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# Effects of Goldthioglucose Lesions on Central Catecholamine Levels in the Mouse

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LORDEN, J. F., R. DAWSON, JR. AND M. CALLAHAN. *Effects of goldthioglucose lesions on central catecholamine levels in the mouse.* PHARMAC. BIOCHEM. BEHAV. 10(1) 165–169, 1979.—Male and female C57 Bl/6J mice were injected with goldthioglucose (GTG) to induce an obesity syndrome. Significant increases in body weight were inversely correlated with pituitary dopamine (DA) levels. Significant reductions in hypothalamic norepinephrine (NE) and DA were also noted; however, these reductions did not appear to be related to body weight gain. The GTG injections did not produce any significant alterations in telencephalic NE or DA. The damage to catecholamine neurons is discussed in relation to the endocrine abnormalities of the GTG mouse and some current hypotheses on the control of food intake.

Goldthioglucose Catecholamines Obesity

IN THE mouse a single injection of goldthioglucose (GTG) can produce both severe obesity and disturbances in endocrine function [8,10]. The obesity syndrome which follows GTG injections is associated with hypothalamic damage and is often assumed to be equivalent to the obesity which follows electrolytic lesions of the ventromedial region of the hypothalamus [8]. Central nervous system damage following GTG injections is, however, not restricted to a particular part of the hypothalamus or to the hypothalamus alone. Damage has also been noted in areas such as the hippocampus and medulla [9]. Thus, the anatomical substrate for the GTG-induced obesity has not been specified.

Several lines of evidence implicate the catecholaminergic systems in the control of food intake and body weight. Stimulation of the hypothalamus with exogenous catecholamines and drugs that act on catecholamine-containing neurons has been effective in manipulating food intake in the rat [20]. Altered levels of central catecholamines have been reported in several types of spontaneously obese rodents [14, 15, 21]. Experimental depletion of hypothalamic norepinephrine (NE) has also been used to produce increases in body weight in rats [1]. Finally, catecholamine metabolism in the rostral and dorsomedial hypothalamus varies with need state in the rat [38].

Catecholamine fluorescence is known to decline in the median eminence following GTG injection [19]. However, due to the difficulty of quantifying fluorescence [18], the magnitude of this effect is not known. Endocrine abnormalities in GTG-obese mice [28, 35, 36] also suggest possible change to catecholamine neurons, since hypothalamic catecholamines are known to be important in controlling the release of anterior pituitary hormones [4,16]. The present experiment was designed to assess damage to catecholamine systems in GTG-induced obesity using quantitative biochemical assay procedures.

## METHOD

### *Animals*

The male and female mice used in the study were bred in the laboratory from C57Bl/6J stock, obtained from Jackson Laboratories (Bar Harbor, ME). A total of 70 females and 95 males were used. All animals were weaned at 21–23 days of age and housed in groups of 6 to 8. The mice were maintained on an ad lib diet of Wayne Lab Blox and water throughout the experiment. The temperature of the colony room was maintained at 23°C and a 12 hr light-dark schedule was in effect.

### *Procedure*

At 60 to 90 days of age, 50 female and 67 male mice received a single injection of GTG (80 mg/cc) in isotonic saline at a dose of 0.01 cc/g of body weight. An additional 20 females and 28 males received equivalent volumes of isotonic saline. Following the injection all animals were weighed every five days. The animals were allowed to survive for over 2 months in order to allow a significant degree of obesity to develop.

After 68 days of observation, all mice were sacrificed by decapitation. The brains were rapidly removed and dissected into hypothalamic and telencephalic sections [21]. The animals were sacrificed in three groups. Telencephalic sections were taken from the last two groups sacrificed. The pituitaries were carefully lifted from the base of the skull and placed in 300 $\mu$l of 0.1 N perchloric acid. All sections were frozen in liquid nitrogen. The hypothalamic and telencephalic sections were weighed while frozen.

The telencephalons were analyzed fluorometrically for catecholamine content by a modification of the method of Hogans [17]. Since the fluorometric techniques are not suffi-

ciently sensitive to measure the small amounts of dopamine (DA) present in the mouse hypothalamus and pituitary, these sections were assayed for catecholamine content by a radioisotopic-enzymatic technique [12]. The Lowry method was used to determine the protein content of the pituitary samples [23]. Data were analyzed by analysis of variance for a 2×2 factorial design (Sex×Injection).

