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Effect of Prolonged Exposure to Nonstimulatory Photoperiods on the Activity of the Neuroendocrine-Testicular Axis of Golden Hamsters

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ABSTRACT

Male golden hamsters were exposed to nonstimulatory short days for 25 weeks, and the effects on gonadal weight and serum gonadotropin levels were assayed at biweekly intervals. Within 11 weeks after animals were transferred from a photostimulatory (LD 14:10) to a nonstimulatory (LD 6:18) light cycle, serum LH and FSH titers, as well as testicular weight had decreased markedly (4-, 5- and 13-fold, respectively). Continued exposure to LD 6:18 resulted in gradual increases in serum gonadotropin levels and subsequently in the complete recrudescence (regrowth) of the testes by 25 weeks. The testis itself does not appear to be involved in the "spontaneous" increase in serum gonadotropin levels since these levels also increased gradually in hamsters that were castrated after 10 weeks of LD 6:18 and then were exposed to LD 6:18 for an additional 17 weeks.

The observation of a gradual increase in the activity of the neuroendocrine-testicular axis during prolonged exposure to LD 6:18 suggested to us that the hamster's reproductive system might become more responsive to photostimulation with increasing length of prior exposure to nonstimulatory photoperiods. To examine this possibility hamsters maintained on LD 6:18 for either 10 or 13.5 weeks were transferred to LD 16:8 for 3.5 weeks, and then sacrificed. In those hamsters exposed to nonstimulatory photoperiods for 13.5 weeks there was a greater increase in testicular size after photostimulation (5.5-fold increase) than in those animals exposed for only 10 weeks (2.2-fold increase). A similar pattern was observed in serum gonadotropin levels, indicating that the neuroendocrine-gonadal axis of the male hamster increases in sensitivity to photostimulation with a longer exposure to nonstimulatory photoperiods.

INTRODUCTION

In numerous mammalian species annual cycles of pituitary gonadotropin release and gonadal development are dependent upon photoperiodic information (van Tienhoven, 1968; Lodge and Salisbury, 1970; Davis and Meyer, 1972; Berndtson and Desjardins, 1974; Turek et al., 1975). Photoperiodic control of the annual testicular cycle has been studied extensively in the golden hamster (Hoffman et al., 1965; Gaston and Menaker, 1967; Reiter, 1972; Elliott et al., 1972). These studies have shown that hamsters normally require long photoperiods (i.e. $\geqslant$12.5 h light/24 h) for the maintenance of testicular size and function, while short photoperiods (i.e. <12 h light/24 h) produce complete testicular regression within 8–10 weeks. However, if male hamsters are held on short photoperiods for an extended period of time, the initial collapse of the testes is eventually followed by recrudescence; complete regrowth of the testes is observed after a 25–30 week exposure to short photoperiods (Hoffman et al., 1965; Reiter, 1972). In light-deprived female hamsters there is an initial decrease in uterine weight and a cessation of vaginal cyclicity that is followed by a "recovery" of uterine weight and a resumption of cyclicity after prolonged light-deprivation (Reiter, 1969; Seegal and Goldman, 1975).

The observation that blinded hamsters experience a similar regression and recrudescence of the gonads (Reiter, 1969) prompted Reiter (1975) to call the phenomenon "spontaneous" regeneration, thus emphasizing that gonadal regrowth during prolonged light deprivation occurs in the apparent absence of photoperiodic photostimulation. The distinction is useful since testicular recrudescence can be induced by long photoperiods well in advance of spontaneous recrudescence (Reiter, 1972; Berndtson and Desjardins, 1974). Reiter (1975) suggested that spontaneous gonadal recrudescence on short photoperiods may be similar to the regrowth of the gonads which occurs in the late stages of winter hibernation under naturally occurring conditions (Mogler, 1958).

Recent studies with golden hamsters have correlated the events of testicular regression

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and photostimulated testicular recrudescence with pituitary gonadotropin release (Berndtson and Desjardins, 1974; Turek et al., 1975). However, despite the interest in spontaneous testicular recrudescence and the pivotal role of the pituitary in regulating gonadal activity, the temporal relationship between pituitary LH and FSH release and spontaneous testicular recrudescence remains unknown. To study this relationship we examined serum gonadotropin levels and testicular activity in hamsters exposed to short days for up to 25 weeks. Furthermore, since little information is available regarding the sensitivity of the hypothalamo-hypophyseal neuroendocrine system to photostimulation as a function of the duration of prior exposure to short photoperiods, we examined the response to photostimulation of hamsters that were previously exposed to short days for either 10 or 13.5 weeks. Our results demonstrate that the hypothalamo-hypophyseal-gonadal axis of the hamster is more sensitive to photostimulation the longer the animals are subjected to nonstimulatory light cycles.

