BIOLOGY OF REPRODUCTION 20, 552–559 (1979)

A Reproductive Endocrine Profile in the Diabetes (*db*) Mutant Mouse[1]

LINDA MARLENE JOHNSON[2] and RICHARD L. SIDMAN

Departments of Neuropathology, Harvard Medical School and Neuroscience, Children's Hospital Medical Center, Boston, Massachusetts, 02115

ABSTRACT

Mice that are homozygous for the autosomal recessive mutation diabetes (*db*) fail to reproduce. We have established that the hypoplastic vaginal epithelia, uteri and ovaries can respond comparably to control tissues on hormonal stimulation. Gonadotropin release from the pituitary gland appeared to be depressed in female mutant mice, but responded normally to exogenous gonadotropin releasing hormone (GNRH) in both sexes. Immature mutant females failed to ovulate on PMS stimulation. Hypothalamic GNRH content was greater than normal in the adult female mutant mice (<0.001), suggesting that in the females, at least, GNRH release may be inadequate, with secondary blunting of pituitary function.

The *db* males appeared to have comparable to normal LH, FSH and GNRH levels and little sign of reproductive tract atrophy. Only the preputial glands were significantly reduced from normal size. The *db* males did fail to show mating behavior. The results substantiate a hypothesis that the presumed single gene mutation, diabetes, acts through a CNS anomaly to cause infertility, as it also may act to cause obesity and thermoregulatory disturbances.

INTRODUCTION

Mice homozygous for the autosomal recessive mutation diabetes (*db*) have physiological abnormalities that include hyperglycemia, hyperinsulinemia, obesity, thermoregulatory disturbances and sterility of both sexes (Hummel et al., 1966). Studies on food intake (Coleman and Hummel, 1969) and body temperature regulation (Yen et al., 1974) have been used to suggest that the brain may be a site of action of the *db* genetic locus. Relatively little interest has been expressed in the sterility aspect of the *db* syndrome and in the possibility that it also may reflect a CNS lesion. Although we have not detected an abnormality in cellular composition or myelination of mutant brains viewed at the light microscopic level (Johnson, 1977), we have gathered endocrine and reproductive data suggesting that abnormal hypothalamic function may be the immediate cause of some of the physiologic abnormalities in *db* mice.

The data presented below include measurements of pituitary and serum LH and FSH concentrations in intact and gonadectomized males and females, serum LH response to GNRH (gonadotropin-releasing hormone) and results concerning ovarian function, uterine growth and vaginal cyclicity in *db* mutants. In addition, GNRH content in the hypothalamus was measured.

MATERIALS AND METHODS

Animals

Two mutant alleles at the *db* locus, *db* and db^{2J} arose separately at the Jackson Laboratories (Bar Harbor, Maine) and are each maintained on several different genetic backgrounds. We reared db^{2J}/db^{2J} and $m+/+db^{2J}$ littermate mice (*m* is misty, a linked coat-color gene on chromosome 4), on the C56Bl/6J inbred background, in our own laboratory and purchased C57Bl/6J-*db*/*db* with +/+ control mice from the Jackson Laboratories. No phenotypic differences have been observed, either previously or in the present studies, between the two alleles when compared on the same inbred genetic background. Animals were weaned after 21 days of age and mutants were recognized by their incipient obesity at 4–6 weeks of age.

Mice were housed at 23°C with lights on at 0700 for 12 h, 2–4 animals per plastic cage (except as noted), with free access to Purina Formulab Chow and water, except when the diet was deliberately restricted. Dieted animals were housed singly, given water *ad lib* and one 3 g food pellet daily.

Accepted September 7, 1978.
Received July 31, 1978.
[1] Supported by NIH grant NS11237.
[2] Please address correspondence and reprint requests to LMJ. Current address: 530 Scaife Hall, Dept. of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Vaginal Smears

Cells obtained before noon by gentle lavage were viewed under phase optics and classified with reference to tables in Green (1968) and Rugh (1972). A total of 26 mutant and 30 control mice were housed singly and checked daily for 1–4 weeks, then 9 were checked during 1 month of diet restriction and 17 during a week of gonadotropin treatment.

