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Effects of Photoperiod on Cyclicity and Serum Gonadotropins in the Syrian Hamster[1]

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Adult female Syrian hamsters were exposed to 10 or 14 hours of illumination daily. Animals on 14 hours of light per day showed consistent 4-day ovulatory cycles, but exposure to 10 hours of illumination daily resulted in acyclicity after about 6 weeks. The acyclic state was accompanied by large diurnal fluctuations in serum gonadotropin concentrations. These fluctuations may be responsible for the inhibition of estrous cyclicity; alternatively, they may be correlated with, but not causal to, the inhibition of estrous cycles.

Variations in illumination play an extremely important role in the regulation of the physiology and behavior of many animals. The presence or absence of light not only provides the cues that regulate many diurnal rhythms, but variations in daylength also provide cues important in the seasonal control of reproduction. This environmental influence on internal processes is clearly evident in the response of the Syrian hamster (*Mesocricetus auratus*) to altered photoperiods. In a series of studies Reiter (1967, 1969) observed that bilateral optic enucleation or exposure to only one hour of light each day resulted in significant decreases in the weight of the uteri of female hamsters when compared with uteri from animals exposed to longer photoperiods. Similarly, male hamsters had atrophied testes and accessory sex organs following exposure to short daily photoperiods (Reiter *et al.* 1970; Reiter, 1972; Reiter and Sorrentino, 1972). Thus, it appears that in this seasonally breeding species the photoperiod may be of primary importance in regulating the annual growth and atrophy of the sex organs.

It seemed likely that in hamsters exposed to a short photoperiod (10L:14D) changes in the normal four day cycle of ovulation might accompany gross changes in reproductive organs and that the disruption of cyclicity might be associated with altered serum levels of gonadotropins. To investigate these possibilities female hamsters were exposed to long and short days, and ovulatory cycles as well as serum gonadotropins were monitored. The results indicate that marked diurnal fluctuations in serum luteinizing hormone (LH) occur during the period of acyclicity induced by the shortened photoperiod.

MATERIALS AND METHODS

General maintenance and photoperiod

All animals were purchased as young adults from the Lakeview Hamster Colony, Newfield, New Jersey, and were temporarily group housed (6–10 animals per cage) in suspended stainless steel cages maintained on 14 hours of light per day (illumination from 5 AM-7 PM). All females were checked daily for external vaginal signs of estrus (Orsini, 1961) and were followed for four normal (4 days in length) cycles before being randomly assigned to the experimental (10L:14D) or control conditions (14L:10D) on the day of estrus. In both cases the onset of the light period occurred at 5 AM each day. All animals were maintained at 20–22°C. Animals under the 10L:14D lighting conditions were either singly or group housed. The communally housed group consisted of 8 animals housed in a single cage; there were 13 singly housed females. All 14L:10D subjects (13 animals) were singly housed, but other observations in our laboratory indicate that hamsters group housed on this regimen show highly regular 4-day cycles with only infrequent periods of acyclicity. These experiments were carried out from March through July, 1973.

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Serum sampling and gonadotropin assays

At designated intervals the animals were briefly anesthetized with ether and 0.7–1.0 ml blood was obtained by cardiac puncture. Bleedings were taken at 9–10 AM and again at 2–3 PM from the same animals. In two experiments a third sample was obtained at 7 PM. Hamsters which were showing estrous cycles were always bled on the second diestrous day (D_2)—i.e. the day prior to proestrus.

The blood was stored at 4°C for 14–26 hours and was then centrifuged at 4–6°C. Serum was stored at −20°C until the time of assay. Serum levels of luteinizing hormone (LH) were determined by a radioimmunoassay carried out with an antiserum raised against ovine LH (Niswender *et al.* 1968) and follicle-stimulating hormone (FSH) assays employed the NIAMD-Rat FSH kit with Anti Rat FSH S-5. LH values are expressed in terms of NIAMD-Rat LH-RP-1; FSH is expressed in terms of NIAMD-Rat FSH-RP-1. These assay systems have been validated for use in the hamster (Bast and Greenwald, 1974; Bex and Goldman, 1974; Blake *et al.*, 1973; Goldman and Porter, 1970).