MATERIALS AND METHODS

Male golden hamsters were reared in our laboratory from animals originally purchased from Lakeview Hamster Colony, Newfield, N. J. Hamsters were housed in groups of 4–6 per cage in a light-controlled room (14 h of light per day; LD 14:10) until experiments were initiated.

Experiment I

Ten week old male hamsters were transferred from a stimulatory photoperiod (LD 14:10) to a nonstimulatory LD 6:18 light cycle. Beginning 3 weeks later, hamsters were killed at biweekly intervals until the last group of animals was sacrificed after a 25 week exposure to LD 6:18. Initial control animals were killed at the time of transfer from LD 14:10 to LD 6:18.

Experiment II

Ten week old male hamsters were transferred from an LD 14:10 room to an LD 6:18 room. After 10 weeks on LD 6:18, intact control animals were sacrificed and half of the remaining animals were castrated under sodium pentobarbital anesthesia. The experiment was continued for an additional 120 days in LD 6:18. Castrated and intact hamsters were killed 3, 10, 20, 40, 60, 90 or 120 days after surgery.

Experiment III

Ten week old male hamsters were transferred from an LD 14:10 room to an LD 6:18 room. Control animals maintained on LD 6:18 were sacrificed 10 (group A), 13.5 (group B), and 17 (group D) weeks after transfer to the nonstimulatory photoperiod. Experimental hamsters were exposed to either 10 (group C) or 13.5 (group E) weeks of LD 6:18, transferred to a photostimulatory LD 16:8 light cycle and then sacrificed after 3.5 weeks.

Animals were killed by decapitation during the light period, the testes were removed and weighed immediately, and trunk blood was collected and treated as previously outlined (Turek et al., 1975). Immunoreactive serum LH and FSH were assayed with reagents supplied by the National Institute of Arthritis and Metabolic Disease Rat Pituitary Hormone Distribution Program (NIAMD). These included NIAMD-: Rat-LH-I-3, Rat-LH-RP-1, A-Rat-LHS-1, Rat-FSH-I-3, Rat-FSH-RP-1 and A-Rat-FSHS-6. The assays were performed according to the accompanying protocol with minor modifications as described elsewhere (Turek et al., 1975). Both LH and FSH radioimmunoassays have been characterized for use in the hamster (Berndtson and Desjardins, 1974; Bast and Greenwald, 1974) and our values are in general agreement with those reported from other laboratories measuring hamster gonadotropins (Goldman and Porter, 1970; Turgeon and Greenwald, 1972; Blake et al., 1973; Varavudhi and Meites, 1974; Berndtson and Desjardins, 1974). Values are expressed in terms of nanogram equivalents of NIAMD-rat-LH-RP-1 (relative potency of 0.02 X NIH-LH-S1) and nanogram equivalents of NIAMD-rat-FSH-RP-1 (relative potency of 2.1 X NIH-FSH-S1) per milliliter of serum. The minimal amount of hormone detectable was 10–15 mg LH-RP-1 per ml serum and 20–25 ng FSH-RP-1 per ml serum. Data were analyzed for significance using the Student's *t* test.

RESULTS

Experiment I

Exposure of male hamsters to an LD 6:18 photoperiod initially suppressed the activity of the neuroendocrine-testicular axis (Fig. 1). Eleven weeks after the transfer from LD 14:10 to LD 6:18, a 13-fold reduction in gonadal weight was observed, as well as 4- and 5-fold decreases for serum LH and FSH respectively. Exposure to LD 6:18 for a total of 25 weeks resulted in complete recrudescence of the testes and a return of serum LH and FSH levels to or above LD 14:10 initial control values. Serum levels of LH remained minimal in animals sacrificed between 7 and 15 weeks, and then began to rise gradually reaching initial control values by week 21. FSH levels began to rise by week 13; at week 17 the values were similar to those observed in the initial control animals, and by the end of the experiment the levels were twice those of the initial controls. A spontaneous increase in serum FSH was noticeable before serum LH levels began to rise and spontaneous increases in gonadal weight were first noticeable at 19 weeks.