Surgery

Adult mice were gonadectomized and killed 6 weeks later unless otherwise stated. Ovary halves were transplanted from 2 db^{2J}/db^{2J} females, age 6 weeks, by placing a half into the emptied ovarian capsule of 8 normal, congenic (C57Bl/6J-A/A) agouti host females. Ovary and oviduct were removed from the opposite side of the recipient. Two or 3 litters of pups were obtained from matings with $m+/+db^{2J}$ males.

Hormonal Injections

Estradiol benzoate (EB, Sigma) in ethanolic saline, pregnant mares' serum gonadotropin (PMS) and human chorionic gonadotropin (hCG, Sigma) in saline and GNRH (NIH, FSH/LH-RF) in 0.05 M phosphate buffered saline (PBS) were injected i.p. in 5 experiments: 1) Twelve days after ovariectomy control mice (14–18 g, 6 weeks old) and mutant mice (15–23 g, 6.5 weeks old) received daily injections for 12 days of vehicle or 5, 10, 20, 40 or 160 ng EB/20 g body weight in 0.05–0.08 ml. Uteri from which the luminal fluid had been extruded were weighed 24 h after the last injection. 2) Intact adult (5–8 months old) db^{2J}/db^{2J} female mice received daily injections for 13 days of 0.05 ml vehicle or 20, 40, 100 or 200 ng EB/20 g body weight. Uteri (minus fluid) were weighed 24 h after the last injection along with those of intact, cycling normal mice. 3) Mutant and control mice (3.5–4.5 months old) received afternoon injections for 2 days of 0.5, 1, 2, 4 or 8 IU PMS plus 7.5 IU hCG on the morning of Day 4. Oviducts were flushed on Day 5 to count released ova. 4) Immature female mutant and normal mice received a single injection of 4 IU PMS at 27 days of age, the lowest dose that reliably induced ovulation in preliminary experiments on controls. Oviducts were flushed at 30 or 31 days of age. If an animal could not yet be classified as mutant or nonmutant by body weight, the oviducts were surgically removed and the animal classified later. 5) Gonadectomized mice, anesthetized with Avertin, were bled via the orbital sinus and then given 50, 100 or 200 ng GNRH/100 g. Fifteen min after injection they were exsanguinated by cardiac puncture. Serum LH was measured by radioimmunoassay.

Tissue Collection

Blood, pituitary and hypothalamus were collected from each mouse for gonadotropin assay. Under Avertin anesthesia, blood was taken from the orbital sinus, clotted at room temperature and refrigerated overnight. The pituitary gland was homogenized in 0.5 ml 1% bovine serum albumin (Armour, fraction V) in 0.05 M PBS, combined with a 0.5 ml rinse and frozen until assay. The hypothalamus-preoptic area, as delineated in Sidman et al. (1971), was homogenized in 0.5 ml 2N acetic acid, rinsed (0.25 ml), refrigerated (21 h), centrifuged (20 min, 10,000 rpm, IEC centrifuge) and the pellet reextracted (0.25 ml) for 1 h at room temperature. Combined supernatants were freeze-dried and the powder dissolved in 0.1% gelatin in PBS.

Radioimmunoassay

LH and FSH were measured with a rat FSH assay system (NIAMDD) and an ovine-ovine LH assay system (Niswender et al., 1968) and are expressed in terms of the RP-1 rat standards. Essentially parallel dilution curves were obtained for samples of the mouse sera and pituitary extracts as compared to RP-1 standards. The anti-FSH serum was FSHS-6 and the anti-ovine LH serum was #15 (Dr. G. Niswender). Highly purified rat FSH (NIAMDD) and ovine LH, LER-1056-C2, were used for iodination. Hypothalamic GNRH was measured by radioimmunoassay, using anti-GNRH serum #42 (Dr. T. Nett) and Beckman LH/FSH-RF. Iodinations were carried out by a choramine T reaction with $Na^{125}I$ (New England Nuclear, NEZ-033L). Binding to excess antiserum reached a maximum of 70%. Interassay variation for assay of LH was 7.4 ± 2.0% and for GNRH assay was 4.7 ± 1.4%.

