AN ABSTRACT OF THE THESIS OF

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The relationship of glucocorticoid secretion to light-induced, early-winter priming of pelage in ranch mink was investigated. Sixteen Standard Dark, mature, female mink were exposed to natural light conditions of 45° North latitude beginning on December 13, 1977. A 2x4 cm area of the right hip of each animal was sheared permitting measurement of changes in fur growth. At biweekly intervals blood samples were obtained by cardiac puncture and the length and width of the vulva measured. On June 26, 1978 the remaining animals were assigned at random to two groups and an additional five animals were placed in each group (total, eight animals per group). Group I animals were housed in a light-control facility and subjected to 6 hours of artificial light daily from June 26 to November 27, 1978. Group II animals served as controls and were exposed to natural light conditions. Blood samples, fur and vulva measurements continued to be collected biweekly. Serum concentrations of total glucocorticoids were determined by competitive protein binding.

Changes in the pelage of mink reared under natural light conditions were typical. The pelage of mink reared under reduced lighting was fully prime by October 30, as compared with the pelage of control animals which was prime by November 27 (time x light regime interaction, $P<.01$). A significant seasonal effect ($P<.01$) on serum total glucocorticoid levels in control mink revealed a pattern of three peaks over the 12-month period. The pattern of glucocorticoid secretion was not affected by exposure of mink to reduced lighting. Maximal concentrations of serum total glucocorticoids occurred on the same date (September 5) in both groups although levels were greater in the light-treated (44.5 ± 10.4 ng/ml) than in the control mink (26.9 ± 5.7 ng/ml). Fur growth was found to be positively correlated with serum total glucocorticoid concentrations in both light-treated and control animals during the period from June 26 to December 21, 1978 (overall $r = .14$, $P<.05$). Both the length and width of the vulva increased ($P<.01$) between February 21 and March 20 in animals reared under natural lighting. No changes were observed in the size of the vulva in control or light-treated mink during the remainder of the year. These data suggest that some light-induced factor acts alone or in concert with glucocorticoids to promote pelage changes in the mink.

Photoperiodic Effects on Serum Glucocorticoids and Fur Growth in Mink

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REVIEW OF LITERATURE

Pelage Cycle of the Mink

Adult mink grow two coats of fur a year which differ in length, density and color. The economically important winter fur growth is normally initiated in early September and the pelage reaches primeness between late November and early December (Bassett and Llewellyn, 1949; Stout et al., 1969). The prime winter pelage is thick and glossy and the skin color is creamy white. The latter characteristic is used as the major criterion of primeness. As winter progresses the pelt fades and the luster of the guard hair decreases and becomes dull. The spring molt is initiated in mid-April and the summer fur growth is completed around mid-July. The summer pelage is less dense than the winter coat and the fur color is lighter and less intense (Bassett and Llewellyn, 1949). Changes that occur during the fur growth cycle are gradual and the various stages which occur in the development of either the winter or summer pelage are difficult to distinguish.

Mink kits, born in late April and early May, exhibit a slightly different molting cycle than the adult mink. Born without fur, mink kits first develop a kit fur before molting and growth of the normal summer pelage. The winter pelage cycle remains the same as in adult mink. Kits demonstrate extremely rapid body growth from birth to maturity. By October they attain almost maximum body size, thereafter gaining only 5 to 10% more weight which mostly represents fat deposition

(Stout and Adair, 1969).

Effects of Light Manipulation on the Pelage Cycle

It has been known for some time that the pelage cycle of the mink is under the influence of day length. Bissonnette and Wilson (1939) demonstrated that changes in the duration of daily exposure to light, irrespective of temperature, conditioned changes in the pelage of mink. Temperature is not a major factor in pelage changes of ferrets and weasels, relatives of the mink (Hammond, 1952; Rust, 1962), although it has been found to have a modifying influence on the speed and nature of the spring pelage change of weasels (Rust, 1962). In weasels, reduction of daily photoperiod was shown to induce molting and growth of fur, together with a color change from the summer to the winter pelage (Bissonnette and Bailey, 1944). An increase in daily photoperiod caused the reverse pelage change from the winter to the summer fur coat. A latent period was observed between the time of increasing light and the shedding and regrowth of hair (Bissonnette and Bailey, 1944).

Domestic animals also demonstrate a photoperiodic influence on hair growth. Cattle shed their coats under the influence of daily photoperiod (Yeates, 1955). In both control and experimental animals in which a six-month shift in the length of daily photoperiod was established using artificial lighting, as light decreased there was shedding and stimulation of new growth, including an increase in the thickness of the coat (Harvey and MacFarlane, 1958). Ewes subjected to eight hours of light year-round produce an increase in wool weight over controls

exposed to natural light (Hart, 1953).

Research was begun in 1965 at Oregon State University to determine whether the winter fur growth cycle of the mink could be shortened appreciably by manipulation of the length of daily photoperiod. Mink exposed to a reduced lighting schedule of one or nine hours of light daily, beginning June 21 showed fur growth as early as August 8 and were fully prime and pelted on October 1. Control animals exposed to natural light conditions during the same period were prime on December 9 (Stout et al., 1969). The body sizes of early-priming animals were 86 and 89% of control and the pelts averaged 30 inches in length (Stout et al., 1969). Further investigations of the effects of artificially controlled photoperiod on both the summer and winter pelage cycles confirmed that abrupt light changes are as effective as gradual light changes in inducing winter fur growth in mink (Duby and Travis, 1970). An extended refractory period was found to exist in the molt from winter to summer pelage whereas a refractory period between completion of the growth of the summer pelage and initiation of the winter molt was undetectable (Duby and Travis, 1970).

