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Hormonal Regulation of the Annual Pelage Color Cycle in the Djungarian Hamster, *Phodopus sungorus*. I. Role of the Gonads and the Pituitary

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ABSTRACT The Djungarian hamster exhibits an agouti pelage in the summer and a predominantly white pelage in the winter. This pelage color cycle is known to be regulated by the length of the daily photoperiod probably acting through the pineal gland, as is the seasonal cycle of reproductive function with which it is closely correlated (Figala et al., '73; Hoffmann, '78b). The possibility of a causal relationship between the decline in gonadal hormone secretion and the coat color change occurring in short photoperiod was examined. Gonadectomized and intact male and female hamsters were exposed to either long (16L:8D) or short (10L:14D) photoperiod for several months. Gonadectomy neither induced the change to the winter pelage color in long photoperiod-housed animals, nor prevented either the change to the winter pelage or the spontaneous return to summer pelage color in short photoperiod-housed animals. Chronic implants of testosterone in castrated males delayed and attenuated the short photoperiod-induced coat color change. Administration of ovine prolactin (100 μg/day) stimulated pigmentation in hamsters with the winter pelage, whereas administration of a αMSH (30 μg/day) was without effect. These results suggest that changes in pelage color may be regulated largely by changes in pituitary prolactin secretion and modified to some extent by changes in gonadal steroid hormone secretion.

The Djungarian hamster, *Phodopus sungorus*, undergoes an annual cycle in pelage color, exhibiting an agouti pelage in the summer and a predominantly white pelage in the winter. This pelage color cycle is known to be partially regulated by the length of the daily photoperiod probably acting through the pineal gland, as is the annual cycle of reproductive function with which it is closely correlated (Figala et al., '73; Hoffmann, '78b). During exposure to long photoperiod, dark coat color and functional gonads are maintained. Short photoperiod exposure induces pelage color lightening and gonadal regression (Figala et al., '73); these effects require the presence of the pineal gland (Hoffmann, '78b). After prolonged exposure to short photoperiod, a spontaneous return to the dark color and functional gonads eventually occurs. The similarities in the regulation and time courses of these two seasonal cycles prompted the investigation of possible mediation of the pelage color cycle by changes in the levels of gonadal hormones.

Further experiments were directed at examining a possible role of pituitary hormones in the regulation of the seasonal pelage color cycle in the Djungarian hamster. The pituitary gland has been implicated to play this role in the short-tailed weasel, *Mustela erminea bangsi*, which also exhibits dark fur in the summer and white fur in the winter (Rust, '65). Hypophysectomized weasels undersent an irregular, asynchronous molt, but grew only white fur, regardless of the season or daylength (Rust, '65). Restoration of the dark fur was brought about by administration of MSH or ACTH (Rust, '65), or by ectopic pituitary grafts thought to hypersecrete MSH (Rust and Meyer, '68). In the

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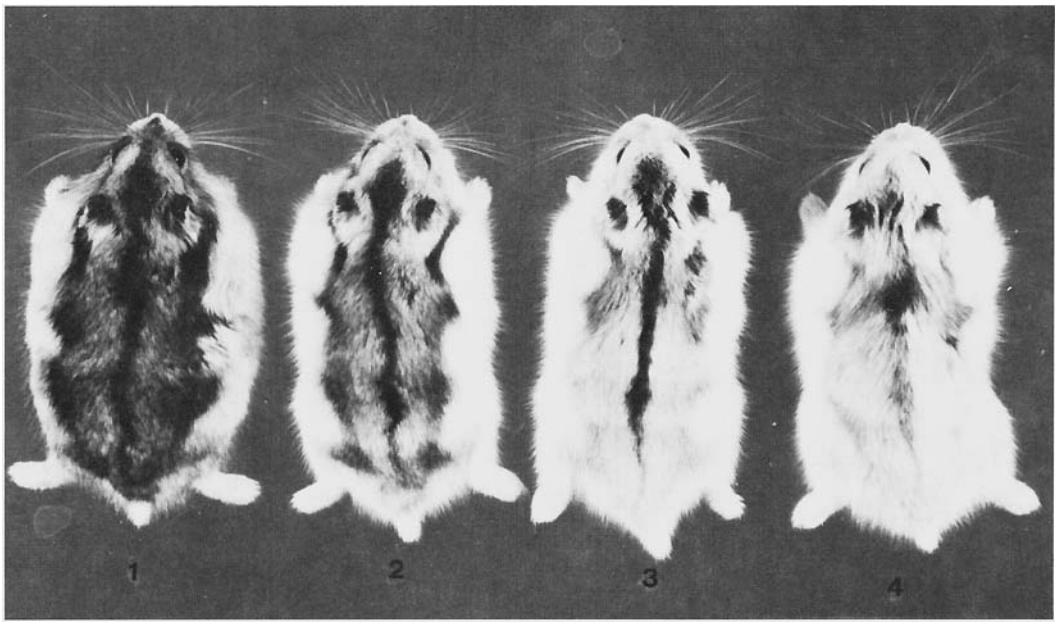

