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# Endogenous circannual rhythms and photorefractoriness of testis activity, moult and prolactin concentrations in mink (*Mustela vison*)

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**Summary.** Mink are seasonal photosensitive breeders; testis activity is triggered when days have less than 10 h light. Increasing and decreasing plasma concentrations of prolactin induce the spring and autumn moults. In a 5 year experiment, males were maintained under short days (8 h light:16 h dark) at 13°C or long days (16 h light:8 h dark) at 21°C, winter and summer conditions, respectively. Under winter and summer conditions, circannual cycles of prolactin secretion and moulting were observed at intervals of about 11 months. Recurrence of testis cycles was not evident. In a second experiment, males were maintained under an 8 h light:16 h dark cycle from the winter solstice or under 10 h light:14 h dark, 12 h light:12 h dark or 14 h light:10 h dark cycles from 10 February. Under 8 h light:16 h dark cycle, testis regression was slightly later than under natural conditions, indicating photorefractoriness. However, mink remained sensitive to light: the longer the photoperiod, the faster the testis regression. In a third experiment, males were transferred under 8 h light:16 h dark or 16 h light:8 h dark from 15 May (group 1), 12 June (group 2) or 4 July (group 3); males submitted to long days received melatonin capsules on the day of transfer. Increasing concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and testis volume were shown by half the males in group 2 and nearly all the males in group 3; the constant release of melatonin from implants was more efficient than short days; but in the three groups, prolactin concentrations decreased in the few days after short-day or melatonin treatment. Overall, the results demonstrate endogenous circannual rhythms of prolactin secretion, body weight and moulting. Although a refractory period to short days was observed, the annual cycle of testis activity totally relies on the annual changes in daylength.

*Keywords:* circannual rhythm; photorefractoriness; melatonin; testis; prolactin; moult; mink

## Introduction

The degree of environmental control of physiological and behavioural seasonality varies among mammals. Endogenous circannual rhythms that persist in the absence of seasonal cues occur in many long-lived mammals. Endogenous rhythms have been reported in body weight (ground squirrel: Zucker & Boshes, 1982; bat: Beasley *et al.*, 1984), reproduction (ewe: Ducker *et al.*, 1973; Malpaux *et al.*, 1989; ram: Howles *et al.*, 1982; ferret: Boissin-Agasse *et al.*, 1985), moulting period (ferret: Rust & Shackelford, 1969; ground squirrel: Joy & Mrosovsky, 1982) and hormone secretion (Howles *et al.*, 1982; Licht *et al.*, 1982; Karsch *et al.*, 1989). In natural conditions, these cycles are adjusted by external factors to a period of 365 days. In mammalian species from temperate and arctic regions, the annual changes in daylength constitute the major zeitgeber.

Unlike the preceding species, small short-lived rodents do not display circannual cycles under constant environmental conditions. In the Syrian hamster, gonadal regression depends on the decrease in daylength after the summer solstice, but resumption of sexual activity occurs spontaneously after 14–16 weeks of short days (Reiter, 1975; Turek *et al.*, 1975).

Photorefractoriness, which appears when animals are no longer able to respond to prevailing stimulatory (Robinson & Karsch, 1984) or inhibitory (Reiter, 1975; Turek *et al.*, 1975) photoperiod, accounts for the expression of an endogenous timing. Photosensitivity is regained after a few weeks of exposure to another photoperiod. In Syrian hamsters, photorefactoriness to the previously inhibitory action of short days on testicular activity is terminated by 11 weeks exposure to long days (Stetson *et al.*, 1977). In the same way, exposure of ewes in December to a long photoperiod for 30 days restores responsiveness to short days (Jackson *et al.*, 1988).

The American mink (*Mustela vison*) is a seasonal photosensitive breeder. Testis recrudescence begins in November. Mating is observed from late February to late March and is followed by a sharp testicular regression and a long period of sexual rest (Duby & Travis, 1972; Boissin-Agasse & Boissin, 1979; Allain *et al.*, 1981; Boissin-Agasse *et al.*, 1982). Lengthening days induce a spring moult from mid-April to late June leading to the growth of a thin summer coat; the autumn moult is triggered by decreasing daylength and establishes the heavy winter coat (Bissonnette & Wilson, 1939; Duby & Travis, 1972; Allain *et al.*, 1981). The annual variation in prolactin secretion paralleling that of photoperiod (Martinet *et al.*, 1982) seems to be at least partly responsible for the initiation of hair follicle activity in both spring and autumn moults (Martinet *et al.*, 1983; Martinet *et al.*, 1984).

If photoperiodic requirements for induction of annual cycles in gonadal activity, moulting and prolactin secretion are documented in male mink, it is not known whether they reflect endogenous rhythms synchronized by photoperiodic signals or if they are partly or totally driven by these signals.

Evidence strongly suggests that the rhythmic secretion of melatonin from the pineal gland mediates the effect of changing daylength on seasonal cycles of reproduction and pelage (for review see Arendt, 1986). In the mink, subcutaneous capsules of melatonin implanted in July reproduce the effect of short days by inhibiting prolactin secretion, inducing autumn moult and stimulating testicular recrudescence (Allain *et al.*, 1981).

Further evidence indicates that photorefractoriness to a fixed photoperiod appears concomitantly to refractoriness to a fixed rhythm of melatonin secretion (Bittman, 1978; Karsch *et al.*, 1986). In the mink, testis activity, which is triggered by melatonin administration, spontaneously regresses after a few months as it does after prolonged exposure to short days (Martinet & Allain, 1985).