## RESULTS

Seventeen female mice (34%) and 27 male mice (40%) survived the GTG injections. Seventeen of the 20 females and all 28 males injected with saline also survived for the complete observation period. Only the data from those mice surviving for a full 68 days has been included here.

Both male and female mice injected with GTG exhibited a comparable and significant increase in body weight in comparison with saline-injected controls (Fig. 1). Within 20 days after the injections, both male and female GTG mice were significantly heavier than the saline-injected controls of the same sex ($p<0.01$). These differences persisted until the time of sacrifice. Toward the end of the observation period, the body weights of the female mice in the GTG group began to reach a plateau. Male mice continued to gain at approximately the same rate.

Of the animals surviving the GTG injections, 18% of the females and 33% of the male mice failed to become obese. That is, the weight gain of these mice over the observation period did not exceed the range of weight gain in the same-sex saline-injected control group (Table 1).

A significant reduction in hypothalamic NE, $F(1,74)=12.15$, $p<0.01$, was observed following GTG treatment (Table 2). There was a 29% decrease in hypothalamic NE in the GTG-injected females and a 12% decrease in the males. Individual comparisons between treatment means indicated that the reduction in hypothalamic NE was statistically significant only for female mice. An 18% decrease in hypothalamic DA was observed in both male and female mice following GTG injections, $F(1,75)=5.76$, $p<0.05$; however, this reduction was significant only in the males. Hypothalamic section weights were similar for all groups.

TABLE 1

CHANGE IN BODY WEIGHT FOLLOWING GTG INJECTIONS IN MICE

| Group | Sex | N | Δ Body Weight (g) (M ± SD) |
|---|---|---|---|
| GTG obese | Female | 14 | 16.1 ± 7.7* |
| GTG non-obese | Female | 3 | 2.0 ± 1.7 |
| Saline | Female | 17 | 2.5 ± 1.4 |
| GTG obese | Male | 18 | 18.2 ± 6.0* |
| GTG non-obese | Male | 9 | 3.9 ± 2.4 |
| Saline | Male | 28 | 4.2 ± 2.0 |

*Differs significantly from same-sex saline control group, $p<0.01$.

Telencephalic NE and DA levels were not significantly altered by GTG treatment (Table 2). There was, however, a sex difference in telencephalic DA. Females had significantly higher DA levels than males, $F(1,46)=4.78$, $p<0.05$. There were no significant differences in telencephalic section weights.

The largest difference between GTG and saline-injected mice was observed in pituitary DA levels (Table 3). Pituitary DA was reduced to 60% of control values in females and 75% of control in males by the GTG treatment, $F(1,83)=18.17$, $p<0.01$. Of the amine measures reported here, only pituitary DA was significantly correlated with body weight change. Correlation coefficients of $-.68$ ($p<0.001$) for females and $-.33$ ($p<0.05$) for males were obtained. No significant differences in pituitary protein content were found.

## DISCUSSION

Peripheral injections of GTG have been found to decrease central catecholamiine levels. The depletion of catecholamines caused by the GTG injection is not a general effect. Hypothalamic and pituitary catecholamines were reduced but telencephalic catecholamines appeared unaf-

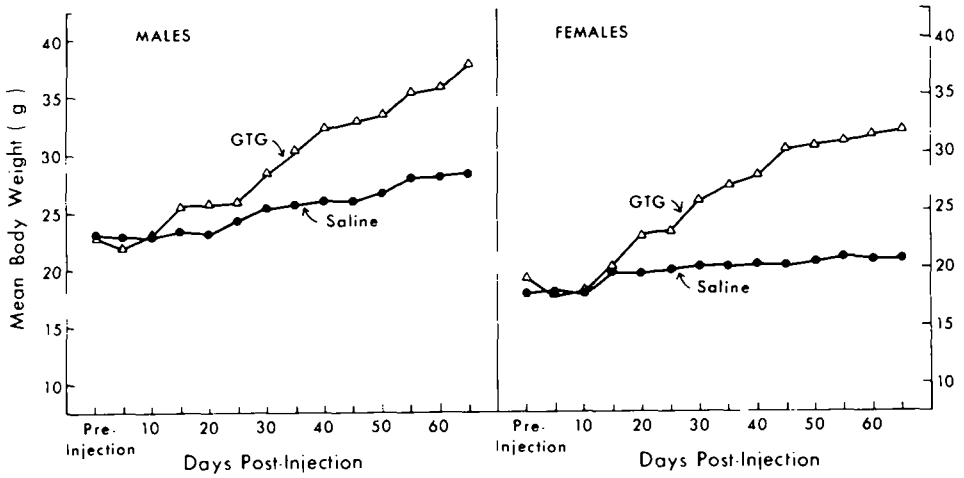


FIG. 1. Mean body weight (g) in male (left panel) and female (right panel) mice after injections of goldthioglucose (GTG) or physiological saline.

