Plasma gonadotrophins and progesterone concentrations during various degrees of underfeeding in pregnant mice

B. A. Rattner*, Sandra D. Michael† and H. J. Brinkley‡

Behavioral Sciences Department, Naval Medical Research Institute, Bethesda, Maryland 20014; †Department of Biological Sciences, State University of New York, Binghamton, New York 13901; and ‡Department of Zoology, University of Maryland, College Park, Maryland 20742, U.S.A.

Summary. Daily reduction of the normal (*ad libitum*) food consumption by as little as 35% significantly reduced ($P < 0.05$) the percentage of mice with implantation sites at Days 7 and 9 of gestation. Underfeeding decreased body weight and reduced the weight of the ovaries and uterus. Plasma progesterone was decreased ($P < 0.05$) as dietary intake was restricted and was associated with regression of the corpora lutea. No significant alterations in the plasma values of LH and FSH were observed in mice underfed between Days 1 and 9 of pregnancy. The decrease in plasma progesterone in the absence of reduced LH values may indicate that progesterone secretion between Days 5 and 9 of gestation is not controlled solely by LH.

Introduction

In the laboratory mouse, implantation may be prevented by short periods of food deprivation (McClure, 1958, 1959; Bruce, 1963; Rattner, Michael & Brinkley, 1978a). A 48-h fast between Days 2 and 4 of gestation in the mouse (vaginal plug = Day 1) suppresses the plasma concentrations of LH and progesterone, and abolishes surges of prolactin (Rattner *et al.*, 1978b). The effects of dietary restriction have been attributed to impaired metabolic, luteotrophic, and steroidal maintenance of pregnancy. Although many investigations have described the effects of dietary protein restriction, restricted food intake, or starvation on plasma gonadotrophins, prolactin, and gonadal steroids in the rat (Howland, 1971; Giannina & Leatham, 1974; Campbell, Kurcz, Marshall & Meites, 1977), relatively little is known about such endocrine values in the mouse. It was the objective of the present study to determine the effects of moderate to severe underfeeding on implantation and the plasma concentrations of LH, FSH, and progesterone in the mouse.

Materials and Methods

Nulliparous mice of the NFR/N strain, 8–10 weeks old, weighing 22–26 g, and maintained in a photoperiod of 14 h light/24 h at $23 \pm 1^\circ\text{C}$, were mated monogamously with proven males. Insemination was verified by inspection of vaginal plugs before 08:00 h on the following morning. The day on which a vaginal plug was first observed was designated as Day 1 of pregnancy. Gravid mice were housed individually in clear plastic cages measuring $30 \times 19 \times 13$ cm.

At 08:00 h the inseminated mice were weighed and assigned to one of 4 groups: those in Group 1 were allowed to eat without restriction (*ad libitum* controls; mean $\pm$ s.e.m. daily consumption was 5.92 ± 0.137 g between Days 1 and 9 of pregnancy); those in Groups 2, 3 and

* Present address: Environmental Physiology and Toxicology Project, Patuxent Wildlife Research Center, Laurel, Maryland 20811, U.S.A.

4 were given 5, 4 or 3 g, respectively, at 08:00 h daily. The protein and fat content of the pelleted food (formula 20 RF: Agway, Inc., Syracuse, New York) was 26.3 and 6%, respectively. All the mice had unrestricted access to water.

The mice were weighed and immediately killed by decapitation at 12:00 h on Days 1, 2, 3, 4, 5, 7 or 9; there were 6 mice killed on each of these days for every treatment group. Blood was collected through heparinized funnels, and the plasma obtained following centrifugation was frozen at -10°C . The ovaries and uterus were dissected and weighed.

The concentrations of LH, FSH and progesterone were measured in duplicate as previously described (Rattner *et al.*, 1978b). The coefficient of variation and sensitivity (95% confidence interval at 100% binding) were 2.44% and 10.1 ng/ml for LH, and 5.48% and 33.1 ng/ml for FSH. Progesterone was extracted with diethyl ether with a mean recovery of 86.2%, and a coefficient of variation of 10.76%. The sensitivity of the progesterone assay was 2.67 ng/ml, and the coefficient of variation was 6.92%. All samples were analysed within single assays.

The experiment was conducted and analysed as a 4×7 factorial (level of feeding by day of killing) with 6 randomized complete blocks. The percentage of mice with implantation sites was compared by contingency analysis (Sokal & Rohlf, 1973). The weight change of each mouse was calculated by determining the difference in body weight on the day of the vaginal plug and at the time of killing. The assay data were processed by using natural logarithm and logit transformations with a weighted linear regression model (Rattner *et al.*, 1978a, b). The protein hormone values were log-transformed to obtain homogeneity of variance before analysis of variance. Log-transformed protein hormone estimates, untransformed steroid hormone estimates, and other variables were tested by analysis of variance, and subsequently by the Newman-Keuls multiple range test.

Results

There was a progressive reduction ($P < 0.05$), directly proportional to the severity of food restriction, in the percentage of mice with implantation sites (Table 1). However, when implantation occurred, relatively constant numbers of embryos were implanted in all groups (means ranged from 9.8 to 10.8 sites). The implantation sites in animals in Group 4 were distinctly smaller than those in other groups (substantiated by the reduction in uterine weight, Table 1).

