Absence of Estrogenic Activity in a Diet that Promotes Estrous Cyclicity in C57BL/6J Mice (43195)

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Abstract. **A breeder diet that shortens estrous cycles in mice has been reported to contain estrogenic substances, based on its ability to increase uterine weight of immature mice. However, the estrogenicity of the diet was inferred from uterine weight gain of immature mice that were intact. The increased uterine weight of mice on the breeder diet could thus have resulted from a precocious pubertal increase of endogenous estrogens induced by the diet rather than estrogenic substances in the diet. We therefore measured the estrogenicity of the breeder diet in ovariectomized animals. C57BL/6J mice were fed the breeder diet or a standard diet for 1 or 4 weeks. The breeder diet failed to increase uterine weights above control values for either treatment interval. Intact mice that were fed the breeder diet had twice the number of cycles of mice fed the standard diet, a confirmation of earlier studies. These results indicate that the breeder diet does not contain biologically significant estrogenic activity, and thus potentiates cyclicity by other means.** [P.S.E.B.M. 1991, Vol 196]

Two isocaloric diets, a breeder diet developed to promote reproduction and a standard maintenance diet, differ markedly in ability to support estrous cyclicity in young mice. Mice on the breeder diet have shorter cycles and fewer acyclic intervals than mice fed the standard diet (1). Neither the dietary component(s) nor the physiologic mechanisms responsible for this potentiation of estrous cyclicity are known.

A report that the breeder diet might contain estrogenic substances (2) was thus of interest, particularly since chronic exposure to low concentrations of estradiol can shorten cycles in mice (3). In that study, intact immature mice fed the breeder diet for 3 to 7 days had significantly greater uterine weights than those of mice fed the standard diet. Since increased uterine weight is a classic bioassay for estrogens (4, 5), the authors concluded that the breeder diet contained estrogenic substances. However, because the mice were intact, their uterine weights could have been greater because the breeder diet induced a precocious pubertal increase in endogenous estrogens. Dietary modulation of the timing of puberty in rodents is well documented (6–8).

The purpose of this study was to determine more conclusively whether the breeder diet contains estrogenic activity. This objective was achieved by measuring the effect of the breeder diet on uterine weight in ovariectomized mice, thus eliminating the potentially confounding influence of the diet on endogenous estrogen secretion.

Materials and Methods

Animals. Female C57BL/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME) as 4- or 8-week-old virgins. They were housed five, four, or three per cage in a limited access colony of aging mice, and were maintained at 23–25°C on a 12-hr light:12-hr dark schedule. Mice had unlimited access to food and to acidified water (pH 2.5). Mice in this colony were routinely screened for common murine viruses and bacteria. All tests were negative during the time mice used in this study were in the colony.

Test Diets. Two diets were used: a closed formula natural-ingredient breeder diet (Purina Mouse Chow 5015; Ralston Purina Co., Richmond, IN), and a closed formula natural-ingredient standard diet (Rodent Laboratory Chow 5001). The two diets are isocaloric [4.31 kcal/g (breeder); 4.25 kcal/g (standard)], but differ in

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fat (11%, breeder; 4.5%, standard), protein (17.5%, breeder; 23.4%, standard), fiber (2.1%, breeder; 5%, standard), as well as micronutrient levels and sources [detailed compositions in Nelson *et al.* (9)]. The same diets, including the source, were used in the earlier study that concluded that the breeder diet contained estrogenic substances (2).

Experiment 1. Five-week-old mice were ovariectomized (OVX) under anesthesia (2,2,2-tribromoethanol; BDH Chemicals, St. Laurent, Quebec, Canada), divided into two groups with similar body weights, and housed five or four per cage. One group was placed on the breeder diet and the other group on the standard diet. After 7 days, the interval used in the earlier examination of this question (2, 10), all mice were weighed and exsanguinated from the right ventricle under anesthesia. Uteri were dissected, trimmed, and blotted as per Thigpen *et al.* (10), and weighed to the nearest 0.1 mg. Vaginal smears were obtained from all mice 2 days prior to sacrifice.

Experiment 2. Four and one-half-month-old mice that had been OVX for 3 months were weighed, separated into two groups, with similar mean body weights, and housed six per cage. One group was fed the breeder diet and the other group the standard diet. Body weights were recorded weekly. After 4 weeks, mice were sacrificed and uteri were collected as in Experiment 1. Vaginal smears were taken from all mice before sacrifice.

Experiment 3. Upon arrival, 8-week-old mice were weighed, separated into two groups with similar mean body weights, housed three per cage, and ear-marked. One group was fed the breeder diet and the other group was fed the standard diet (six mice per group), Daily vaginal smears were obtained from both groups for a period of 38 days as described previously (11). The vaginal cytology during the first 6 days after placing mice on the respective diets was not included in the cycle analysis in order to allow animals to adapt to the handling and to the new diets. Cycle frequency and length were determined as described previously (11). Only cycles that were equal to or longer than 3 days and equal to or shorter than 6 days were used in the analysis; partial cycles were not counted.

Additional 8-week-old mice were weighed, separated into two groups with similar mean body weights, ovariectomized, ear-marked, and housed three or four per cage. One group was placed on the breeder diet and the other group on the standard diet. Body weights were recorded weekly and vaginal smears were obtained twice a week. After 4 weeks, mice were sacrificed and uteri were collected and weighed as in Experiment 1.

Statistical Analysis. Data are expressed as mean ± SE, and differences between groups were compared by *t* test. Differences with *P* values <0.05 were considered significant.