The present study addressed three related questions. (i) Do circannual endogenous rhythms of body weight, testis size, moult and prolactin secretion exist in the mink? (ii) Is increasing daylength in late winter inhibiting testis activity or is photorefractoriness to the stimulatory action of short days developing? (iii) In this latter case, once photorefractoriness is established, how do animals regain their photosensitivity to short days or to melatonin administration?

## Materials and Methods

### Animals

Male mink born in the laboratory colony (48°N latitude) were housed out of doors in individual wire pens. A commercial diet and water were available *ad libitum*.

### Experiment 1

Adult males born in May 1983 were caged either under a short photoperiod (8 h light:16 h dark; lights on at 08:00 h; $n = 8$) and an ambient temperature of 12–14°C from December 1983 to November 1988 or under a long photoperiod (16 h light:8 h dark; lights on at 05·00 h; $n = 8$) and at 20–22°C from June 1984 to March 1988. Seven males were left outside as controls.

## Experiment 2

Twenty-four adult males born in the preceding spring were maintained from the winter solstice under an 8 h light: 16 h dark ($n = 6$) daily light cycle or from 10 January under 10 h light:14 h dark ($n = 6$), 12 h light:12 h dark ($n = 6$) or 14 h light:10 h dark ($n = 6$) cycles (lights on at 08:00, 07:00, 06:00 and 05:00 h, respectively). At latitude 48°N, daylight lasts for 8 h at the winter solstice and for 10 h in mid-February. Six males remained outside as controls.

## Experiment 3

Forty-five males born the preceding year were transferred on 15 May (group 1; $n = 16$), 12 June (group 2; $n = 15$) or 3 July (group 3; $n = 14$) into short (8 h light:16 h dark, lights on at 08:00 h; group 1: $n = 10$; group 2: $n = 10$; group 3: $n = 8$) or long (16 h light:8 h dark, lights on at 05:00 h; group 1: $n = 6$; group 2: $n = 5$; group 3: $n = 6$) days. Males of the long day groups were subcutaneously implanted with melatonin capsules on the day of transfer.

## Experimental rooms

Rooms were illuminated by cool fluorescent white tubes. Light intensity at cage level was approximately 300 lx. Light failures were prevented by using an electric generating set. During Expts 2 and 3, ambient temperature ranged from 18 to 20°C.

## Procedure

Animals, lightly anaesthetized by an i.m. injection of ketamine hydrochloride (0·5 ml Imalgène 500; Mérieux, France), were observed every two weeks in Expt 1 and at weekly intervals in Expts 2 and 3. Body weight was recorded and testis diameter measured through the scrotum wall with a caliper. The stages of fur development were checked on the head, flank and tail by looking for the blue pigmentation of the skin corresponding to melanogenesis renewal during the phase of hair follicle activity. The spring moult begins on the head and progresses towards the tail whereas autumn moult passes from the tail to the head (Allain & Rougeot, 1980).

Blood samples were collected by jugular venepuncture between 09:00 and 11:00 h and the plasma (Expts 1 and 2) or serum (Expt 3) were stored at −20°C until required for hormone determinations.

## Radioimmunoassay

Prolactin concentrations were measured using porcine prolactin (NIH SP162C) as a tracer and standard and a guinea-pig antiserum to ovine prolactin (Martinet *et al.*, 1982). In each experiment, all samples were assayed during the same incubation. The results were expressed in terms of NIH porcine prolactin SP162C standards. The sensitivity of the assay was 2 ng $ml^{-1}$. The intra-assay coefficients of variation were 9·2, 11·3 and 8·9% in Expts 1, 2 and 3, respectively.

Serum FSH concentrations were measured, as previously described for foxes (Mondain-Monval *et al.*, 1988) and hares (Caillol *et al.*, 1990), using ovine FSH (LER 1976-A2) for iodination, a rabbit antiserum against human FSH (M91/1) and a crude mink pituitary extract as reference preparation after comparison with a highly purified rat FSH (NIADDK RP2). The antiserum crossreacts with FSH of several species, but does not crossreact with LH (McNeilly *et al.*, 1976). In mink serum, binding was not affected by high concentrations of LH (4·7–6·8 ng $ml^{-1}$ LH versus undetectable concentrations of FSH). The specificity of the assay was determined by validation studies, using mink pituitary extract and serum which resulted in parallel displacement of $^{125}$I-labelled ovine FSH. The sensitivity of the assay was 1 ng $ml^{-1}$. The intra-assay coefficient of variation calculated between duplicates of 1·0–10·0 ng $ml^{-1}$ ranged from 3·8 to 13·9%. The inter-assay coefficient of variation of four pooled mink serum samples containing 2·9–14·7 ng $ml^{-1}$ ranged from 9·8 to 14·6% in four different assays.

Serum LH concentrations were measured according to Mondain-Monval *et al.* (1984) with minor modifications, using highly purified ovine LH (LER 1056-C2) for iodination, a rabbit antiserum to rat LH (CSU 120) and a crude mink pituitary extract as reference preparation after comparison with a highly purified rat LH (NIAMDD AFP 5666C). The antiserum crossreacts with ovine, canine and rat LH, but not with rat FSH (0·06%; NIADDK FSH) or thyroid-stimulating hormone (TSH) (0·03%; NIADDK TSH). Furthermore, in mink, serum binding was not affected by high concentrations of FSH (9·2–15·4 ng $ml^{-1}$ FSH versus undetectable concentrations of LH). The specificity was established by validation studies using mink pituitary extract and serum which resulted in parallel displacement of $^{125}$I-labelled ovine LH. The sensitivity of the assay was 0·25 ng $ml^{-1}$. The intra-assay coefficient of variation for duplicates of 0·6 ng $ml^{-1}$ was below 10% and the inter-assay coefficient of variation of pooled mink serum samples in three different assays was 11·8%.