TABLE 2

HYPOTHALAMIC AND TELENCEPHALIC CATECHOLAMINE LEVELS IN GTG- AND SALINE-INJECTED C57B1/6J MICE

| Section | N | Mean Brain Section Weight (g ± SD) | Mean Catecholamine Levels NE (μg/g ± SD) | DA (μg/g ± SD) |
|---|---|---|---|---|
| Hypothalamus | | | | |
| Female GTG | 17 | 0.013 ± 0.004 | 1.037 ± 0.398* | 0.390 ± 0.202 |
| Female Saline | 14 | 0.013 ± 0.004 | 1.463 ± 0.278 | 0.477 ± 0.198 |
| Male GTG | 23 | 0.012 ± 0.002 | 1.313 ± 0.324 | 0.382 ± 0.099* |
| Male Saline | 25 | 0.014 ± 0.003 | 1.439 ± 0.361 | 0.468 ± 0.147 |
| Telencephalon | | | | |
| Female GTG | 13 | 0.221 ± 0.024 | 0.327 ± 0.084 | 1.513 ± 0.392 |
| Female Saline | 15 | 0.227 ± 0.017 | 0.295 ± 0.076 | 1.452 ± 0.278 |
| Male GTG | 13 | 0.218 ± 0.019 | 0.306 ± 0.093 | 1.316 ± 0.468 |
| Male Saline | 20 | 0.226 ± 0.012 | 0.347 ± 0.072 | 1.094 ± 0.290 |

*Differs from same-sex saline-injected control group, $p<0.01$.

TABLE 3

PITUITARY PROTEIN CONTENT AND DA LEVELS IN GTG- AND SALINE-INJECTED C57B1/6J MICE

| Group | N | Mean Protein Content (mg ± SD) | Mean DA Levels (ng/g protein ± SD) |
|---|---|---|---|
| Female GTG | 17 | 0.210 ± 0.031 | 0.778 ± 0.553* |
| Female Saline | 17 | 0.212 ± 0.046 | 1.291 ± 0.481 |
| Male GTG | 27 | 0.220 ± 0.032 | 1.000 ± 0.410* |
| Male Saline | 28 | 0.218 ± 0.031 | 1.331 ± 0.375 |

*Differs significantly from same-sex, saline-injected control group, $p<0.01$.

fected. Pituitary DA depletion was inversely correlated with weight gain; however, no similar relationship between weight gain and hypothalamic amine levels was noted.

Using the histochemical fluorescence technique, Lefranc and his co-workers [19] examined changes in catecholamine fluorescence in the brains of male mice 13 weeks after a GTG injection. These authors found a reduction in fluorescence in the median eminence and the arcuate nucleus. A sharp decrease in fluorescence was noted in the region of termination of the tubero-infundibular tract but the aminergic innervation of the rest of the hypothalamus appeared unchanged. The results of the present study lack the anatomical resolution of fluorescence histochemistry but tend to confirm the findings of Lefranc *et al.* of a fairly circumscribed reduction in hypothalamic catecholamine content. In addition we report that a loss of catecholamine content occurs in females as well as males and can be measured as early as nine weeks after a GTG injection.

Depletion of DA in the tubero-infundibular system as described by Lefranc *et al.* [19] could account for the loss in hypothalamic DA observed in the data reported here. The loss of pituitary DA may also represent damage to the tubero-infundibular system. The source of pituitary DA is unknown; but it is presumed to be of neural origin. In the rat the tubero-infundibular DA system is known to innervate the intermediate and posterior lobes of the pituitary [7,27]. Fluorescent fibers have also been observed in the infundibulum of the mouse; but few fluorescent fibers appear in the pituitary itself [6]. However, DA has also been measured in the capillaries of the hypophysial portal system [5]. DA of hypothalamic origin can reach the pituitary through the blood as well as through a direct neural connection. Thus, the reduction in pituitary DA following GTG injections may represent damage to the tubero-infundibular system at the level of the hypothalamus or median eminence. The noradrenergic innervation of the hypothalamus is provided by neurons with cell bodies in the posterior brain stem that send axons through the medial forebrain bundle [37]. The reduction in hypothalamic NE observed in GTG treated mice may reflect damage to the axons or terminals of the ventral noradrenergic bundle.