Reproductive Cycle of the Mink

In addition to a photoperiodically-cued pelage cycle, mink display a seasonal estrous cycle. Gonadal development begins in early January and is completed in late February or early March. The breeding season lasts until late March when the gonads begin to regress.

The effect of light on estrus in mink and related species is well documented. Artificial light treatment causes alterations in the time

of gonad activity in both ferrets (Bissonnette, 1932; Hammond, 1951; Donovan and Harris, 1954; Herbert, 1969) and mink (Hammond, 1951; Duby and Travis, 1970). By increasing the photoperiod, the onset of estrus can be induced to occur during the nonbreeding season in these species. The effect of light on estrus is not a result of direct stimulation of the gonads as its effects depend upon the presence of an intact pituitary gland (Hill and Parkes, 1933) and the integrity of the hypophysial portal blood vessels (Donovan and Harris, 1954). The degree of gonadal stimulation attained using artificial light appears to be proportional to the length of the photoperiod above a threshold level (Hammond, 1953).

The Pineal as Mediator of Photoperiodic Responses

The role of the mammalian pineal gland and its major secretory product, melatonin, as the mediator of photoperiodic responses is well documented in ferrets (Herbert, 1969; Herbert, 1972), hamsters (Hoffman and Reiter, 1965; Reiter, 1973), hares (Lincoln, 1976) and ewes (Rollag et al., 1978). Melatonin, a methoxyindole, was first isolated and identified by Lerner et al. (1958, 1959, 1960) from the bovine. It is synthesized by the pineal in response to post-sympathetic nervous release of norepinephrine (Axelrod and Wurtman, 1967). Levels of norepinephrine decrease as light activates photoreceptors in the retina. The ganglion layer of the retina is essential for the gonad stimulating influence of light in the ferret (Farner, 1958). Retinal projections directly to the suprachiasmatic nucleus have been demonstrated in rats and ferrets (Mason, 1975; Thorpe, 1975). The major tract involved is

the inferior accessory optic tract which terminates in the suprachiasmatic nucleus. Small lesions in the suprachiasmatic region in rats interfere with light influenced estrous responses (Critchlow, 1963). Fibers originating from this point synapse within the superior cervical ganglia. The cell bodies of the sympathetic nerves that innervate the pineal lie within the superior cervical ganglia. The axons of these cell bodies make very close contact at their terminus with the pinealocytes (Axelrod and Wurtman, 1967). Superior cervical ganglionectomy blocks the pineal response to light (Wurtman and Anton-Tay, 1969) and also blocks the gonadal response of ferrets (Herbert, 1969).

The synthesis of melatonin by the pinealocytes requires the transfer of a methyl group from s-adenosyl-methionine to the 5-hydroxy of the indole, n-acetylserotonin. The enzyme involved in this reaction, hydroxyindole-o-methyl-transferase (HIOMT), is the rate-limiting enzyme in the synthesis of melatonin (Wurtman et al., 1963). Recently, N-acetyltransferase, another important enzyme involved in the synthesis of melatonin, has been suggested to play a rate-limiting role in addition to HIOMT (Binkley, 1979). The activity of HIOMT appears to be regulated by photoperiod, increasing when rats are maintained in constant darkness and decreasing under constant illumination (Wurtman et al., 1963). Thus, constant light inhibits melatonin synthesis in the pineal (Wurtman et al., 1963) by way of its effects on HIOMT activity.

The gonadal, and possibly the pelage cycle, photo-response of mink may be mediated by changes in melatonin secretion from the pineal (Wurtman et al., 1964a) and the subsequent action of this hormone on the hypothalamic-pituitary axis (Hill and Parkes, 1933; Donovan and Harris,

1954). Organs containing endocrine or nervous elements, such as the ovary and pituitary, have the capacity to concentrate melatonin several-fold from the circulation (Wurtman et al., 1964b). Melatonin has been shown to deplete pituitary stores of melanocyte-stimulating hormone (MSH) in rats (Kastin and Schally, 1966) and pinealectomy has the opposite effect (Kastin et al., 1967). Melatonin administered to weasels was found to inhibit testicular growth and development of summer pelage (Rust and Meyer, 1969). In contrast, daily administration of melatonin to ferrets during anestrus advanced the onset of estrous activity (Thorpe and Herbert, 1974) and pinealectomy abolished the gonadal response of ferrets to increased lighting (Herbert, 1969). The apparent contradictory actions of melatonin in these experiments can be explained by the inclusion of a daily critical period in the scheme for photoperiodic control of reproduction and possibly fur growth. If the time of day during which melatonin concentrations are elevated, rather than the duration of increased melatonin concentrations, acts as the cue for photo-induced changes these results are no longer incongruent. If this premise is true, melatonin injected during different times of the day could produce these paradoxical results. This is supported by the observation that melatonin injections in the morning did not affect reproductive competence in hamsters, whereas injections in the afternoon caused gonadal regression (Tamarkin et al., 1976).

No neural or vascular connections between the pineal gland and the hypothalamus have yet been observed therefore the route of melatonin secretion and its transport to the hypothalamus is not known with

certainty. The pineal gland is located in the roof of the third ventricle and melatonin has been found in the cerebrospinal fluid in concentrations greater than that of the circulation (Rollag et al., 1978). The walls of the third ventricle contain cells, called tanycytes, with long processes that terminate on the portal blood vessels (Martin et al., 1977). These cells appear to be transport cells as they display the typical characteristics of such cells; smooth endoplasmic reticulum and prominent Golgi bodies. Melatonin may therefore be secreted by the pineal gland into the cerebrospinal fluid of the third ventricle from whence it is transported to the portal blood system of the hypothalamic-pituitary axis by the tanycytes.