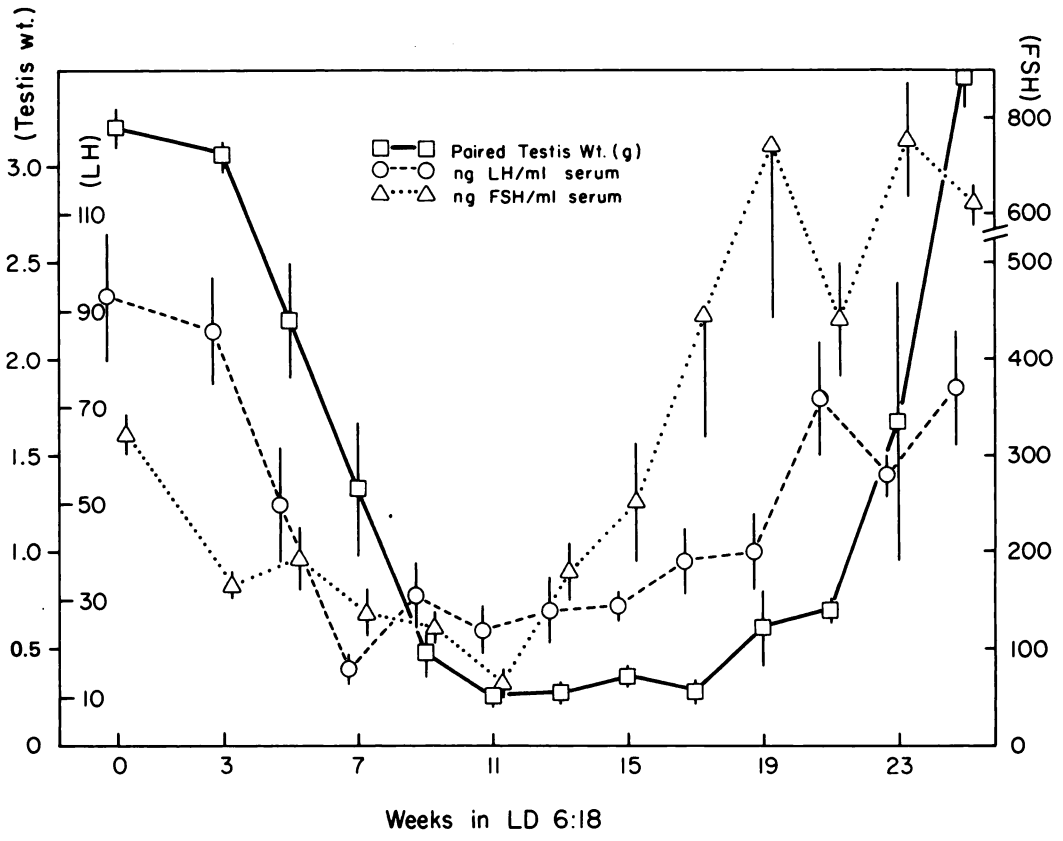

FIG. 1. Mean (± S.E.) testicular weight (□——□) and concentration of immunoreactive LH (○ – – – ○) and FSH (△ • • • • △) in serum of adult male hamsters (5–6 animals/group) that were transferred at time 0 from LD 14:10 to LD 6:18. There is an initial decline in gonadal weight and serum gonadotropin levels following exposure to the nonstimulatory LD 6:18 photoperiod. After prolonged exposure to LD 6:18 gradual increases in testicular weight and serum LH and FSH concentrations are observed.

Experiment II

Serum gonadotropin data obtained from hamsters previously exposed to LD 6:18 for 10 weeks, castrated and then held on LD 6:18 for an additional 120 days is summarized in Fig. 2. Data from the intact hamsters in this experiment have been omitted since the temporal patterns of serum gonadotropin levels and testicular weight were similar to those observed in Experiment I. Serum LH and FSH concentration remained similar to initial intact control hamsters (i.e. hamsters exposed to LD 6:18 for 10 weeks) for the first 40 days following castration. By day 60, all the castrated hamsters had elevated serum FSH levels while only two of six of these animals had elevated LH levels. This suggests that during a prolonged exposure to LD 6:18, serum FSH levels increase sooner than LH levels in castrate as well as intact hamsters (cf Fig. 1). By day 90, serum LH and FSH levels in castrates had increased dramatically to 8 and 36 times the levels observed in initial control animals. After approximately 27 weeks exposure to LD 6:18 (120 days of which were postcastration), serum LH and FSH levels in castrates were about 3 and 9 times, respectively, that found in intact animals exposed to 25 weeks of LD 6:18 (Fig. 1). These results demonstrate that castration of hamsters previously exposed to LD 6:18 for 10 weeks does not interfere with the subsequent increases in pituitary gonadotropin release that are associated with spontaneous testicular recrudescence in intact hamsters. Castrated hamsters experience an increase in serum gonadotropin levels at about the same time (i.e. after 15–23 weeks of exposure to LD 6:18) as do intact animals (cf Figs. 1, 2).

