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## Effects of Continuous Light on Hypothalamic FSH-Releasing Factor and Pituitary FSH Levels in Rats* (32792)

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Exposure of female rats to continuous illumination has been reported to induce prolonged or persistent vaginal cornification (1,2). Fiske (3,4) concluded that pituitaries from female rats on constant light had more FSH and less LH activity than rats on constant darkness. Maric *et al.* (5) found that total pituitary gonadotropin content was not changed after 50 or 100 days of constant illumination, whereas pituitary LH content and concentration were markedly reduced. Lawton and Schwartz (6) and Bradshaw and Critchlow (7) also reported decreased LH content in rats with persistent estrus induced by continuous light. It was our purpose to elucidate the role of the FSH-releasing factor (FSH-RF) in the hypothalamus on the pituitary FSH response to constant light.

*Materials and Methods.* Female Sprague-Dawley rats (Holtzman, Madison, Wisc.) about 3 months old and weighing 200–300 gm each were used in these experiments. Two separate groups of rats were kept under continuous illumination for 21 days. Continuous light was provided by four 100-W white light bulbs, placed 4 feet above the wire-topped cages. Controls were housed under standard light conditions (14 hours of light daily). All rats were maintained on a diet of Wayne Lab Blox pellets (Allied Mills, Chicago, Ill.) and fed *ad libitum*. Vaginal smears were taken daily in both experimental and control groups. After the first 10 days of constant illumination, all rats showed a proestrous or estrous type of smear. Control animals showed normal cycling patterns. On day 22, the rats were killed by guillotine and the hypothalami and anterior pituitaries were removed. The hypothalami were placed in ice cold 0.1 *N* HCl (0.2 ml per hypothalamus) and stored at −20°C. The pituitaries were weighed individually and stored at −20°C. A few days later, the pituitaries were homogenized in saline with a glass homogenizer and assayed for FSH activity. Ovaries and uteri were cleaned, weighed, and processed for histological study. Differences in organ weights were analyzed by Student's *t* test.

*Preparation of hypothalamic extracts.* Hypothalami were homogenized in chilled 0.1 *N* HCl and centrifuged at 12,000*g* for 40 min at 4°C just prior to use. The supernatants were placed in protein free medium 199 (Difco Labs, Detroit, Mich.) and the pH was adjusted to 7.4 by adding a drop at a time of 1 *N* NaOH and testing with glass electrodes.

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[1] Kellogg Foundation Fellow.

TABLE I. Effect of Constant Light on Hypothalamic FSH-RF Content.

| Expt. no. | Treatment and no. of rats | Hypothalamic equivalents/incubated pituitary | FSH released ($\mu$g/mg of pituitary[a,b]) | Relative potency[b,c] | $\lambda$[d] |
|---|---|---|---|---|---|
| 1 | Standard light (20) | 0.5 | 8.66 ( 7.54– 9.94) | | |
| | Constant light (20) | 0.5 | 13.26 (11.55–15.22) | 1.48 (1.29–1.70) | 0.076 |
| 2 | Standard light (30) | 0.5 | 7.54 ( 6.52– 8.72) | | |
| | Constant light (30) | 0.5 | 12.44 (10.14–15.26) | 1.70 (1.39–2.07) | 0.082 |

[a] Expressed as $\mu$g equivalents of NIH-FSH-S3.
[b] Mean and 95% confidence limits.
[c] Experimental vs control.
[d] Index of precision.

TABLE II. Effect of Constant Light on Pituitary FSH Content and Concentration.

| Treatment and no. of rats | FSH ($\mu$g/pituitary[a,b]) | FSH ($\mu$g/mg of wet pituitary[a,b]) | $\lambda$[c] |
|---|---|---|---|
| Standard light (30) | 28.46 (20.62–39.27) | 2.94 (2.13–4.06) | |
| Constant light (30) | 27.16 (19.68–37.48) | 2.42 (1.75–3.34) | 0.152 |

[a] Expressed as $\mu$g equivalents of NIH-FSH-S3.
[b] Mean and 95% confidence limits.
[c] Index of precision.

