a recent study indicate a highly significant relationship between testosterone production rate and a number of hostility and aggression indicators in normal young men (Persky, Smith, & Basu, 1971). These behavioral findings in conjunction with the neuroendocrine data suggest the possibility of an alteration in testosterone secretion to account for the aggressiveness of the VMH animal.

## The Present Investigation

On the basis of the previous review, it is evident that a number of issues remain unresolved. It is well documented that lesions of the VMH produce several endocrine and metabolic disorders. Since parasagittal knife cuts placed between the VMH and LH are effective in producing the behavioral and bodily changes associated with the VMH syndrome, although to a lesser degree, it is of interest to determine the metabolic and endocrine profile of animals subjected to such knife cuts. In particular, do parasagittal knife cuts lateral to the VMH affect the secretion of plasma insulin, glucose,

free fatty acids, and growth hormone in a manner similar to that of VMH lesions? Also, is an alteration in testosterone secretion responsible for the characteristic appearance of aggressive behavior following interruption of VMH function? And, thirdly, what are the metabolic and endocrine consequences of overeating?

In order to study the above questions, male rats were subjected to parasagittal knife cuts that separated the medial from the lateral hypothalamic areas. Behavioral, somatic, metabolic, and endocrine measures were taken at appropriate times, before and after surgery, and the effects of knife cuts and overeating were examined.

## METHOD

### Subjects and Design

Twenty-one male Long-Evans hooded rats, weighing 250-300 gm., served as subjects. They were individually housed in wire-mesh cages and maintained on a 12-hr. light-dark cycle (11 p.m.-11 a.m., light; 11 a.m.-11 p.m., dark) in a constant-temperature (23° C) room.

The experiment lasted for a period of 31 days, during which time various behavioral, somatic, metabolic, and endocrine measures were taken. Surgery was performed on the 10th day. At the time of surgery the subjects were divided into four groups: (1) experimental-restricted food intake (EXP-REST), (2) experimental-food ad libitum (EXP-AD LIB), (3) control-restricted food intake (CONT-REST), and (4) control-food ad lib (CONT-AD LIB), matched as closely as possible on two measures--food intake and body weight --taken during the preoperative period. This 2 x 2 design permitted an examination of the independent and interactional effects of knife cuts and overeating.

Food Intake, Water Intake, Body Weight, and Linear Growth

Food intake, water intake, and body weight were recorded daily for a period of 10 days prior to surgery and for 21 days postoperatively. Daily food intake was measured to the nearest 0.1 gm. by giving each rat a weighed quantity of Purina Lab Chow pellets every day and, at the end of 24 hr., weighing the remaining quantity; papers were placed under each cage to catch spillage. During the preoperative period all animals had food available ad libitum. Following surgery each rat in the restricted food intake groups was offered an amount of food equal to its preoperative mean daily food intake; thus rats in the restricted food intake groups were not allowed to overeat. All subjects in the food ad lib groups had food available ad libitum. Fresh tap water was continuously available in Richter tubes and daily water intake was recorded to the nearest 1 ml. Linear growth was assessed by measuring, under light ether anaesthesia, naso-anal length prior to surgery and again on the 20th postoperative day.

Aggression

Two types of aggressive responses were examined; irritability or affective attack and predatory attack. Measures of irritability were taken at the same time each day during the light portion of the cycle in the rat's home cage. Each rat was rated on a three-point scale on the following three categories: (1) biting reaction to visual presentation of a gloved hand, (2) biting reaction to prodding of snout with a gloved hand, and (3) vocalization. Scores of from 0 (no response) to 2 (maximum response) were given for each of the three categories.

Mouse killing, as a form of predatory attack, was measured during the dark cycle on two successive days preoperatively and on alternate days post-

operatively. A mouse was introduced into the home cage of each rat in a manner so as to disturb the rats as little as possible. The mouse was left in the cage for a period of 20 min. and instances of killing were recorded. If a mouse was killed during the test it was removed immediately to prevent the rat from eating it.

Blood Sampling

All blood samples were collected following a 12-hr. period of food deprivation (11 p.m.-11 a.m.). Blood was collected from the tail of each rat on three occasions during the experiment: four days prior to surgery, 36 hr. postoperatively, and 10 days postoperatively. The rat was wrapped in a towel and it's tail warmed for 30 sec. Approximately 0.5 mm. of the tip of the tail was amputated with a scalpel and blood (0.5-1.0 ml.) was collected into heparinized tubes and centrifuged immediately. Plasma was separated and stored at -20°C for subsequent assay of glucose and insulin. At the termination of the experiment (21st postoperative day), the rats were sacrificed by decapitation within 30-60 sec. after removal from the cage and trunk blood was collected into heparinized tubes and centrifuged. Fresh plasma was utilized for the free fatty acids determination and the remaining plasma was kept frozen for later analysis of growth hormone, testosterone, insulin, and glucose.

Metabolic and Endocrine Tests

Plasma glucose was determined by the glucose oxidase method (Glucostat, Worthington Biochemicals, Freehold, New Jersey).

Free fatty acids (FFA) were measured according to the method of Laurell and Tibbling (1967), which is a colorimetric micromethod based on the

formation of FFA-Cu soaps.

Insulin was measured by a radioimmunoassay procedure utilizing $^{131}I$ and employing dextran-coated charcoal to separate the free and bound components (Hillman & Colle, 1969). Porcine insulin was used as a standard.

Plasma growth hormone (GH) was determined by radioimmunoassay using purified rat GH(NIAMD-GH-I-1) for radiolabelling with $^{125}I$ and for standards (Martin, 1972). All samples were measured in duplicate and the assay was sufficiently sensitive to measure plasma GH levels of 1 ng/ml.

Testosterone concentration was determined by a competitive protein-binding method which combines the protein-binding technique of Mayes and Nugent (1968) and the extraction, paper chromatography, and elution techniques of Maeda, Okamoto, Wegienka, and Forsham (1969).

The determinations of plasma glucose, free fatty acids, and insulin were made by the author, and those of plasma growth hormone and testosterone were made by others. All determinations were made blind without knowledge of the rat's behavioral or somatic results.