Fig. 1. Pelage color stages. Stage 1 (summer pelage): agouti fur on dorsal surface and light gray or white fur on lateral and ventral surfaces. Stage 2 (early development of winter pelage): white fur on posterior dorsal surface and face. Stage 3 (more advanced winter pelage): white fur on mid to rear dorsal and lateral surfaces. Stage 4 (complete winter pelage): white fur covering body except for mid-dorsal stripe and neck area.

Djungarian hamster, ectopic pituitary grafts maintained the dark pelage during exposure to short photoperiod (Duncan, '80). Furthermore, these grafts maintained high circulating levels of prolactin, whereas exposure to short photoperiod normally suppresses prolactin levels (Duncan, '80). In the present study, we describe the effects of MSH and prolactin administration on pigmentation of the winter pelage.

MATERIALS AND METHODS

Animals

Djungarian hamsters used for these experiments were 60 to 80 days old and were raised in our breeding colony, which was derived from animals provided by Dr. Klaus Hoffmann (Max-Planck Institut fur Verhaltensphysiologie, Andechs, F.D.R.). All animals were group housed in large plastic tubs ($18 \times 10 \times 12$-in.^{3}) containing cedar shavings. Water and Wayne F6 Lab Blox were provided ad libitum. Animal rooms were maintained at a constant temperature ($22 \pm 1°C$) and provided either a long day photoperiod (16L:8D, lights on at 0200) or a short day photoperiod (10L:14D, lights on at 0500). Prior to experimentation, animals were maintained under long photoperiod and had the dark agouti pelage.

Pelage color stages

In order to monitor pelage color changes a color scale of four stages was established as shown in Figure 1.

Stage 1 (summer pelage). Agouti fur on the dorsal surface included a prominent medial black stripe, light gray or white underfur penetrated by agouti guard hairs on the lateral surface, and light gray fur on the ventral surface.

Stage 2 (first intermediate in the winter molt). White fur on the ventral and lateral surfaces had an absence of dark guard hairs, as well as a region of light-colored fur on the posterior dorsal surface, and the appearance of white fur around the eyes and behind the ears.

Stage 3 (second intermediate). White fur was noted in the regions listed in stage 2 and light gray or white fur extended from the tail to the mid-dorsal region.

Stage 4 (winter pelage). White fur was noted all over the body except the mid-dorsal stripe and the area just posterior to the neck and forepaws, which remained dark. The white color seen on the dorsal surfaces in stage 3 and stage 4 was due primarily to the white guard hairs. The underfur often remained gray.

To determine the reliability of pelage color scoring, multiple scorings were conducted on the same group of animals. Inter-rating reliability was >98% accurate.

Statistical analysis

Group comparisons of pelage color stage were compared by the Kruskal-Wallis one-way analysis of variance by ranks (experiment 1) or the Mann-Whitney U Test (experiment 2). Group differences in fur color after plucking were determined by χ^2 tests (experiment 4). The level of significance used was $P < 0.05$.