Table 1. Effect of underfeeding of mice on implantation, uterine weight, plasma gonadotrophins and progesterone (mean $\pm$ s.e.m.)

Group	Proportion with implantation sites on Days 7 + 9	Uterine wt at Days 7 + 9* (mg)	Hormone concentrations (ng/ml)†		
			LH	FSH	Progesterone
1	12/12 ^a	347.6 $\pm$ 34.81 ^a	44.5 $\pm$ 10.71	137.6 $\pm$ 13.20	85.9 $\pm$ 5.82 ^a
2	11/12 ^{a,b}	328.7 $\pm$ 36.84 ^a	42.0 $\pm$ 10.23	135.2 $\pm$ 15.49	81.0 $\pm$ 6.05 ^{a,b}
3	9/12 ^{b,c}	290.2 $\pm$ 42.28 ^a	26.3 $\pm$ 4.11	138.4 $\pm$ 9.76	67.5 $\pm$ 5.95 ^c
4	6/12 ^c	239.5 $\pm$ 43.44 ^b	29.2 $\pm$ 5.66	164.9 $\pm$ 13.96	57.4 $\pm$ 5.97 ^c

Values with different superscripts are significantly different ($P < 0.05$).

* Animals with implantation sites.

† Treatment means pooled across sampling times; the 6 observations/day for Days 1–9 for each treatment group were combined, and the mean therefore represents 42 observations/treatment group.

The weights of the ovaries in Groups 3 and 4 were reduced slightly, but not significantly, on Days 7 and 9. Examination of the ovaries of Group 4 mice under the dissecting microscope frequently revealed chalk-white corpora lutea on Days 7 and 9, although small hyperaemic corpora lutea were observed in animals with implantation sites. There were hyperaemic corpora lutea in the mice in Groups 1, 2 and 3 on Days 4, 5, 7 and 9.

Body weight gain (Text-fig. 1) generally increased with day of pregnancy in Group 1, and was paralleled at a significantly lower level ($P < 0.01$) by that in Group 2 on Days 7 and 9. Body weight in Group 3 mice was maintained at or just below the initial body weight, which was significantly less ($P < 0.01$) than the corresponding day means of Group 1 mice on Days 3, 5, 7 and 9. In Group 4 body weight was significantly lower ($P < 0.01$) than that in all other groups on Days 4–9.

Text-fig. 1. Daily mean $\pm$ s.e.m. body weight changes of the mice ($N = 6$ at each point) in Group 1 (control, food *ad libitum*), Group 2 (5 g/day), Group 3 (4 g/day) and Group 4 (3 g/day). The Day 1 body weight (mean $\pm$ s.e.m.) was 24.5 ± 0.25 in Group 1, 24.6 ± 0.24 in Group 2, 24.2 ± 0.24 in Group 3 and 24.8 ± 0.28 in Group 4.

Plasma LH concentrations (Text-fig. 2) were low on Days 1–4 of pregnancy in Group 1, and gradually rose on Days 5, 7, and 9. In Groups 2, 3 and 4 the levels of LH were similar to those in Group 1, except on Days 7 and 9 when they were lower. The treatment means (level of feeding) pooled across sampling time (day of killing) (Table 1) were greater in Groups 1 and 2 than in Groups 3 and 4, but the difference was not significant. Analysis of variance of the log-transformed LH values indicated significant effects of day ($P < 0.001$) and the interaction of treatment and day ($P < 0.05$). However, no significant differences were detected between group means by the multiple range test on any single day.

Plasma FSH concentration (Text-fig. 2) was elevated on Day 1 and remained low on subsequent days in all groups. Analysis of variance indicated only significant day effects ($P < 0.001$), with the Day 1 mean pooled across treatment being greater ($P < 0.01$) than all other day means.

Plasma progesterone concentration (Text-fig. 2) rose between Days 1 and 4 in all groups, and remained elevated in Groups 1, 2 and 3 on Days 5, 7 and 9. Significant treatment and day effects ($P < 0.001$), and the interaction of treatment and day ($P < 0.01$) were detected. Treatment means pooled across time (Table 1) were lower ($P < 0.05$) in Groups 3 and 4 than in Groups 1 and 2. Plasma progesterone concentration was relatively low on Days 5, 7 and 9 in Group 4, and was significantly less ($P < 0.05$) than the values in Groups 1 and 2 on Day 5.

Text-fig. 2. Mean plasma LH, FSH and progesterone concentrations in mice in Group 1 (control, food *ad libitum*), Group 2 (5 g/day), Group 3 (4 g/day) and Group 4 (3 g/day). The ranges of the s.e.m. (for 6 mice at each point) were 0.90–35.81 ng LH/ml, 7.12–82.29 ng FSH/ml and 4.32–24.77 ng progesterone/ml.