RESULTS

Inspection of approximately 200 mutant females indicated that their infertility was associated with extremely small uteri, small vaginal openings and diestrous vaginal acyclicity or occasionally (5–10%) metestrous acyclicity. Under these same conditions, littermate control females were fertile and had vaginal smears typical of cycling females. Diet restriction that reduced body weight of the mutants from an initial 40-65 g to a final 30–40 g did not initiate vaginal cycling. Gonadotropin injections did lead to production of proestrous and/or estrous smears in 9 of 17 mutants treated with a sequence of 2.5 IU PMS and 5 IU hCG twice in a week. Three nonresponsive mutants had predominantly metestrous smears in the week before (67%) and during (57%) treatment, but the other 14 had 99% diestrous smears before and only 42% during treatment.

Male Mutants

Even though male mutants are all infertile, at autopsy a number of db^{2J}/db^{2J} mice appeared on inspection to have normal reproductive tracts. When organ weights were taken for seminal vesicles and testes of 6 mutants and 6 heterozygous controls, 4 pairs of which were littermates, they were normal (Table 1). In addition, numerous motile sperm filled the ducti deferentes in each animal. The only difference from normal found on inspection of

TABLE 1. Weights of testes, seminal vesicles and preputial glands in mutant male mice and their normal male littermates.[a]

Genotype	n	Body weight gm	Testis mg	Seminal vesicle mg	n	Preputial gland mg
db^{2J}/db^{2J}	6	41 (34–54)	93 ± 14	165 ± 9.6	3	29.5 ± 3.0*
$m+/+db^{2J}$	6	24 (22–27)	105 ± 9	171 ± 14	3	55.3 ± 3.6

[a]Mean weights are given, with either (range) or ±SEM.

*$P<0.001$.

the reproductive tract and accessory organs was that of the preputial glands, which looked small in all 6 mutants. Three pairs were weighed and found to be half the normal weight (Table 1).

This finding of an essentially normal reproductive tract offers no clue as to the cause of infertility in male *db* mutant mice, but the 6 mutant males plus 4 others were also observed in their behavior toward normal receptive females (tested for receptivity by a short screening test with a fertile male). The behavior of all mutant males over a 15 min period (in the home cage or a neutral cage) was limited to sniffing the female entrant and to some grooming, whereas normal males mounted these females within 1–3 min. An additional 4 mutants, which had been constrained in their food intake since the onset of plumpness to maintain normal body weights, were periodically left overnight in a cage with 1–3 estrous females (no food or 1 pellet/animal available). No vaginal plugs were found on the following mornings, nor did any of the females become pregnant.

Uterine Weight

The dose response curves for uterine weight gain were indistinguishable for mutant and normal immature, ovariectomized mice (Fig. 1). Similarly when EB was given to intact, extremely obese mutant mice, uterine weight increased to the normal range found in cycling mice (Table 2).

Ovary Transplantation

A previous study by Hummel et al. (1966) indicated that *db/db* ovaries function successfully when transplanted to normal hosts. Similar results were obtained for db^{2J}/db^{2J} ovaries. Four of 8 recipient females bore litters with black pups, of which about half became obese. The success rate for transplants compared favorably with rates typical in a laboratory using the technique routinely (Beamer, 1972/73).

Ovulation in situ

The capacity of mutant ovaries to function *in situ* was tested with PMS, which has a dose dependent effect on the quantity of ova released in normal mice (Table 3). hCG was given to provide a uniform ovulatory stimulus. The

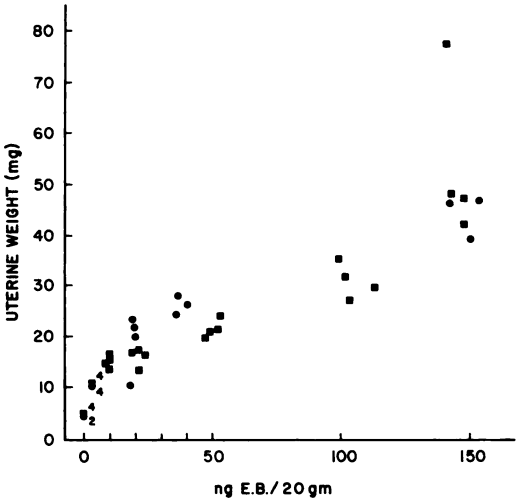

FIG. 1. Response of uterine weight to estrogen. Mutant (circle) and control (square) mice were ovariectomized at 5.5 to 6.5 weeks of age and treated after 12 days with 12 daily EB injections. Although intact, untreated mutants at this age had 8.0 ± 0.74 mg uteri compared to the normal 20 to 60 mg uteri, ovariectomized mutant and control mice responded similarly to exogenously administered estrogen. Symbols represent a single uterine weight, except for the 0 and lowest dose where overlap is too great for separate plotting and number of animals is indicated.

