EFFECT OF HYPOTHYROIDISM, DIET AND LITTER SIZE ON SUPEROVULATION IN THE MOUSE AND RAT*

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Summary. Litter size in mice influences the body growth of the young under normal conditions, on inadequate diet and under propylthiouracil (PTU) treatment. Invariably the larger litter size of seven showed poorer growth curves when compared with the litter size of four. A significant increase in the relative ovarian weight was noted in the litter size of four when treated with PTU, but this was not seen in the litter size of seven. No significant differences were noted in the number of ova released in the mice under various conditions of diet, goitrogen administration and litter size. Increasing dosages of goitrogen caused both a decrease in body weight and in ova release in the rat. The number of ova fell from a count of thirty-five in normal rats to eighteen in rats on 0.01 % PTU and to no ova in rats on 0.05 and 0.1 % PTU in the diet.

INTRODUCTION

Numerous factors have been shown to influence superovulation. Among these may be listed the dosage of gonadotrophin (Rowlands, 1944; Wilson & Zarrow, 1962) type of gonadotrophin (Pincus, 1940), age of animal (Gates & Runner, 1957; Zarrow & Wilson, 1961), species (Wilson & Zarrow, 1962), strain (Wilson & Zarrow, unpublished), time interval for injection of hormone (Zarrow, Caldwell, Hafez & Pincus, 1958) and levels of thyroid activity (Wilson & Chai, 1962). Even though superovulation can be induced with comparative ease in many animals, the high degree of variation in the numbers of ova released following treatment with a constant dosage of gonadotrophin demonstrates the need for further examination of additional factors that might influence superovulation.

The purpose of this experiment was to evaluate the effect of experimental hypothyroidism, inadequate diet and litter size on superovulation in the mouse and rat. The possibility that these variables might influence the number of ova released was explored.

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MATERIALS AND METHODS

The mice for this investigation were obtained from one cross of two inbred strains raised at the Jackson Memorial Laboratory. The cross made was BALB/c females $\times$ 129 males (F_1). The rats used were of the Wistar strain raised at Purdue University.

The animals were fed Purina Laboratory Chow and water *ad libitum* during their gestation period and both light and temperature were maintained relatively constant. The Purina pellets were removed from the food hopper at the time of parturition, and small food containers from which the mother could obtain ground food were placed inside the cages. The following diets were used: (1) a standard diet of ground Purina Laboratory Chow, (2) a diluted diet containing 60 % ground Purina Laboratory Chow and 40 % cellulose (alphacel), (3) a standard diet of ground Purina Laboratory Chow or the diluted diet with 0.1 % propylthiouracil (PTU) added was fed to mice and the standard diet with 0.01, 0.05 or 0.1% PTU added was fed to rats. At parturition each litter was reduced in size to either four or seven for mice and four or eight for rats. The maximum number of females was retained in each litter.

Ovulation was induced in the weanling female mice by the procedure of Gates & Runner (1957). The BALB/c female $\times$ 129 male F_1 hybrid animals were treated with 4 i.u. pregnant mares' serum injected intraperitoneally at 5.00 p.m. on the 25th day of age, and 44 hr later were given 2 i.u. of human chorionic gonadotrophin intraperitoneally. The animals were killed 18 hr following the ovulatory injection. The rats were induced to ovulate at 35 days of age by the procedure of Zarrow *et al.* (1958).

The mother and young mice were weighed on Days 8, 15, 22, 25 and were autopsied on Day 28. The rats were weighed on Days 8, 15, 22, 29 and were autopsied on Day 35. The young remained with their mothers until autopsy at which time the oviducts were carefully removed and the ova observed in the swollen ampulla of the oviduct with the aid of a dissecting microscope. The ampulla was pricked with a dissecting needle and the ova were expressed and spread out for counting (Rowlands, 1942). In addition, the ovaries, uteri and thyroids of representative animals from each group were weighed on a Roller-Smith torsion balance (sensitive to 0.1 mg).

RESULTS

MICE

A graphic representation of the growth curve for young females (BALB/c females $\times$ 129 males F_1) from litters of four and seven which had been on four different diets is presented (Text-fig. 1). The females from litters of four on standard diet grew steadily from 6.2 g at 8 days of age to 15.8 g at 28 days of age. Females from litters of four placed on a diluted diet were approximately 2 g smaller at all points on the growth curve than were females maintained on standard food. Propylthiouracil exerted its greatest influence on retarding body weight of animals between the ages of 15 and 22 days. This effect appears

to occur at approximately the age that the young animals start eating solid food.

The effect of litter size on weight gain may best be seen by comparing the growth curves of females on standard diet from litters of four and from litters of seven (Text-fig. 1). The slope of the curves is similar, but the curve for litters of four starts out 1.5 g higher than the one for litters of seven.

TEXT-FIG. 1. Effect of litter size, food dilution and 0.1% PTU on the body weight of immature BALB/c $\times$ 129 F₁ hybrids. The numbers in parenthesis represent adjusted size of litters.

TABLE 1

EFFECT ON DIET, LITTER SIZE AND GOITROGENIC AGENT ON SUPEROVULATION, AND OVARIAN, UTERINE AND THYROID WEIGHTS (IN MG/100 G BODY WEIGHT) IN BALB/C FEMALE $\times$ 129 MALE F₁ MICE ($\pm$ S.E.)