Melatonin was assayed in serum according to Tillet *et al.* (1989) following extraction with dichloromethane by using tritiated melatonin (Amersham, UK) and a sheep anti-melatonin antiserum (Arendt G/7/704-6483).

### Statistical analysis

In Expt 1, a peak was defined as the median highest value corresponding to an increase of at least 200 g body weight or 4 ng $ml^{-1}$ prolactin compared with the lowest preceding value. Means ± SEM for intervals between two peaks (Expt 1) of testis volume and plasma prolactin (Expts 2 and 3), FSH and LH (Expt 3) concentrations were calculated. In Expts 2 and 3, the effects of treatment on testis volume and concentrations of prolactin were estimated by analysis of variance for repeated measures. Concentrations of LH and FSH were compared by one-way analysis of variance followed by the Duncan test (SAS procedures, SAS/STAT Guide for Personal Computers, 1987).

## Results

### Experiment 1

Males maintained under natural environmental conditions exhibited dramatic annual variations of body weight (Fig. 1) and testis volume (Fig. 2) which peaked in February. Plasma concentrations of prolactin rose concomitantly with the testis regression, then decreased rapidly after the summer solstice (Fig. 3). The spring moult was observed from mid-April to late June and the autumn moult from late August to early December (Fig. 3).

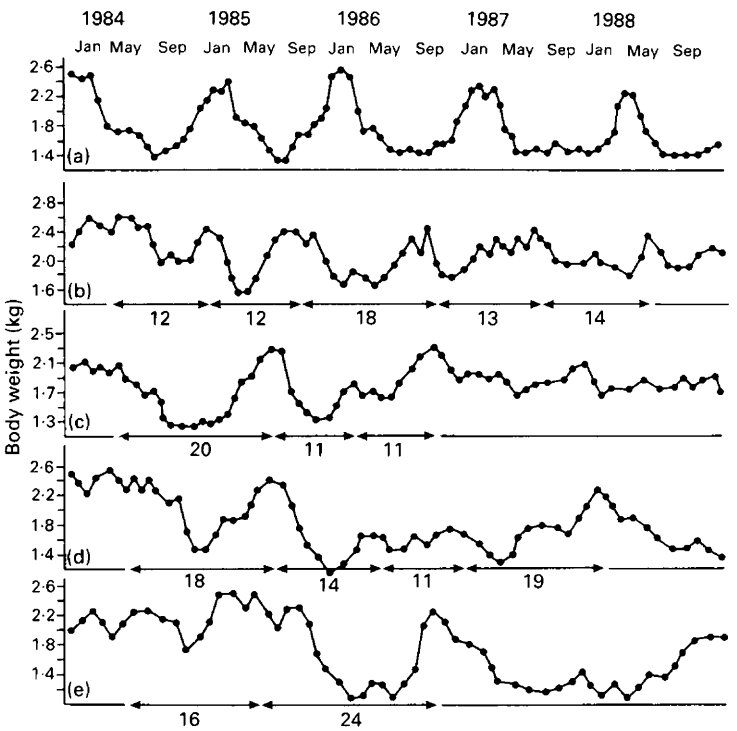


**Fig. 1.** (a) Mean body weight of seven male mink maintained outside and (b–e) individual plots of body weight of four males kept under 8 h light:16 h dark at 13°C. Figures represent the intervals in months between two successive peaks.

Six out of eight males transferred in December under 8 h light:16 h dark at 12–14°C stayed alive throughout the 5 year experimental period. After transfer to short days, body weight and testis size steadily increased to reach peak values in February–March 1984 as in outside controls. All the experimental males then showed a prolonged phase of high weight and reproductive condition. They entered the phase of decrease in both body weight and testis size two to three months later

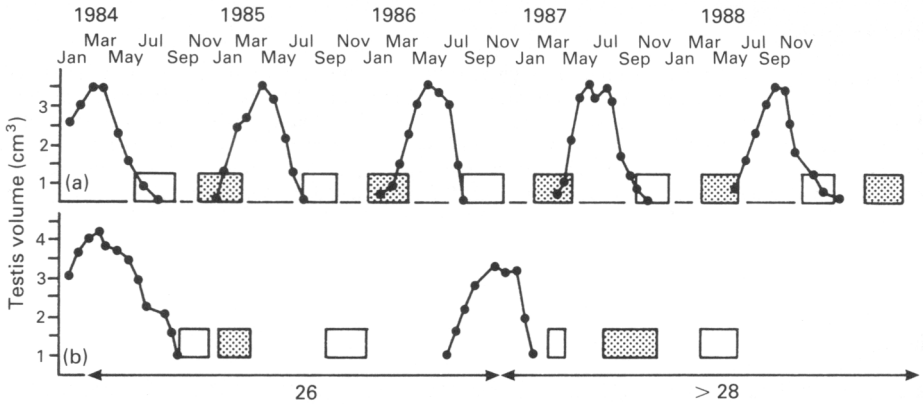


**Fig. 2.** Mean testis volume in (a) seven mink maintained outside and (b) in one male kept under 8 h light:16 h dark at 13°C. Periods where no data are given correspond to the periods when testis in abdominal position could not be measured. Periods of (□) spring and (▨) autumn moults.