Experiment 1: Role of the gonads in pelage color change

Eight groups of six or seven animals each were established as follows: (1) intact males, long photoperiod; (2) castrated males, long photoperiod; (3) intact males, short photoperiod; (4) castrated males, short photoperiod; (5) intact females, long photoperiod; (6) ovariectomized females, long photoperiod; (7) intact females, short photoperiod; and (8) ovariectomized females, short photoperiod.

Animals were observed for changes in pelage color at 2-week intervals. Color scoring was conducted from week 0 to 14 for groups in long photoperiod and from weeks 0 to 14 and 18 to 30 for groups in short photoperiod.

Experiment 2: Effect of testosterone on pelage color change

Thirty male hamsters were castrated and divided into two groups of 15 each. Animals in the first group were implanted with silastic capsules containing testosterone; those in the second group were implanted with empty silastic capsules. Capsules were made by cutting Dow Corning silastic tubing (inner diameter .078 in., outer diameter .125 in.) into 10-mm lengths and filling them with crystalline testosterone or leaving them empty. Ends were sealed with silastic cement. Capsules were soaked overnight in physiological saline before subcutaneous implantation. Preliminary experiments had indicated that 10-mm testosterone implants in castrated Djungarian hamsters maintain approximately physiological levels of testosterone. Serum concentrations of testosterone, as measured by radioimmunoassay (Pang and Johnson, '73), averaged 1.48 ± 0.26 ng/ml in castrated males with 10-mm implants and 1.38 ± 0.44 ng/ml in intact males without implants (Duncan, '80). After implantation, all animals were transferred to short photoperiod. Pelage color was scored at 2-week intervals from week 8 to week 20.

Experiment 3: Effect of αMSH injections on pelage color

Fourteen male hamsters were transferred to short photoperiod. After 12 weeks, all animals exhibited pelage color stage 2 or 3 and had regressed testes as determined by palpation. A patch of fur was plucked from the mid-right dorsum of each animal. Beginning the following day, animals received daily subcutaneous injections of either 30 μg αMSH (Bachem) dissolved in 0.9% saline or vehicle alone. Injections were given at 1200 hr and were continued for 12 days. One week after the last injection, the color of the fur grown in the plucked area was recorded.

Experiment 4: The effect of prolactin injections on fur color

Hamsters of both sexes were exposed to short photoperiod. After 14 weeks, 20 males and 16 females, which had molted to stage 3 or 4, were selected for the experiment. All males had regressed testes as revealed by palpation. A patch of fur was plucked and daily injections of either 100 μg ovine prolactin (NIAMDD-o-PRL-14, 29.5 IU/mg) suspended in 0.9% saline or vehicle were administered at 1200 hr for 21 days. Color of the newly grown fur was recorded.

RESULTS

Experiment 1: Role of the gonads in pelage color change

During weeks 8 to 14, intact and gonadectomized hamsters in short photoperiod molted to the winter pelage, in contrast to intact and gonadectomized hamsters in long photoperiod (Figs. 2 and 3). By week 14, there was a significant difference among groups of males ($P < 0.05$, $H_2 = 8.5$) and among groups of females ($P < 0.01$, $H_2 = 12.05$). At this time, the intact females exhibited greater variability in pelage color stage than other groups. This has not been a consistent find-