Ovariectomy

To assess possible interactions between the respective roles of photoperiod and gonadal steroids in the control of gonadotropin secretion, six of the singly-housed hamsters in 10L:14D and eight females in 14L:10D were selected (randomly) for bilateral ovariectomies. The operations were performed under ether anesthesia and were carried out following six weeks exposure to the respective photoperiods. Blood samples were obtained 3.5 weeks after ovariectomy and intact, singly-housed females were bled at the same time (i.e. after 9.5 weeks exposure to the 10L:14D or 14L:10D regimen).

Acyclicity during 14L:10D

Female hamsters maintained on 14L:10D in our laboratory occasionally become acyclic. Such animals usually remain acyclic for several weeks. It was of interest to determine whether the pattern of gonadotropin secretion in such animals was similar to that displayed by females induced to become acyclic by exposure to short photoperiod. Therefore, a group of hamsters which had become "spontaneously" acyclic on 14L:10D were bled serially at 10 AM, 4 PM, and 7 PM. Six to eight days following bleeding the animals were ovariectomized, and after a period of 3–5 days the hamsters were bled at the same times. Cycling hamsters on 14L:10D were treated in the same way for comparison. As before, the cycling animals were sampled on D_2. This study was carried out in April–May, 1974.

RESULTS

Cyclicity

Four of the 13 females exposed to 10L:14D stopped showing vaginal cycles within 3 weeks, and 11 of the females exposed to this regimen failed to show estrous vaginal discharges after 6 weeks. In contrast, most of the 14L:10D females continued to cycle throughout the experiment. The exceptions were two females which became acyclic for 3 and 4 weeks, respectively (Fig. 1). There were no effects of housing (grouped vs. isolated) on the length of exposure to short photoperiod required to induce acyclicity, and the 10L females began to show a spontaneous return to the normal 4-day estrous cycle after approximately 20 weeks (singly housed animals). The group housed females were transferred from 10L:14D to 14L:10D after 7 weeks exposure to the short photoperiod. At the time of transfer 7 of the 8 hamsters were acyclic. Cyclicity was resumed in these animals after 3–5 weeks on the 14L:10D regimen.

Serum gonadotropins

Since the time of light onset has a crucial role in the timing of the proestrous surge and because of a possible diurnal rhythm in LH secretion in ovariectomized hamsters (Goldman *et al.* 1971) it was felt that valuable information might be gained by examining gonadotropin levels at different times of the day. Data from bleedings taken at 9 AM and 3 PM during the acyclic phase indicated a strong circadian rhythm for serum LH levels in 10L:14D housed animals (Fig. 2), with serum LH concentrations 10–20 fold higher at 3 PM than at 9 AM ($p < .001$). Although this effect is clear in the intact animal the surge is even more pronounced in the ovariectomized preparation (i.e. Serum LH concentrations were greater at 3 PM in ovariectomized hamsters as compared to intact animals, $p < .05$). Diurnal variations in FSH were noted only in intact hamsters maintained on the 10 hour photoperiod. Blood samples were not obtained from the two females which failed to become acyclic during exposure to 10L:14D. No difference between morning and afternoon serum LH concentrations was observed for cycling hamsters maintained on 14L:10D and sampled on D_2.

