This is to certify that the

dissertation entitled

Brain and Pineal Gland Mediation of

Photoperiod-Induced Hormone Concentrations in Cattle

presented by

Edward Peter Stanisiewski

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Animal Science

H. Allen Tucker
Major professor

Date 6/25/1987

BRAIN AND PINEAL GLAND MEDIATION OF PHOTOPERIOD-INDUCED HORMONE CONCENTRATIONS IN CATTLE

By

Edward Peter Stanisiewski

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirement
for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Science

1987

ABSTRACT

BRAIN AND PINEAL GLAND MEDIATION OF PHOTOPERIOD-INDUCED HORMONE CONCENTRATIONS IN CATTLE

by

Edward Peter Stanisiewski

Concentrations of prolactin (PRL) in serum were 2 to 3-fold greater in prepubertal bulls exposed to 24 (24 Light:0 Dark) or 16 (16L:8D) h of daily light as compared with 8L:16D. However, PRL attained maximum concentrations approximately 3 wk later in calves exposed to 24L:0D.

In other bulls, PRL was 2.5-fold greater after 6-wk exposure to 16L:8D compared with 8L:16D, while testosterone was concurrently increased 2-fold by 16L:8D. However, after 6 additional wk of repetitive application of 16L:8D bulls became refractory in terms of PRL while testosterone concentrations plateaued. Photoperiod did not affect serum luteinizing hormone concentrations. Shifting from 8L:16D to 16l:8D increased serum concentrations of PRL equally in castrated and gonadally intact males.

Dopamine concentrations in anterior pituitary glands were greater in calves exposed to 8L:16D compared with 16L:8D, while plasma catecholamine concentrations were not affected by photoperiod.

In other experiments, serum melatonin concentrations varied episodically and were 60 to 405% greater during dark than light periods, while 24L:0D abolished the nocturnal

increase. Exposure to 8-h of darkness coincidently elevated serum melatonin, whereas melatonin is elevated for only the first 12 to 14-h of a 16-h dark period. Serum melatonin is probably not responsible for photoperiod-induced changes in serum PRL since PRL concentrations are equivalent in bulls infused daily with melatonin for 8 or 16-h.

Pinealectomy (PX) of bull calves does not abolish photoperiod-induced changes in serum PRL concentrations; however, testosterone concentrations in PX bulls are lower at 20 wk of age than in sham-PX bulls.

It is concluded that photoperiod-induced changes in PRL concentrations does not involve melatonin, the pineal gland or the testes, but may be mediated by dopamine in prepubertal bull calves. Serum PRL concentrations in calves become refractory to prolonged 16L:8D photo-stimulation. Finally, long-days hasten puberty onset in bulls and the pineal gland may be involved.

ACKNOWLEDGMENTS

I wish to express appreciation to my major professor, Dr. H. A. Tucker, as well as the other members of my committee, Drs. T. A. Ferris, R. W. Mellenberger, R. A. Merkel and D. R. Romsos, for their assistance and guidance in transforming a few ideas into the substance of this dissertation.

I also wish to thank Drs. N. K. Ames, K. Demarest, J. L. Gill, F. J. Karsch and Mr. L. T. Chapin for their invaluable help with methololoqies and loqistics. To Miss B. A. Sheathelm, thank you once again for your time and effort in typing this dissertation.

TABLE OF CONTENTS

List of Tables

List of Figures

INTRODUCTION

Researchers in animal science generally follow two basic ideologies in terms of their rationale for conducting research. The first is to gain knowledge which can immediately benefit animal health and production and the second is to gain knowledge for the sake of knowledge. I chose to deal primarily with the latter aspect.

The focus of this thesis pertains to the effects of daily duration of light (photoperiod) on endocrine function. Many have demonstrated the benefits of photoperiod on increasing milk yield in cattle (Peters et al., 1978, 1981; Stanisiewski et al., 1985), growth rates in cattle (Petitclerc et al., 1983a; Tucker et al., 1984) and sheep (Schanbacher and Crouse, 1981) as well as shortening the duration from birth to puberty in cattle (Hansen et al., 1983, Petitclerc et al., 1983a) and in swine (Mahone et al., 1979, Ntunde et al., 1979). Yet, very little is known about the mechanism through which photoperiod affects these parameters; particularly, in cattle. Photoperiodic hormone regulation is achieved through the pineal gland and its' secretions in birds, hamsters and sheep. Whether this is also true in cattle is unknown.