TABLE 2. Uterine weight gain in intact, adult female mutant mice when estradiol benzoate was administered i.p. for 13 days. Comparison is made with mutants ovariectomized for 13 weeks and with cycling and ovariectomized normal mice.[a]

Genotype	Age (mo)	n	Cycle stage	Dosage ug/100 gm	Uterine weight (mg)
db^{2J}/db^{2J}	6.5	3	anestrus	alcoholic saline	20 ± 3.7
	6.5	3		0.1	43 ± 14
	6.0	3		0.2	36 ± 2.9
	6.0	3		0.5	63 ± 15
	7.5	3		1.0	93 ± 13
	4.5	4	ovariectomized	...	14.4 ± 2.7
$m+/+db^{2J}$	5.0	4	diestrus/metestrus	...	49 ± 1.5
and m/m		3	estrus	...	93 ± 9
$m+/+db^{2J}$	4.5	4	ovariectomized	...	6.1 ± 1.0

[a] ±SEM.

response of mutant mice was similar in this experiment to that of the normal mice. In both genotypes the ovaries were heavily luteinized, and the uteri of mutant mice had enlarged to weights equivalent (0.5 and 2.0 IU) or nearly equivalent (1.0 IU) to those in control mice.

A single injection of PMS in prepuberal mice after 21 days of age initiates an estrous cycle by eliciting estrogen secretion and an LH surge. In this experiment, mutant as well as normal mice had vaginal opening, vaginal estrus and uterine growth indicative of effective stimulation of estrogen production. Ovulation occurred in 20 of 22 control mice, yielding from 1 to 16 eggs per animal. In contrast, none of the 9 mutants ovulated, even though large, Graafian follicles were present on their ovaries.

LH and FSH

The LH and FSH measurements obtained for

TABLE 3. Dose effectiveness of PMS treatment on ovulation. All mice were given PMS at 1600 h on Days 1 and 2, 7.5 IU hCG at approximately 1000 h on Day 4. Oviducts were flushed on Day 5. Mutant and normal mice responded similarly, both in terms of percent of mice ovulating and the number of eggs per ovulator for each dose of PMS studied.[a]

IU PMS	Parameter	n	Mutant	n	Normal
0.5*	% ovulating	6	83	7	86
	# eggs/ovulator	5	4.2 ± 0.2	6	4.5 ± 0.8
1	% ovulating	6	83	5	60
	# eggs/ovulator	5	2.6 ± 0.5	3	4.7 ± 0.3
2	% ovulating	6	100	7	100
	# eggs/ovulator	6	10.0 ± 2.4	7	14.3 ± 2.2
4	% ovulating	4	100	4	100
	# eggs/ovulator	4	14.7 ± 1.3	4	14.7 ± 2.7
8	% ovulating	4	75	4	100
	# eggs/ovulator	4	12.0 ± 7.0	4	7.3 ± 2.4

[a] ±SEM.

*IU/mouse.

normal mice, intact and castrated, were in general agreement with the findings of others (Parlow, 1964; Kovacic and Parlow, 1972; Beamer et al., 1972; Beamer, personal communication). Only enough serum was obtained from intact female mice to measure LH or FSH and the former was given priority. Data from intact mutants are summarized in Fig. 2. Serum gonadotropin measurements did not significantly differ from normal, but it should be noted that control mice had been selected in diestrus. Their mean serum LH thus represents the low extreme for normal mice. Even so, mutant females chosen at random had mean serum LH concentrations just below the normal minimum. The only significant difference from normal seen in intact mutant mice was that of increased pituitary content of FSH ($P<0.001$).