Hormonal Effects on Pelage

The mechanisms by which photoperiodic changes in melatonin concentration produce photo-induced responses in mink are unknown. The cyclic molting and regrowth of hair in ferrets, weasels and mink requires the presence of the pituitary gland (Bissonnette, 1935; Rust and Meyer, 1969; Rust et al., 1965). Hypophysectomized animals in which fur growth was initiated by plucking always regrew winter coats regardless of the length of the photoperiod (Rust et al., 1965). Administration of luteinizing hormone (LH), follicle-stimulating hormone (FSH) or thyroid-stimulating hormone (TSH) to hypophysectomized animals had no effect whereas adrenocorticotropin (ACTH) or melanocyte-stimulating hormone (MSH) administered to these animals restored the normal change from the winter to the summer pelage after growth was initiated (Rust et al., 1965). Administration of ovine gonadotropin along with ACTH to hypophysectomized weasels

stimulated molting in addition to restoring the normal pelage changes (Rust, 1965). Administration of melatonin or its precursors has been reported to inhibit the secretion of ACTH by the anterior pituitary (Butte et al., 1976). Thus, the effects of photoperiod and the pineal on the pelage cycle of the mink may be mediated through the secretion of ACTH by the anterior pituitary.

Adrenocorticotropin may possibly be acting directly on the hair follicles to stimulate melanogenesis or it may be acting indirectly via its effects on adrenal corticoid secretion. This is somewhat paradoxical as the effects of ACTH are stimulatory whereas the adrenal gland appears to have a retarding influence on hair growth. Hair growth is accelerated by adrenalectomy in the rat (Butcher, 1937; Dieke, 1948) and local application of adrenal corticoids retards hair growth (Baker, 1951). In mink, bilateral adrenalectomy hastens the spring pelage change while administration of hydrocortisone to intact mink before or during the spring molt inhibits the change (Rust et al., 1965).

There is some evidence that melatonin may directly modulate adrenal steroidogenesis. Melatonin has been shown to inhibit corticosteroid biosynthesis by preparations of bovine adrenal cortex in vitro (Mehdi and Sandor, 1977). Melatonin has also been shown to stimulate 5α-reductase activity in rat adrenals in vitro so that 5α-dihydro and 3β,5α-tetrahydrocorticosterone increase with a proportionate concomitant decline in the secretion of corticosterone (Ogle and Kitay, 1978). The direct effects of melatonin in suppressing adrenal steroidogenesis most likely occur coincident with its ability to inhibit secretion of ACTH by the pituitary. This latter effect of melatonin probably

contributes to the overall reduction in corticosteroid secretion.

Hormonal Effects on Reproduction

As with the pelage cycle, photoperiodic and pineal influenced changes in the reproductive cycle of the mink require the presence of the pituitary (Hill and Parkes, 1933). The role of the pituitary in mediating reproductive responses to light is uncertain. Secretion of prolactin (PRL) by the pituitary appears to be particularly sensitive to changes in photoperiod. Kamberi et al. (1971) observed a dose-dependent increase in plasma PRL concentration in rats following injection of melatonin into the third ventricle. Intravenous injection of melatonin or its precursor, 5-hydroxytryptophan, also produced a significant increase in serum PRL in rats and mice (Lu and Meites, 1973; Larson et al., 1977). The rat pituitary appears to have a tendency to release PRL spontaneously in a circadian pattern (Tindal, 1974). Rats kept in constant darkness have higher circulating PRL concentrations than rats kept in a light-dark regimen or constant light (Relkin, 1972). This effect is abolished after pinealectomy (Relkin et al., 1972). Meier et al. (1969, 1971b), utilizing the white-throated sparrow as a model, found that reproduction, and a number of other seasonally conditioned physiological events in this species appears to be regulated by circannian rhythms of PRL release. These rhythms are a result of an annual modulation of the daily cycle of PRL secretion which itself is regulated in some way by the temporal synergism with corticosterone secretion. It is possible that photoperiodic regulation of the breeding and pelage cycle of the mink occurs in a similar manner;

through the seasonal release and temporal synergism of PRL and glucocorticoids or ACTH.

STATEMENT OF THE PROBLEM

A mink with a fully prime pelt and with almost normal body size can be produced by October by manipulation of the light environment to which the animal is exposed. Acceleration of the normal furring cycle is possible for the following reasons. Mink kits, born in late April and early May, reach approximately mature body size by September after which the animal gains only 5 to 10% more body weight, primarily in the form of fat deposition. The fur growth cycle is initiated in early September and pelage growth is completed between mid-November and early December. In addition, the pelage cycle is regulated in some way by the decrease in daylight which occurs at this time of year.

The economic potential of early-winter priming to the mink producer is very great. Feed, labor, storage and equipment costs can be substantially reduced if 45 to 60 days can be eliminated from the time required to produce a prime pelt. Mortality as a result of adverse weather conditions can be diminished and disease loss reduced due to the shortened lifespan of the animal.

The major problems involved in the use of artificial illumination to induce early-winter priming are the costs of construction of special facilities or modification of existing facilities and the uncertain effects on the breeding cycle of potential breeders so exposed. The investigation of the physiological and endocrinological factors which underline the phenomenon of early-winter priming might lead to the development of methods which would eliminate these problems.