At present, it appears the popular trend among consumers of animal products is to demand a healthful product free of additives such as exogenous hormones and other growth promotants. By better understanding the mechanism through which photoperiod affects the endocrine milieu in cattle, researchers may someday have the capability to consistently increase production efficiency and alter carcass and milk composition (less carcass fat and milk fat), using an acceptable agent to the consumer (light).

Review of Literature

A. INTRODUCTION

It has long been known that season of the year as well as daily duration of light exposure (photoperiod) can effect several physiological parameters in mammals including reproductive cycles, growth rates and lactational performance (Wylie, 1925; Marshall, 1937; Peters et al., 1978, 1981; Forbes, 1982). Manipulation of such parameters using photoperiod can be an economical tool for the animal industry to increase performance and efficiency of domestic livestock. It is the purpose of this review of literature to examine several biological factors which can be altered with photoperiod. The factors I selected are by no means all inclusive, but were selected for their potential (as judged by myself) for economic benefit. Specific introductions are given preceding each chapter of experiments, however, this overall review of literature should provide useful background information which is intended to embellish the justification for following certain protocols.

B. SEASONAL AND PHOTOPERIODIC INDUCED CHANGES IN HORMONES OF THE ANTERIOR PITUITARY

1. PROLACTIN

Of the known non-gonadotropic, hypophyseal hormones, prolactin (PRL) is most affected by season of the year in all mammals in which it has been measured including seasonal as well as non-seasonal breeders. Concentrations of PRL in blood are 2 to 4 fold greater during the summer months in cattle (Schams, 1972; Kensinger et al., 1979; Tucker, 1982), goats (Buttle, 1974; Muduuli et al., 1979; Howland et al., 1985), sheep (Rhind et al., 1980; Kennaway et al., 1981),and deer (VanMourik and Stelmasiak, 1985; Webster and Barrell, 1985) than in blood collected during winter months. Photoperiod is one component of season which follows a consistent pattern from year to year whereas, ambient temperature tends to be more variable. Within limits, both duration of daily light exposure and ambient temperature are positively correlated with concentrations of PRL in serum of cattle (Wettemann and Tucker, 1974; Leining et al., 1979), goats (Muduuli et al., 1979) and sheep (Pelletier, 1973). Several researchers have attempted to separate the effects of photoperiod and ambient temperature on PRL concentrations in blood. In heifers exposed to a constant photoperiod of 12 h light: 12 h dark (12L:12D), increasing ambient temperature from 21 to 32 C results in a linear increase in PRL (1.2 ng/ml$\cdot$C^{-1}); whereas, decreasing temperature from 21 C to 4.5 C results in decreased PRL (.6 ng/ml$\cdot$C^{-1}; Tucker and Wettemann, 1976). A similar study using steers resulted in a 4.2 ng/ml$\cdot$C^{-1} increase in PRL when ambient temperature

increased from 20 to 40 C while reducing temperatures from 20 C to 4 C resulted in a 1.85 ng/ml·C^{-1} decrease (Smith et al., 1977). Animal variation may account for the absolute response differences of PRL to ambient temperature but temperature appears to control serum PRL concentrations on a minute to minute basis.

On the other hand, PRL responses to photoperiod tend to be more sluggish. For instance, it takes 4 to 6 wk for PRL concentrations in sera to increase in bull calves when photoperiod is switched from 8L:16D to 16L:8D (Stanisiewski et al., 1984). Similar response patterns are seen in rams (Lincoln et al., 1978). Given the positive correlation between temperature and day length on PRL release, it is possible to account (using covariance analysis) for the effects of each factor in animals maintained under natural environment (seasonal) conditions. Temperature and photoperiod account for most of the seasonal pattern of PRL secretion in cattle, however, a small percentage of the seasonal variation may be attributable to an endogenous rhythm of secretion or to other unknown environmental cues (Petitclerc et al., 1983b).