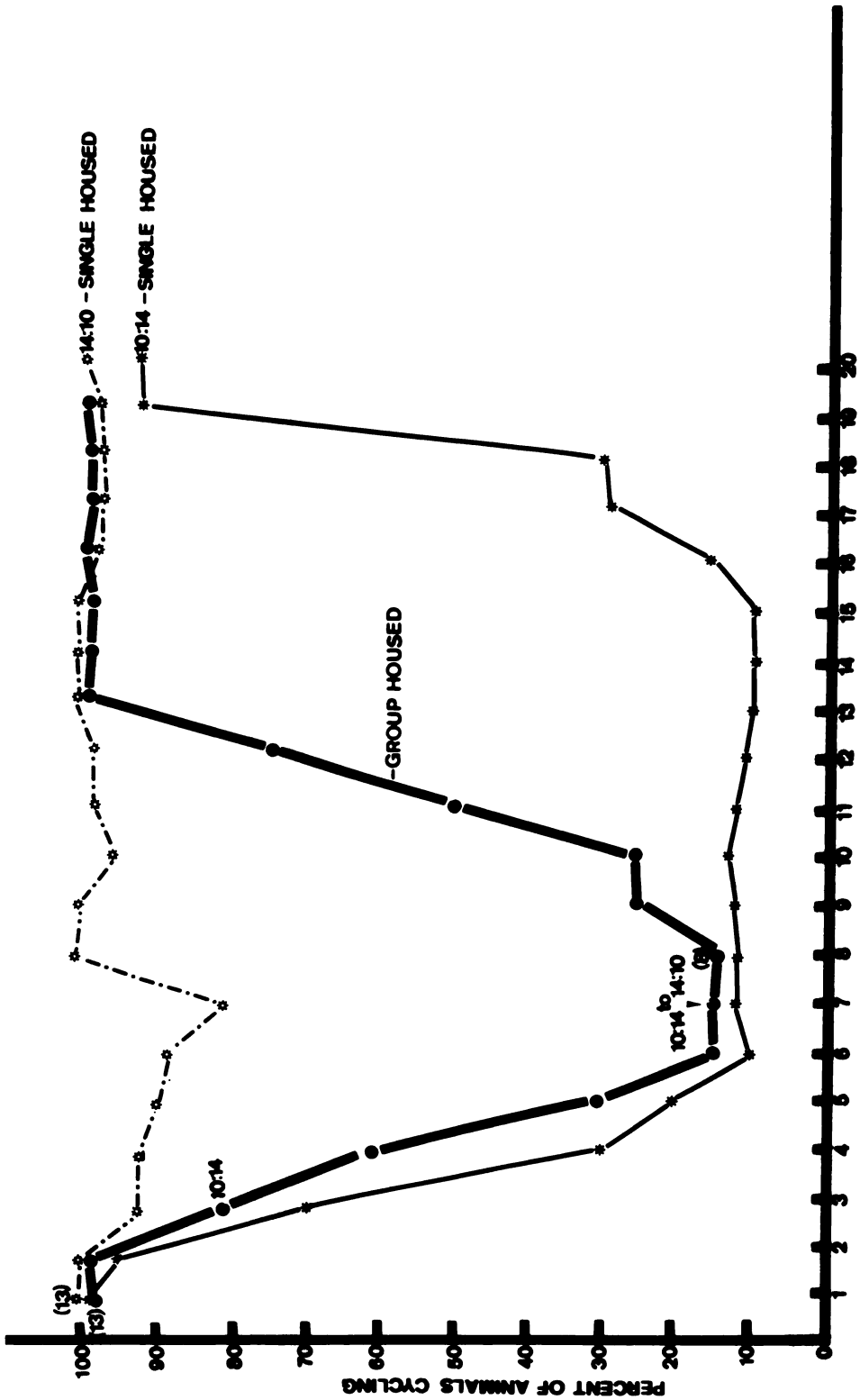

FIG. 1. Estrous cyclicity in hamsters maintained on long and short daily photoperiods. The percentage of animals displaying regular vaginal estrus is plotted as a function of the duration of exposure to either 14L:10D or 10L:14D. The number of animals represented is shown next to some of the points. Note that the group housed females were placed on 14L:10D after 7 weeks exposure to 10L:14D.

