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Influence of photoperiod and gonadal steroids on hibernation in the European hamster

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Summary. Torpor was monitored daily in adult male and female European hamsters (*Cricetus cricetus*) induced to hibernate by exposure to a cold environment (6 °C). The effect of photoperiodic manipulations or administration of exogenous gonadal steroids was examined in gonadectomized or intact hamsters.

1. Gonadal regression occurred in all short day, but only in some long day, cold-exposed hamsters. Entry into hibernation was not observed until reproductive regression had occurred. Thus, gonadal atrophy appears to be a necessary precondition for hibernation.

2. Castrated hamsters in the short day cold condition showed a significantly greater incidence of torpor than those in the long day cold condition. Hence, photoperiod affected torpor independently of its effect on the gonadal cycle.

3. Testosterone, when administered via silastic capsules at near physiological levels, completely inhibited torpor in gonadectomized male and female hamsters hibernating in the short day cold condition.

4. In ovariectomized females, torpor was unaffected by progesterone treatment, but partially inhibited by estradiol. A greater inhibition of torpor was observed when estradiol-primed females were administered both estradiol and progesterone simultaneously. Thus, the effect of both hormones may be functionally comparable to that of the single testicular hormone.

5. Estradiol inhibited torpor to a greater extent in intact and ovariectomized female hamsters hibernating in long days than those in short days, suggesting an effect of photoperiod on responsiveness to estradiol.

These results indicate an inverse relationship between the gonadal and hibernation cycles, and a probable role for gonadal steroids to influence the timing of the hibernation season. However, non-gonadal factors must also be involved in controlling hibernation, since photoperiod affected the incidence of torpor in gonadectomized animals and because hamsters were able to terminate hibernation in the absence of gonadal hormones.

Introduction

Hibernation is a thermoregulatory adaptation observed among a variety of temperate zone mammals to conserve energy during the winter season. In many long-day breeding rodents, the hibernation season lasts for 4–6 months, coincident with reproductive quiescence. In the Turkish hamster, hibernation is characterized by alternating bouts of torpor, lasting 4–5 days, and intervals of spontaneous arousal from torpor, lasting 1–2 days (Hall 1981). During torpor, a dramatic decline in core body temperature occurs, by as much as 30 °C, as well as substantial reductions in metabolic, respiratory and heart rates (Lyman and Chatfield 1955). In several hamster species, a relationship has been suggested between the annual gonadal cycle and the hibernation cycle, since exogenous gonadal steroids, particularly testosterone, inhibit torpor when administered to gonadectomized hibernating Turkish hamsters, *Mesocricetus brandti*

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(Hall and Goldman 1980), Syrian hamsters, *Mesocricetus auratus* (Jansky et al. 1984) and Djungarian hamsters, *Phodopus sungorus* (Vitale et al. 1985). In male Turkish hamsters, testicular regression and the decline in serum testosterone which occur in response to short-day photoperiods, appear to be prerequisites for entry into hibernation (Darrow et al. 1987). Furthermore, after long term exposure to short days, regrowth of the gonads occurs coincident with the end of the hibernation season (Goldman 1980; Hall et al. 1982; Darrow et al. 1987).

In the present study, we were interested in examining the relationship between the gonadal and hibernation cycles in another genus of hamsters, the European hamster (*Cricetus cricetus*). We investigated the effects of exogenous gonadal steroids, gonadectomy and photoperiodic manipulations on hibernation in both male and female European hamsters.

Materials and methods

Animals. Adult European hamsters (*Cricetus cricetus*) were raised from birth in our laboratory on an LD 16:8 photoperiod at 22 °C. Food (Purina Formulab Chow) and water were available ad libitum. Animals selected for experiments were housed individually in 26 × 45 cm polyethylene cages with wire lids. When transferred to a cold room for hibernation studies, individuals were supplied with a mixture of pine shavings, crushed corn cobs and cotton nesting material. The temperature of the cold rooms was maintained at 6±1 °C and light cycles were either LD 16:8 or LD 10:14, identical in phase to separate long and short-day photoperiod rooms maintained at 22 °C.

Hibernation was monitored by daily midday observation of each animal. Torpid animals were easily distinguished from those in arousal by their decreased respiratory rate, characteristic nesting posture and lack of response to a puff of air directed at the back. This method was corroborated in a preliminary study by telemetric recording of body temperature in European hamsters bearing subcutaneous transmitters (Minimitter Corporation, Indianapolis, Indiana). Those animals judged to be torpid by visual inspection showed body temperatures of 7 °C to 10 °C, as measured by telemetry. The incidence of torpor (number of days torpid per 15 or 30 day interval, expressed as percent torpor) was calculated for each individual. Group means were statistically analyzed using the SPSS computer programs for Anova and one-way analysis of variance, followed by Duncan and Scheffe posteriori tests.

Reproductive state was monitored in male hamsters by testis length measurements, as described previously (Hall et al. 1982), and in females by examination of vaginal state. Females with closed vaginae were classified as anovulatory, and those with open vaginae generally showed exfoliated vaginal cell types similar to those seen in cyclic female rats. The handling of hibernating animals, including cage changes, was restricted to days of spontaneous arousal from torpor.