Results

Experiment 1. Mean body weights, uterine weights, and uterine to body weight ratios of 5-week-old OVX mice fed the standard or breeder diet for 7 days are compared in Table I. Weights of mice on the two diets did not differ. Uterine weights and, consequently, uterine to body weight ratios also did not differ between the two groups. Vaginal smears were leukocytic in all mice.

Experiment 2. Because subtle effects of dietary estrogens might have been missed due to the short duration of dietary exposure in the first experiment, OVX mice were exposed to the two diets for a longer interval (4 weeks) (Table II). Mice fed the breeder diet weighed more than mice fed the standard diet ($P < 0.001$), but the breeder diet had no significant effect on uterine weight or the uterine to body weight ratio. Moreover, vaginal smears obtained from both groups contained only leukocytes.

Experiment 3. Although different batches of the breeder diet contain the same percentage of nutrients, they may differ in the sources of the nutrients (Ralston-Purina, personal communication). Consequently, estrogenic substances could have been present in the earlier formulations of the diet that potentiated cyclicity (1, 9), even though they were no longer present in the current formulations. Thus, before concluding that the breeder diet potentiates cyclicity through means other than an intrinsic estrogenicity, it was necessary to es-

Table I. Mean Body Weights, Uterine Weights, and Uterine to Body Weight Ratios of 5-Week-Old Acutely OVX Mice Fed a Breeder (No. 5015) or Standard (No. 5001) Diet for 7 Days (Experiment 1)

	Breeder diet	Standard diet	*P*
Body weight (g)	16.3 ± 0.3	16.6 ± 0.4	NS[a]
Uterine weight (mg)	8.1 ± 0.7	8.2 ± 0.7	NS
Uterine to body weight	0.49 ± 0.03	0.49 ± 0.04	NS
No. of mice	9	8	

[a] NS, not significant.

Table II. Mean Body Weights, Uterine Weights, and Uterine to Body Weight Ratios of 4.5-Month-Old OVX Mice Fed a Breeder (No. 5015) or a Standard (No. 5001) Diet for 4 Weeks (Experiment 2)

	Breeder diet	Standard diet	*P*
Body weight (g)	31.2 ± 0.5	24.2 ± 0.3	NS[a]
Uterine weight (mg)	7.2 ± 0.8	7.4 ± 1.7	NS
Uterine to body weight	0.23 ± 0.03	0.30 ± 0.07	NS
No. of mice	5	6	

[a] NS, not significant.

tablish that the current formulation of the diet still enhanced cyclicity.

Cycle frequency in mice fed the breeder diet (5.67 ± 0.49 cycles/32 days) was was 2-fold higher than that of mice on the standard diet (2.67 ± 0.76, $P < 0.01$, t test). This difference was due to both decreased cycle length and the absence of acyclic mice (data not shown). Mean body weights, uterine weights, and uterine to body weight ratios of 8-week-old OVX mice fed the breeder or standard diet are summarized in Table III. Again, none of these parameters differed between the two groups. In addition, vaginal smears were leukocytic in all OVX mice on the two diets.

Discussion

The major finding of this study was that a breeder diet that doubles the number of cycles in intact mice does not increase uterine weights in ovariectomized mice relative to those of mice fed the standard diet. We therefore conclude that the breeder diet does not contain biologically significant amounts of estrogenic substances, and thus must enhance estrous cyclicity by other means.

Our conclusion that the breeder diet does not have estrogenic activity differs from that of an earlier study in which the same diet increased uterine weight (2). However, that study was conducted with immature animals that were intact. The breeder diet has a higher fat content (9) and accelerates weight gain (9) relative to the standard diet, and it may have increased uterine weight by triggering a precocious pubertal increase of endogenous estrogens. High fat diets can accelerate puberty in rats, and this may be partly associated with an earlier attainment of a threshold body weight (8). This confound was avoided in the present study by measuring the effect of the diet in ovariectomized mice. Because a different strain of mouse was used in the earlier study, we cannot completely exclude the possibility that the different outcomes of the two studies reflect strain differences in sensitivity to estrogens. However, we believe that this is unlikely since C57BL/6J mice are sensitive to very small changes in circulating levels of estradiol (3). Clearly, however, the breeder diet is not estrogenic in C57BL/6J mice.

In two longitudinal studies spanning a period of 6 years, we observed that the breeder diet reduced cycle length and increased cycle frequency in C57BL/6J mice (1, 9). In the present study, the breeder diet continued to potentiate cyclicity, indicating that this is a robust property of the diet. Since the breeder diet does not appear to contain estrogenic substances, it must promote shorter and more frequent estrous cycles by other means. The 2-fold higher fat content of the breeder diet may be involved, since young rats fed a high fat diet had shorter cycles than animals fed a low fat diet (12). Schneider and Wade (13) recently reported evidence that estrous cyclicity in hamsters is governed by the general availability of metabolic fuels, rather than the selective availability of fatty acids or glucose. Since mice fed the breeder diet gain weight faster than mice fed the standard diet (9), the availability of metabolic fuels may be higher in mice on the breeder diet. Examining the role of differences in fat content and availability of metabolic fuels on the potentiation of cyclicity may thus be useful approaches to understanding the basis for the influence of these two diets on estrous cyclicity.

Table III. Mean Body Weights, Uterine Weights, and Uterine to Body Weight Ratios of 8-Week-Old OVX Mice Fed a Breeder (No. 5015) or a Standard (No. 5001) Diet for 4 Weeks (Experiment 3)

	Breeder diet	Standard diet	*P*
Body weight (g)	22.5 ± 0.6	23.2 ± 0.5	NS[a]
Uterine weight (mg)	11.9 ± 0.4	12.2 ± 0.5	NS
Uterine to body weight	0.53 ± 0.02	0.53 ± 0.03	NS
No. of mice	5	6	

[a] NS, not significant.

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