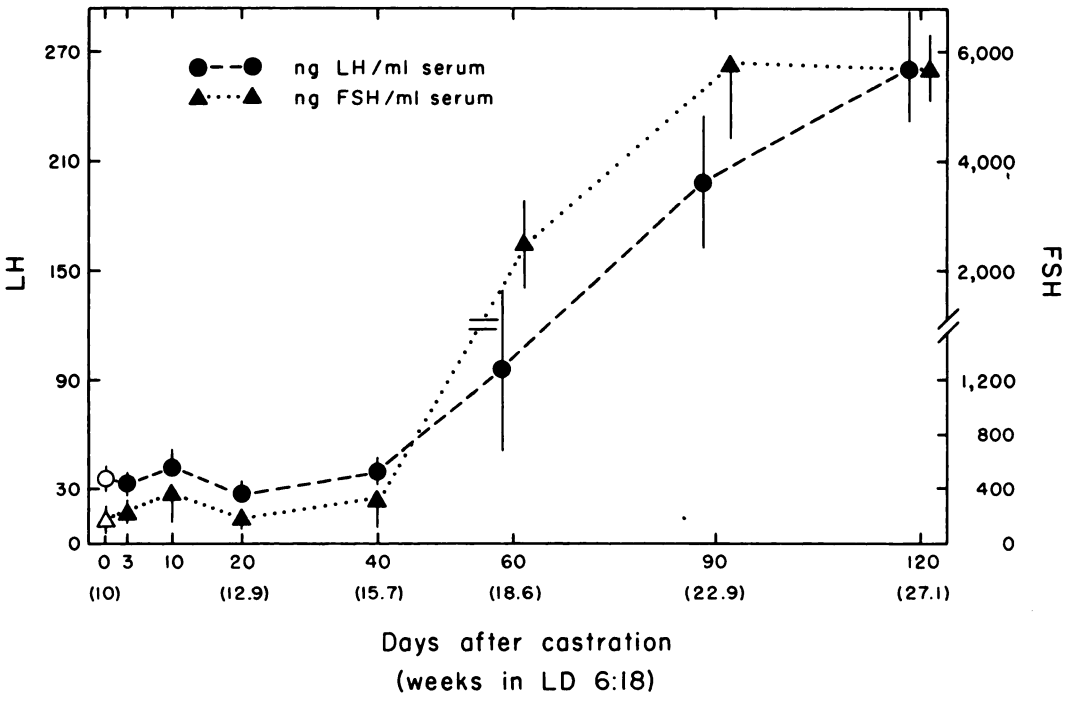

FIG. 2. (Mean ± S.E.) concentration of immunoreactive LH (● – – – ●) and FSH (▲ • • • • ▲) in serum of adult male hamsters (5–6 animals/group) castrated on day 0 after previous exposure to LD 6:18 for 10 weeks. Day 0 values are for intact initial control animals. There is a spontaneous increase in serum LH and FSH which coincides temporally with the increase observed in intact hamsters (cf Fig. 1). A significant rise in serum FSH occurs by day 60 while a significant rise in serum LH is not observed until day 90.

Experiment III

Animals in groups A, B and D (control groups) were maintained on LD 6:18 throughout the experiment while animals in groups C and E received 3.5 weeks of photostimulation (i.e. exposure to LD 16:8) before sacrifice. The mean testicular weight of group C animals increased only 2.2-fold compared to a 5.5-fold increase for animals in group E (Fig. 3a). These two groups differed by the length of their prior exposure to the LD 6:18 photoperiod (10 weeks for group C, 13.5 weeks for group E). If the slight increase in gonadal weight attributable to spontaneous recrudescence is taken into consideration (this increase can be seen in the control groups A, B and D), a net gain of 200 mg due to photostimulation can be calculated for group C (by subtracting B from C), compared to a net gain of 949 mg for group E (obtained by subtracting D from E). The net gain in gonadal weight in animals in group E is significantly greater ($P<.001$) than the net gain in animals in group C. Furthermore, the pattern of gonadotropin release by the pituitary in response to photostimulation (Fig. 3b and c) is qualitatively similar to the pattern described for the gonadal response. The net increase in serum LH and FSH is significantly greater ($P<.02$) in photostimulated hamsters that were previously exposed to LD 6:18 for 13.5 weeks than in hamsters previously exposed to LD 6:18 for only 10 weeks. The data presented in Fig. 3 demonstrate a differential response of the hypophyseal-gonadal axis to photostimulation, depending on the length of previous exposure to nonstimulatory photoperiods.