Hormone treatment and assays. Testosterone (ICN Pharmaceuticals), estradiol-17β (Steraloids) and progesterone (Becton Dickson) were administered via subcutaneous insertion of hormone-filled silastic capsules. The capsules were prepared by packing the crystalline hormone into 20 mm lengths of silastic tubing (Dow-Corning, 0.078″ i.d. × 0.125″ o.d.) and sealing both ends with silastic adhesive. Capsules were rinsed in ethanol and presoaked in saline prior to use. Empty capsules were prepared for use as blanks. Hamsters to receive an implant were removed from the cold room for 5 to 10 min on a day of spontaneous arousal from a bout of torpor and placed under brief ether anesthesia for implantation of the capsule. Hormone treatments lasted for at least 30 days, after which time animals were ether-anesthetized, blood samples were obtained by cardiac puncture and capsules were either removed or replaced. Control blood samples were obtained from either blank-implanted hamsters, or from hormone-treated hamsters at least one month after hormone capsule removal. All blood sampling and capsule manipulations occurred on days of spontaneous arousal from torpor, between 3–6 h before lights-off.

Serum testosterone levels were determined by radioimmunoassay (Bartke et al. 1973) without Sephadex LH-20 column separation of the diethyl ether-extracted samples. The antiserum used in this assay system cross-reacted with dihydrotestosterone and androstenedione. Therefore, results are expressed as total serum androgen. Ovarian steroid hormones were measured by radioimmunoassay (Leipheimer et al. 1984) of benzene-extracted samples for estrogen (100% recovery) and isooctane-extracted samples for progesterone (85–88% recovery). The antiserum for estradiol (provided by Drs. D.C. Collins and K. Wright) cross reacts with 6-ketoestradiol (40%) and 6α-hydroxyestradiol (40%). The antiserum for progesterone (provided by Dr. M.J. Field) cross-reacts with Δ^5 pregnenalone (20%), 5β-pregnanedione (25%) and 5α-pregnanedione (43%). Results are expressed as serum estrogen and progestin. Assay systems were validated for use with European hamster serum by tests for parallelism and quantitative recovery.

Experiment I. Effect of photoperiod on hibernation in intact and gonadectomized hamsters. This study involved gonadectomized and intact adult hamsters of each sex, exposed to either short or long day photoperiods. Sample size was 6–10 animals per treatment group. The short day females were exposed to LD 10:14 for 9 weeks prior to cold exposure, and gonadectomies performed three weeks prior to cold exposure. Pretreatment of males was similar except for a 6-week prior exposure to short photoperiod and a shorter recovery time (several days) after castration before transfer to the cold. All animals were checked daily for torpor over a five-month period in the cold, from August to December.

Experiment II. Effect of exogenous testosterone on hibernation in gonadectomized, short day hamsters. Eight weeks prior to the beginning of the experiment, reproductively competent adult hamsters (9 females and 15 males, 4–5 months of age) were transferred to a short-day photoschedule (LD 10:14) to induce gonadal regression. Hamsters were then gonadectomized under sodium pentobarbital anesthesia, allowed to recover for one week and transferred to a short-day cold room (LD 10:14, 6 °C). Within a few days, most animals had entered hibernation; those displaying torpor for at least 15 of 30 days were selected for gonadal steroid implantation. After completion of hormone treatment, daily observations continued until the hibernation cycle of most hamsters had terminated spontaneously. The cold room portion of this experiment ran from December through August.

Experiment III. Effects of ovarian steroids on hibernation in female hamsters.

Experiment *III-A.* The effect of progesterone, estradiol or a combination of the two ovarian steroids was tested in ovariec-

tomized females ($n=17$) concomitantly with Experiment II above. The prehistory of the animals and ovariectomy schedule was the same as for Experiment II females.

Experiment *III-B*. Effect of estradiol on ovariectomized females hibernating on long day vs. short day photoperiods. Prior to the beginning of the experiment, one group of adult female hamsters ($n=11$) was transferred to short days, 22 °C for 8 weeks, while the other group ($n=13$) was maintained on long days. All subjects were then ovariectomized, allowed to recover for one week and transferred to cold rooms (6 °C) of the corresponding photoperiod. Individuals showing at least 50% torpor during the first 30-day interval were selected for hormone treatment ($n=10$ in short days, $n=7$ in long days) and received a single 20 mm estradiol capsule. After 30 days, the experiment was terminated for the long photoperiod group, and single implants of the short day females were replaced by triple estradiol implants. Blood samples were obtained by cardiac puncture after 30 days treatment with triple implants and at the termination of the long photoperiod group. The cold room portion of this experiment ran from October to February.

Experiment *III-C*. Effect of estradiol on intact females hibernating in long days. The adult females selected for this study all had closed vaginae, despite long-term exposure to LD 16:8 at 22 °C. On day 0, they were transferred to the long day cold room. About 30 days later, those showing hibernation were implanted with either a 20 mm estradiol capsule or an empty capsule. After 30 days, vaginal condition was checked and treatment was reversed for another 30-day interval. Torpor was monitored daily during the experiment, from March to July.