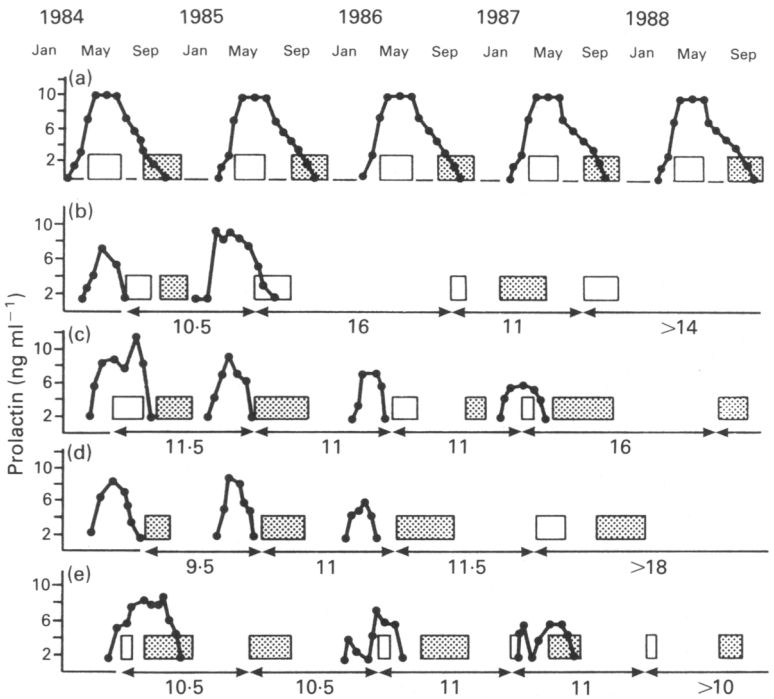


**Fig. 3.** (a) Mean concentrations of prolactin in plasma of seven males maintained outside and (b) prolactin concentrations in four males kept under 8 h light:16 h dark at 13°C. Periods where no data are given correspond to periods when prolactin could not be detected. Periods of (□) spring and (▨) autumn moults. Figures represent the intervals in month between two successive onsets of spring or autumn moults.

than controls (Figs 1 and 2). A similar delay was observed in the onset of increasing concentration of prolactin and spring moult (Fig. 3).

Body weight cycles were evident in four out of the six males that survived throughout the experiment; but these variations were less year after year (Fig. 1). The period of the body weight

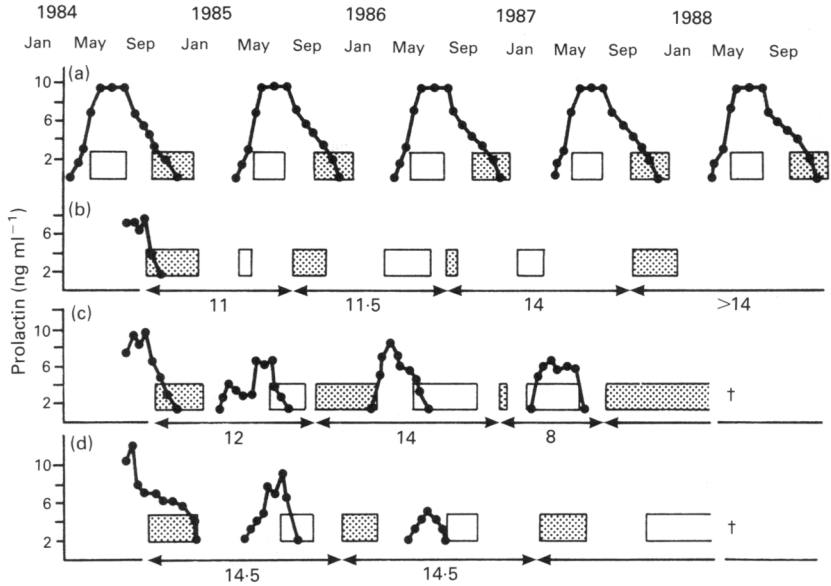


**Fig. 4.** (a) Mean concentrations of plasma prolactin in seven males maintained outside and (b–d) prolactin concentrations in three males kept under 16 h light:8 h dark at 21°C. Periods where no data are given correspond to periods when prolactin could not be detected. Periods of (□) spring and (▨) autumn moult. Figures represent the intervals between two successive onsets of spring and autumn moults. † dead.

cycle measured as the interval between two successive peaks ranged from 11 to 24 months (15·2 ± 1·1 months; $n = 14$). In five out of six males, testis recrudescence was never observed; in the other male, a normal cycle of growth and regression occurred once from March to July 1986, that is 26 months after the preceding one (Fig. 2).

Circannual cycles of spring and autumn moults persisted in four males; the period of the cycles ranged from 9·5 to >18 months (11·6 ± 0·5 in 14 completed cycles); in the two other males (not shown here), brief and partial moults were observed every 4–7 months, probably corresponding to the succession of spring and autumn moults; thus in these two males the period of the rhythm should have varied between 8 and 14 months. Under winter conditions the spring moult was missing in six out of 17 cycles; the autumn moult was missing only once (Fig. 3). Circannual cycles of plasma prolactin concentrations were also evident, the highest concentrations generally occurring before or during the spring moult (Fig. 3). The period of the rhythm oscillated from 9·5 to 13·5 (11·5 ± 0·6; $n = 6$) months in males 1, 2 and 3 and 19·3 to 21·7 in male 4.

When males were transferred in July under summer conditions, body weight and testis size were at their lowest levels, concentrations of plasma prolactin peaked and the spring moult was completed. Only five males out of eight survived for more than 2 years. In these males, body weights exhibited very low amplitude variations so that cycles could not be clearly seen. Testis recrudescence (Fig. 2) never occurred.