INTRODUCTION

Mink exhibit a seasonally determined pelage cycle. It is possible to induce early winter priming (in mid-October) by exposing mink to a reduced photoperiod beginning in June (Stout et al., 1969). The physiological pathways by which light may regulate pelage are not known but may involve the pineal, pituitary and adrenal glands. It has been demonstrated that duration of light exposure markedly affects the concentration of melatonin in the pineal gland (Wurtman and Axelrod, 1967). Administration of melatonin or its precursors has been reported to inhibit the secretion of adrenocorticotropin (ACTH) by the anterior pituitary (Butte et al., 1976). It is conceivable that seasonal variations in the secretion of ACTH may be related to changes in the pelage. Rust et al. (1965) found that hypophysectomized mink and weasels exhibit a series of asynchronous molts always followed by regrowth of the winter pelage. Administration of ACTH to hypophysectomized animals inhibited normal winter pelage changes (Rust et al., 1965). These data suggest that the winter pelage may be the prevailing fur and that ACTH either directly or indirectly by way of its stimulated increase in the secretion of corticoids, is required for the normal molting process and development of the summer fur. The absence of this hormone as a consequence of hypophysectomy or decreased photoperiod results in the growth of winter fur.

The present study was conducted to determine whether changes in the secretion of glucocorticoids are associated with observed changes in pelage. Variations in vulva diameters were also noted to determine if these could be used as an indication of the sexual maturation of the animals.

MATERIALS AND METHODS

Beginning December 13, 1977, sixteen Standard Dark, mature, female mink were exposed to natural light conditions of 45° North latitude (range in day length, 8.75 hr Dec to 15.5 hr June). A 2x4 cm area of the right hip of each animal was sheared permitting measurement of fur growth at biweekly intervals. Concomitant with measurements of fur growth, blood samples were obtained between 1200 and 1400 hr by cardiac puncture under mild anesthesia (.6 cc Ketamine hydrochloride, IM) for subsequent determination of serum glucocorticoids. Preliminary investigations revealed the optimum time for blood collection to be 12 minutes following administration of anesthesia. Although serum glucocorticoid concentrations in response to anesthetic stress attained maximal levels between 8 and 12 minutes following injection, the least amount of variability among animals ($P<.005$) was exhibited at 12 minutes. Samples of blood were allowed to clot and after 24 hr were centrifuged at 4°C for 10 minutes. The resulting sera were frozen at -20°C until assayed. Measurements of the length and width of the vulva were also obtained biweekly as an indication of the reproductive condition of the animals (Pilbeam et al., 1979).

On June 26, 1978 the remaining six animals (ten had died as a result of cardiac puncture) were assigned at random to two groups and an additional five animals were placed in each group (total, eight animals per group). Group I animals were housed in a light-control facility (Stout et al., 1969) and subjected to six hours of artificial light daily from June to November 27, 1978. Group II animals served as controls and were exposed to natural light conditions. Blood samples

continued to be collected biweekly along with fur and vulva measurements.

A modification of the competitive protein binding (CPB) procedure of Murphy (1967) was used for determination of serum total glucocorticoids. Corticosteroid-binding globulin (0.5% CBG) was prepared using human, male serum (0.5 ml) and 2.22×10^7 dpm ^{3}H-cortisol (10 µCi/mmol, New England Nuclear) made up to 100 ml in distilled water. Total binding of CBG averaged 58.2% (N=5). Human CBG has been shown to cross-react most appreciably with progesterone (Murphy, 1967). A method of extraction was employed which removed an average of 97.0 $\pm$ 0.8% (N=3) of the progesterone from the serum. Eleven thousand dpm ^{3}H-cortisol (10 µCi/mmol, New England Nuclear) was added to 1.0 ml serum to facilitate the determination of procedural losses. The samples were extracted twice with 5 ml redistilled iso-octane, and the organic solvent phase containing most of the progesterone was discarded. Samples were then extracted twice with 5 ml of redistilled dichloromethane and the combined extract was evaporated to dryness and reconstituted to a volume of 3 ml with dichloromethane. The efficiency of extraction, as determined by the recovery of labelled cortisol, averaged 67.2 $\pm$ 0.5% (N=7). One milliliter of each extract was assayed in duplicate. Two sets of standard tubes containing 0, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0 and 10.0 ng cortisol in ethanol were included in each assay. The sensitivity of the assay was determined to be 2.5 ng/ml ($P<.01$, N=9) and solvent blanks always averaged less than this value. Sample and standard tubes were evaporated to dryness and the CBG-^{3}H-cortisol was added in a volume of 1000 microliters. Samples and standards were

incubated in a water bath at 45-52°C for five minutes followed immediately by a ten minute incubation on ice. Separation of free and CBG-bound glucocorticoids was accomplished by the addition of 80 mg of activated Florisil, 60/100 mesh (Fisher Scientific Co.). A 0.3 ml aliquot of the supernatant was counted in 2.2 ml of scintillation fluid (7 gm/l; 2,5-diphenyloxazole in Toluene:Triton X-100, 2:1).

Accuracy of the glucocorticoid CPB was determined by adding known amounts of cortisol (2.0 and 6.0 ng/250 µl, N=5) to a serum pool obtained from female mink (7.3 ± 0.3 ng/250 µl, N=5). After CPB and the subtraction of the glucocorticoid concentrations of the pooled serum, the following amounts were recovered: 2.7 ± 1.5 and 5.1 ± 1.3 ng/250 microliters. The intra- and interassay coefficients of variation were 9.1% and 14.9%, respectively.

Although the experiment was designed to utilize a split-plot analysis of variance, due to the large loss of animals as a result of the sampling method, 12-month data for mink housed under natural light were first analyzed by use of one-way analysis of variance. Differences between means were tested for significance using the least-significant difference test. It is recognized that this method does not account for the error which is derived from repeated measures on the same animal. Data obtained during the period from June 26 to November 27, 1978, from animals reared under natural light and those reared under reduced light were analyzed by least-squares analysis of variance. Correlations between fur growth and total serum glucocorticoid concentrations were calculated.