Surgery

The subjects were given atropine sulphate (o.6 mg / ml ) and anaesthetized with sodium pentobarbital (60 mg /ml ) followed by chloral hydrate (300 mg /ml ), all administered intraperitoneally. Thirteen rats received bilateral parasagittal knife cuts using a retractable wire knife similar to the one developed by Sclafani and Grossman (1969). It consisted of a 30-gauge stainless steel guide cannula constructed from a hypodermic needle. The tip of the needle was bent at one end so that a 0.13 mm.-diam. tungsten wire pushed through the cannula would extend in the direction of the curved tip. The knife assembly was attached to the stereotaxic holder and inserted

into the brain at 0.3 mm. anterior to bregma and 1.0 mm. lateral to the midline, with the incisor bar at 3.7 mm. below horizontal. The guide cannula was lowered 7.5 mm. below the skull surface. The inner wire was then extended by pushing it through the cannula and out the curved tip in a sagittal plane 3.0 mm. posterior to the tip. The extended wire was lowered approximately 2.2 mm. to the base of the brain, thus producing a para-sagittal knife cut 3.0 mm. long with a dorso-ventral extent of 2.2 mm. The wire was retracted into the cannula and the guide cannula was withdrawn from the brain.

Eight rats served as sham-operated controls. Sham operations consisted of anaesthetizing the rat, placing it in the stereotaxic instrument, exposing the skull and drilling holes to the dura. The brain was left intact.

Statistical Analysis

Five experimental rats died following surgery and two control rats were eliminated from the experiment because of illness. All data analyses pertains to the remaining 14 animals. An analysis of variance (ANOVA), using an unweighted means solution, was performed on glucose and insulin levels across days (repeated measures) to assess the effects of knife cuts and overeating. The significance of the difference between all pairs of means across time was evaluated using the Newman-Keuls test (Winer, 1971). A multivariate analysis of variance followed by univariate ANOVA's was performed on final levels of food intake, body weight, water intake, linear growth, insulin, glucose, growth hormone, testosterone, and free fatty acids.

## Histology

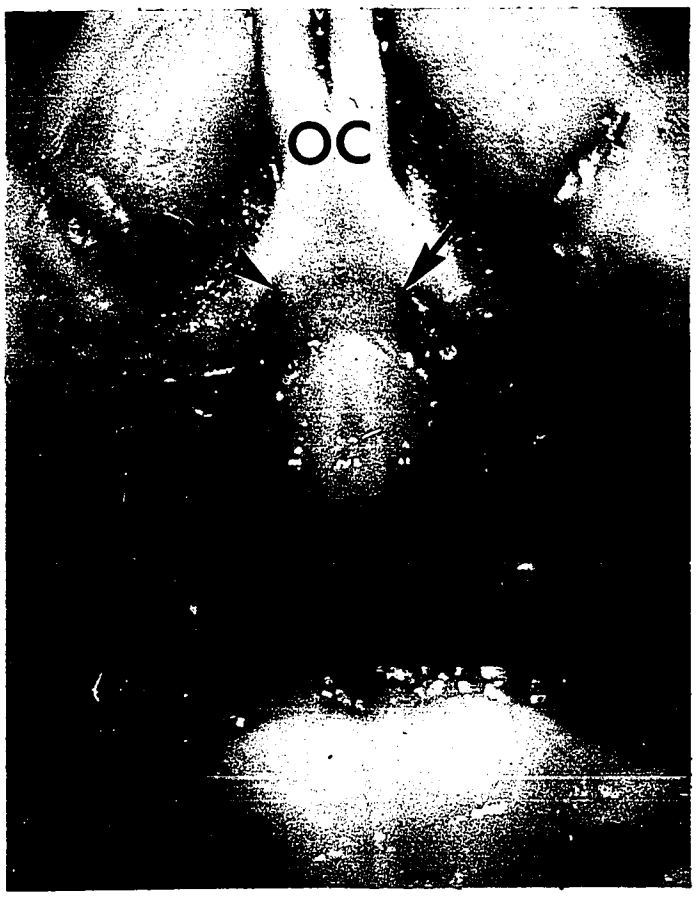
Following decapitation, the brains were removed and fixed in 10% formalin. Coronal sections were obtained by cutting at a thickness of 30μ and every third section was stained with thionin. Knife cuts were localized according to the atlas of Pellegrino and Cushman (1967).

# RESULTS

## Histological Findings

Figure 1 illustrates the type of bilateral parasagittal knife cuts made in this experiment, as seen from the ventral side of the brain. Figure 2 shows the knife cuts in three anterior-posterior coronal sections from the brain of a rat representative of the experimental subjects. While a slight degree of asymmetry was noted, it was not marked in any case. The 3 mm. long cuts extended from the preoptic area just rostral to the anterior hypothalamus to the lateral mamillary nucleus just caudal to the posterior hypothalamus, paralleling the entire length of the VMN. The approximate anterior-posterior extent of the cut is from 7.4 to 4.4 in the atlas of Pellegrino and Cushman (1967). The cuts were located lateral, or medial, to the fornix and extended from the level of the anterior commissure dorsally to the base of the brain. In most rats the extreme lateral tips of the VMN were clipped. Consequently, all neural connections between the medial and lateral hypothalamus were severed in addition to some slight separation of the medial from the lateral preoptic area. One subject (Rat 23), whose cut was slightly more anterior (A-P, 7.6), exhibited damage to the supraoptic nuclei.

Figure 1. Parasagittal knife cuts as seen from the ventral side of the brain of an experimental rat. Arrows indicate the anterior-posterior extent of the cuts. OC: optic chiasm.