Ovariectomy in normal mice caused serum LH to increase from 43 ± 8 ng/ml to 922 ± 87 ng/ml. Serum FSH made a similarly dramatic increase. Pituitary content increased for both LH ($P<0.02$) and FSH ($P<0.001$). Ovariectomized mutant mice had only a third the normal increase in serum LH and FSH, even though pituitary content of LH increased ($P<0.02$) as in normal mice and pituitary FSH increased ($P<0.02$) to remain greater than in normal, ovariectomized mice ($P<0.001$). Responses to orchidectomy that were similar for mutant and control mice included an approximately two-fold increase in serum LH, no change in serum FSH and no change in the highly variable pituitary LH contents. Mean pituitary FSH content of normal male mice decreased after castration ($P<0.05$), but the decrease was not significant in mutant males ($P>0.1$) (Fig. 3).

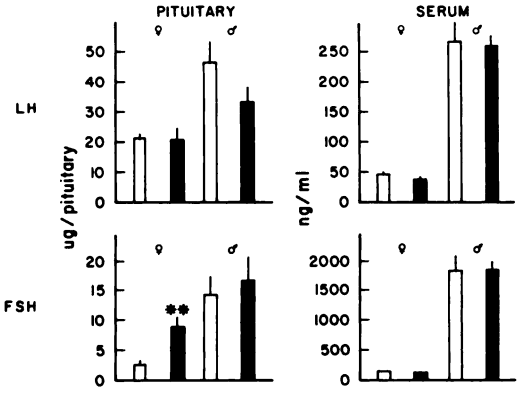

FIG. 2. LH and FSH in intact mutants. Pituitary content of FSH was increased in mutant female mice. Diestrous control mice were used for comparison of serum gonadotrophins. Serum FSH in females was below the detection limit. Open bars are for control and closed bars for mutant mice. **$P<0.001$. Vertical lines = SEM.

GNRH

Intact male mutants had 1.4 times the hypothalamic GNRH content of normal mice, but this increase was not statistically significant. Intact mutant females had a significant increase in GNRH for each of 4 experiments ($P<0.001$) (Fig. 4). Data from these experiments were not pooled because the mean content of GNRH varied greatly between experiments. The experiments differed only in the date of sacrifice of animals, and the samples were reassayed in a single RIA to confirm the differences.

Ovariectomized mutants (10) had 1.31 ± 0.25 ng/hypothalamus, while ovariectomized, normal mice had only 0.72 ± 0.1 ng/hypothalamus ($P<0.02$). Castrate male mutants had 1.33 ± 0.21 ng/hypothalamus and controls had 0.92 ± 0.15 ng/hypothalamus, again in the direction of an increase, but not significantly so.

Exogenous GNRH

Mice that were bled before and after i.p. injection of GNRH responded to the 100 and

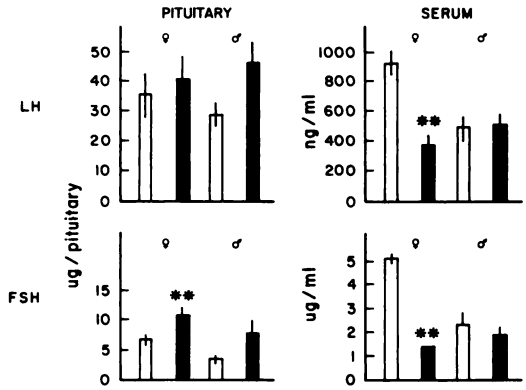

FIG. 3. LH and FSH in castrate mutants. Castration increased the serum LH and FSH of female mice, but not to as high a level in mutant as in normal mice. Ovariectomy did not alter the tendency toward a greater content of pituitary FSH in mutant than in normal mice. Orchidectomy lowered pituitary FSH in normal mice, while in mutant male mice no significant decrease occurred. Open bars are for control and closed bars for mutant mice. **$P<0.001$. Vertical lines = SEM.

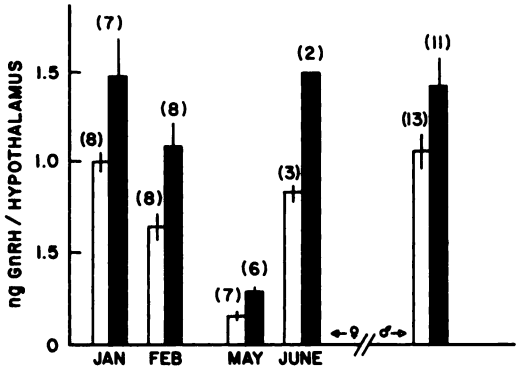

FIG. 4. Hypothalamic GNRH content. For female mice the mean hypothalamic GNRH content varied on different dates of sacrifice, but on each day mutants had more GNRH than controls ($P<0.001$). The difference for male mice was not statistically significant. Open bars are for control and closed bars for mutant mice. Vertical lines = SEM.