Results

Effect of photoperiod on hibernation in intact and gonadectomized hamsters

The pattern of torpor observed during the early and mid hibernation season is presented in Fig. 1 for male European hamsters exposed to either short day or long day cold. Short day males were generally good hibernators, as evidenced by the high incidence of torpor (>60%) from Day 60 to Day 150. These males had undergone testicular regression during the 6-week pre-exposure to short days, and some were then castrated several days prior to cold exposure. In contrast to the short day castrated males, gonadectomized males maintained on long days were slower to enter hibernation, showing significantly lower levels of torpor ($P<0.05$) until Day 120. Testis-intact long day males varied in their response to the cold, and are presented as two groups. One group had undergone testicular regression by Day 60 (Fig. 1, Regressed) and displayed a pattern of torpor no different than for short day intact males. The other group did not undergo gonadal regression during the study (Fig. 1, Nonregressed), and never displayed torpor for the entire 4 months exposure to long day cold. Interestingly, upon subsequent transfer of these nonregressed males to short day cold (data not shown), testicular regression was

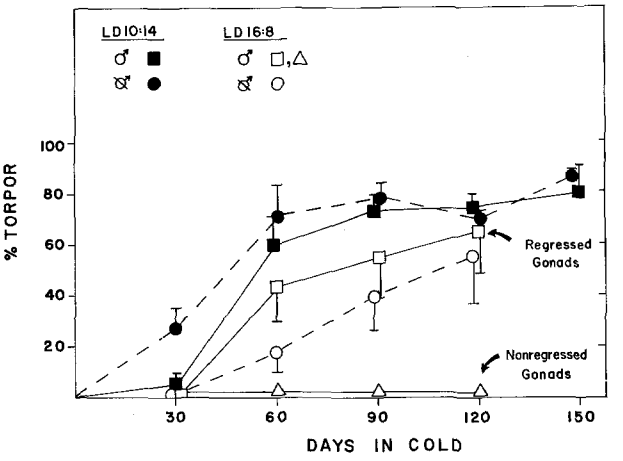

Fig. 1. Mean incidence of torpor in male European hamsters exposed to long day or short day cold. On Day 0, animals were transferred from 22 °C to a 6±1 °C coldroom of the indicated photoperiod. Short-day animals (dark symbols) were transferred to LD 10:14 six weeks prior to cold exposure and either castrated (●, $n=7$) or not (■, $n=6$) four days prior. Open symbols present three groups of long-day males: intact males showing complete gonadal regression by Day 60 (□, $n=4$, Regressed), intact males showing no testicular regression throughout long-day cold exposure (△, $n=4$ Nonregressed) and males castrated 4 days prior to cold exposure (○, $n=9$). Percent torpor was calculated for each hamster as (number of days in torpor/30 days) × 100. Symbols denote mean values; vertical lines denote SEM

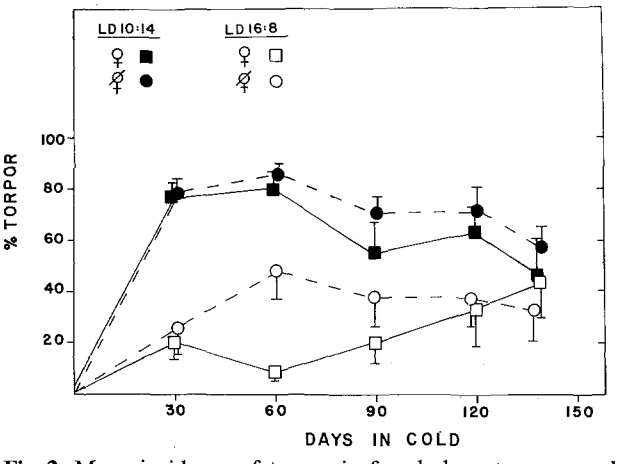

Fig. 2. Mean incidence of torpor in female hamsters exposed to long day or short day cold. Data are plotted as in Fig. 1. Short day groups were transferred to LD 10:14 9 weeks prior to cold exposure and were either unoperated (■, $n=5$) or ovariectomized (●, $n=9$) three weeks prior to cold exposure. Open symbols depict long day females: intact (□, $n=5$) or ovariectomized three weeks before cold exposure (○, $n=9$)

observed by 5 weeks and all were hibernating by 7 weeks after the transfer.

Female European hamsters (Fig. 2) displayed a pattern of hibernation generally comparable to that of the males. In short-day females, hibernation began within the first months of cold exposure and