The changes in hypothalamic and pituitary catecholamines which follow GTG injection are interesting in the light of the endocrine abnormalities which accompany GTG lesions. In the GTG obesity syndrome, prolactin (PRL) and growth hormone (GH) secretion are both substantially altered [28, 35, 36]. Serum levels of PRL and GH are reduced as early as 2 weeks after GTG injection. Pituitary levels of

PRL increase as obesity develops and administration of a DA receptor blocker causes the release of large amounts of PRL in comparisons with controls. Pituitary levels of GH are relatively unaffected by GTG; and PRL and GH release approach normal after weight reduction [36]. These results suggest that some of the endocrine abnormalities of the GTG mouse are a consequence of obesity and not the cause. However, even in GTG-injected mice that do not become obese, some increase in the perphenazine-stimulated release of PRL can be observed. Thus, part of the response may be due to a change in hypothalamic control of PRL secretion. Many experiments suggest that DA functions physiologically as a PRL inhibitory factor (e.g., [13, 24, 25]) and both NE and DA are thought to be involved in control of GH [26]. The decreases observed in hypothalamic and pituitary DA in GTG-treated mice may contribute to the changes in PRL and GH release. It is also possible, however, that early hormonal changes brought about by GTG lesion damage to non-catecholaminergic systems may alter the synthesis and release of catecholamines [3]. In future experiments on GTG-injected mice, the rate of amine turnover, the time course of changes in amine systems, and response of these systems to body weight reduction should be evaluated.

Alteration of central catecholamine levels is not an uncommon correlate of obesity. However, the pattern of changes associated with the GTG syndrome distinguishes GTG obesity from other obesity syndromes. For example, reduction in hypothalamic DA, but not NE, has been noted in rats made obese by neonatal injections of monosodium L-glutamate [29]. In the Zucker fatty rat, a spontaneously obese rodent, no significant changes in hypothalamic DA have been observed; but NE is reduced in the paraventricular nucleus [14,15]. The genetic obesities of the *obob* and *dbdb* mice are characterized by elevations in hypothalamic NE in comparison with lean controls. The *obob* mouse also shows elevated levels of pituitary DA [22]. It is worth noting that in the *obob* syndrome, in contrast to the GTG syndrome, both pituitary and serum levels of PRL are low and no PRL release can be stimulated with perphenazine [35]. The pituitary hormones have not been studied exhaustively in all of the different animal models of obesity; however, the different patterns of abnormalities in catecholamine levels may reflect the presence of unique patterns of endocrine disturbance.

The diversity of changes and abnormalities in central catecholamine systems in different types of obesity makes it unlikely that any single unifying catecholamine hypothesis will be able to account for all forms of obesity. Nonetheless, lesions which deplete hypothalamic NE in rats are associated with increases in food intake and body weight [1], although NE depletion alone has not been proven a sufficient cause [30]. In addition, data from chemical stimulation experiments argue for the existence of hypothalamic NE feeding and both NE and DA satiety mechanisms [20]. In the present study, a relationship was found to exist between pituitary DA and body weight. The failure to demonstrate a comparable relationship between hypothalamic NE or DA and body weight may have been due to the size of the sample measured. If, as one would predict on the basis of the chemical stimulation studies, only particular nuclei are involved in feeding or satiety effects, a relationship between hypothalamic NE or DA and body weight could be obscured by the inclusion of other unrelated tissue.

In some forms of obesity, abnormalities in central catecholamine systems may influence food intake and body weight indirectly by causing alterations in hormonal systems. At least some of the catecholamine feeding effects appear to depend on the pituitary. Hypophysectomy blocks the obesity syndrome associated with NE depletion and also blocks NE-induced feeding [2,20]. In the Zucker fatty rat, the *obob* mouse, and the GTG mouse, all syndromes in which altered catecholamine levels have been observed, hypophysectomy halts weight gain [32, 33, 34]. This is not true in other obesity syndromes such as the A$^y$/a mouse or the ventromedial hypothalamic rat [11,31]. The fact that hypophysectomy blocks weight gain in several types of obesity does not necessarily indicate a common etiology for all these syndromes. If pituitary DA proves to be a determinant of GTG obesity, another explanation may account for the obesity of the *obob* mouse in which pituitary DA levels are elevated [22].