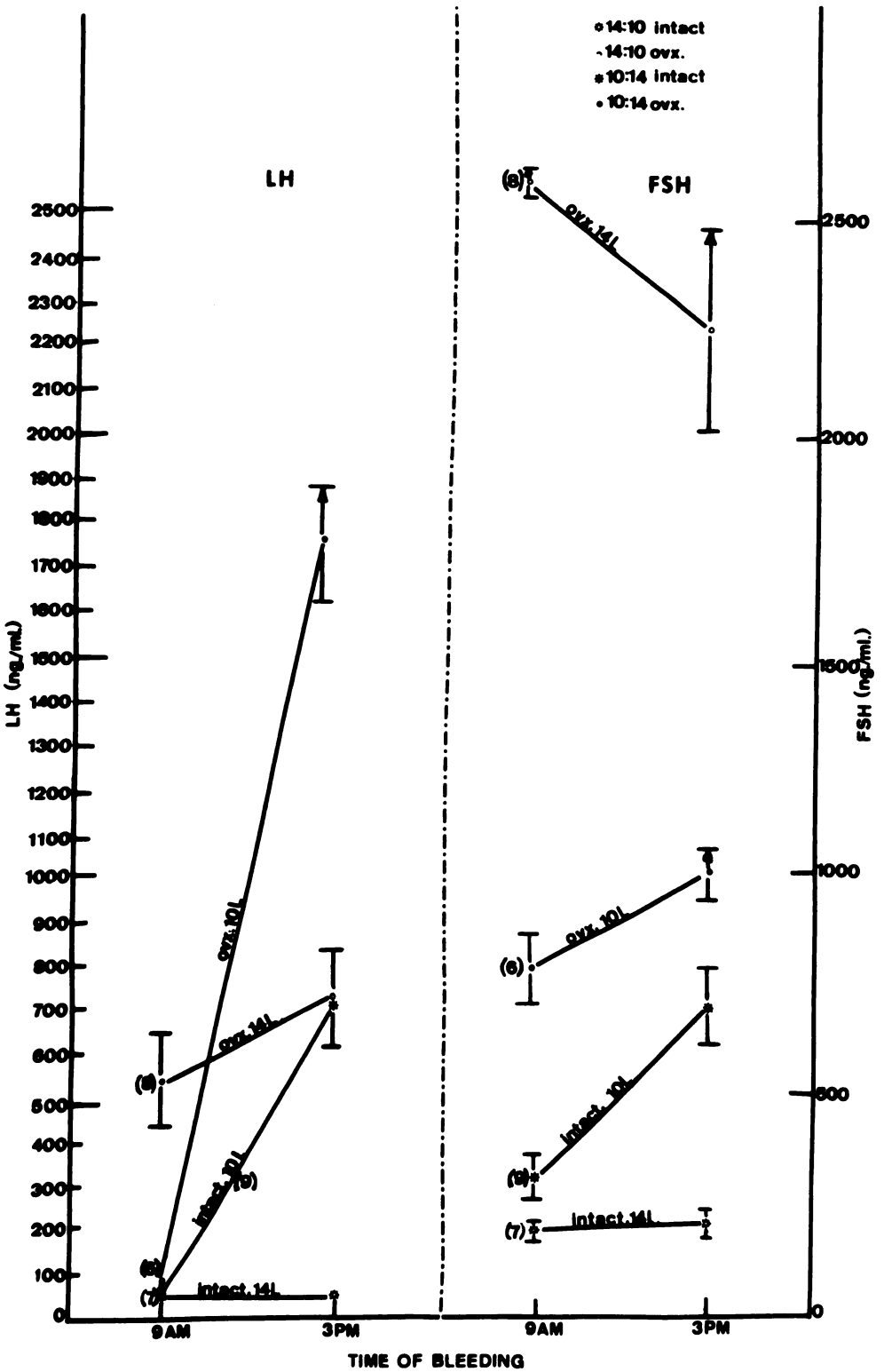

FIG. 2. Serum LH and FSH concentrations in intact and ovariectomized (OVX) hamsters maintained on long and short photoperiods. Sequential blood samples were taken at 9 AM and 3 PM after approximately 9.5 weeks exposure to the respective photoperiods (i.e. 14L:10D or 10L:14D). Values are plotted as mean ± standard error. The N for each group is shown in parentheses.

In order to more closely map the daily variations in serum gonadotropins, intact 10L acyclic hamsters (group housed) were bled at 9 AM, 2 PM and 7 PM. These bleedings were carried out after 6 weeks exposure to the 10L:14D regimen. The serum gonadotropin concentrations at 9 AM, 2 PM and 7 PM, respectively, are shown in Figure 3. In this