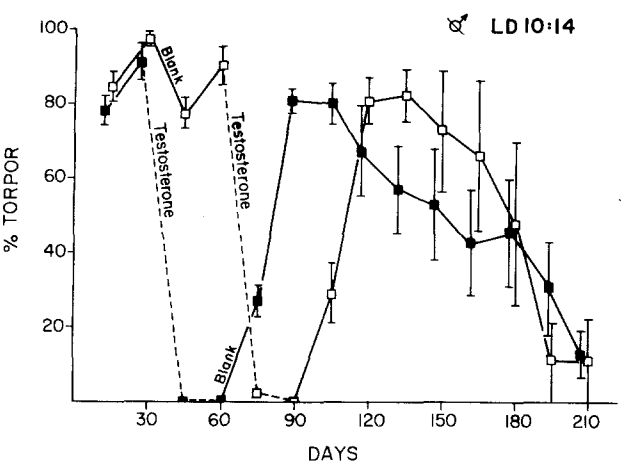

Fig. 3. Effect of testosterone on hibernating, gonadectomized males. At Day 0, male hamsters which had been exposed to short days for 8 weeks prior and castrated one week prior were transferred to the cold (6 °C). At approximately Day 30, one group of males (■, $n=8$) received a testosterone implant (20 mm) for 30 days, after which time the implant was replaced with an empty capsule (blank). Treatment of the second group (□, $n=6$) occurred in reverse order. Dotted lines denote hormone treatment. After 90 days in the cold, all implants were removed and the animals were observed until spontaneous cessation of hibernation had occurred. Incidence of torpor is presented as (number of days in torpor/15 days) $\times 100$

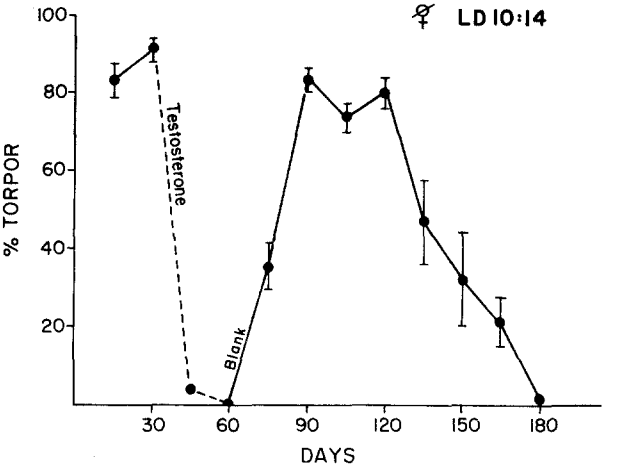

Fig. 4. Effect of testosterone on hibernating gonadectomized females. At Day 0, female hamsters which had been exposed to short days or 8 weeks prior and ovariectomized one week prior, were transferred to the cold (6 °C). At approximately Day 30, 9 females received a 20 mm testosterone implant for 30 days, followed by an empty capsule for 30 days. Data are presented as in Fig. 3. Observations of torpor continued after the cessation of hormone treatment until hibernation was spontaneously terminated

the incidence of torpor remained high (>50%) for the remainder of the study. In contrast, long-day females tended to enter hibernation less readily. Ovariectomized females on long days showed significantly lower levels of torpor ($P<0.05$) than did ovariectomized short day females until Day 140. The incidence of torpor for intact long day females was less than 10% during the second month in the cold, significantly lower than for intact short day females ($P<0.01$). At this time (Day 60) only 2 of the 5 long day females had closed vaginae, indicative of a regressed reproductive system. By Day 120, when the mean level of torpor had increased somewhat (to 33%), and was not significantly different from the short day groups, 4 of the 5 females were anovulatory; the one animal with an open vagina had not yet begun to hibernate. Thus as in the male hamsters, entry into hibernation appeared to depend on reproductive regression, but even in gonadectomized animals an effect of photoperiod was observed.

Effect of testosterone on hibernation in gonadectomized hamsters

Castrated male hamsters displayed a dramatic and immediate cessation of hibernation after treatment with exogenous testosterone (Fig. 3). The hormone was administered via constant release, silastic capsules (20 mm in length) subcutaneously implanted on the first day of spontaneous arousal from a torpor bout. In the first group of males to receive a testosterone implant, no individual was observed to re-enter torpor during the entire 30-day interval of hormone treatment. In contrast, the group of hamsters concomitantly receiving an empty silastic capsule maintained an incidence of torpor above 70%, not different from pre-implantation levels. When the blank capsule of these animals was replaced by a testosterone implant, the incidence of torpor dropped precipitously from 90.0% to 2.3% ($P<0.01$). The essentially total inhibition of hibernation observed in both testosterone-treated groups was reversed within 30 days after removal of the hormone or its replacement with an empty capsule. Subsequent observation of these males revealed a continuation of hibernation, with mean incidence of torpor above 50%, for about 6 months, after which time most individuals had completed the hibernation season.

The effect of testosterone in gonadectomized females was equally as dramatic as that seen in males and followed a parallel time course (Fig. 4). After implantation of a 20 mm testosterone capsule, the incidence of torpor decreased from more than 80% to less than 5% ($P<0.01$). Upon replacement of the testosterone implant with an empty capsule, the percentage of time spent in torpor returned to pre-implantation levels within 30 days. Subsequently, a gradual decline in torpor was

Table 1. Serum androgen titers

	Androgen (ng/ml)	*n*
Castrated males (of Fig. 3)		
With blank capsule	0.084±0.023	(8)
With tesosterone capsule (20 mm)	5.790±1.380**	(6)
Ovariectomized females (of Fig. 4)		
With blank capsule	0.269±0.080	(6)
With testosterone capsule (20 mm)	0.964±0.138**	(6)
Intact males		
With regressed testes	0.375±0.080	(8)
With intermediate testes	0.312±0.052	(4)
With mature testes	2.540±0.672**	(6)

Mean±SEM
** Adjacent mean values differ at the $P<0.01$ level

observed, and all females of this group had spontaneously terminated the hibernation season by 6 months in the cold.