OC

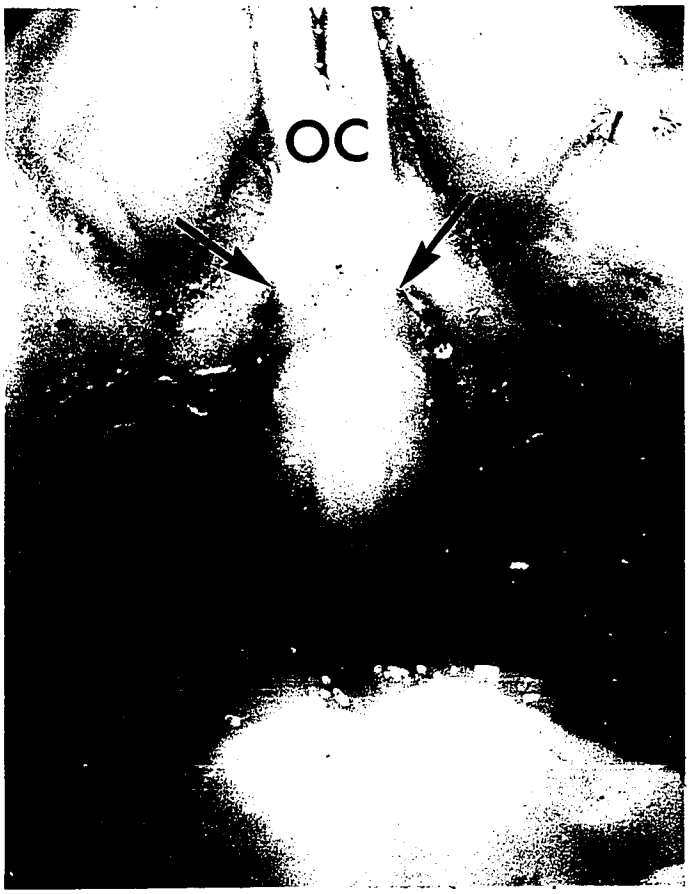
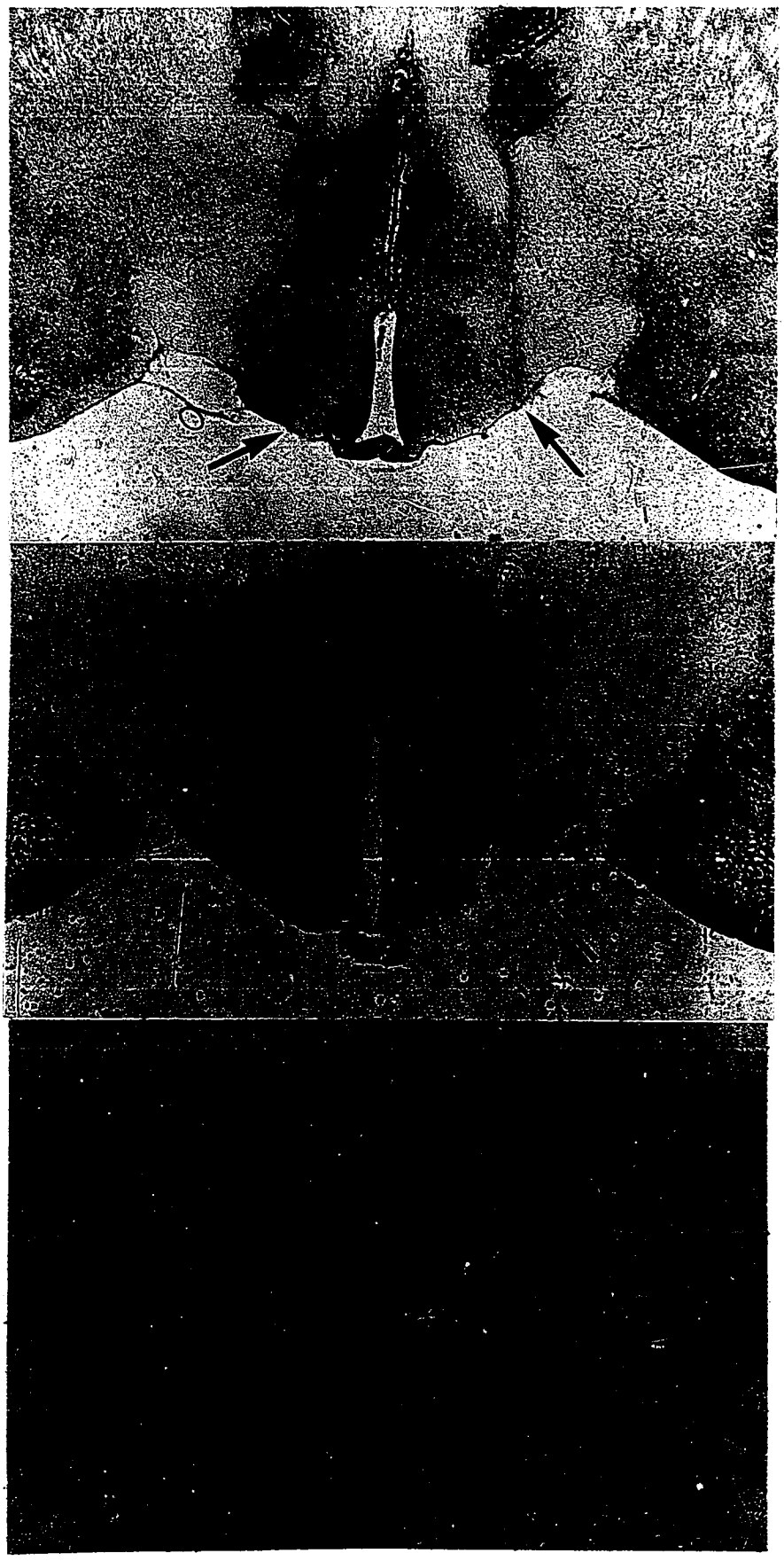
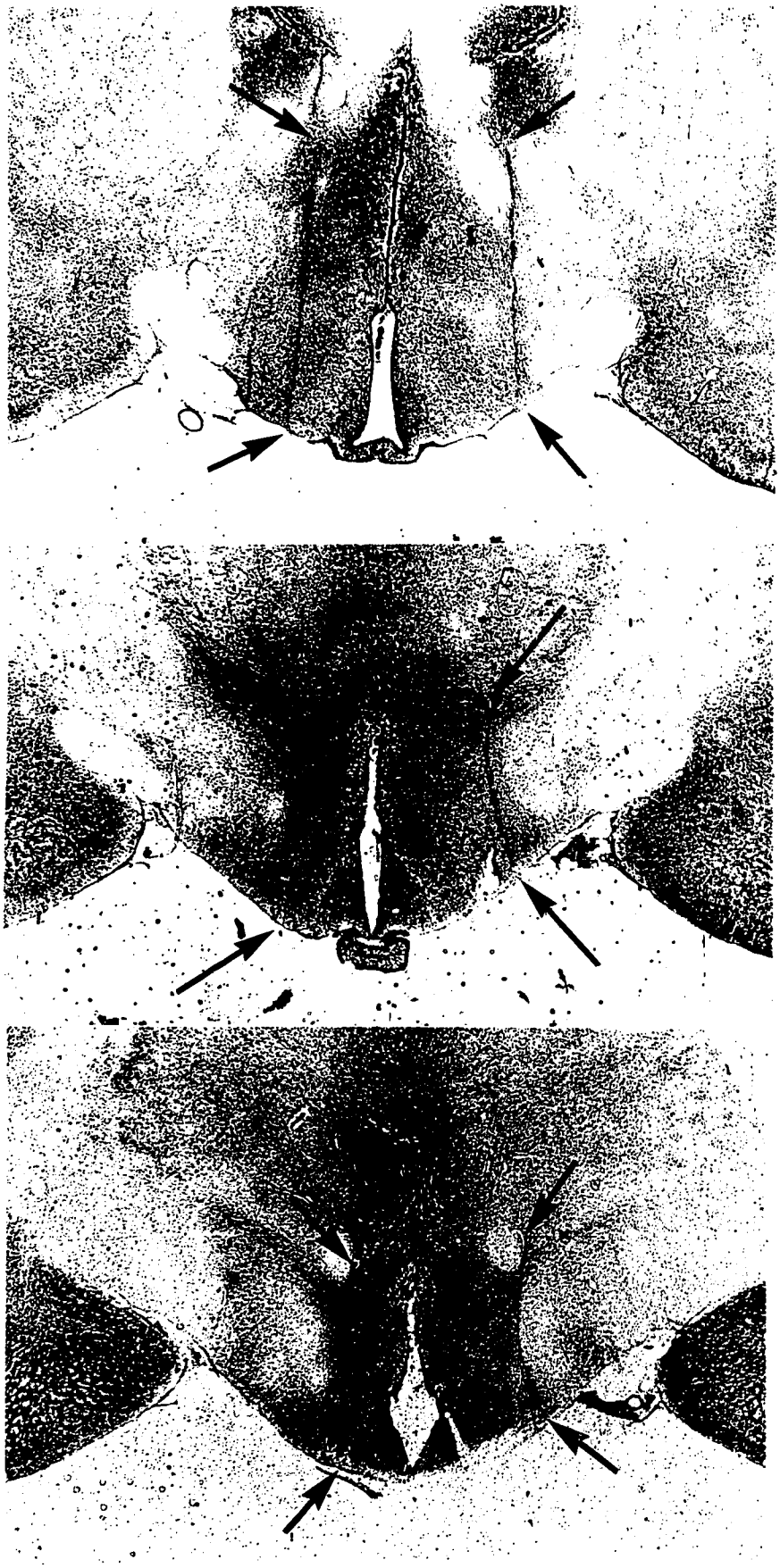