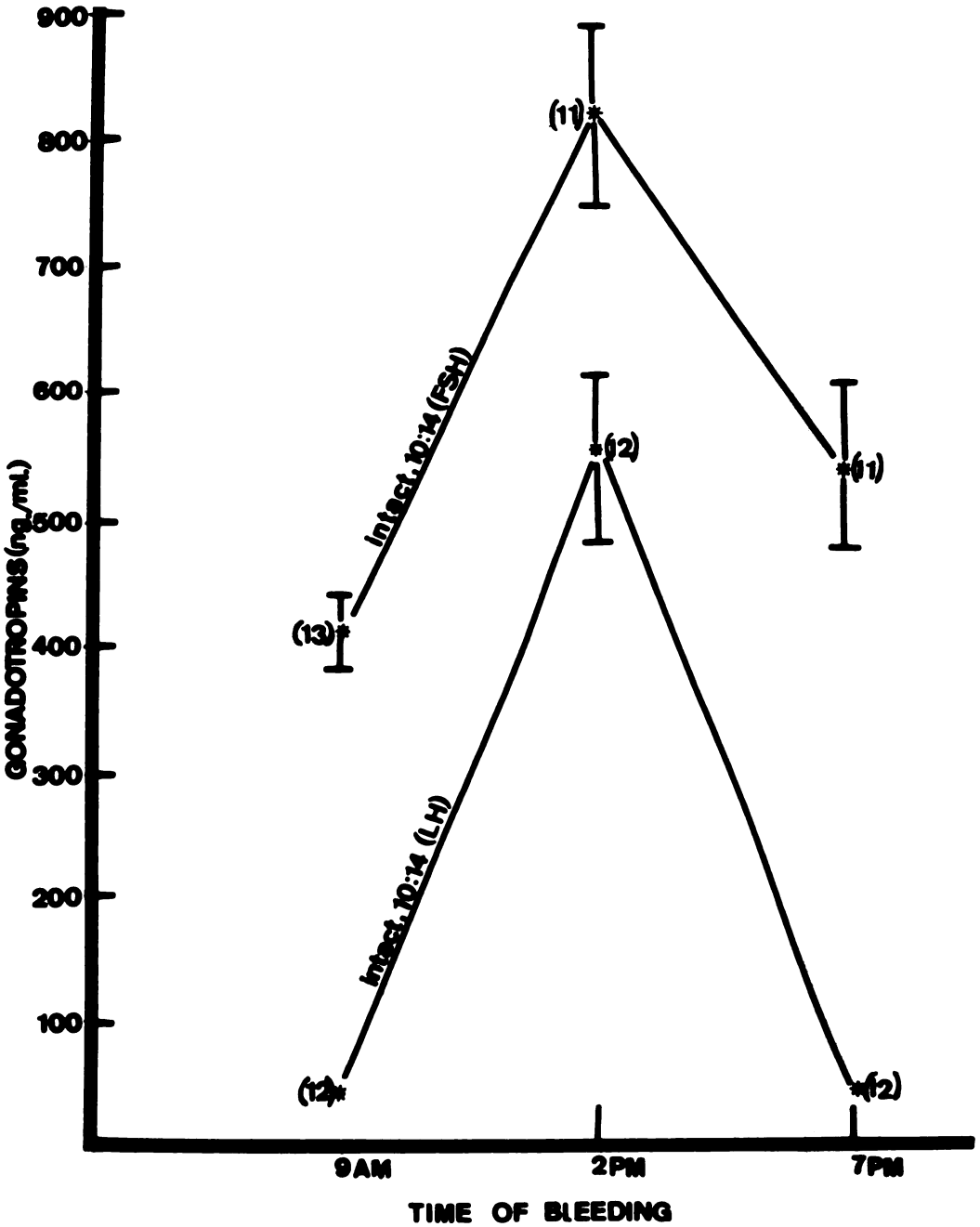

FIG. 3. Diurnal rhythm in serum LH and FSH in intact hamsters maintained on a short photoperiod (10L:14D). Blood samples were obtained after 6 weeks exposure to the lighting regimen and only animals which were acyclic at that time are included here. At 9 AM and 7 PM most of the animals failed to show detectable serum LH (<25 ng/ml).

experiment significant fluctuations in both LH and FSH were again observed. However, whereas LH concentrations at 2 PM were at least 15–20 fold higher than the morning and evening levels, the serum FSH concentration was elevated by only about 2-fold at the same time.

LH levels were also measured in ovariectomized hamsters maintained on 10L:14D shortly after their intact counterparts had resumed cycling (i.e. after 20 weeks exposure to 10L:14D). At this time it was found that serum LH had "stabilized" at a level typical of long-term ovariectomized animals maintained on 14L:10D (>600 ng/ml serum) and, most notably, no diurnal rhythm in serum LH was detected.

Female hamsters which had become acyclic during maintenance on 14L:10D showed a similar diurnal rhythm in serum LH concentrations as did animals induced to become acyclic by exposure to a short photoperiod, and this rhythm persisted after ovariectomy (Fig. 4). However, the afternoon LH values before and after removal of the ovaries, respectively, were not statistically different from each other in this experiment.