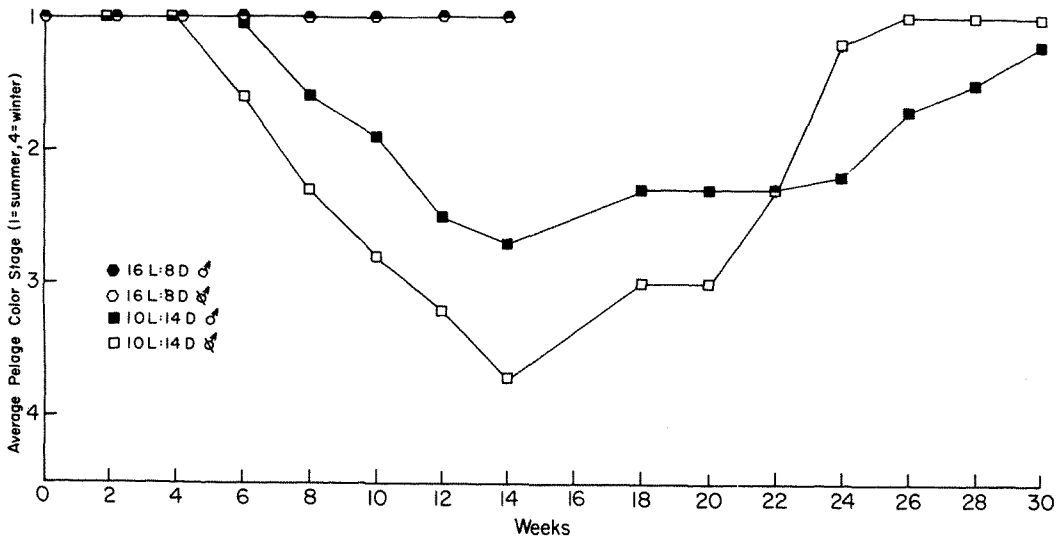

Fig. 2. Pelage color in intact and castrated Djungarian hamsters exposed to long (16L:8D) or short (10L:14D) photoperiod. Pelage color stage ranged from 1 = summer pelage to 4 = complete winter pelage. Each point represents the mean pelage color stage. N = 5–7 for each group. Intact and castrated hamsters in 10L:14D acquired the winter pelage by week 14 and molted back to the summer pelage by week 30.

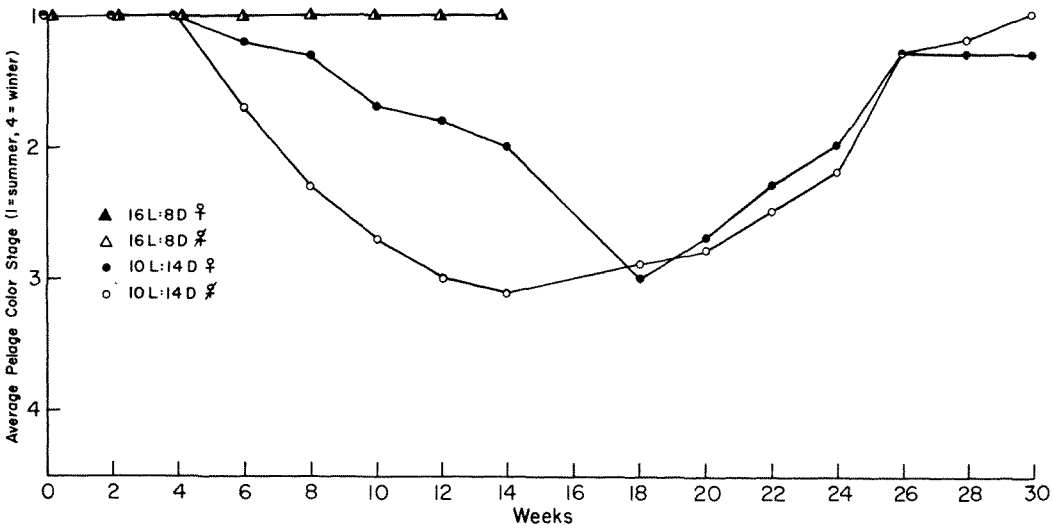

Fig. 3. Pelage color in intact and ovariectomized Djungarian hamsters exposed to long (16L:8D) or short (10L:14D) photoperiod. Pelage color stage ranged from 1 = summer pelage to 4 = complete winter pelage. Each point represents the mean ± S.E.M. N = 5–7 for each group during weeks 0–14. Only 3 intact, short-day females were studied during weeks 16–30; N = 6 for the ovariectomized females during this time. Intact and ovariectomized hamsters in 10L:14D acquired the winter pelage by week 14 and molted back to the summer pelage by week 30.