OC

Figure 2. Microphotographs (x10) of parasagittal knife cuts in three anterior-posterior coronal sections from the brain of a rat representative of the experimental subjects. Arrows indicate the dorso-ventral extent of the cuts.





Food Intake, Water Intake, Body Weight, and Linear Growth

The mean daily food intake, water intake, and body weight of each group are shown in Figure 3. Following surgery, all rats in the EXP-AD LIB group were hyperphagic and became obese. They ate twice as much and gained weight three times as rapidly as the control animals. Their water intake also increased but the water/food ratio remained at its preoperative value of 1.2 indicating that the increase in water intake paralleled the increase in food intake. The EXP-REST group exhibited no difference in food intake or body weight as compared to the corresponding control animals. They drank about the same amount of water except for one subject (Rat 23) whose water intake increased five-fold following surgery. Histological examination revealed that this subject had sustained damage to the supraoptic nuclei and as a consequence exhibited massive polydipsia (>200 ml /day) and polyuria.

The naso-anal length of all animals increased approximately 0.5-1.0 mm. during the postoperative period.

For quick easy reference, the mean final levels of all dependent variables for the four groups of rats are shown in Table 1. The multivariate ANOVA on the final-level data yielded the results shown in Table 2. The knife cuts produced a highly significant effect on food intake ($p < 0.0001$) and body weight ($p < 0.001$). As expected, the effect of overeating on these two measures was also highly significant. The analysis showed a significant interaction between the two main effects on both food intake and body weight. There was no significant effect of either knife cut or overeating on water intake and linear growth.

Aggression

Prior to surgery there were no differences among the groups in their

Figure 3. Mean daily food intake, water intake, and body weight of the four groups of rats before and after parasagittal knife cuts and sham operations. Asterisks indicate days on which the animals were 12-hr. food-deprived.