DISCUSSION

Cyclicity

The time course for cessation of vaginal cyclicity in hamsters exposed to a reduced photoperiod (Fig. 1) is similar to the time course for decreases in uterine weight in light-deprived hamsters (Reiter, 1969). However, in the present experiments cyclicity was resumed after about 20 weeks exposure to 10L:14D as compared to approximately 27 weeks required for "recovery" of the uterus in light-deprived hamsters. The shorter period required for resumption of cycles in hamsters maintained on 10L as compared to animals which are totally light-deprived (i.e. total darkness produces a more prolonged effect than 10L:14D) suggests that hamsters may be somewhat responsive to photoperiod even during the period of acyclicity. The observation that a resumption of cyclicity could be hastened by returning the 10L females to a 14L:10D regimen (Fig. 1) is further evidence that the hamster continues to be responsive to the photoperiod following cessation of reproductive function.

Serum gonadotropins

The observation of marked diurnal fluctuations in serum gonadotropins during the acyclic period in 10L females was unexpected. The timing (i.e. mid-afternoon) and magnitude of the LH/FSH peaks in these animals is suggestive of the proestrous LH/FSH surge (Bast and Greenwald, 1974; Bex and Goldman, 1974; Goldman and Porter, 1971; Goldman *et al.*, 1971; Turgeon and Greenwald, 1972). A mid-afternoon rise in serum LH of similar time course but of much lesser magnitude has been observed within one day following ovariectomy in cycling hamsters (Goldman *et al.*, 1971). Cycling hamsters did not show elevated LH levels during the afternoon of the second diestrous day. Samples were not obtained on proestrus in these animals in order to avoid including values obtained during the ovulatory surge. Other data from our laboratory suggest that afternoon "surges" of LH do not occur on any day except proestrus (Bex and Goldman, 1974).

Norman *et al.* (1973) observed successive mid-afternoon LH surges in ovariectomized hamsters treated with a large dose of estrogen. These fluctuations were not observed unless estrogen was given. Thus, our failure to observe a significant diurnal fluctuation in gonadotropins in ovariectomized 14L females is in agreement with the findings of Norman *et al.* In the present experiments the diurnal fluctuations in 10L hamsters were not dependent upon ovarian estrogen since even greater LH peaks were observed 3.5 weeks after ovariectomy in 10L females (Fig. 2). It is tempting to speculate that there may be a common pathway in the mechanisms by which either reduced photoperiod or estrogen treatment can induce rhythmic LH secretion in the ovariectomized hamster.

Since the diurnal pattern of LH release in 10L hamsters appeared to be relatively inde-