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## REFERENCES

1. Ahlskog, J. E. Food intake and amphetamine anorexia after selective forebrain norepinephrine loss. *Brain Res.* **82:** 211–240, 1974.
2. Ahlskog, J. E., B. G. Hoebel and S. T. Breisch. Hyperphagia following lesions of the noradrenergic pathway is prevented by hypophysectomy. *Fedn Proc.* **33:** 463, 1974.
3. Annunziato, L. and K. E. Moore. Prolactin in CSF selectively increases dopamine turnover in the median eminence. *Life Sci.* **22:** 2037–2041, 1978.
4. Anton-Tay, F. and R. J. Wurtman. Brain monoamines and endocrine function. In: *Frontiers in Neuroendocrinology*, edited by L. Martini and W. F. Ganong. New York: Oxford University Press, 1971, pp. 45–66.
5. Ben-Jonathan, N., C. Oliver, H. J. Weiner, R. S. Mical and J. C. Porter. Dopamine in the hypophysial portal plasma of the rat during the estrous cycle and throughout pregnancy. *Endocrinology* **100:** 452–458, 1977.
6. Björklund, A., A. Enemar and B. Falck. Monoamines in the hypothalamo-hypophyseal system of the mouse with special reference to the ontogenetic aspects. *Z. Zellforsch.* **89:** 590–607, 1968.
7. Björklund, A., R. Y. Moore, A. Nobin and U. Stenevi. The organization of tubero-hypophyseal and reticulo-infundibular catecholamine systems in the rat brain. *Brain Res.* **51:** 171–191, 1973.
8. Bray, G. A. and D. York. Genetically transmitted obesity in rodents. *Physiol. Rev.* **51:** 598–646, 1971.
9. Brecher, G., G. L. Laqueur, E. P. Cronkite, P. M. Edelman and I. L. Schwartz. The brain lesion of goldthioglucose obesity. *J. exp. Med.* **121:** 395–401, 1965.
10. Brecher, G. and S. H. Waxler. Obesity in albino mice due to single injection of goldthioglucose. *Proc. Soc. exp. Biol. Med.* **70:** 498–501, 1949.