In castrated males bearing a 20 mm testosterone capsule, the mean serum androgen titer (Table 1) was about two fold higher than observed in a separate group of intact males with mature testes ($P<0.01$), and more than 50-fold higher than in blank implanted, castrated males ($P<0.01$). Testosterone implants in females produced an approximately 3-fold increase in serum androgen titers compared to ovariectomized levels ($P<0.01$), but circulating androgen titers were considerably lower than in males bearing the same size testosterone implant ($P<0.01$), indicating a more rapid clearance of exogenous testosterone from the circulation in females.

Effect of estradiol and progesterone on hibernation in female hamsters

Short-day ovariectomized females (Experiment III-A) showed no change in the incidence of torpor after treatment with progesterone-filled silastic capsules (Fig. 5, group 1). There was some effect of estradiol alone (Fig. 5, group 2), but this was marginally significant; i.e., although torpor values for the estradiol treatment group at 45 and 60 days were significantly less than the blank-implanted controls ($P<0.01$), these levels did not represent a significant decline when compared to pre-treatment levels within the group at 15 and 30 days. When estradiol treated females subsequently received both a progesterone and an estradiol capsule, the incidence of torpor declined significantly (to 23% at Day 75, $P<0.01$), clearly a greater effect than with either hormone alone. However, by Day 90, the incidence of torpor was not significantly less than at Day 60, prior to dual hormone treatment. Subsequent to Day 90 (Fig. 5) when the combined estradiol and progesterone implants were removed, a return to high levels of torpor was not observed (as had been evident in testosterone-treated females of Fig. 4). At this time, however, the group 1 females (those previously receiving only progesterone) were showing a spontaneous decline in the incidence of torpor. Mean levels of torpor in both groups were similar from Day 105 on, and most females had stopped hibernating by Day 180.

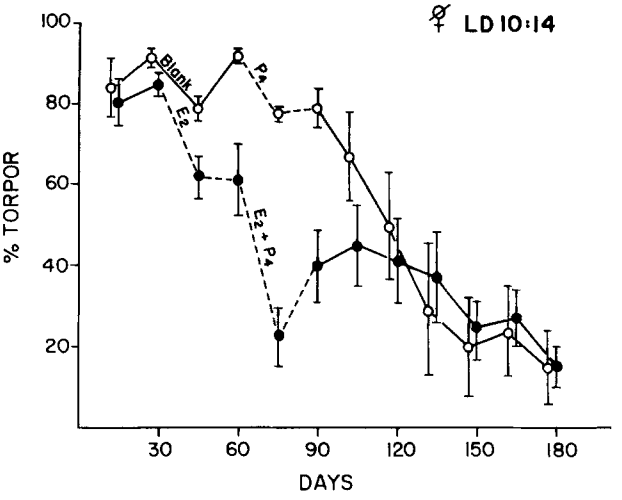

Fig. 5. Effect of ovarian steroids on hibernating gonadectomized females. After induced to hibernate, female hamsters of group 1 (o, $n=7$) were implanted with a blank capsule for 30 days, followed by a 20 mm progesterone (P_4) implant for 30 days. Group 2 (●, $n=10$) received a 20 mm estradiol (E_2) capsule, which was replaced at the end of 30 days with an E_2 and a P_4 capsule, each 20 mm in length. Pre-treatment of females and data presentation are as described for Fig. 4

In a subsequent study (Experiment III-B), the effect of exogenous estradiol was compared in ovariectomized females hibernating on long vs. short days (Fig. 6). Long day females showed a rapid and substantial response to estradiol, such that the incidence of torpor had decreased significantly within 15 days of hormone treatment (from 75% at Day 30 to 25% at Day 45, $P<0.01$), and remained low thereafter. In contrast, the same dose of estradiol was less effective in short day females, with no significant decline in torpor observed until Day 60 ($P<0.01$ compared to Day 30). When short-day females received a 3-fold higher dose of estradiol beginning at Day 60, hibernation was inhibited to the same extent as for singly-implanted long day females.

In comparison to ovariectomized long day females, hibernation in ovary-intact, anestrous females on long days was also strongly affected by

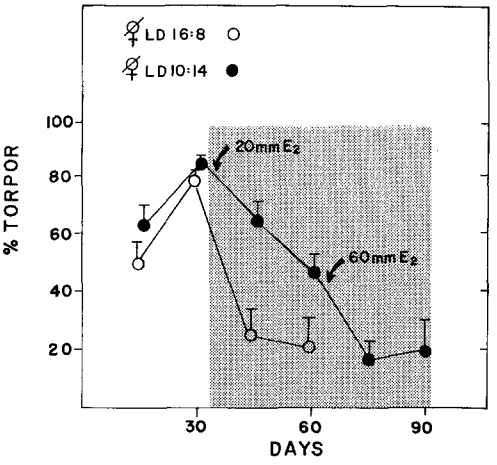

Fig. 6. Effect of estradiol on ovariectomized females hibernating on long or short day photoperiods. Females on each photoperiod received one 20 mm E_2 capsule after 30 days in the cold (●, short day, $n=10$; ○, long day, $n=7$). At Day 60, the single E_2 implant of the short day group was replaced by triple E_2 implants. The shaded area depicts approximate time of hormone treatment. Ovariectomy occurred 1 week before transfer to the cold (Day 0); short day exposure began eight weeks prior