200 ng/100 g dose. The changes in mean serum LH concentrations in mutants were at least as great as normal (Table 4). The decrease in serum LH concentration after saline treatment may be a response to stress (Moore, 1966).

DISCUSSION

A principle question in regard to the diabetes syndrome has been whether other anomalies, such as infertility, occur as a result of the metabolic difficulties. In these studies, the reproductive tracts of intact mutant female mice appeared to be unstimulated, yet the response of target tissues to estrogen was normal. The ovaries, whether transplanted to control females or exposed *in situ* to exogenous PMS, appeared to have normal sensitivity to gonadotropic stimulation. Even so, the immature mutants failed to ovulate in an experiment which required that animals produce an endogenous surge of LH. On the basis of these observations, we would conclude that infertility of *db* mutant female mice results from inadequate gonadotropic stimulation and not from an unresponsive reproductive tract.

Direct measurements of gonadotropins lent support to that conclusion. Intact mutant female mice had minimal diestrous concentrations of serum LH. Whether intact or ovariectomized, they had a normal pituitary content of LH and an increased onctent of FSH, yet neither LH nor FSH concentration in serum increased after gonadectomy as much as it did in normal mice. This reduction in the serum response, along with the increased GNRH content found in the hypothalamus of intact and ovariectomized mutants, suggests the possibility of a defect in the mechanism for release of gonadotropins. Although theoretically such a defect could occur in either the CNS or the pituitary gland, the finding of equivalent increases in serum LH for mutant and control

TABLE 4. Serum LH in adult gonadectomized mice before and 15 min after i.p. saline or graded doses of GNRH (NIH, LH/FSH-RF).[a]

Sex	Genotype	n	Before ng/ml	GNRH ng/100 gm	n	After ng/ml
male	$m+/+db^{2J}$	11	229 ± 28	saline	3	111 ± 11
				50	3	107 ± 24
				100	3	345 ± 32
				200	3	652 ± 51
	db^{2J}/db^{2J}	12	199 ± 24	saline	0	. . .
				50	2	179
				100	3	430 ± 55
				200	3	661 ± 72
female	$m+/+db^{2J}$	12	475 ± 45	saline	4	129 ± 36
				50	4	467 ± 125
				100	2	761
				200	3	927 ± 54
	db^{2J}/db^{2J}	10	140 ± 27	saline	2	<87
				50	3	399 ± 23
				100	2	555
				200	3	673 ± 207

[a] ±SEM.

mice after GNRH administration makes it seem more likely that the problem relates to inadequate GNRH release from the brain.

For male mutant mice these experiments did not demonstrate a reduction in gonadotropin secretion, yet the failure to display copulatory behavior, which Batt and Harrison (1963) also observed in db^{ad}/db^{ad} mice, is *prima facie* cause for infertility. This behavioral anomaly could be another consequence of a CNS lesion.

The reproductive tract, which has been widely assumed in diabetics to lose the ability to respond to gonadotropins, is in all likelihood vindicated in at least the *db/db* mouse, the *ob/ob* mouse (Swerdloff et al., 1976) and in rats made insulin deficient with alloxan (Kirchick et al., 1978a). For alloxan-diabetic rats, the pituitary gland has a reduced capacity to respond to GNRH, although the responsiveness can apparently be restored sufficiently for ovulation to occur when the diabetes is well controlled by insulin replacement therapy (Kirchick et al., 1978a,b).

The obese (*ob*) mutation in mice is of particular interest in relation to *db*, because the 2 mutations, found on different chromosomes, produce similar syndromes of obesity and insulin-resistant diabetes. The female *ob/ob* mice are all infertile, but 20% of the males are fertile when fed *ad lib* (Lane, 1959), in contrast to *db/db* males. Swerdloff et al. (1978) studied intact male *ob/ob* mice (no distinction was made between fertile and infertile mutants) and found that the pituitary response to GNRH is diminished in the mutants. They concluded that the pituitary gland is a more likely site of abnormality than is the CNS in the *ob/ob* mouse. The apparent contrast between these results and ours on the *db* mouse parallels that shown by Coleman (1973) concerning food intake regulation. He hypothesized that the diabetes mutation involves a CNS anomaly, but that the obese mutation produces a peripheral disorder.