11. Cox, V. C., J. W. Kakolewski and E. S. Valenstein. Effects of ventromedial hypothalamic damage in hypophysectomized rats. *J. comp. physiol. Psychol.* **65:** 145–148, 1968.
12. Coyle, J. T. and D. Henry. Catecholamines in fetal and newborn rat brain. *J. Neurochem.* **21:** 61–67, 1973.
13. Cronin, M. J., J. M. Roberts and R. I. Weiner. Dopamine and dihydroergocryptine binding to the anterior pituitary and other brain areas of the rat and sheep. *Endocrinology* **103:** 302–309, 1978.
14. Cruce, J. A. F., N. B. Thoa and D. M. Jacobowitz. Catecholamines in the brains of genetically obese rats. *Brain Res.* **101:** 165–170, 1976.
15. Cruce, J. A. F., N. B. Thoa and D. M. Jacobowitz. Catecholamines in discrete areas of the hypothalamus of obese and castrated male rats. *Pharmac. Biochem. Behav.* **8:** 287–289, 1978.
16. Hökfelt, T. and K. Fuxe. On the morphology and the neuroendocrine role of the hypothalamic catecholamine neurons. In: *Brain-Endocrine Interaction*, edited by K. M. Knigge, D. E. Scott and A. Weindl. Basel: Karger, 1972, pp. 181–223.
17. Jacobowitz, D. M. and J. Richardson. Method for the rapid determination of norepinephrine, dopamine, and serotonin in the same brain region. *Pharmac. Biochem. Behav.* **8:** 515–519, 1978.
18. Kopin, I. J., M. Palkovits, R. M. Kobayashi and D. M. Jacobowitz. Quantitative relationship of catecholamine content and histofluorescence in brain of rats. *Brain Res.* **80:** 229–235, 1974.
19. Lefranc, G., A. L'Hermite and S. Kenerve. Charge en catécholamines de l'eminence médiane de souris mâles devenues obèses après administration d'auro-thioglucose. *C. r. Séanc Soc. Biol.* **164:** 1627–1629, 1970.
20. Leibowitz, S. F. Brain catecholaminergic mechanisms for control of hunger. In: *Hunger: Basic Mechanisms and Clinical Implications*, edited by D. Novin, W. Wyrwicka and G. A. Bray. New York: Raven Press, 1976, pp. 1–18.
21. Lorden, J. F., G. A. Oltmans and D. L. Margules. Central catecholamine levels in genetically obese mice (*obob* and *dbdb*). *Brain Res.* **96:** 390–394, 1975.
22. Lorden, J. F. and G. A. Oltmans. Hypothalamic and pituitary catecholamine levels in genetically obese mice (*obob*). *Brain Res.* **131:** 162–166, 1977.
23. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193:** 265–275, 1951.
24. MacLeod, R. M. and S. W. Lamberts. The biphasic regulation of prolactin secretion by dopamine agonist-antagonists. *Endocrinology* **103:** 200–203, 1978.
25. MacLeod, R. M. and J. E. Lehmeyer. Studies on the mechanism of the dopamine mediated inhibition of prolactin secretion. *Endocrinology* **94:** 1077–1085, 1974.
26. Martin, J. B. Brain regulation of growth hormone secretion. In: *Frontiers in Neuroendocrinology*, edited by L. Martini and W. F. Ganong. New York: Raven Press, 1976, pp. 129–168.
27. Moore, R. Y. and F. E. Bloom. Central catecholamine systems: anatomy and physiology of the dopamine systems. *A. Rev. Neurosci.* **1:** 129–169, 1978.
28. Müller, E. E., D. Miedico, G. Giustina, A. Pecile, D. Cocchi and V. Mandelli. Impaired secretion of growth hormone in goldthioglucose-obese mice. *Endocrinology* **89:** 56–62, 1971.
29. Nemeroff, C. B., R. J. Konkol, G. Bissette, W. Youngblood, J. B. Martin, P. Brazeau, M. S. Rone, A. J. Prange, Jr., G. R. Breese and J. S. Kizer. Analysis of the disruption in hypothalamic-pituitary regulation in rats treated neonatally with monosodium L-glutamate (MSG). Evidence for involvement of tubero-infundibular cholinergic and dopaminergic systems in neuroendocrine regulation. *Endocrinology* **101:** 613–622, 1977.
30. Oltmans, G. A., J. F. Lorden and D. L. Margules. Food intake and body weight: effects of specific and non-specific lesions in the midbrain path of the ascending noradrenergic neurons of the rat. *Brain Res.* **128:** 293–308, 1977.
31. Plocher, T. A. and T. L. Powley. Effect of hypophysectomy on weight gain and body composition in the genetically obese yellow ($A^y$/a) mouse. *Metabolism* **25:** 593–602, 1976.
32. Plocher, T. A. and T. L. Powley. Maintenance of obesity following hypophysectomy in the obese-hyperglycemic mouse (*ob/ob*). *Yale J. Biol. Med.* **50:** 291–300, 1977.
33. Powley, T. L. and S. A. Morton. Hypophysectomy and regulation of body weight in the genetically obese Zucker rat. *Am. J. Physiol.* **230:** 982–987, 1976.
34. Redding, T. W., C. Y. Bowers and A. V. Schally. Effects of hypophysectomy on hypothalamic obesity in CBA mice. *Proc. Soc. exp. Biol. Med.* **121:** 726–729, 1966.
35. Sinha, Y. N., C. B. Salocks and W. P. VanderLaan. Prolactin and growth hormone secretion in chemically induced and genetically obese mice. *Endocrinology* **97:** 1386–1393, 1975.
36. Sinha, Y. N., C. B. Salocks and W. P. VanderLaan. Control of prolactin and growth hormone secretion in mice by obesity. *Endocrinology* **99:** 881–886, 1976.
37. Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta physiol. Scand. Suppl.* **367:** 1–48, 1971.
38. Van Der Gugten, J., E. R. De Kloet, D. H. G. Versteeg and J. L. Slangen. Regional hypothalamic catecholamine metabolism and food intake regulation in the rat. *Brain Res.* **135:** 325–336, 1977.