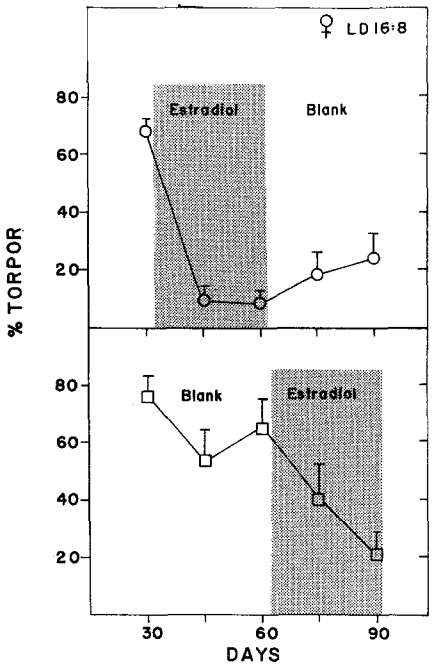

Fig. 7. Effects of estradiol on intact females exposed to long day cold. Two groups of females received a single 20 mm E_2 capsule, either followed by (○, $n=9$) or preceded by (□, $n=9$) implantation of a blank capsule. Torpor data are presented as in previous figures. Transfer to the cold occurred on Day 0. Shaded areas depict intervals of hormone treatment

estradiol treatment (Fig. 7, Experiment III-C). For the first group implanted with a 20 mm estradiol capsule (upper panel), the incidence of torpor declined dramatically from 69% to 9% ($P<0.01$) within 15 days. Torpor remained low even after the estradiol capsule was replaced with a blank capsule. In the second group to receive estradiol a month later (lower panel), inhibition of torpor was not as rapid, but after 30 days of hormone treatment, levels were significantly reduced ($P<0.01$). The females chosen for this study had closed vaginae prior to transfer to the long-day cold room. After 30 days of estradiol treatment, all females of both groups had open vaginae.

Serum estrogen and progestin titers (Table 2) were significantly increased by the 20 mm estradiol or progesterone capsules, in comparison to samples taken from ovariectomized females bearing no implants. An approximate tripling of serum estrogen levels was observed when the 60 mm vs 20 mm estradiol implant conditions were compared. Estradiol levels were significantly higher ($P<0.01$) in intact females with closed vaginae (anestrous females) than in ovariectomized females. Progestin levels in intact anestrous females were quite variable, but did not differ significantly from those of ovariectomized females. In females bearing only a progesterone capsule, circulating levels of proges-

Table 2. Serum estrogen and progestin titers

	Estrogen (pg/ml)	*n*	Progestin (ng/ml)	*n*
Ovariectomized females (of Fig. 5)				
With E_2 and P_4 capsules (20 mm each)	236±17	(9)	3.70±0.60	(9)
With P_4 capsule (20 mm)	**		9.40±1.80**	(9)
With no capsule	13± 3	(7)	0.61±0.15**	(8)
Ovariectomized females (of Fig. 6)				
With triple E_2 capsules (60 mm)	951±116	(6)	–	
With single E_2 capsule (20 mm)	244± 20**	(7)	–	
With no capsule	13± 2**	(9)	1.00±0.02	(7)
Intact anestrous females	35± 13*	(9)	8.8 ±6.9	(8)

Adjacent mean values differ at the * $P<0.05$ or ** $P<0.01$ level

tins were higher than when estradiol was administered in conjunction with progesterone. Intact females undergoing estrous cycles have unfortunately not been available for a comparison of ovarian steroid levels.

Discussion

Results of these studies generally confirm an interrelation between the annual reproductive cycle and hibernation in both male and female European hamsters. Female hamsters did not display torpor until the occurrence of vaginal closure, a condition indicating atrophy of the reproductive system. Male hamsters which failed to undergo testicular regression in a long-day, cold environment, also failed to hibernate. Thus, gonadal atrophy appears to be a necessary precondition for entry into hibernation. These results are consistent with studies of male European hamsters which show a decline in serum testosterone prior to hibernation (Buijs et al. 1986) and which demonstrate reduced testicular weight, incomplete spermatogenesis and other histological indications of gonadal atrophy during hibernation (Reznick-Schuler and Reznick 1973). In other hamster species as well, an inverse relationship between gonadal function and hibernation has been observed, e.g. in Syrian hamsters (Smit-Vis 1972; Jansky et al. 1984), Turkish hamsters (Darrow et al. 1987; Hall et al. 1982) and Djungarian hamsters (Vitale et al. 1985). In male Turkish hamsters, testicular regression and a decline in serum androgen levels have been shown to precede entry into torpor, whereas gonadal regrowth and a resurgence of androgen secretion commenced toward the end of the hibernation season (Darrow et al. 1987). Reduced serum androgen levels throughout hibernation have also been reported for the golden-mantled ground squirrel (Barnes 1986), the edible dormouse (Jallageas and Assenmacher 1983) and the Eastern chipmunk (Scott et al. 1981).