Regular recurrence of spring and autumn moults was observed in three males and short phases of hair loss and regrowth in the other two. The period between two successive onsets of autumn moults varied from 8 to 14·5 months (12·4 ± 0·8 months; $n = 8$). Two males exhibited episodic variations in prolactin concentrations; the intervals between successive peaks were 11·8 ± 0·6 months ($n = 5$) (Fig. 4).

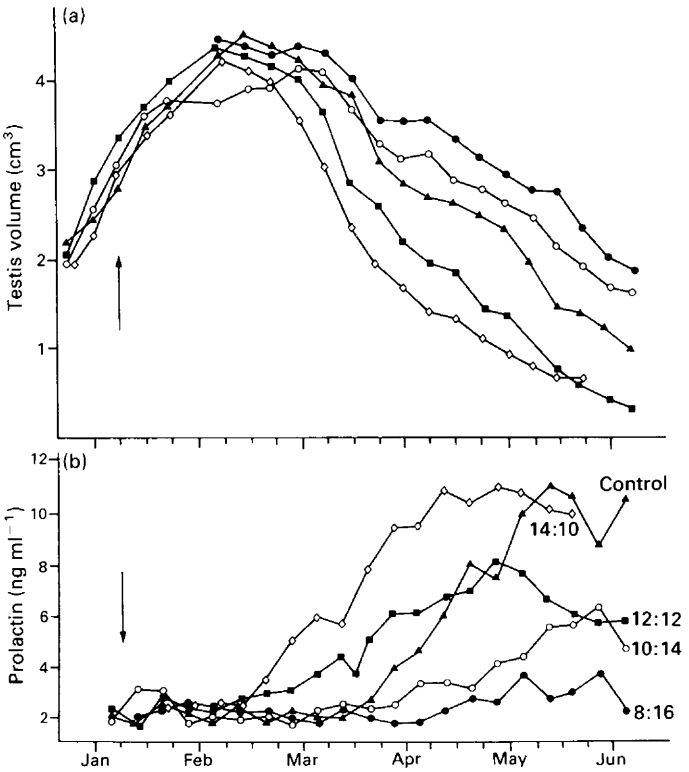


**Fig. 5.** (a) Mean testis volume and (b) concentrations of plasma prolactin in mink kept (▲) outside (control) from 10 February. (●) 8 h light:16 h dark from 21 December; (○)10 h light: 14 h dark from 10 February; (■) 12 h light:12 h dark from 10 February; (◇) 14 h light:10 h dark from 10 February.

## Experiment 2

In control males left under natural conditions, testis volume peaked in mid-February then rapidly decreased (Fig. 2). In males transferred to 8 h light:16 h dark from December, testis size also increased up to February, but regression was delayed until late March, then progressed more slowly than in control mink ($P < 0{\cdot}01$ from 1 April). In the group maintained under 10 h light:14 h dark from 10 February, testis regression was slightly delayed and slower compared with the control group ($P < 0{\cdot}05$ from 7 May). On the contrary, regression was significantly hastened in males transferred to 12 or 14 h of light per day ($P < 0{\cdot}01$ from 15 March and 1 March, respectively) compared with the control group (Fig. 5).

The concentrations of plasma prolactin remained very low in outside males until the spring equinox; they then increased rapidly to reach their highest levels in May (Fig. 5). In the experimental group, the longer the photoperiod, the earlier and faster the rise of prolactin concentrations. Furthermore the peak concentrations were related to the length of the photoperiod ($P < 0{\cdot}01$: control and 14 h light:10 h dark groups versus 12 h light:12 h dark, 10 h light:14 h dark and 8 h light:16 h dark groups (Fig. 5)). In all groups, onset of the spring moult followed the rise in prolactin concentrations, that is on 21 March, 1 April, 15 May and 10 June in 14 h light:10 h dark, 12 h light:12 h dark, 10 h light:14 h dark and 8 h light:16 h dark groups, respectively.

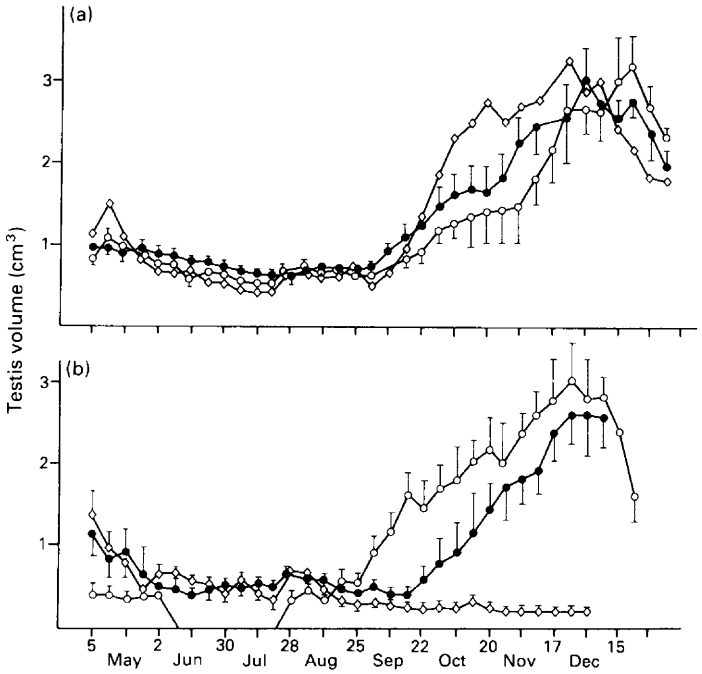


**Fig. 6.** Testis volume (mean ± SEM) of mink triggered (a) by transfer under short days (8 h light: 16 h dark) on 15 May (◇; $n = 1$), 12 June (●; $n = 5$) or 4 July (○; $n = 7$) or (b) by melatonin implants after transfer to long days on 12 June (●; $n = 3$) or 4 July (○; $n = 5$).