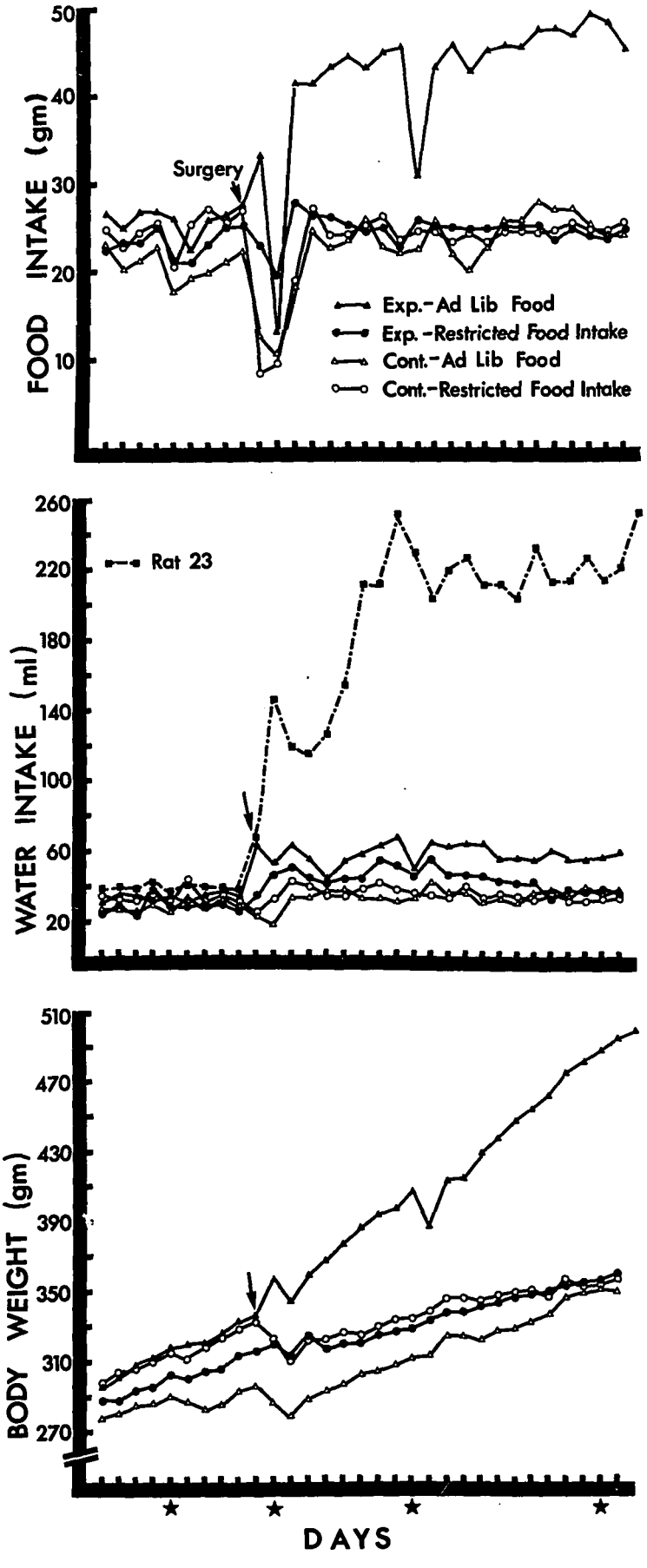

FOOD INTAKE (gm)
50
40
30
20
10
Surgery
Exp.-Ad Lib Food
Exp.-Restricted Food Intake
Cont.-Ad Lib Food
Cont.-Restricted Food Intake
WATER INTAKE (ml)
260
220
180
140
100
60
20
Rat 23
BODY WEIGHT (gm)
510
470
430
390
350
310
270
DAYS

TABLE 1

Final Levels of Behavioral, Somatic, Metabolic, and Endocrine Parameters for the Four Groups of Rats

| PARAMETER | Group | | | |
|---|---|---|---|---|
| | EXP-REST (4)[a] | EXP-AD LIB (4) | CONT-REST (3) | CONT-AD LIB (3) |
| Food Intake (gm/day) | 25.0±1.1 | 50.1±0.4 | 25.8±1.4 | 25.7±1.5 |
| Body Weight (gm) | 360.5±9.6 | 494.0±12.6 | 362.6±14.9 | 348.0±25.7 |
| Water Intake (ml/day) | 98.0±52.3 | 60.0±4.3 | 34.3±3.3 | 37.6±2.9 |
| Naso-Anal Length (mm) | 23.4±0.2 | 24.6±0.1 | 24.3±0.2 | 23.5±0.1 |
| Plasma Insulin (μU/ml) | 22.2±2.9 | 33.2±1.5 | 9.0±2.6 | 8.3±2.6 |
| Plasma Glucose (mg/100 ml) | 145.0±7.1 | 144.5±3.8 | 129.3±7.1 | 127.6±11.3 |
| Free Fatty Acids (meq/L) | 0.18±0.02 | 0.26±0.01 | 0.17±0.01 | 0.18±0.02 |
| Growth Hormone (ng/ml) | 6.7±5.4 | 0.5±0.0 | 27.3±14.5 | 1.8±0.7 |
| Testosterone (mμg/100 ml) | 35.1±7.2 | 54.1±11.6 | 35.9±15.8 | 96.7±34.6 |

Note.- Values are means ±S.E.M.

[a] Number of rats in each group

TABLE 2

Multivariate Analysis of Variance of
Final Levels of Behavioral, Somatic,
Metabolic, and Endocrine Parameters

| Multivariate Analysis | | | Univariate Analyses | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Food Intake | | Water Intake | | Body Weight | | Linear Growth | |
| Source | df | F | df | MS | F | MS | F | MS | F | MS | F |
| Knife Cut (C) | 9 | 595.14** | 1 | 475.71 | 114.13*** | 6339.42 | 1.90 | 17732.58 | 21.27*** | 0.019 | 0.14 |
| Overeating (F) | 9 | 356.94** | 1 | 717.14 | 172.06*** | 1440.28 | 0.43 | 17150.03 | 20.57** | 0.378 | 2.89 |
| C x F | 9 | 124.83** | 1 | 545.40 | 130.85*** | 1464.38 | 0.43 | 18817.11 | 22.57*** | 3.54 | 27.11*** |
| Error | 2 | | 10 | | | | | | | | |

* $p<0.05$

** $p<0.01$

*** $p<0.001$

(continued on p. 16d)

TABLE 2 (cont'd.)