The fact that reproductive regression occurred in some of the European hamsters exposed to long-day cold (i.e. in the absence of a short-day cue) suggests that these hamsters may respond to temperature as well as photoperiodic cues. A similar response has been observed in Turkish hamsters (Hall et al. 1982). Interestingly, during extended continual exposure to long-day warm conditions, a cyclical loss and resumption of reproductive capacity have been observed in European hamsters (T. Lee and I. Zucker, personal communication) and in Turkish hamsters (Darrow et al. 1986). In contrast, Djungarian hamsters do not show reproductive regression when exposed to long-day cold (Vitale et al. 1985), nor during prolonged exposure to long-day warm conditions.

Compatible with the inverse relationship of gonadal activity and hibernation in European hamsters were the effects of gonadal steroids, particularly testosterone, to inhibit torpor. When administered at near physiological levels to hibernating male European hamsters, testosterone totally and immediately blocked re-entry into torpor. The same dose of testosterone produced an equally dramatic inhibition of hibernation in female hamsters, such that in both sexes the incidence of torpor dropped from more than 70% to less than 5%. Inhibition was constant during hormone treatment, but reversible once the hormone capsules were removed. A strong testosterone-induced inhibition of torpor has also been observed in Turkish hamsters (Hall and Goldman 1980), Syrian hamsters (Jansky et al. 1984) and Djungarian hamsters (Vitale et al. 1985). In the Turkish hamster, the threshold dose of testosterone for inhibiting torpor in castrated males resulted in serum androgen titers comparable to those seen in intact male hamsters with intermediate-sized testes (Hall and Goldman 1980), further supporting an inverse relationship between gonadal function and hibernation.

Because of this dose relationship and the strength of the testosterone effect on torpor, one might presume a causal role for testosterone in determining the onset and/or termination of the hibernation season in male hamsters. A reduction of serum testosterone levels in the fall would be permissive to the initiation of torpor, whereas spontaneous gonadal recrudescence and increased androgen secretion in the spring might result in termination of the hibernation season. However, a recent body of evidence, including some of the data presented here, indicate that the situation is not this straightforward. (1) With regard to the initiation of hibernation, a decline in gonadal hormones appears not to be a sufficient condition for the entry into torpor. Castrated male European hamsters exposed to long-day cold were slower to enter hibernation and showed a generally lower incidence of torpor than castrates on short days. A similar effect of photoperiod, independent of the gonadal cycle, has been observed in Turkish hamsters (Goldman and Darrow 1987) and in Djungarian hamsters (Vitale et al. 1985). Furthermore, intact Turkish males which failed to hibernate in long-day cold underwent a decline in serum testosterone levels which was similar in magnitude and timecourse to that observed in short-day males which did hibernate (Darrow et al. 1987). Thus,

although gonadal atrophy may be required for the initiation of torpor, other factors must also be involved. (2) With regard to the termination of hibernation, it is now well established that gonadal hormones need not be present. In Turkish hamsters, where the timing of hibernation has been best studied, most castrated males hibernate for only a few weeks longer than testis-intact males (Darrow et al. 1987; Goldman and Darrow 1987), although marked prolongation of the hibernation season has been observed for a few individuals (Hall and Goldman 1980; Goldman 1980). Only slight differences in the duration of the hibernation season have been detected between castrated vs. intact groups of Syrian hamsters (Jansky et al. 1984) and Djungarian hamsters (Elliott et al. 1987). In the present study, all but a few of the castrated males terminated hibernation by 7 months in the cold (Fig. 3), well after the effects of exogenous testosterone had subsided. Thus, gonadal factors are apparently not essential for the termination of torpor in these species. Instead, the strong action of testosterone which has now been observed in a number of hamster species may reflect some sort of redundancy or back-up system in the mechanism controlling hibernation, possibly to modulate or fine-tune the timing of the hibernation season.

Interpretation of the results with female European hamsters is somewhat more complicated than for the males, but a generally similar pattern has emerged. An inverse relationship between ovarian function and hibernation has already been mentioned for the female hamsters, in that hibernation did not begin until vaginal closure occurred. Similarly in Turkish hamsters, estrous cyclicity was observed to terminate shortly before the hibernation season commenced, resuming within a few days after the conclusion of hibernation (Darrow, unpublished observations). In female European hamsters, as in males, photoperiod affected torpor independently of its effect on the gonadal cycle, such that ovariectomized females on long days showed a lower incidence of torpor than those on short days. The ovaries were apparently not required for the termination of hibernation, since all but a few ovariectomized European hamsters ended hibernation after 6 months in the cold. Furthermore, no striking differences in duration of the hibernation season have been reported for ovariectomized vs intact Turkish or Djungarian hamsters (Hall and Goldman 1982; Elliott et al. 1987). Thus as in male hamsters, gonadal factors are apparently not obligatory for the termination of hibernation.