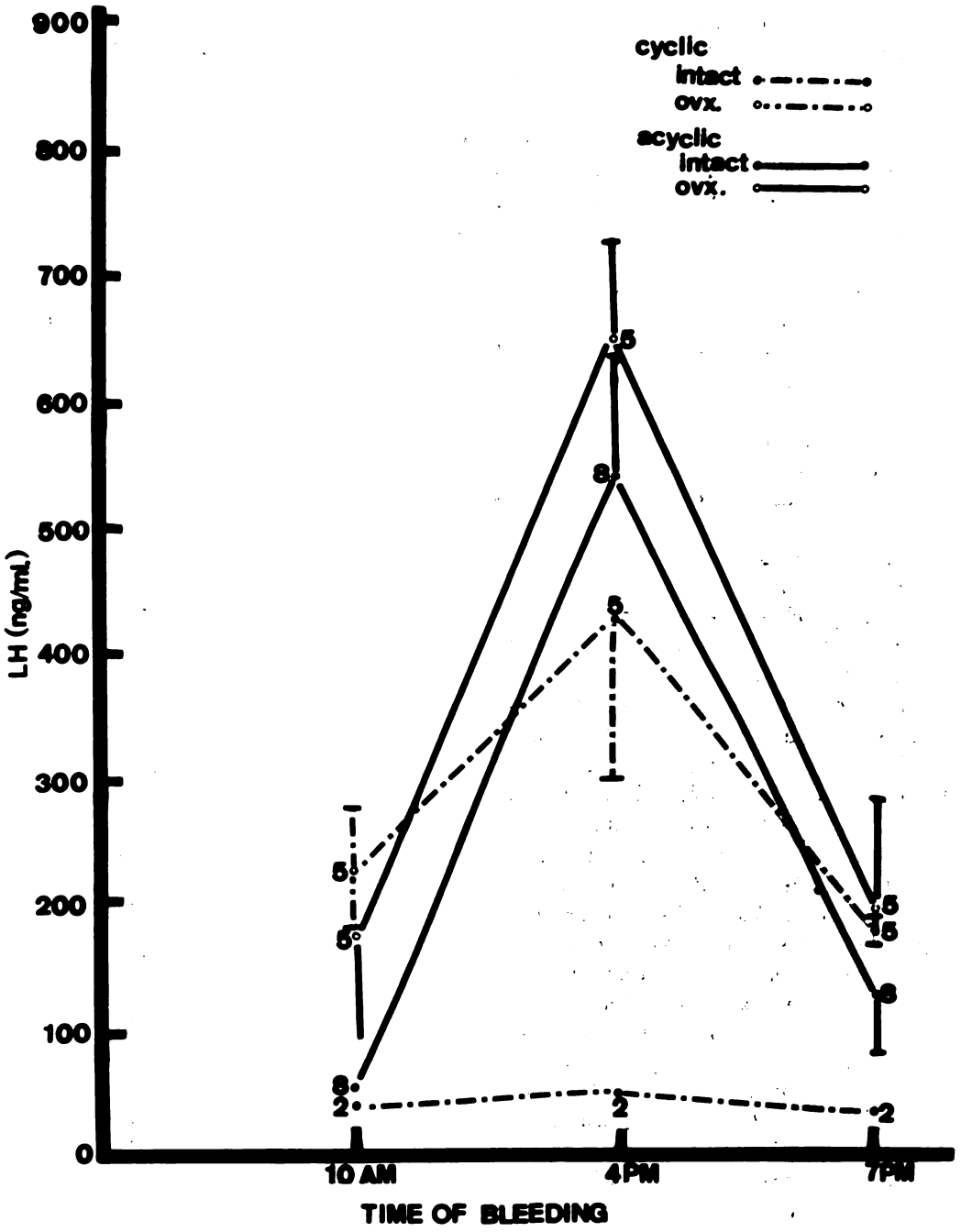

FIG. 4. Diurnal rhythm in serum LH in hamsters which became acyclic during maintenance on 14L:10D. Values are shown for studies performed before and after ovariectomy. Values for cycling females bled during the second day of diestrus and again following ovariectomy, are shown for comparison.

pendent of ovarian influence we wished to determine whether the loss of this rhythmicity (i.e. females resume normal estrous cycles after 20 weeks on 10L:14D) was also independent of ovarian hormones. Serum LH concentrations were elevated in both the morning and afternoon in 10L hamsters ovariectomized during the acyclic phase and stud-

ied after 20 weeks exposure to this photoperiod (i.e. shortly after their intact counterparts had resumed estrous cycles). The LH concentrations were >600 ng/ml at both times with no evidence of a diurnal rhythm. Thus, it appears that the "spontaneous" loss of diurnal rhythmicity in LH secretion in 10L females does occur independently of gonadal influences.

It seems that the failure to show external signs of ovulation under a short photoperiod may be due to the large, daily fluctuations in gonadotropins. However, this hypothesis is quite tentative; i.e., the anti-reproductive effects of short photoperiods may be exerted initially at *either* the gonadal *or* the pituitary level. In particular, the role of FSH in this process is uncertain as the serum FSH concentrations observed in the acyclic 10L:14D hamsters are intermediate between the lowest and highest FSH concentrations observed in our laboratory in cycling females (Bex and Goldman, unpublished). Nevertheless, once the hamster has become acyclic the daily fluctuations in gonadotropin secretion appear to be independent of gonadal control as seen both by (a) the presence of the diurnal rhythm for LH even following ovariectomy and (b) the spontaneous loss of the LH rhythm in ovariectomized females after 20 weeks exposure to the short photoperiod. Furthermore, the present results indicate that, at least in the hamster, the acyclic state is not accompanied by a relatively quiescent state of the pituitary gonadotrophs; rather, these cells are actively releasing gonadotropins in a dynamic pattern.