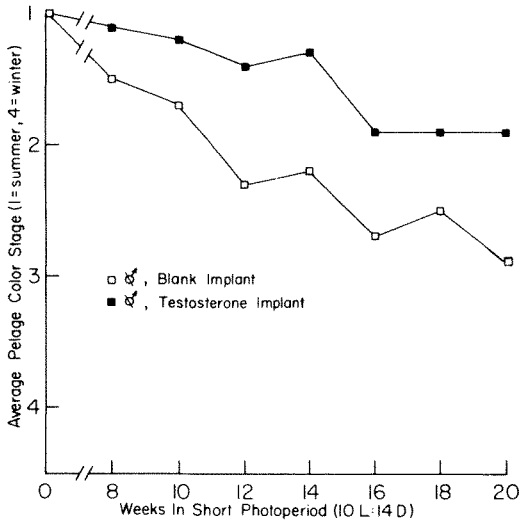

Fig. 4. Pelage color in castrated, blank-implanted and castrated, testosterone-implanted (10-mm) hamsters exposed to short photoperiod (10L:14D). Pelage color stage ranged from 1 = summer pealge to 4 = complete winter pelage. Each point represents the mean pelage color stage (N = 15 for each group). The winter pelage color change, exhibited by the blank-implanted hamsters, was partially inhibited in the testosterone-implanted hamsters.

ing, however, in subsequent experiments (Duncan, unpublished).

By week 30, all gonadectomized males (Fig. 2) and females (Fig. 3) exposed to short photoperiod had regained the summer pelage. There was a tendency for gonadectomized animals in short photoperiod to undergo both the winter and the spring molt earlier than intact animals, but this difference was not always statistically significant. Intact males in short photoperiod had regressed testes, as determined by palpation, from week 8 through weeks 23 to 28. Time of recrudescence varied greatly among individuals.

Experiment 2: Effect of testosterone on pelage color change

Testosterone implants delayed and attenuated the short photoperiod-induced pelage color change (Fig. 4). A significant difference ($P < 0.05$, $U = 54.5$) between the testosterone-implanted animals and the blank-implanted animals was apparent by week 12. Although testosterone-implanted males, on the average, did not reach a winter pelage color stage as advanced as that of blank-implanted males, most of them did show at least a partial change to the winter pelage (stage 2): 67% of the castrated, testosterone-implanted males acquired pelage stage 2 or greater, similar to 87% of the castrated, blank-implanted males.

TABLE 1. Effect of prolactin upon pigmentation in male and female Djungarian hamsters exposed to short photoperiod for 17 weeks

Sex	N	Saline	Prolactin
Male	20	0[1]	80
Female	16	0	50

[1]Percentage of animals that grew pigmented fur after plucking during week 14.

Experiment 3: The effect of αMSH injections on fur color

In both the αMSH- and saline-treated groups, all animals exhibited white and/or light gray fur in the plucked region 7 days after the last injection. In all cases, the newly grown fur appeared to be of the same color or lighter than the fur on the left, unplucked dorsal surface.

Experiment 4: The effect of prolactin injections on fur color

Injections of prolactin, but not saline, led to the growth of pigmented fur after plucking (Table 1). A significant difference in the proportion of animals that grew pigmented fur was seen between the prolactin-injected males and the saline-injected males ($P < 0.05$, $\chi_1^2 = 13.33$) and the prolactin-injected females and the saline-injected females ($P < 0.05$, $\chi_1^2 = 5.33$).

DISCUSSION

The results of these experiments indicated that the seasonal cycle of gonadal activity exerts, at most, only a modulating effect on the seasonal cycle of pelage color in the Djungarian hamster. Gonadectomy did not induce the winter pelage color change in long photoperiod-housed male or female Djungarian hamsters. Thus, a decrease in gonadal hormone secretion does not appear to be the primary stimulus for the change to the white pelage. Furthermore, gonadal recrudescence was not essential for the change to the summer pelage since this molt occurred in gonadectomized as well as intact animals after 24–30 weeks exposure to short photoperiod. This finding agrees with observations made on other mammals exhibiting seasonal pelage changes: Gonadectomy does not prevent

the normal spring pelage change in the mink (Rust et al., '65) or the varying hare (Lyman, '43).