RESULTS

Changes in pelage of mink reared under natural light conditions were typical of the annual furring cycle for this species (Fig. 1A). Development of the summer pelage began in late March and was completed by early July. Development of the winter pelage in those animals reared under reduced photoperiod (Group I) as well as controls (Group II) began at the same time (mid-August). However, time required to attain prime pelage (October 31 vs. November 13) was less in animals subjected to a reduced photoperiod than control animals (time x light regime interaction, $P<.05$). Length of prime pelage did not differ between groups of mink.

The patterns of glucocorticoid secretion in mink reared under natural lighting and reduced photoperiod are depicted in Fig. 1B. A significant seasonal effect ($P<.01$) on serum total glucocorticoid levels in control mink revealed a pattern of three peaks over the 12-month period. The first peak occurred in January (14.9 ± 6.4 ng/ml) followed by a smaller peak on May 1 (9.1 ± 1.1 ng/ml) and a third peak in levels between August 23 and October 2 (26.9 ± 5.7 ng/ml). The pattern of glucocorticoid secretion was not affected by exposure of mink to reduced photoperiod. Animals reared under reduced photoperiod exhibited a similar increase in serum total glucocorticoid concentrations between August 23 and October 2. Overall glucocorticoid levels were significantly greater ($P<.05$) in the reduced-photoperiod mink than in the control mink with maximal concentrations of 44.5 ± 10.4 and 26.9 ± 5.7 ng/ml, respectively. Fur growth was found to be positively correlated with glucocorticoid concentrations in both reduced-photoperiod

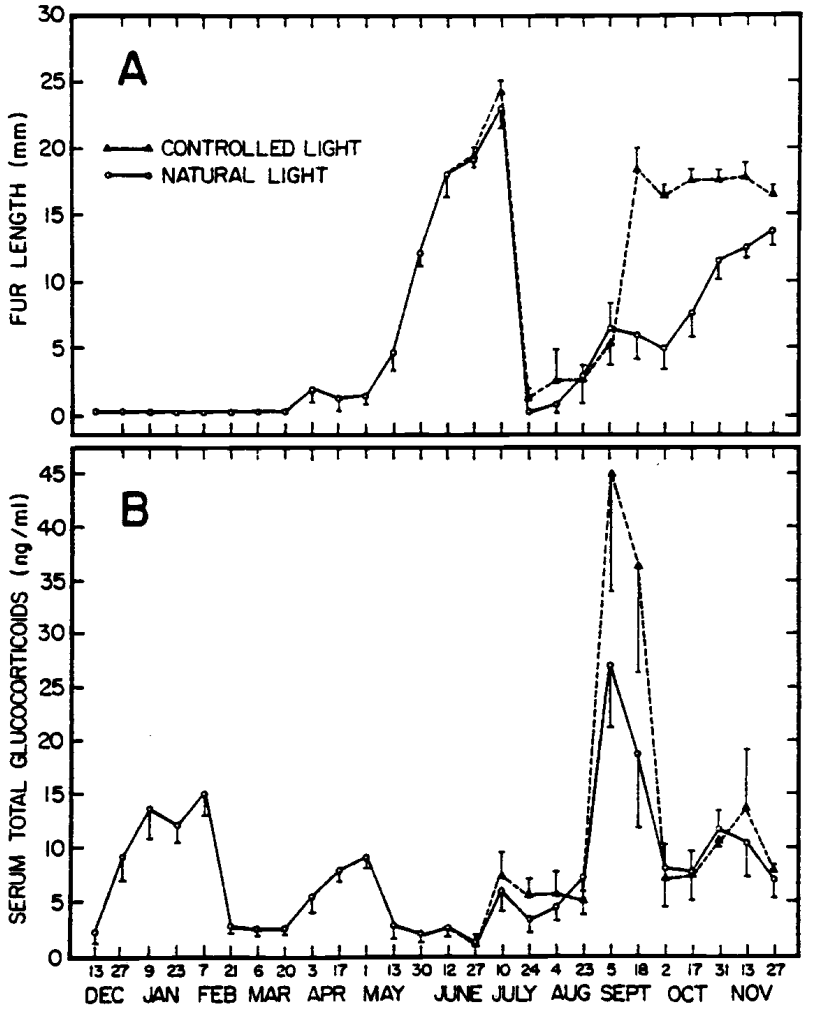

Figure 1. Changes in fur growth (A) and serum total glucocorticoid concentrations (B) of mink exposed to natural light conditions from December 13, 1977 to November 27, 1978 and of controlled-light mink from June 26 to November 27, 1978. The marked decrease in fur length on July 10, in both groups, indicates the date that the 2x4 cm area of the summer pelage (which was fully developed) was sheared. Each point represents the mean $\pm$ SE.

and control animals during the period from June 26 to November 27, 1978 (overall $r=.14$, $P<.05$).

Both the length and width of the vulva were significantly increased ($P<.01$) throughout the period from February 21 to March 20 in animals reared under natural lighting (Fig. 2). No changes were observed in the size of the vulva in either the control or the reduced-photoperiod mink during the remainder of the year.