In contrast to the complete inhibition of torpor by testosterone, only a partial inhibition by estradiol and no effect of progesterone were detected, when these hormones were administered singly to ovariectomized European hamsters hibernating on short days. However, when progesterone was given in combination with estradiol to estradiol-primed females, a substantial reduction in the incidence of torpor was observed. These results suggest that the two ovarian steroid hormones, if administered in the appropriate sequence and/or dose relationship, might have a functionally comparable effect on torpor in females as testosterone alone did in males. Although dual estradiol/progesterone treatment was not tested in ovariectomized Turkish hamsters, in intact Turkish females hibernating on short days, it led to a reduction in torpor from 81 to 42%; a greater effect than with either hormone alone (Hall 1981). The lack of a complete and constant inhibition of torpor by dual estradiol/progesterone treatment may be explicable in terms of the dynamic interaction of these two hormones at the cellular receptor level. This complex interaction has been well characterized for uterine receptor regulation in Syrian hamsters during sustained exposure to ovarian steroids (Okulicz 1986). If a similar mechanism were operating at the steroid hormone target site(s) involved in the control of torpor, one might expect a robust but transient effect of combined estradiol/progesterone implants. It might be that ovarian steroids presented in cyclical fashion, as during the estrous cycle, would be more likely to cause a strong and continual inhibition of torpor than when administered tonically via silastic capsules. Further work is needed to characterize the fluctuations in circulating levels of ovarian hormones (as well as levels of endogenous androgens) throughout the estrous cycle in this species.

The most substantial inhibition of torpor observed in the female European hamster studies was during estradiol treatment of ovary-intact, anovulatory females hibernating on long days (Fig. 7). In this case, the inhibition (to $<10\%$ torpor) continued even after hormone implant removal. The concomitant change in vaginal condition observed in these females indicated an estradiol-induced, long-lasting activation of the reproductive axis, possibly affecting ovarian function. In addition to the presence of intact ovaries, exposure to long days undoubtedly contributed to the strength of the estradiol effect in this experiment. In a separate study, ovariectomized females hibernating on long days were more responsive to the inhibitory action of estradiol than those on short days (Fig. 6). There is precedence for a photoperiodic modula-

tion of dose response to gonadal steroids in Syrian hamsters, in that short day photoperiods alter the sensitivity of the hypothalamic/pituitary axis to negative feedback by testosterone (Ellis and Turek 1979). The possibility that responsiveness to estradiol might change during the course of the hibernation season (as suggested by Fig. 7) has also been indicated in the Turkish hamster hibernation data (Hall and Goldman 1980) and is consistent with the pattern observed in male Syrian hamsters (Ellis and Turek 1979) in which the threshold for testosterone action is altered at the beginning and at the end of the winter period of gonadal regression.

Little is known about the mechanism of action by which gonadal steroids affect torpor, although the site(s) of action presumably reside within the CNS. A number of brain areas have been implicated in the control of hibernation, including the limbic system, involved in arousal from torpor (cf. Heller 1979), the hypothalamus, involved in temperature regulation (Malan 1969; Raths and Bohn 1975) and the anterior medial raphe nucleus, known to be required for hibernation in European hamsters (Canguilhem et al. 1986). Furthermore, gonadal steroids are known to stimulate increased locomotor activity in rodents (Blizard 1983; Gentry and Wade 1976) and may be acting at brain sites to affect activity/rest cycles or general level of activity. It is known from work with Turkish hamsters that enzymatic aromatization of testosterone to estrogen is not needed for hormone action, because dihydrotestosterone is equally effective in blocking torpor as testosterone (Hall and Goldman 1980). This conclusion has been indirectly confirmed for European hamsters in the present study since, if aromatization were required for androgen action, one would not predict the more robust action of testosterone to inhibit torpor, as compared to estradiol.

Most tests of steroid action on torpor have been performed by administering hormones during intervals of spontaneous arousal from torpor. Whether the hormone signal is perceived by target site(s) during a bout of torpor has been difficult to ascertain, because administration of vehicle alone is often a sufficient disturbance to artificially arouse the animal. Preliminary results suggested a lowered response to testosterone in torpid vs aroused Turkish hamsters (Hall 1981). In the first study to quantify steroid hormone receptor levels during hibernation, it is now known that in Turkish hamsters, uterine responsiveness to estradiol is greatly attenuated during bouts of torpor, as compared to arousal (Okulicz et al. 1988). Furthermore, recent evidence has indicated that secretion of a variety of hormones is greater during bouts of arousal than during torpor in Turkish hamsters (Darrow et al. 1986; Darrow et al. 1987), as well as in other rodents (Barnes 1986; Jallageas and Assenmacher 1983), and that slight increases in gonadal steroids may occur prior to the end of the hibernation season for both male (Hall et al. 1982; Darrow et al. 1987) and female Turkish hamsters (Okulicz et al. 1988). Since bouts of arousal become more frequent toward the end of the hibernation season (Darrow et al. 1987), one might predict increased exposure to the steroids during times when reception of the hormonal signal is possible. This increased exposure, together with a possible change in sensitivity to steroids, might allow for gonadal hormone action, even though circulating hormone titers are low. Thus, in hibernating hamsters, gonadal steroids might play a role in synchronizing the activation of the reproductive axis with emergence from hibernation.

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