### Experiment 3

In males transferred to short days on 15 May, testis regrowth was observed from early September in only one of ten males. Five out of 10 males and seven out of eight males in groups transferred under short days on 12 June and 4 July, respectively, displayed testis recrudescence beginning between 25 August and 10 October (Fig. 6). Recrudescence was preceded by 2–3 weeks by a significant rise in the concentrations of serum FSH and LH (Table 1). Such an increase was not observed in males that did not display testis regrowth. Whatever the period and the testis response to short days, transfer to 8 h light:16 h dark induced a rapid and highly significant decrease ($P < 0{\cdot}01$) in the concentrations of serum prolactin followed 3 weeks later by the onset of the autumn moult (Fig. 7).

Similar results were obtained in males transferred under long days and implanted with melatonin. The concentrations of melatonin in blood sampled during the day ranged from 20 to 85 pg $ml^{-1}$ in control males and 350 to 880 pg $ml^{-1}$ in males bearing an implant, indicating that all the capsules were releasing melatonin. Testis recrudescence did not occur in the six males of group 1, but was observed in three out of five and five out of six males in groups 2 and 3, respectively. The response was faster when melatonin was given from 4 July than from the 12 June (Fig. 6). An increase in the concentrations of serum FSH and LH ($P < 0{\cdot}05$) was observed only in the males exibiting testis recrudescence (Table 1). Melatonin administration was followed in all the males by a sharp decrease in prolactin concentrations and the onset of the autumn moult (Fig. 7).

## Discussion

In the American mink, the annual cycles of body weight, moult and prolactin secretion that persist in the absence of seasonal cues rely on self-sustained endogenous rhythms. But the annual cycle of

**Table 1.** Serum concentrations ($ng\,ml^{-1}$) of follicule-stimulating hormone (FSH) and luteinizing hormone (LH) in males transferred under short days or long days and given melatonin from 12 June (group 2) or 3 July (group 3). Results are given only for males that showed testis recrudescence

| | 8 h light:16 h dark | | 16 h light:8 h dark + melatonin | |
|---|---|---|---|---|
| Date | Group 2 serum concentration ($ng\,ml^{-1}$) | Group 3 serum concentration ($ng\,ml^{-1}$) | Group 2 serum concentration ($ng\,ml^{-1}$) | Group 3 serum concentration ($ng\,ml^{-1}$) |
| FSH | | | | |
| 2 May – 12 June | $3{\cdot}8 \pm 0{\cdot}4$ | $2{\cdot}6 \pm 0{\cdot}2^a$ | $2{\cdot}7 \pm 0{\cdot}2^a$ | $2{\cdot}9 \pm 0{\cdot}2^a$ |
| 19 June – 3 July | $2{\cdot}8 \pm 0{\cdot}3^a$ | $2{\cdot}7 \pm 0{\cdot}3^a$ | $2{\cdot}1 \pm 0{\cdot}1^a$ | $1{\cdot}6 \pm 0{\cdot}1^a$ |
| 10 July – 12 August | $2{\cdot}1 \pm 0{\cdot}3^a$ | $2{\cdot}4 \pm 0{\cdot}2^a$ | $2{\cdot}8 \pm 0{\cdot}3^a$ | $2{\cdot}5 \pm 0{\cdot}1^a$ |
| 20 August – 18 September | $4{\cdot}7 \pm 0{\cdot}6^b$ | $3{\cdot}2 \pm 0{\cdot}3^a$ | $5{\cdot}2 \pm 0{\cdot}4^b$ | $3{\cdot}9 \pm 0{\cdot}3^b$ |
| 22 September – 17 October | $5{\cdot}3 \pm 0{\cdot}6^b$ | $4{\cdot}8 \pm 0{\cdot}4^b$ | $4{\cdot}9 \pm 0{\cdot}4^b$ | $4{\cdot}8 \pm 0{\cdot}4^b$ |
| 23 October – 22 November | $3{\cdot}6 \pm 0{\cdot}3$ | $5{\cdot}1 \pm 0{\cdot}3^b$ | $3{\cdot}7 \pm 0{\cdot}6$ | $4{\cdot}4 \pm 0{\cdot}3^b$ |
| LH | | | | |
| 2 May – 12 June | $1{\cdot}6 \pm 0{\cdot}1^a$ | $1{\cdot}3 \pm 0{\cdot}1^a$ | $1{\cdot}5 \pm 0{\cdot}1^a$ | $1{\cdot}4 \pm 0{\cdot}1^a$ |
| 19 June – 3 July | $1{\cdot}5 \pm 0{\cdot}1^a$ | $1{\cdot}4 \pm 0{\cdot}1^a$ | $1{\cdot}6 \pm 0{\cdot}1^a$ | $1{\cdot}4 \pm 0{\cdot}1^a$ |
| 10 July – 12 August | $1{\cdot}6 \pm 0{\cdot}1^a$ | $1{\cdot}4 \pm 0{\cdot}1^a$ | $1{\cdot}6 \pm 0{\cdot}1^a$ | $1{\cdot}4 \pm 0{\cdot}2^a$ |
| 20 August – 18 September | $2{\cdot}2 \pm 0{\cdot}1^b$ | $1{\cdot}4 \pm 0{\cdot}1^a$ | $2{\cdot}1 \pm 0{\cdot}2^b$ | $1{\cdot}9 \pm 0{\cdot}1$ |
| 22 September – 17 October | $2{\cdot}0 \pm 0{\cdot}1^b$ | $1{\cdot}8 \pm 0{\cdot}2$ | $2{\cdot}3 \pm 0{\cdot}2^b$ | $1{\cdot}8 \pm 0{\cdot}2$ |
| 23 October – 22 November | $2{\cdot}2 \pm 0{\cdot}1^b$ | $2{\cdot}1 \pm 0{\cdot}2^b$ | $2{\cdot}0 \pm 0{\cdot}1$ | $2{\cdot}0 \pm 0{\cdot}2^b$ |

Values are means ($\pm$ SEM). [a]Values are statistically significant from [b]values ($P < 0{\cdot}05$)

gonadal activity seems passively driven by the changes in daylength since it disappears under constant environmental conditions. Furthermore, alternation of periods of sensitivity and insensitivity to the prevailing photoperiod characterized this cycle.