*Incubation.* The FSH-RF was measured by the method of Mittler and Meites (8). Adult male rats of the same strain were killed rapidly by decapitation. Each anterior pituitary was separated from the posterior lobe and hemisected. One-half was placed in a control flask and the other half in an experimental flask. The equivalent of four anterior pituitaries (8 halves) was placed in each 25-ml flask. Incubations were carried out in a Dubnoff metabolic shaker (60 cycles/min) under constant gassing with 95% $O_2$, 5% $CO_2$ at 37°C. The pituitaries were preincubated in medium 199 for one-half hour, and the medium was discarded. Fresh medium containing the neutralized acid extract was then added rapidly to the flasks. Six hours later, the incubation was terminated. The incubated pituitary tissue was weighed and the medium was stored at —20°C until assayed a few days later for FSH.

*FSH bioassay.* The FSH activity was measured by the HCG augmentation method of Steelman and Pohley (9) as modified by Parlow and Reichert (10). Each assay included two doses of the control and experimental unknowns, as well as two doses of the reference standard (NIH-FSH-S3)[2], 70 and 140 $\mu$g. The difference between doses was twofold and 4 rats were injected per dose. The statistical methods of Bliss (11) were used to compute the potencies of the unknowns, ratios of relative potencies between control and experimental preparations, 95% confidence limits and any departures from parallelism. Further details for assaying FSH-RF were reported previously (8).

*Results.* 1. *Effect of constant light on hypothalamic FSH-RF and pituitary FSH levels.* In two independent experiments, anterior pituitary halves incubated with hypothalamic extracts from constant light treated rats released significantly more FSH into the medium than the corresponding anterior pituitary halves incubated with hypothalamic extracts from randomly cycling control animals (Table I). This indicates that constant light increased the FSH-RF content of the hypothalamus. It can be seen in Table II that

[2] The FSH-S3 was kindly supplied by the Endocrinology Study Section, NIH.

TABLE III. Organ Weights of Rats under Standard and Constant Light.

| Treatment and no. of rats | Body wt. (gm)[a] | Pituitary (mg)[a] | Uterus (mg)[a] | Ovaries (mg)[a] |
|---|---|---|---|---|
| Standard light (30) | 235.2 ± 3.8 | 9.67 ± 0.26 | 432.40 ± 13.00 | 68.14 ± 1.71 |
| Constant light (30) | 229.6 ± 4.6 | 11.65 ± 0.32[b] | 652.00 ± 26.33[b] | 52.31 ± 2.31[b] |

[a] Mean ± SE.

[b] Experimental vs control: $p < 0.001$.

pituitary content and concentration of FSH were similar in both control and experimental groups.

2. *Organ weights.* Table III shows that continuous illumination produced a significant ($p<0.001$) increase in pituitary and uterine weights, and a significant decrease in ovarian weight ($p<0.001$). The ovaries from rats under standard light showed follicles in different stages of development and moderately to well-developed corpora lutea (Fig. 1), whereas the ovaries of constant light treated animals had a large number of well-developed follicles and few small corpora lutea (Fig. 2). The uteri of the constant light treated rats had increased vascularity, a marked augmentation of muscle mass, and increase in number of glands and greater height of the epithelium (Fig. 4). These histological observations are indicative of prolonged estrogen action, and are in contrast to the lesser development seen in the uteri of the control rats (Fig. 3).

*Discussion.* The results presented here demonstrate that constant light increased FSH-RF content in the hypothalamus. Previous observations from our laboratory (12) also showed that constant light can increase blood plasma levels of FSH-RF in hypophysectomized male rats. Although pituitary FSH content and concentration remained unchanged in the rats under constant light, there can be no doubt that release of this hormone was increased, as indicated by the marked growth of the ovarian follicles and stimulation of uterine development. The increased pituitary weight can be accounted for by enhanced estrogen secretion from the ovaries.