Multivariate Analysis of Variance of
Final Levels of Behavioral, Somatic,
Metabolic, and Endocrine Parameters

| Multivariate Analysis | | | Univariate Analyses | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Insulin | | Glucose | | Free Fatty Acids | | Growth Hormone | | Testosterone | |
| Source | df | F | df | MS | F | MS | F | MS | F | MS | F | MS | F |
| Knife Cut (C) | 9 | 595.14** | 1 | 1248.59 | 57.76*** | 905.36 | 4.87 | 0.0069 | 4.66 | 411.71 | 2.53 | 1610.76 | 1.47 |
| Overeating (F) | 9 | 356.94** | 1 | 125.99 | 5.83* | 3.50 | 0.02 | 0.0093 | 6.22* | 735.87 | 4.51 | 4776.70 | 4.36 |
| C x F | 9 | 124.83** | 1 | 116.67 | 5.40* | 1.16 | 0.006 | 0.0053 | 3.53 | 317.62 | 1.95 | 1494.05 | 1.36 |
| Error | 2 | | 10 | | | | | | | | | | |

* $p<0.05$

** $p<0.01$

*** $p<0.001$

TABLE 2

Multivariate Analysis of Variance of
Final Levels of Behavioral, Somatic,
Metabolic, and Endocrine Parameters

| Multivariate Analysis | | | Univariate Analyses | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Food Intake | | Water Intake | | Body Weight | | Linear Growth | |
| Source | df | F | df | MS | F | MS | F | MS | F | MS | F |
| Knife Cut (C) | 9 | 595.14** | 1 | 475.71 | 114.13*** | 6339.42 | 1.90 | 17732.58 | 21.27*** | 0.019 | 0.14 |
| Overeating (F) | 9 | 356.94** | 1 | 717.14 | 172.06*** | 1440.28 | 0.43 | 17150.03 | 20.57** | 0.378 | 2.89 |
| C x F | 9 | 124.83** | 1 | 545.40 | 130.85*** | 1464.38 | 0.43 | 18817.11 | 22.57*** | 3.54 | 27.11*** |
| Error | 2 | | 10 | | | | | | | | |

* $p<0.05$

** $p<0.01$

*** $p<0.001$

(continued on p. 16d)

TABLE 2 (cont'd.)

Multivariate Analysis of Variance of
Final Levels of Behavioral, Somatic,
Metabolic, and Endocrine Parameters

| Multivariate Analysis | | | Univariate Analyses | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Insulin | | Glucose | | Free Fatty Acids | | Growth Hormone | | Testosterone | |
| Source | df | F | df | MS | F | MS | F | MS | F | MS | F | MS | F |
| Knife Cut (C) | 9 | 595.14** | 1 | 1248.59 | 57.76*** | 905.36 | 4.87 | 0.0069 | 4.66 | 411.71 | 2.53 | 1610.76 | 1.47 |
| Overeating (F) | 9 | 356.94** | 1 | 125.99 | 5.83* | 3.50 | 0.02 | 0.0093 | 6.22* | 735.87 | 4.51 | 4776.70 | 4.36 |
| C x F | 9 | 124.83** | 1 | 116.67 | 5.40* | 1.16 | 0.006 | 0.0053 | 3.53 | 317.62 | 1.95 | 1494.05 | 1.36 |
| Error | 2 | | 10 | | | | | | | | | | |

* $p<0.05$

** $p<0.01$

*** $p<0.001$

irritability ratings. None of the rats bit the gloved hand and there were no vocalizations. Following surgery seven out of eight rats which had received knife cuts became irritable and hyperreactive to normal handling. They bit the gloved hand in each of the 21 postoperative tests and no decline in irritability was noted throughout the postoperative period. The amount of vocalization did not change. The rat which did not exhibit any irritability was Rat 23 whose knife cut was slightly more anterior than that of the others. The irritability ratings of the EXP-REST group did not differ from those of the EXP-AD LIB group. The irritability scores of the control groups did not change over the experimental period.

Preoperatively, none of the experimental rats and only one control rat killed mice on two successive tests. One day after surgery, four out of the eight experimental animals started killing and continued to kill mice when tested on the third and fifth postoperative day. The percentage of killers in the experimental groups increased by 50% as a consequence of the knife cut operation. However, this effect was a transient one since these animals failed to kill on subsequent tests throughout the experiment. No changes were noted in the control groups.

Metabolic and Endocrine Findings

Figure 4 shows the mean plasma insulin and glucose levels in the four groups before and after surgery, and Table 3 provides a summary of the analysis of variance of these two measures over time. The mean plasma insulin levels of the four groups of rats were similar prior to surgery. About 36 hr. after the knife cuts the plasma insulin levels of both experimental groups were markedly elevated. The mean insulin level of the EXP-REST group was more than double that of the CONT-REST group and remained consistently elevated on all subsequent measurements. Since both groups

Figure 4. Mean plasma insulin and plasma glucose levels of the four groups of rats before and after parasagittal knife cuts and sham operations. Vertical lines represent SEM.