In the long-term experiment during which photoperiod, light intensity, ambient temperature and food were constant, the amplitude in body weight variations was similar in males kept under winter conditions or left in a natural environment, but was attenuated or suppressed under summer conditions. In a hibernator, *Citellus tridecemlineatus*, endogenous circannual rhythms of body weight are expressed under constant environmental conditions at 22°C (Armitage & Shulenberger, 1972), but not at 4 or 11°C (Mrosovsky, 1978). Although amplitude of the circannual cycle of body weight is decreased after gonadectomy, gonadal hormones are not necessary for the generation of these cycles in ground squirrels (Zucker & Boshes, 1982) or pallid bats (Beasley & Zucker, 1986). In the mink, the absence of a cycle of testis activity in addition to summer temperature may decrease the body weight variations. When body weight cycles were observed, they recurred with a period close to one year (eight out of 14 cycles) or longer. The large variation in the periods of recurrence may be due also to the lack of gonadal hormone. Indeed, in ground squirrels, the timing of body weight seems to be modified by gonadal hormones (Zucker & Boshes, 1982).

The cycles of prolactin and moult that were observed under winter and summer conditions recur most frequently with a period close to one year. It must be pointed out that the amplitude in the cycles of prolactin concentrations was similar under constant long or short days, although in the mink, as in other photosensitive mammals, prolactin secretion is reduced by decreasing daylength (Martinet *et al.*, 1984). Circannual rhythms of prolactin also persist in ewes maintained for 5 years in short days (Karsch *et al.*, 1989); however, the rhythmicity seems to disappear in rams maintained under short days (Howles *et al.*, 1982).

Endogenous circannual cycles of moulting have been reported in several species. In the thirteen-lined ground squirrel, moulting cycles that occur in the field during the weight gain phase of the body weight cycle do not depend on the body weight cycle (Joy & Mrosovsky, 1982). In mink, under natural conditions, the spring moult followed the sharp decrease in body weight at the end of

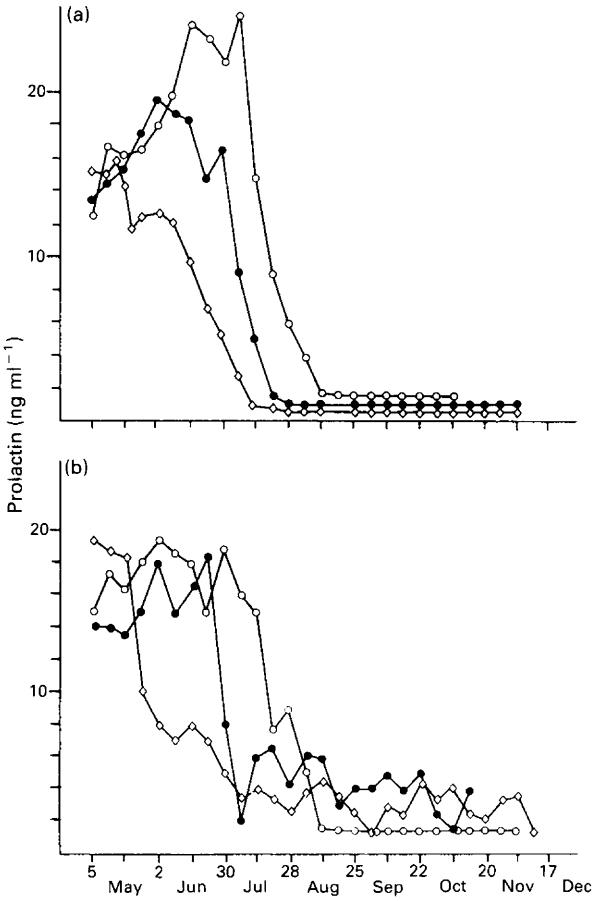


**Fig. 7.** Mean concentrations of prolactin in mink transferred under (a) short days (8 h light:16 h dark) or (b) long days (16 h light:8 h dark) and given melatonin implants. Transfer on (◇) 15 May, (●) 12 June, and (○) 4 July.

winter and the autumn moult occurred during the weight gain phase. Under constant laboratory conditions, this phase relationship was gradually lost. The persistence of moulting period in mink with permanently suppressed gonadal activity supports a previous demonstration of the lack of regulation of annual changes in the pelage of mink by gonadal hormones (Allain & Martinet, 1986).

Endogenous circannual cycles of body weight, prolactin secretion and moulting are also displayed by pinealectomized (Martinet & Allain, 1985; Boissin-Agasse *et al.*, 1988) or ganglionectomized (Martinet *et al.*, in press) mink. The pineal gland, which mediates, through its rhythmic secretion of melatonin, the entrainment of endogenous cycles of prolactin secretion, body weight and pelage changes by the annual changes in daylength, is therefore not essential for the generation of circannual rhythms, although it seems to modulate their period (Zucker, 1985).