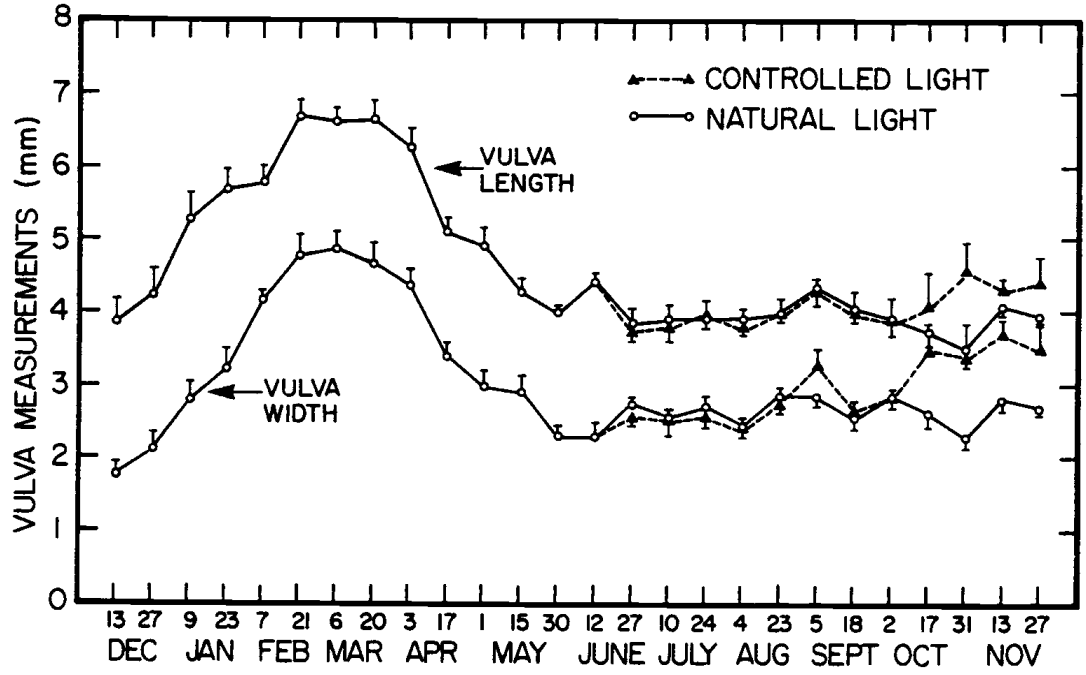

Figure 2. Changes in vulva length and width of mink exposed to natural light conditions from December 13, 1977 to November 27, 1978 and of controlled-light mink from June 26 to November 27, 1978. Each point represents the mean $\pm$ SE.

DISCUSSION

Changes in the patterns of fur growth observed in this experiment in both the animals subjected to a reduced period of lighting and control animals were similar to the changes described by previous investigators (Stout et al., 1969). Serum total glucocorticoid concentrations were found to vary seasonally which is in agreement with data reported for the rat (Butte et al., 1976) and white-throated sparrow (Dusseau and Meier, 1971). The largest increase in glucocorticoid concentrations occurred in September, at a time when daily photoperiod is decreasing. In those animals subjected to experimentally reduced periods of light (Group I) the increase in glucocorticoids which occurred in September was markedly greater than that of controls. Thus, the relationship between photoperiod and glucocorticoid concentrations observed in mink during September or when mink are exposed to reduced periods of light experimentally is opposite to that reported in rats (Butte et al., 1976) in which decreasing photoperiod was associated with decreasing glucocorticoids.

Two smaller peak increases in glucocorticoid concentrations occurred in mink housed under natural light conditions in January and May, coincident with increases in length of daily photoperiod. The peak increase in glucocorticoids that occurred in January was greater than that which occurred in May (14.93 ± 6.35 ng/ml vs. 9.12 ± 1.05 ng/ml). It appears that a reduction in photoperiod does not affect the date at which increases in glucocorticoids occur but does affect the concentration of glucocorticoids secreted. Some endogenous factor(s), other than light, may affect the seasonal variation in

increases in glucocorticoids. There would appear to be no direct interaction between photoperiod and this "factor," as manipulation of the photoperiod had no affect on the date at which increases in glucocorticoids occurred. The existence of temporal synergisms between endogenous hormones has been suggested by Meier et al., (1971a) in the control of gonadal growth in sparrows. These investigators demonstrated that photoperiodically-induced changes in corticosterone concentrations induced conditions of testicular sensitivity and insensitivity to prolactin. A temporal synergism of prolactin and the adrenal steroids also has been shown to be involved in regulation of other vertebrate systems. Meier et al. (1971b) observed that daily variations in the accumulation of fat stores as a result of exogenous PRL administration in fish and pigeons may be phased by injections of adrenal steroids. Furthermore, PRL secretion is less dependent than other photoperiodically-cued hormones on the light-dark cycle for its rhythmicity. Mann et al. (1977) observed a PRL surge in ovariectomized rats maintained in constant light. These data concur with my comments concerning an unknown factor that appears to be involved in photoperiodically-induced increases in glucocorticoids. Prolactin may be acting synergistically with glucocorticoids (or ACTH) on the hair follicles to control fur growth. Alternatively, glucocorticoids may be acting alone but changes in sensitivity of the hypothalamus-pituitary-adrenal cortex axis to the effects of different photoperiods on the animal may be mediated by prolactin. There is presently no evidence to support the latter premise.

The peak increases in glucocorticoids that occurred in January

and May were not associated with fur growth changes whereas the large increase in hormone concentration in September was associated with growth of the winter pelage in both the animals subjected to a reduced photoperiod and the control group (r=.14). These data are in partial agreement with those of Rust et al. (1965) who concluded that ACTH or adrenal hormones are involved in regulating the growth of normal winter pelage. Our results indicate that ACTH or adrenal corticoids may be involved in stimulation of winter pelage, whereas Rust proposed that the winter fur is predominant and administration of ACTH is necessary to induce the change from winter to summer pelage in hypophysectomized mink. Therefore, though there was no direct correlation between fur growth and the glucocorticoid increases observed in January and May, these increases may be necessary for the normal changes from winter to summer pelage to occur.

The results of the present study suggest that glucocorticoids (or ACTH) secretion is related to early-winter priming as a result of decreased photoperiod. For normal winter pelage growth to begin in October an increase in corticoids may be required in September. The initiation of this increase (and other increases observed throughout the year) appears to be regulated by some unknown endogenous factor(s). In September, as compared with other times of the year, the effect of decreasing light, acting in conjunction with this factor, may cause increased glucocorticoid secretion to reach a threshold that induces changes in winter fur growth.