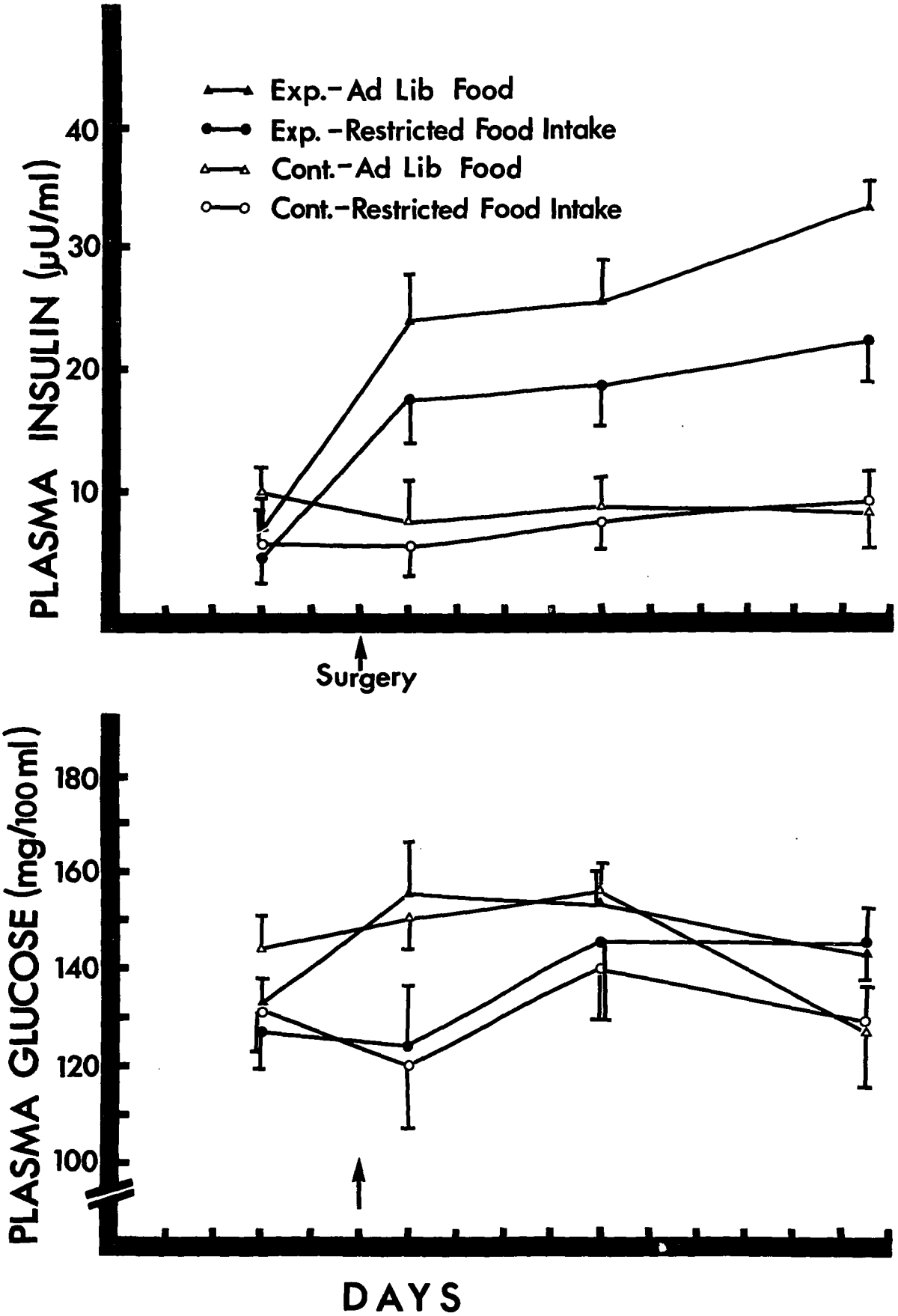

Exp.-Ad Lib Food
Exp.-Restricted Food Intake
Cont.-Ad Lib Food
Cont.-Restricted Food Intake
PLASMA INSULIN (μU/ml)
40
30
20
10
Surgery
PLASMA GLUCOSE (mg/100ml)
180
160
140
120
100
DAYS

TABLE 3

Analysis of Variance of Plasma Insulin
and Plasma Glucose Levels Over Time

| | | Insulin | | Glucose | |
|---|---|---|---|---|---|
| Source | df | MS | F | MS | F |
| Knife Cut (C) | 1 | 1761.52 | 59.57*** | 224.02 | 0.51 |
| Overeating (F) | 1 | 238.09 | 8.05* | 2236.72 | 5.08* |
| C x F | 1 | 91.52 | 3.09 | 49.29 | 0.11 |
| Error | 10 | 29.56 | | 440.15 | |
| Time (T) | 3 | 319.57 | 12.36** | 612.09 | 2.50 |
| C x T | 3 | 292.81 | 11.32** | 322.67 | 1.32 |
| F x T | 3 | 1.84 | 0.71 | 638.85 | 2.61 |
| C x F x T | 3 | 26.50 | 1.02 | 24.09 | 0.98 |
| Error | 30 | 25.84 | | 244.87 | |

* $p<0.05$

** $p<0.01$

*** $p<0.001$

ate approximately the same quantity of food the difference in insulin levels must be attributed to the surgical intervention. The effect of the knife cut operation on plasma insulin levels was highly significant ($p < 0.001$). A Newman-Keuls test was used to test the difference between all pairs of means over time. The preoperative mean insulin level was significantly different from the 36 hr. postoperative level ($q_3 = 3.5527$, df = 30, $p < 0.05$), the 10-day postoperative level ($q_3 = 4.30$, df = 30, $p < 0.05$), and the 21-day postoperative level ($q_3 = 5.88$, df = 30, $p < 0.01$). The postoperative levels did not differ significantly from each other. The highest insulin levels were exhibited by rats of the EXP-AD LIB group which were overeating and becoming obese. The effect of increased food intake on insulin levels was significant ($p < 0.05$). The only significant interaction was Cut x Time ($p < 0.01$).

The analysis showed no significant effect of the knife cut operation on plasma glucose levels. However, the plasma glucose levels of the rats eating ad libitum appeared higher than those of rats on a restricted diet. The effect of overeating on plasma glucose was significant at the 0.05 level.

The results of the multivariate ANOVA on levels of plasma growth hormone, free fatty acids, and testosterone are listed in Table 2. The knife cut operation did not affect the circulating levels of these variables to a significant degree. In testing the effect of overeating, the analysis revealed a significant effect on plasma free fatty acid levels ($p < 0.05$) but no effect on growth hormone or testosterone levels. However, it is interesting to note that the subjects of the EXP-AD LIB group exhibited the lowest growth hormone levels (less than 1 ng /ml , the minimum detectable amount of hormone in the assay).

## DISCUSSION

The behavioral and somatic results of the present study confirmed the recent findings of Paxinos and Bindra (1972) that parasagittal knife cuts lateral to the VMH produce hyperphagia, hyperdipsia, obesity, and hyperirritability in the male rat. In addition, the finding of the present investigation that such cuts produce a transient increase in mouse-killing behavior suggests the importance of medio-lateral hypothalamic connections in the control of predatory attack. Procedural differences may account for the failure to find any change in mouse-killing behavior in the previous report (see Paxinos, 1972, for elaboration of this point). With respect to linear growth, the present data indicate that such knife cuts do not affect body length.

Hyperphagia and obesity have generally been attributed to the destruction of inhibitory connections from a VMH "satiety center" and the consequent release of a lateral hypothalamic "feeding center" (Albert & Storlien, 1969; Sclafani & Grossman, 1969; Gold, 1970). This conclusion must be considered tentative, for surgical interruption of connections between medial and lateral hypothalamic areas may produce disturbances in endocrine and metabolic systems relevant to the regulation of food intake. In fact, just such a disturbance--damage to the supraoptico-hypophyseal tract which transports vasopressin to the posterior pituitary--may account for the excessive water intake observed in one subject.