The absence of circannual rhythms of testis activity in mink reared in constant conditions should be emphasized. In many mammals maintained in constant conditions, phases of testis growth and regression leading to cycles of approximately one year have been observed (Howles *et al.*, 1982; Licht *et al.*, 1982; Beasley & Zucker, 1986). In the closely related ferret kept in constant light and temperature, the testis size displays spontaneous variations with a period slightly shorter than one year (Boissin-Agasse *et al.*, 1985); in blind or pinealectomized ferret females, recurrence of

oestrus was observed, but without a fixed periodicity (Herbert *et al.*, 1978). One possibility to explain the lack of an endogenous circannual cycle of testis activity in mink is that it can be expressed only within a narrow range of photoperiods, as reported for cycles of antler growth in the Sika deer (Goss, 1984). If an endogenous cycle in testicular activity exists, it needs some kind of photoperiodic signal to be expressed since it does not appear in pinealectomized mink (Boissin-Agasse *et al.*, 1988; Martinet *et al.*, in press).

Seasonal cycles of body weight, prolactin secretion, moulting and testis activity observed in mink caged under natural environmental conditions may be entrained by the annual changes in daylength or temperature or both. In fact, high temperature, which increases prolactin secretion (Tucker & Wetterman, 1976), could trigger the onset of autumn moult in minks (Martinet *et al.*, 1984). However, two observations are not consistent with this role of temperature. First, the timing of seasonal cycles of moulting and testis activity is identical in mink farms, whatever the latitude, for example from northern Scandinavia to Spain. Second, decreasing temperature from 22 to 12°C or increasing it from 12 to 22°C did not alter changes in hair follicles and testis activity of mink maintained under long or short days, respectively (Allain & Martinet, unpublished observation).

Under natural conditions, regression was observed from mid-February when daylength went beyond 10 h of light in 24 h which seems to be the critical threshold for induction of a functional testis (Boissin-Agasse *et al.*, 1982). In the second experiment, the testis regression that was delayed, but not suppressed, by keeping males under winter solstice conditions showed photorefractoriness to short days. However, this photorefractoriness demonstrated under artificial photoperiod is probably not used under natural conditions. Indeed, after the winter solstice, males remained photodependent since regression could be hastened by photoperiods longer than the natural one. We therefore suggest that, in minks, cessation of breeding may reflect photosuppression by increasing daylength rather than photorefractoriness to short days. The onset of testis regression was not related to prolactin secretion, since it was observed whatever the photoperiod and the prolactin concentrations; the same observation has already been reported in ewes (Worthy & Haresign, 1983; Curlewis *et al.*, 1991). Testicular regression resulting from photorefractoriness to short days was identical to that reported in males given melatonin capsules in January (Allain *et al.*, 1981). In sheep (Karsch *et al.*, 1986) and hamsters (Bittman, 1978), gonadal photorefractoriness is caused by loss of response of target cells to the melatonin signal.

The results obtained in the third experiment do not allow us to rule out the development of photorefractoriness to the stimulatory action of short days on the testis. Indeed, the later the transfer under short days in the spring, the higher the number of males responding by a rise in FSH and LH secretion and testis recrudescence. As in seasonal mammals such as foxes (Smith *et al.*, 1987) or rams (Lincoln, 1989), the cycle of testis activity is dictated by the changes in the secretion of FSH and LH; there is a close relationship between the increase of both hormones and the testis regrowth induced by a switch from long to short days or by treatment with melatonin which mimicks the effects of short days (Lincoln & McNeilly, 1989).

Ninety per cent of the males submitted to short days from early July responded. As 12 h light in 24 h is read as a long day by the pituitary–gonadal axis in mink (Boissin-Agasse *et al.*, 1982), this species might need 12–15 weeks of long days to regain their sensitivity to the stimulatory action of short days. Hamsters also need 12–18 weeks of long days to restore responsiveness to the inhibitory action of short days on the maintenance of testis activity (Stetson *et al.*, 1977). In ewes, only thirty days of an opposite photoperiod seem sufficient to break refractoriness to long or short days (Jackson *et al.*, 1988).

Contrary to the response of gonadotrophins, transfer to short days was always followed by a sharp decrease in concentration of peripheral prolactin and the onset of an autumn moult; prolactin did not seem to interfere with the timing of gonadal activity.

The effects of shortened photoperiod on the concentrations of serum FSH, LH and prolactin, onset of moult and testis recrudescence were perfectly mimicked by melatonin capsules in mink maintained under long days. This similarity supports the role of the pineal gland in transducing the

effect of photoperiod as an endocrine message. Furthermore, our data corroborate results obtained in ewes (Karsch *et al.*, 1986), namely the loss of response to prevailing photoperiod seems to result from a loss of sensitivity of the target organ to melatonin (Bittman, 1978). The continuous release of melatonin by subcutaneous capsules is not identical to the rhythmic secretion of melatonin by the pineal gland; in the mink (Bonnefond *et al.*, 1991) as in other mammals (Arendt, 1986), the duration of melatonin secretion is a critical parameter for transducing photoperiodic information to the hypothalamo–pituitary axis. The results observed with melatonin implants suggest that rhythmic secretion is not necessary but that melatonin has to be present every day for a minimum duration.

In conclusion, the annual cycles of prolactin secretion, body weight and pelage changes exhibited by male mink under natural conditions reflect the existence of endogenous circannual rhythms entrained by increasing and decreasing daylength. In contrast, the annual cycle of testis activity could rely almost totally on photoperiodism. Photorefractoriness or inhibition by increasing days in late winter or both could contribute to the cessation of the breeding season under natural environmental conditions.

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