These experimental results support the hypothesis that the diabetes (*db*) mutation may act through the CNS to cause infertility. Both the defect in food intake regulation (Coleman and Hummel, 1969) and the reproductive neuroendocrine defect could point to a common hypothalamic disorder, or other CNS sites could play more fundamental roles, with the hypothalamic-pituitary-gonadal axis being further downstream in the complex.

REFERENCES

Batt, R.A.L. and Harrison, G.A. (1963). The reproductive system of the adipose mouse, J Hered 54, 135–138.

Beamer, T. C. (1972/1973). Pathological changes associated with ovarian transplantation, 44th Ann Report Jackson Lab. p. 104.

Beamer, W. G., Murr, S. M. and Geshwind, I. I. (1972). Radioimmunoassay of mouse luteinizing and follicle stimulating hormones. Endocrinology 90, 823–827.

Coleman, D. L. and Hummel, K. P. (1969). Effects of parabiosis of normal with genetically diabetic mice. Amer. J. Physiol. 217, 1298–1304.

Green, M. (1968). Biology of the Laboratory Mouse. 2nd ed. (E. Green, ed.). McGraw-Hill, N.Y. pp. 188–191.

Hummel, K. P., Dickie, M. M. and Coleman, D. L. (1966). Diabetes, a new mutation in the mouse. Science 153, 1127–1128.

Lane, P. W. (1959). The pituitary-gonad response of genetically obese mice in parabiosis with thin and obese siblings. Endocrinology 65, 863–868.

Johnson, L. M. (1977). Endocrine and neural factors in sterility of diabetes mutant mice. Doctoral thesis, Harvard University.

Kirchick, H. J., Keyes, P. L. and Frye, B. E. (1978a). Etiology of anovulation in the immature alloxan-diabetic rat treated with pregnant mare's serum gonadotrophin: absence of the preovulatory luteinizing hormone surge. Endocrinology 102, 1867–1873.

Kirchick, H. J., Keyes, P. L. and Frye, B. E. (1978b). Hypothalamic GnRH depletion and pituitary response to GnRH in alloxandiabetic rats. 11th Annual Meeting of the Society for the Study of Reproduction. Abstr.

Kovacic, N. and Parlow, A. (1972). Alterations in serum FSH/LH ratios in relation to the estrous cycle, pseudopregnancy, and gonadectomy in the mouse. Endocrinology 91, 910–915.

Moore, W. W. (1966). Effect of stress on plasma LH in ovariectomized rats. Physiologist 9, 248.

Niswender, G. D., Midgley, Jr., A. R., Monroe, S. C., Reichert, Jr., L. E. (1968). Radioimmunoassay for rat luteinizing hormone with antiovine LH serum and ovine LH-^{131} I. Proc. Soc. Exp. Biol. Med. 128, 807–811.

Parlow, A. F. (1964). Effect of ovariectomy on pituitary and serum gonadotrophins in the mouse. Endocrinology 74, 102–107.

Rugh, R. (1972). The Mouse–its Reproduction and Development. Burgess Publ. Co., Minneapolis. p. 42.

Sidman, R. L., Angevine, Jr., R. L. and Pierce, E. T. (1971). Atlas of the Mouse Brain and Spinal Cord. Harvard University Press, Cambridge, pp. 31–44.

Swerdloff, R. S., Batt, R. A. and Bray, G. A. (1976). Reproductive hormonal function in the genetically obese (ob/ob) mouse. Endocrinology 98, 1359.

Swerdloff, R. S., Peterson, M., Vera, A., Batt, R. A. L., Heber, D. and Bray, G. A. (1978). The hypothalamic-pituitary axis in genetically obese (ob/ob) mice: response to luteinizing hormone-releasing hormone. Endocrinology 103, 542–547.

Yen, T.T.T., Steinmetz, J. and Simpson, P. J. (1974). The response of obese (*ob/ob*) and diabetic (*db/db*) mice to treatments that influence body temperature. Comp. Bioc. Phys. 49A, 377–385.