The present results indicate that rats subjected to parasagittal knife cuts exhibit elevated plasma insulin levels as early as 36 hr. after surgery, in spite of normal food intake and normal plasma glucose levels. This finding is similar to the hyperinsulinemia seen after VMH lesions

(Martin & Bouman, 1971; Hustvedt & Løvø, 1972) and suggests that the axons mediating this neuroendocrine response course laterally from the VMH. Two possible mechanisms may account for the hypothalamic effect on insulin secretion; it may be mediated directly through neural pathways or indirectly through humoral factors. Recent anatomical studies provide evidence for direct connections between both the VMH and LH and the autonomic centers in the brain stem (Ban, 1966), and lend support to the hypothesis that the effect is mediated by the autonomic nervous system. One such pathway is the medial forebrain bundle which carries fibers from the LH to the mesencephalon and rhombencephalon. Since it has been established that the secretion of insulin is increased by stimulation of both the right and left vagus nerves (Kaneto, Kosaka, & Nakao, 1967; Frohman, Ezlindi, & Javid, 1967), one possible explanation is that increased activity in LH (following removal of the restraining influence of VMH) leads to a neurogenic stimulation of insulin via the vagus nerves (Bernardis & Frohman, 1971b). Evidence for a humoral mechanism is provided by the studies of Idahl and Martin (1971) which demonstrated the existence of a ventrolateral hypothalamic factor that stimulates insulin release. They observed a consistent stimulation of insulin release in pancreatic islets submitted to the effluent from the ventrolateral hypothalamus, while no stimulation was observed in islets exposed to other tissue portions. The exact mechanism of the hypothalamic influence on insulin secretion remains conjectural at the present time.

It is well documented that the metabolic effects of increased insulin levels resemble those of hypothalamic hyperphagia (MacKay, Callaway, & Barnes, 1940; May & Beaton, 1968; Booth & Brookover, 1968). May and Beaton

(1968) report that rats given daily injections of protamine-zinc-insulin exhibited hyperphagia, increased body-weight gain, and increased lipogenesis. Hustvedt and Løvø (1972) noted a positive correlation between the increase in plasma insulin levels and the subsequent gain in body weight. The important role of insulin in the pathogenesis of the VMH syndrome is clearly demonstrated by the studies of York and Bray (1972). They examined the effects of weight gain after VMH lesions in rats in which the secretory function of the pancreatic β cells had been destroyed with streptozotocin, and they concluded "that an increased circulating level of insulin is required for the appearance of hyperphagia and for the progression of obesity resulting from hypothalamic damage of adult female rats [p. 893]."

The finding that parasagittal knife cuts lateral to the VMH do not alter growth hormone secretion provides evidence for the hypothesis of Bernardis and Frohman (1970) that the axons concerned with growth hormone secretion travel in a different direction from those concerned with insulin secretion. It is likely that the axons of neurons involved in growth hormone secretion project from the ventromedial nucleus to the arcuate nucleus and terminate in the median eminence, where growth hormone-releasing factor is released into the hypophyseal portal vessels for transport to the anterior pituitary. The present data indicate that it is possible to dissociate the VMH effects on growth hormone and insulin and suggest that these are two independent functions of the ventromedial nucleus.

Growth hormone is known to influence the intermediary metabolism of lipids; it induces mobilization of fat from adipose tissue, and impaired growth hormone secretion could result in a greater accumulation of fat

(Knobil & Hotchkiss, 1964). Since lesions of the VMH do result in growth hormone deficiency (Frohman & Bernardis, 1968), it is hypothesized that the greater degree of weight gain observed in VMH-lesioned animals than in rats subjected to knife cuts,may be attributed to the depressed growth hormone levels seen only in the former.

Testosterone levels were not significantly affected by the knife cut operation. On the basis of the present data it may be concluded that an alteration in testosterone secretion is not necessary for the characteristic appearance of aggressive behavior following damage to the VMH. Other evidence, however, does suggest that gonadal hormones are important in the neonatal development of brain mechanisms for aggression (Conner & Levine, 1969; Edwards, 1970; Peters, Bronson, & Whitsett, 1972). A study involving the examination of aggressive behavior following knife cuts in adult rats which were neonatally castrated would be of value in determining whether testosterone plays a role in the development of those neural pathways affected by the knife cuts.

The metabolic and endocrine disturbances observed in the obese rats of the present study include hyperinsulinemia, elevated free fatty acids, depressed growth hormone levels, and mild hyperglycemia. This profile is similar to that of human obesity (Sims & Horton, 1968), as well as of other experimental animals such as the mouse (Malaisse, Malaisse-Lagae, & Coleman, 1968) and the genetically obese rat (Zucker & Antoniades, 1972). Recent studies on man have suggested that these abnormalities may be a consequence of the obesity rather than its cause (Sims, Horton, & Salans, 1971). The results reported here indicate that hyperinsulinemia, at least, can develop in the absence of overeating and obesity. Further research is

needed to determine whether these metabolic and hormonal changes play a causal or compensatory role in the genesis of obesity.

In conclusion, the findings of the present investigation suggest that the role assigned to the ventromedial hypothalamus in the VMH syndrome, as well as in the overall regulation of food intake, must be modified to include neuroendocrinological mechanisms.

## SUMMARY

Metabolic and endocrine factors were examined in relation to the behavioral syndrome (hyperphagia, hyperdipsia, and increased aggression) associated with ventromedial hypothalamic damage. Male rats were subjected to parasagittal knife cuts that separated the medial from the lateral hypothalamic areas; following surgery, some rats were given free access to food while others were restricted to normal quantities of food. Compared to control animals, the restricted-food rats exhibited hyperinsulinemia as early as 36 hr. after surgery, without any change in plasma glucose levels. Growth hormone secretion was not significantly altered. The blood samples of free-food rats, which had become obese, showed hyperinsulinemia, mild hyperglycemia, and elevated levels of free fatty acids. These results suggest that the interruption of the medial hypothalamic influence on the lateral hypothalamus produces hyperinsulinemia directly, and that further increases in insulin, glucose, and free fatty acid levels are caused by overeating. The surgical cuts produced an increase in aggressive behavior but no change in the circulating levels of testosterone. Possible neural pathways that mediate endocrine changes are discussed.

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