DISCUSSION

Depriving hamsters of photoperiodic photostimulation by exposure to short photoperiods or by blinding promotes testicular involution followed by spontaneous recrudescence of the testes after 25–30 weeks (Reiter, 1975). Our results are consistent with previous findings and document, for the first time, the temporal relationship between pituitary gonadotropin release and gonadal development during spontaneous testicular recrudescence. In Experiments

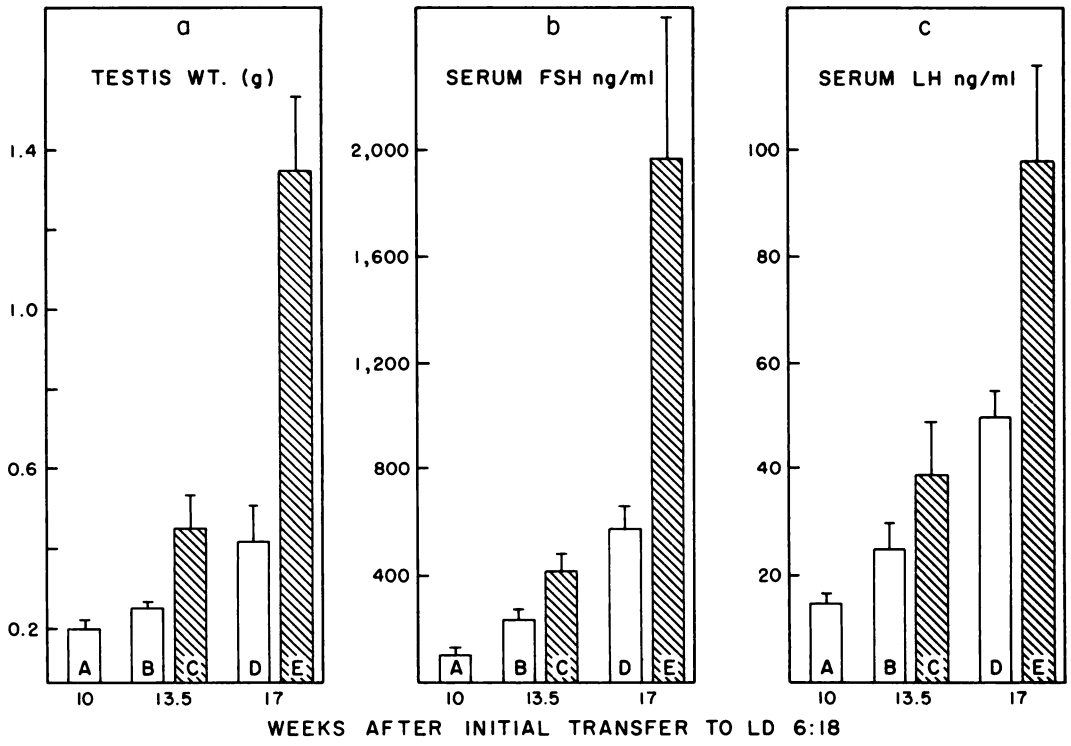

FIG. 3. Mean (± S.E.) testicular weight (a) and concentration of immunoreactive FSH (b) and LH (c) in serum of adult male hamsters (5–8 animals/group) that were transferred from LD 14:10 to LD 6:18 and killed 10 (A), 13.5 (B, C) or 17 (D, E) weeks later. Open bars: animals that remained on the nonstimulatory LD 6:18 photoperiod for the duration of the experiment. Hatched bars: animals that were transferred to and maintained on a photostimulatory LD 16:8 photoperiod for 3.5 weeks prior to sacrifice. The response of the reproductive system to 3.5 weeks of photostimulation depends markedly on the length of prior exposure to LD 6:18.