A decline in gonadal hormone secretion may have a permissive effect on the winter pelage color change, since this change occurred more slowly and less completely in testosterone-implanted males than in blank-implanted males. This effect of testosterone may reflect either inhibition of the molting process or stimulation of pigmentation, or both. Seasonal molts are thought to involve a synchronization of hair follicles such that hair replacement progresses across the body as a wave (Ling, '72). Age-related molts in the rat and mouse have been shown to be inhibited by testosterone (Johnson, '58; Houssay, '49). In the mink, seasonal molts occur at times of the year when circulating testosterone levels are decreased (Boissin-Agasse et al., '81). Stimulation of pigment synthesis by testosterone has been demonstrated in guinea pig skin (Mondal and Banerjee, '81).

In the present study, administration of 100 μg ovine prolactin/day induced pigmentation of the fur in short photoperiod-housed Djungarian hamsters. It seems probable that this treatment produced supraphysiological levels of circulating prolactin. Experiments are currently being conducted in our laboratory to investigate whether a more physiological dose of prolactin will also stimulate pigmentation of the winter pelage. Preliminary data suggest that chronic infusion of 10 or 20 μg ovine prolactin/day, doses shown to produce physiological levels of circulating prolactin (as measured by a highly specific radioimmunoassay), also have this effect.

To our knowledge, this is the first report that prolactin is capable of stimulating pigmentation in a mammal. In the viable yellow (A^{vy}) mouse, which shows a developmental darkening of the pelage, Levitin and colleagues ('79) concluded that prolactin did not stimulate pigmentation since ectopic grafts of the anterior pituitary (presumed to hypersecrete prolactin) did not lead to growth of dark fur after plucking. Stimulation of pigmentation by prolactin has been reported in fish (Pickford and Kosto, '57; Kosto et al., '59; Sage, '70).

In constrast to administration of prolactin, administration of MSH (30 μg/day) to short photoperiod-housed Djungarian hamsters did not stimulate pigmentation of the winter pelage. This result is in agreement with an earlier brief report. However, in the earlier study MSH did increase hair follicle melanin content in hamsters transferred from short to long photoperiod (Mistry and Weatherhead, '76).

MSH has also been shown to stimulate melanogenesis in short-term cultures of Djungarian hamster hair follicles, but coincubation with an equimolar amount of melatonin blocked this effect (Logan and Weatherhead, '80b, '81). In the present study, it is possible that endogenous melatonin prevented MSH stimulation of pigmentation. This effect of melatonin, together with the fact that melatonin secretion is prolonged during short photoperiod (Goldman et al., '81), suggests that a possible winter decrease in pituitary MSH secretion (Logan and Weatherhead, '80a) may not be necessary for the winter pelage whitening in the Djungarian hamster. A study in the varying hare also reported that MSH failed to stimulate pigmentation of the winter pelage, perhaps because the follicular melanocytes were immature at this time (Keogh, '69).

The significance of the present findings is best seen in the context of earlier studies on pelage color changes in the short-tailed weasel (Rust, '65; Rust and Meyer, '68) and the Djungarian hamster (Hoffmann, '78b, '81). In neither species do the gonads seem to play a determining role in pelage color changes. This information is especially intriguing in the Djungarian hamster, since the role of the pineal in seasonal pelage and reproductive changes has been demonstrated in this species (Hoffmann, '78b). This finding further supports the hypothesis that the pineal may mediate seasonal physiological changes that are not stimulated by changes in gonadal function; thus, the pineal is not merely a regulator of reproduction (Hoffmann, '81; Goldman et al., '82). Other documented extrareproductive effects of the pineal in mammals are concerned with body weight regulation in the Djungarian hamster (Hoffman, '78a) and thermoregulation in the Djungarian hamster (Heldmaier et al., '81) and the white-footed mouse (Glass and Lynch, '82).

Furthermore, the pituitary gland appears to participate in the regulation of the seasonal pelage color cycle. In constrast to the short-tailed weasel, in which MSH has been suggested to regulate seasonal color changes by stimulating pigmentation (Rust, '65; Rust and Meyer, '68), prolactin may be the pitui-

tary hormone serving this function in the Djungarian hamster. Consistent with this hypothesis is the finding that circulating prolactin levels are suppressed in short photoperiod during the time when the winter pelage is exhibited (Duncan and Goldman, '83).

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