It is possible that pituitary FSH synthesis and release were augmented to an equal degree by constant light, and hence no change was seen in pituitary content or concentration. Fiske (3,4) concluded that pituitary FSH was greater in rats maintained under constant light as compared to rats maintained under constant darkness, but a specific FSH bioassay was not employed. A previous study from our laboratory (13) showed that constant light did not alter pituitary FSH content in rats even though growth of ovarian follicles was greatly stimulated. More recently, Singh and Greenwald (14) also reported that constant light failed to alter pituitary FSH content and concentration in the female rat.

Pituitary LH and hypothalamic LRF levels were not measured in these rats. However, if these were decreased in rats under constant light, as suggested by other investigators (3–7,14), then they also may have contributed to the results observed in the present study. The increase in number and size of ovarian follicles, together with the increased weight of the uterus and pituitary of rats on constant light, could be due in part to continuous stimulation of follicular growth by FSH without hinderance from LH-induced ovulation to form corpora lutea. However, the present study indicates that constant light enhances pituitary FSH secretion by augmenting FSH-RF release from the hypothalamus, and this is probably the principal mechanism for increased follicular stimulation.

*Summary.* The effects of 21 days of constant illumination on the hypothalamic content of FSH-RF and on pituitary content and concentration of FSH were studied in two groups of intact adult female rats. Control rats were housed under standard light conditions (14 hours of light daily). In the rats under constant light, hypothalamic FSH-RF content was significantly increased whereas pituitary FSH content and concentration remained unchanged. Pituitary and uterine