BIBLIOGRAPHY

Axelrod, J. and R. J. Wurtman. 1967. Photic and neural control of indolamine metabolism in the rat pineal gland. Advances in Pharmacology 6A:157-167.

Baker, B. L. 1951. The relationship of the adrenal, thyroid and pituitary glands to the growth of hair. Annals of the New York Academy of Science 53:690-707.

Bassett, C. F. and L. M. Llewellyn. 1949. The molting and fur growth pattern in the adult mink. American Midland Naturalist 42:751-756.

Binkley, S. 1979. A timekeeping enzyme in the pineal gland. Scientific American 240:66-71.

Bissonnette, T. H. 1932. Modification of mammalian sexual cycles. Reactions of ferrets of both sexes to electric light added after dark in November and December. Proceedings of the Royal Society of London, Series B 110:322-336.

Bissonnette, T. H. 1935. Relation of hair cycles in ferrets to changes in the anterior hypophysis and to light cycles. Anatomical Record 63:159-168.

Bissonnette, T. H. and E. E. Bailey. 1944. Experimental modification and control of molts and changes of coat-color in weasels by controlled lighting. Annals of the New York Academy of Science 45:221-260.

Bissonnette, T. H. and E. Wilson. 1939. Shortening daylight periods between May 15 and September 12 and the pelt cycle of the mink. Science 89:418-419.

Butcher, E. O. 1937. Hair growth in adrenalectomized and adrenalectomized thyroxin-treated rats. American Journal of Physiology 120:427-434.

Butte, J. C., R. Kakihana and E. P. Noble. 1976. Circadian rhythm of corticosterone levels in rat brain. Journal of Endocrinology 68:235-239.

Critchlow, V. 1963. The Role of Light in the Neuroendocrine System. in: A. V. Nalbandov (ed.), Advances in Neuroendocrinology, University of Illinois Press, Urbana. pp. 377-402.

Dieke, S. H. 1948. The effect of removing various endocrine glands on the hair growth cycles of black rats. Endocrinology 42:315-319.

Donovan, B. J. and G. W. Harris. 1954. The effect of pituitary stalk section on light-induced estrus in the ferret. Nature 174:503-504.

Duby, R. T. and H. F. Travis. 1970. Photoperiodic control of fur growth and reproduction in the mink (Mustela vison). Journal of Experimental Zoology 182:217-226.

Dusseau, J. W. and A. H. Meier. 1971. Diurnal and seasonal variations of plasma adrenal steroid hormone in the white-throated sparrow, Zonotrichia albicollis. General and Comparative Endocrinology 16:399-408.

Farner, D. S. 1958. Photoperiodism in animals with special reference to avian testicular cycles. Photobiology. 19th Annual Biological Colloquium, Oregon State College. 19:17.

Hammond, J., Jr. 1951. Control by light of reproduction in ferrets and mink. Nature 167:150-151.

Hammond, J., Jr. 1952. Control of reproductive and pelt changes in ferrets: some experiments with animals kept entirely upon artificial light. Journal of Agricultural Science 42:293-303.

Hammond, J., Jr. 1953. Effects of Artificial Lighting on the Reproductive and Pelt Cycles of Mink. Hefner and Sons, Ltd. Cambridge, England. pp. 1-16.

Hart, D. S. 1953. Additional photoperiodic response in sheep. Nature 171:133-134.

Harvey, N. B. and W. V. MacFarlane. 1958. The effects of daylength on the coat-shedding cycles, bodyweight and reproduction of the ferret. Australian Journal of Biological Science 11:187-199.

Herbert, J. 1969. The pineal gland and light-induced estrus in ferrets. Journal of Endocrinology 43:625-636.

Herbert, J. 1972. Initial observations on pinealectomized ferrets kept for long periods in either daylight or artificial illumination. Journal of Endocrinology 55:591-597.

Hill, M. and A. S. Parkes. 1933. Studies on the hypophysectomized ferret. V. Effect of hypophysectomy on the response of the female ferret to additional illumination during anestrus. Proceedings of the Royal Society of London, Series B 113:537.

Hoffman, R. A. and R. J. Reiter. 1965. Pineal gland: Influence on gonads of male hamsters. Science 148:1609-1611.

Kamberi, I. A., R. S. Mical and J. C. Porter. 1971. Effects of melatonin and serotonin on the release of FSH and prolactin. Endocrinology 88:1288-1293.

Kastin, A. J. and A. V. Schally. 1966. In vivo assay for melanocyte lightening substances. Experimentia 22:389-396.

Kastin, A. J., A. V. Schally and T. W. Redding. 1967. MSH activity in rat pituitaries after pinealectomy. Proceedings of the Society of Experimental Biology and Medicine 124:1275-1277.

Larson, B. A., Y. N. Sinha and W. P. Vanderlaan. 1977. Effect of 5-hydroxytryptophan on prolactin secretion in the mouse. Journal of Endocrinology 74:153-154.

Lerner, A. B., J. D. Case and R. V. Heinzelman. 1959. Structure of melatonin. Journal of the American Chemical Society 31:6084.

Lerner, A. B., J. D. Case and Y. Takahashi. 1960. Isolation of melatonin and 5-methoxyindole-3-acetic acid. Journal of Biological Chemistry 235:1992-1997.

Lerner, A. B., J. D. Case, Y. Takahashi, T. H. Lee and W. Mori. 1958. Isolation of melatonin, the pineal gland factor that lightens melanocytes. Journal of the American Chemical Society 80:2587.

Lincoln, G. A. 1976. Seasonal changes in the pineal gland related to the reproductive cycle in the male hare, Lepus europaeus. Journal of Reproduction and Fertility 46:489-491.