No. females	Litter size	Ovary	Uterus	Thyroid	Average No. ova	Diet
12	4	64.0 $\pm$ 4.91	222.0 $\pm$ 15.59	12.1 $\pm$ 3.4	42.3 $\pm$ 8.9	Standard
10	4	71.7 $\pm$ 8.3	204.8 $\pm$ 7.0	10.2 $\pm$ 1.8	57.8 $\pm$ 5.0	Dilute
12	4	92.5 $\pm$ 2.6	264.4 $\pm$ 6.7	82.8 $\pm$ 6.2	45.0 $\pm$ 6.3	Standard + 0.1% PTU
10	4	88.2 $\pm$ 3.9	277.0 $\pm$ 12.0	138.2 $\pm$ 9.3	31.8 $\pm$ 6.5	Dilute + 0.1% PTU
11	7	75.2 $\pm$ 3.1	215.4 $\pm$ 9.4	11.3 $\pm$ 0.95	51.2 $\pm$ 5.9	Standard
12	7	71.0 $\pm$ 7.3	221.0 $\pm$ 8.4	11.0 $\pm$ 0.69	42.2 $\pm$ 7.6	Dilute
9	7	77.9 $\pm$ 4.0	260.9 $\pm$ 11.7	74.9 $\pm$ 16.0	35.4 $\pm$ 6.9	Standard + 0.1% PTU
9	7	80.4 $\pm$ 13.5	251.8 $\pm$ 15.0	86.0 $\pm$ 6.7	41.2 $\pm$ 12.3	Dilute + 0.1% PTU

The weights of the ovaries, uterus and thyroids at 28 days of age are recorded as mg/100 g body weight (Table 1). As expected, PTU produced a marked increase in thyroid weight. A 13-fold increase in the relative weight of the thyroid gland was noted in mice from litters of four and fed a diluted diet

plus 0.1 % PTU as compared with animals from litters of four fed the standard diet. An eightfold increase in relative thyroid weight was noted in animals from litters of seven that were fed 0.1 % PTU and diluted diet as compared with control animals on the standard diet.

The averages of egg counts are also recorded (Table 1). Due to the small number of animals available for this experiment, the standard error of the mean was quite large. BALB/c females $\times$ 129 males F₁ females from litters of four fed standard food were induced to ovulate 42.3 ova. The ovum count increased from 42.3 to 57.8 in the animals with litters of four that were maintained on diluted food. However, a decrease from 51.2 ova on a standard diet

TEXT-FIG. 2. Effect of litter size, dilution of food and 0.1% PTU on the body weight of immature Wistar rats. The numbers in parenthesis represent adjusted size of litters.

to 42.2 on a diluted diet was obtained with litters of seven. Propylthiouracil caused no change in number of ova released or a decrease when added either to the standard or diluted diet.

RATS

A graphic representation of the growth curve for young females from litters of four and eight that have been on four different diets is represented in Text-fig. 2. The body weight of rats receiving PTU as a part of their diet was markedly reduced.

Text-fig. 3 shows the effect of increasing doses of PTU on the body weight, relative thyroid weight and the average number of ova released following gonadotrophic treatment. All levels of PTU administered reduced average ovum counts below control values. Propylthiouracil also caused a reduction in body weight and a corresponding increase in thyroid weight at all dose levels. Ovarian and uterine weights were not obtained in this part of the experiment.

DISCUSSION

This experiment was designed primarily to determine the ability of litter size, inadequate diet and a goitrogenic agent (PTU) to alter the superovulatory response in the mouse and rat. It is possible that any one of the factors could increase, decrease or have no effect on the number of ova shed following a standard gonadotrophic regimen to induce superovulation. From our data it is apparent that the 0.1 % PTU was extremely toxic when fed to rats and produced a growth-deterrent effect in mice. The ovulatory response, however, needs considerable explanation. In the rat, the hypothyroid condition produced by 0.1 % PTU reduced the ovarian response to zero and it appears to be a graded response as indicated from the 13.3 ova in animals receiving only 0.01 % PTU in their diet (Text-fig. 3). On the other hand, the ovulatory response

TEXT-FIG. 3. Effect of PTU in a standard diet on body weight, ovulation and thyroid weight in immature rats from litters of eight.

- ▨ = Body weight (g).
- = Average ovum count.
- = Thyroid weight in mg/100 g body weight.

in mice from litters of four maintained on a standard diet does not appear to be markedly affected by the 0.1 % PTU (Table 1). However, when the 0.1 % PTU is combined with a diluted diet, there is a significant decrease in mouse ovum count. It has been reported that short-term-PTU treatment in mice increases ovum counts (Wilson & Chai, 1962). This discrepancy might be due to strain differences or to the length of treatment (Janes, 1954). The mouse picture is further complicated by the known change in ovarian response to gonadotrophins at different age intervals between 18 and 30 days (Gates & Runner, 1957; Zarrow & Wilson, 1961). The peak ovarian response in these hybrid mice has been shown to occur at 22 days of age. Since in this present experiment gonadotrophic treatment started at Day 25, it is possible that any factor which retards maturation would shift the ovulatory response to the left on the age curve. Therefore, any treatment which would cause inanition or reduction in metabolic activity could produce these same effects. This same rationale, however, does not explain our results obtained in the rat. These animals were 35 days of age when gonadotrophic treatment began which was

10 days beyond the peak response as shown by Zarrow & Wilson (1961).

It is apparent that all the factors individually retard body growth in both mice and rats. The diluted diet had no effect on ovarian or uterine weight in the mouse, but the goitrogen produced an increase in both ovarian and uterine weights. This same response of the ovary following a short-term administration of 0.1 % PTU has been reported (Wilson & Chai, 1962). The uterus, however, was unaffected by this short-term treatment.

Current studies are under way which will more specifically delineate the effects of hypothyroidism on the ovulatory capacity of immature mice and rats.

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