Discussion

In the mouse, moderate to severe underfeeding (35 to 65% reduction of ad-libitum consumption) decreases the weight of the ovaries and uterus, and may interrupt implantation and terminate pregnancy. The morphological changes of the corpora lutea in Groups 3 and 4, and the absence of implantation sites are associated with the reduction of plasma progesterone, the steroid required for the maintenance of pregnancy (Amoroso & Finn, 1962). Fasting mice for short periods (6–48 h) during early pregnancy causes similar contragestational effects and uniformly suppresses the concentration of LH to less than 10 ng/ml within 6 h of the onset of fasting (Rattner *et al.*, 1978a, b). However, it is apparent from the present investigation that moderate to severe restriction of food intake during Days 1–9 of gestation in the mouse does not uniformly suppress the circulating levels of LH. The inability of chronic underfeeding to suppress plasma LH concentration in mice is in contrast to the reduction of plasma LH observed in underfed male and female rats (Howland, 1971; Campbell *et al.*, 1977), but is similar to findings in starved female hamsters (Howland & Skinner, 1973).

As previously described (Murr, Bradford & Geschwind, 1974), plasma FSH concentration was elevated on Day 1 of pregnancy, and then remained low. The plasma profile of FSH was not

altered by underfeeding, and is similar to findings in the rat (Howland, 1971; Campbell *et al.*, 1977).

LH-stimulated secretion of progesterone during early pregnancy in the mouse has been described (Bindon, 1971; Munshi, Purandare & Rao, 1972; Murr *et al.*, 1974). The fasting of mice during early pregnancy reduced plasma progesterone about 24 h after the suppression of LH values (Rattner *et al.*, 1978b), and indicated that the corpus luteum can function for short periods in the presence of reduced levels of LH. The present study confirmed that plasma progesterone concentrations were reduced by underfeeding (Table 2, Text-fig. 2), particularly after Day 4 of pregnancy (Text-fig. 2), although LH values were not concomitantly reduced. This may indicate that the regulation of progesterone secretion between Days 5 and 9 of gestation in the mouse is not controlled solely by LH. Prolactin, perhaps in combination with LH, might be necessary for sustained progesterone secretion at this time.

B.A.R. was supported by a NRC Postdoctoral Associateship. This work was supported in part by USPHS Grant RRO-7042-11, MH 28286 and the University of Maryland Computer Science Center. We are deeply indebted to Ms F. R. Koplin and Mr B. T. Macmillan.

The opinions and assertions herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

References

- Amoroso, E.C. & Finn, C.A.** (1962) Ovarian activity during gestation, ovum transport and implantation. In *The Ovary*, 1st edn, pp. 451–537. Ed. S. Zuckerman. Academic Press, New York.
- Bindon, B.M.** (1971) Gonadotrophin requirements for implantation in the mouse. *J. Endocr.* **50**, 19–27.
- Bruce, H.M.** (1963) A comparison of olfactory and nutritional stress as pregnancy-blocking agents in mice. *J. Reprod. Fert.* **6**, 221–227.
- Campbell, G.A., Kurcz, M., Marshall, S. & Meites, J.** (1977) Effect of starvation in rats on serum levels of follicle stimulating hormone, luteinizing hormone, thyrotropin, growth hormone and prolactin: Response to LH-releasing hormone and thyrotropin-releasing hormone. *Endocrinology* **100**, 580–587.
- Giannina, T. & Leatham, J.H.** (1974) Serum progesterone levels in pregnant rats fed a protein-free diet. *Proc. Soc. exp. Biol. Med.* **146**, 957–960.
- Howland, B.E.** (1971) Gonadotrophin levels in female rats subjected to restricted feed intake. *J. Reprod. Fert.* **27**, 467–470.
- Howland, B.E. & Skinner, K.R.** (1973) Effect of starvation on LH levels in male and female hamsters. *J. Reprod. Fert.* **32**, 505–507.
- McClure, T.J.** (1958) Temporary nutritional stress and infertility in mice. *Nature, Lond.* **181**, 1132.
- McClure, T.J.** (1959) Temporary nutritional stress and infertility in female mice. *J. Physiol., Lond.* **147**, 221–225.
- Munshi, S.R., Purandare, T.V. & Rao, S.S.** (1972) Effect of antisera to ovine luteinizing hormone on corpus luteum function in mice. *J. Reprod. Fert.* **30**, 7–12.
- Murr, S.M., Bradford, G.E. & Geschwind, I.I.** (1974) Plasma luteinizing hormone, follicle stimulating hormone, and prolactin during pregnancy in the mouse. *Endocrinology* **94**, 112–116.
- Rattner, B.A., Michael, S.D. & Brinkley, H.J.** (1978a) Embryonic implantation, dietary intake and plasma GH in pregnant mice exposed to hypoxia. *Aviat. Space Environ. Med.* **49**, 687–691.
- Rattner, B.A., Michael, S.D. & Brinkley, H.J.** (1978b) Plasma gonadotrophins, prolactin and progesterone at the time of implantation in the mouse: effects of hypoxia and restricted dietary intake. *Biol. Reprod.* **19**, 558–565.
- Sokal, R.R. & Rohlf, E.J.** (1973) *Introduction to Biostatistics*, pp. 296–303. W. H. Freeman and Co., San Francisco.

Received 15 November 1978