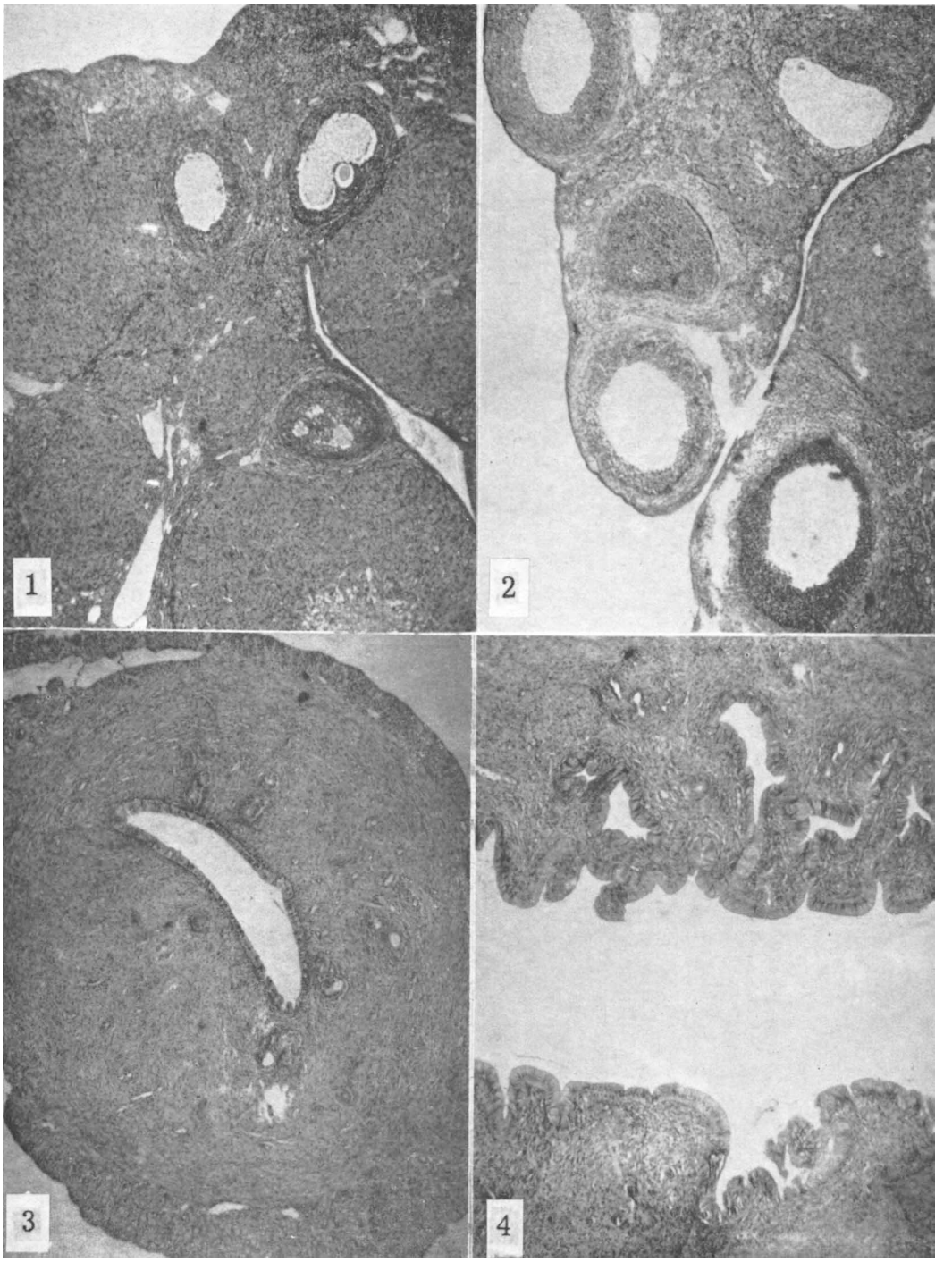


FIG. 1. Ovarian section from rat under standard light conditions. × 30.
FIG. 2. Ovarian section from rat under constant light. × 30.
FIG. 3. Uterus from rat under standard light conditions. × 30.
FIG. 4. Uterus from rat under constant light. × 30.

weights were increased, and ovarian weight was decreased. Histological examination of the ovaries of the constant light treated rats showed large numbers of well-developed follicles with few small corpora lutea. The uteri of these rats showed a marked augmentation in vascularity, muscle mass, number of glands in the endometrium, and increased height of the epithelium. These results suggest that constant light stimulates increased release of FSH from the pituitary by increasing hypothalamic production of FSH-RF.

*Addendum.* We have recently observed that FSH-RF content of the hypothalamus of female rats is high during diestrus and in the morning of proestrus, and low in the afternoon of proestrus and during estrus (Negro-Vilar, Sar and Meites, unpublished observations). This suggests that if all the control rats had been in the estrus phase of the cycle, their hypothalamic FSH-RF content would have been even lower than reported here, and the difference from the rats under constant light would have been greater.

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## Congenital Lymphocytic Choriomeningitis Virus Infection in Gnotobiotic Mice* (32793)

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Lymphocytic choriomeningitis (LCM) virus is unique in that it is disseminated congenitally to progeny, in which it resides for long periods without distinct symptoms (1,2). The LCM virus inoculated in mice at birth remained in the circulatory system; and the animals manifested as part of the disease, what appeared to be an accelerated aging process (3,4).

When mature mice were inoculated with LCM virus the appearance of disease and death was related to the immunological status of the hosts: they were spared if pretreated by X-rays (5), by amethopterin (6), or by neonatal thymectomy (7), otherwise they died.

Thus far, evidence of two types of viral agents have been reported in gnotobiotic Lobund mice: leukemia virus has been detected in the thymic tissues of six mouse strains, and lymphatic leukemia appeared in significant numbers of them following whole-body exposures to small doses of X-rays (8). In addition, strain AKR mice carry leukemia virus and have manifested the same pattern of leukemic disease as the conventional stock from which they were derived (9). During 6 years, seven gnotobiotic Lobund mice (Balb/c, C3H/f and Swiss-Webster strains) developed spontaneous breast carcinomas, and B-type virus particles were detected in two

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