At any given time we estimate that about 5% of the female hamsters maintained on 14L:10D in our laboratory will be acyclic. Since the state of acyclicity generally persists for several weeks it does not appear to be comparable to the pseudopregnant condition, which lasts for only 10–12 days in this species. Since the pattern of LH secretion is similar in animals which become acyclic during maintenance on 14L:10D and those exposed to 10L:14D there may be a similarity in the mechanisms involved in the respective acyclic situations. The failure of acyclic 14L:10D females to show an accentuated LH rhythm (higher afternoon LH levels) following ovariectomy as compared to intact animals may have been a consequence of the shorter time between ovariectomy and blood sampling in this study (3–5 days) as compared to the study in 10L:14D females (3.5 weeks).

Although it is clear that long-term exposure to 10L:14D lighting conditions greatly affects the hypothalamic-pituitary-gonadal axis, the problem of determining the primary sites of action of altered photoperiods on gonadal function is still unresolved. Present effort now involves parallel studies in male hamsters and will extend to an examination of pineal and neural involvement in both sexes.

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REFERENCES

BAST, J. D., AND GREENWALD, G. S. (1974) Serum profiles of follicle-stimulating hormone, luteinizing hormone and prolactin during the estrous cycle of the hamster. *Endocrinology* **94**, 1295–1299.

BEX, F. J., AND GOLDMAN, B. D. (1974) Serum gonadotropins in the hamster: Effect of unilateral ovariectomy. Program of the Fifty-Sixth Meeting of the Endocrine Society, Atlanta, Georgia, June 12–14, 1974, p. A-198.

BLAKE, C. A., NORMAN, F. L., AND SAWYER, C. H. (1973). Validation of an ovine-ovine LH radioimmunoassay for use in the hamster. *Biol Reprod* **8**, 299–305.

GOLDMAN, B. D., AND PORTER, J. C. (1970) Serum LH levels in intact and castrated golden hamsters. *Endocrinology* **87**, 676–679.

GOLDMAN, B. D., MAHESH, V. B., AND PORTER, J. C. (1971) The role of the ovary in control of cyclic LH release in the hamster, *Mesocricetus auratus*. *Biol Reprod* **4**, 57–65.

NISWENDER, G. D., MIDGLEY, A. R. JR., MONROE, S. E., AND REICHERT, L. E. JR. (1968) Radioimmunoassay for rat luteinizing hormone with antiovine LH (luteinizing hormone) serum and ovine LH-I^{131}. *Proc. Soc. Exp. Biol. Med.* **128**, 807–811.

NORMAN, R. L., BLAKE, C. A., AND SAWYER, C. H.

(1973) Estrogen dependent twenty-four hour periodicity in pituitary LH release in the female hamster. *Endocrinology* **93,** 965–970.

ORSINI, M. W. (1961) The external vaginal phenomena characterizing the stage of the estrous cycle, pregnancy, pseudopregnancy, lactation and anestrous hamster. *Proc. Animal Care Panel,* **II,** 193–206.

REITER, R. J. (1967) Changes in the reproductive organs of cold exposed and light deprived female hamsters (*Mesocricetus auratus*). *J. Reprod. Fert.* **16,** 217–222.

REITER, R. J. (1969) Pineal function in long term blinded male and female golden hamsters. *Gen. Comp. Endocrinol.* **12,** 460–468.

REITER, R. J., SORRENTINO, S., JR., AND HOFFMAN, R. A. (1970) Early photoperiodic conditions and pineal antigonadal function in male hamsters. *Int. J. Fert.* **15,** (3), 163–170.

REITER, R. J. (1972) Surgical procedures involving the pineal gland which prevent gonadal degeneration in adult male hamster. *Ann. J. Endocrinol.* **33,** (6) 571–581.

REITER, R. J., AND SORRENTINO, S., JR. (1972) Prevention of pineal mediated reproductive responses in light deprived hamsters by partial or total isolation of the medial basal hypothalamus. *J. Neuro. Visc. Rel.* **32,** 355–367.

TURGEON, J., AND GREENWALD, G. S. (1972) Preovulatory levels of plasma LH in the cyclic hamster. *Endocrinology* **90,** 657–662.

RECOMMENDED REVIEWS

Reiter, R. J. (1973) Comparative Physiology: Pineal Gland. *Ann. Rev. Physiol.* **35,** 305–328.

Schwartz, N. B. (1969) A model for the regulation of ovulation in the rat. Rec. *Prog. Horm. Res.* **25,** 1–55.