Lu, K. H. and J. Meites. 1973. Effects of serotonin precursors and melatonin on serum prolactin release in rats. Endocrinology 93:152-155.

Mann, D. R., M. G. Cost, C. D. Jacobson and L. A. MacFarland. 1977. Adrenal gland rhythmicity and pituitary regulation of adrenal steroid secretion. Proceedings of the Society for Experimental Biology and Medicine 156:441-445.

Martin, J. B., S. Reichlin and G. M. Brown. 1977. Clinical Neuroendocrinology. F. A. Davis Company. Philadelphia, Pennsylvania. p. 28.

Mason, C. A. 1975. Delineation of the rat visual system by the axonal iontophoresis-cobalt sulphide precipitation technique. Brain Research 85:287-293.

Mehdi, A. Z. and T. Sandor. 1977. The effect of melatonin on the biosynthesis of corticosteroids in beef adrenal preparations in vitro. Journal of Steroid Biochemistry 8:821-823.

Meier, A. H., J. T. Burns and J. W. Dusseau. 1969. Seasonal variations in the diurnal rhythm of pituitary prolactin content in the white-throated sparrow, Zonotrichia albicollis. General and Comparative Endocrinology 12:282-289.

Meier, A. H., D. S. Martin and R. MacGregor, III. 1971a. Temporal synergism of corticosterone and prolactin controlling gonadal growth in sparrows. Science 173:1240-1242.

Meier, A. H., T. N. Trobec, N. M. Joseph and T. M. John. 1971b. Temporal synergism of prolactin and adrenal steroids in the regulation of fat stores. Proceedings of the Society for Experimental Biology and Medicine 137:408-415.

Murphy, B. E. P. 1967. Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radioassay. Journal of Clinical Endocrinology 27:973-990.

Ogle, T. F. and J. I. Kitay. 1978. In vitro effects of melatonin and serotonin on adrenal steroidogenesis. Proceedings of the Society for Experimental Biology and Medicine 157:103-105.

Pilbeam, J. E., P. W. Concannon and H. F. Travis. 1979. The annual reproductive cycle of mink (Mustela vison). Journal of Animal Science 48:578-584.

Reiter, R. J. 1973. Pineal control of a seasonal reproductive rhythm in male golden hamsters exposed to natural daylight and temperature. Endocrinology 92:423-430.

Relkin, R. 1972. Rat pituitary and plasma prolactin levels after pinealectomy. Journal of Endocrinology 53:179-180.

Relkin, R., M. Adachi and S. A. Kahan. 1972. Effects of pinealectomy and constant light and darkness on prolactin levels in the pituitary and plasma and on pituitary ultrastructure of the rat. Journal of Endocrinology 54:263.

Rollag, M. D., R. J. Morgan and G. D. Niswender. 1978. Route of melatonin secretion in sheep. Endocrinology 102:1-8.

Rust, C. C. 1962. Temperature as a modifying factor in the spring pelage change of short-tailed weasels. Journal of Mammology 43:323-328.

Rust, C. C. 1965. Hormonal control of pelage cycles in the short-tailed weasel (Mustela erminea bangsi). General and Comparative Endocrinology 5:222-231.

Rust, C. C. and R. K. Meyer. 1969. Hair color, molt, and testis size in male, short-tailed weasels treated with melatonin. Science 165:921-922.

Rust, C. C., R. M. Shackelford and R. K. Meyer. 1965. Hormonal control of pelage cycles in the mink. Journal of Mammalogy 46:549-565.

Stout, F. M. and J. Adair. 1969. Induced early fur growth in pelter mink by light regulation. National Fur News, April, p. 16.

Stout, F. M., J. Adair, G. E. Costley and J. E. Oldfield. 1969. Induced early fur growth in mink by light regulation. Journal of Animal Science 29:117. (Abstract).

Tamarkin, L., W. K. Westrom, A. I. Hamill and B. D. Goldman. 1976. Effects of melatonin on the reproductive systems of male and female Syrian hamsters: A diurnal rhythm in sensitivity to melatonin. Endocrinology 99:1534-1541.

Tindal, J. S. 1974. Hypothalamic control of secretion and release of prolactin. Journal of Reproduction and Fertility 39:437-461.

Thorpe, P. A. 1975. The presence of a retinohypothalamic projection in the ferret. Brain Research 85:343-346.

Thorpe, P. A. and J. Herbert. 1974. The role of the pineal gland in the response of ferrets to artificially restricted photoperiods. Journal of Endocrinology 63:56-57P. (Abstract).

Wurtman, R. J. and F. Anton-Tay. 1969. The mammalian pineal as a neuroendocrine transducer. Recent Progress in Hormone Research 25:493.

Wurtman, R. J. and J. Axelrod. 1967. The formation, metabolism and physiologic effects of melatonin. Advances in Pharmacology 6A:141-151.

Wurtman, R. J., J. Axelrod and J. E. Fischer. 1964a. Melatonin synthesis in the pineal gland: Effect of light mediated by the sympathetic nervous system. Science 143:1329-1330.

Wurtman, R. J., J. Axelrod and L. S. Phillips. 1963. Melatonin synthesis in the pineal gland: Control by light. Science 142:1071-1073.

Wurtman, R. J., J. Axelrod and L. T. Potter. 1964b. The uptake of ^{3}H-melatonin in endocrine and nervous tissues and the effects of constant light exposure. Journal of Pharmacology and Experimental Therapeutics 143:314-318.

Yeates, N. T. M. 1955. Photoperiodicity in cattle. I. Seasonal changes in coat character and their importance in heat regulation. Australian Journal of Agricultural Research 6:891-902.