I and II a spontaneous increase in serum FSH was noted before an increase in serum LH in intact animals. A temporal dissociation in the circulatory levels of LH and FSH has also been observed during sexual maturation in mice and rats (Swerdloff et al., 1971; Stiff et al., 1974). This temporal dissociation in the increase of serum gonadotropins observed in intact hamsters may also occcur in castrated hamsters since each of the six animals sacrificed 60 days after castration had elevated serum FSH levels, while only two of these six animals had elevated LH levels. However, further experiments are necessary before anything definite can be said regarding the role of the testis in the temporal dissociation of the spontaneous increase in serum LH and FSH levels.

The mechanism by which the hypothalamo-hypophyseal system spontaneously increases its activity is not understood, although the pineal gland may well be involved. The collapse of the reproductive organs in response to nonstimulatory photoperiods is thought to be mediated by the pineal gland, since pinealectomy prevents dark induced gonadal regression (Reiter, 1972). Reiter has suggested that during spontaneous gonadal growth, the hypothalamo-hypophyseal gonadal axis may lose its sensitivity to the pineal antigonadal factor, or that the pineal may discontinue secretion of this factor.

The results from castrated hamsters (Fig. 2) suggest that the increased pituitary gonadotropin release associated with spontaneous testicular recrudescence (Fig. 1) is not dependent upon the testis or its secretion since serum gonadotropin levels increase spontaneously in castrate as well as intact animals exposed to LD 6:18. The absence of an initial rise of serum gonadotropins following castration of animals held on short days is in agreement with our earlier observation that castration after a 60 day exposure to LD 6:18 has no immediate effect on serum LH and FSH levels in hamsters (Turek et al., 1975). On the other hand, in that

study we also reported that when hamsters held on long days are castrated, dramatic increases in serum LH and FSH levels are noted within 3 days. These earlier findings led us to suggest that after a 60 day exposure to short photoperiods, the hypothalamic-hypophyseal neuroendocrine system governing gonadotropin release becomes relatively insensitive to the effects of castration. In the present study serum gonadotropin levels were significantly higher ($P<.001$) in castrate hamsters exposed to LD 6:18 for 23–27 weeks than in intact animals exposed to the same photoperiod for 23–25 weeks (compare Figs. 1 and 2). These results indicate that during a prolonged exposure to short photoperiods, the neuroendocrine system eventually regains sensitivity to the absence of testicular secretions.

The data presented in Fig. 3 demonstrate that the hypophyseal-gonadal axis of the hamster becomes increasingly sensitive to photostimulation with continued exposure to nonstimulatory photoperiods. The mechanism controlling this altered responsiveness is unknown, but appears to be operating at or above the level of the pituitary gland, since serum gonadotropin levels exhibited the same temporal pattern as did the gonads. Increased gonadal response to stimulatory photoperiods after prolonged exposure to short days has been observed in birds (Farner and Follett, 1966; Turek, 1975). Whatever the mechanisms underlying the increased responsiveness of the hypophyseal-gonadal axis to photostimulation, the results obtained in Experiment I suggest that this increased responsiveness may be casually related to events which culminate in spontaneous testicular recrudescence.

Serum levels of FSH can rise to an average of about 2,000 ng/ml in hamsters that have been photostimulated for 3.5 weeks (Fig. 3b, group E) while serum FSH concentration is normally about 300–350 ng/ml in hamsters that have been maintained on LD 14:10 for a much longer time (cf Turek et al., 1975; and Fig. 1, initial controls). Other observations (Turek, unpublished) from this laboratory indicate that serum FSH levels in photostimulated hamsters are very high soon after photostimulation but eventually decline once the testes have attained full size. A similar response in serum LH levels has not been observed. These data taken together suggest that the fully developed testis may be producing a substance inhibitory to FSH synthesis or release, and that during early photostimulated gonadal development very high levels of serum FSH are observed because there is little of this inhibitory substance present. These observations are consistent with the suggestion of others that the germinal epithelium may be a source of feedback control of FSH (Swerdloff et al., 1971; Debeljuk et al., 1973; Gomes et al., 1973).

We have demonstrated that spontaneous testicular growth in hamsters maintained on short photoperiods for an extended period of time is associated with spontaneous increases in serum gonadotropin levels. In this paper, and in previous reports (Berndtson and Desjardins, 1974; Turek et al., 1975) photostimulated testicular activity in the hamster has also been shown to be correlated to increased serum LH and FSH levels. However, at the present time we do not know if the physiological mechanism governing spontaneous testicular recrudescence and photostimulated testicular recrudescence is the same.

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