2. GONADOTROPINS

Other hormones of the anterior pituitary gland are subject to regulation by season and photoperiod in some species. Most notable are changes in gonadotropin secretion associated with onset of reproductive activity in seasonally breeding species such as goats, sheep and horses. Most

sheep and goats are reproductively quiescent during the non-breeding season of spring/summer. But, in response to reduction in day length , or to abruptly decreasing day length from 16 to 8 h per day, plasma luteinizing hormone (LH) and follicle stimulating hormone (FSH) begin an initial increase within 2 to 4 wk after a photoperiod switch, with peak responses occurring 1 to 5 wk later, again demonstrating a sluggish hormonal response to photoperiod (Lincoln and Davidson, 1977). Peak endogenous pulses of LH and FSH are 3-and 10-fold greater; respectively, when compared with the quiescent period in rams. Likewise, LH in serum of ewes are approximately 12-fold (6 vs .5 ng/ml) greater during the breeding season than during the non-breeding season (Bittman et al., 1983). Pulsatile activity of LH increases from 3.6 to 5.4 pulses/24h in rams during the breeding season when compared with the non-breeding season (Schanbacher and Ford, 1976) or from 1 to 10 pulses/24h when comparing long days with short days (Lincoln et al., 1977). LH concentrations in another short day breeder, the pygmy goat, follow a seasonal pattern similar to that seen in sheep (Muduuli et al., 1979; Howland et al., 1985). Reproductive activity ceases in short-day breeders in response to increasing day lengths.

In contrast, horses are long-day breeders, and increasing duration of daily light from 9 to 16 h induces 100 to 400 fold increases in serum LH in mares within 7 to 14 wk (Oxender et al., 1977). Seasonal changes in

circulating gonadotropin concentrations are positively correlated with pituitary gonadotropin content indicating that changes in hormone synthesis rates are probably occurring in the brain (Berndtson and Desjardins, 1974). In cattle, a non-seasonal breeder, LH and FSH are unaffected by photoperiod (Rzepkowski et al., 1982).

3. STEROID HORMONES

In general, gonadal steroid release is under direct control of the gonadotropins, therefore, reproductive steroids tend to follow a similar circannual pattern to the gonadotropins in seasonally breeding mammals. Breeding season induced rises in LH and FSH are followed immediately by increased testosterone or estradiol concentrations in serum of rams, (Lincoln and Davidson, 1977) ewes (Legan and Karsch, 1979) and pygmy goats (Muduuli et al., 1979). Progesterone and estradiol serum concentrations are greater during spring/summer months in mares, and can be elevated during winter months using a 16L:8D photoperiod (Oxender et al., 1977). Furthermore, serum testosterone in stallions is greatest in May (3.2 ng/ml) and lowest during December (1.5 ng/ml) and is correlated with the normal spring/summer breeding season (Berndtson et al., 1974). An important sex difference exists however, in that the hypothalamo-hypophyseal-ovarian axis remains functional (though anovulatory) in ewes during the anestrous season which is in contrast to rams in which gonadal regression takes place. Changes in feedback

sensitivity are perhaps responsible for the ewes inability to demonstrate preovulatory LH surges under long days. In fact, anestrus in the ewe may be due to a long-day induced increase in sensitivity to estradiol negative feedback on LH secretion (Legan and Karsch, 1979).

Lincoln et al. (1977) demonstrated circadian changes in testosterone concentrations in sexually active rams with highest concentrations tending to occur at night (between 1700 and 2000h). This was attributable to increased frequency of gonadotropin pulses occurring at night, a phenomenon which has also been described in men (Boyer et al., 1972) and rats (terHaar et al., 1974).

Glucocorticords do not appear to vary with any seasonal or photoperiod-induced rhythmicity in cattle (Leining et al., 1980), white-tailed deer (Bubenik et al., 1975) or rams (Lincoln et al., 1982). However, in male pygmy goats, cortisol concentrations in serum change with season and at times are directly correlated with pulsatile changes in testosterone (Howland et al., 1985).

C. HYPOTHALAMIC CONTROL OF PROLACTIN SECRETION

PRL is secreted episodically into blood of most species including cattle (Stanisiewski et al., 1984). Furthermore, PRL increases in blood within a few minutes by stimuli such as stress (Johke, 1970; Dunn et al., 1972) and rising ambient temperature (Tucker and Wettemann, 1976). In addition, PRL release is controlled by less acute means, such as in response to changing day lengths.

Several hypothalamic PRL-release (PRF) and-release inhibiting factors (PIF) are presumed to directly control PRL synthesis and (or) release from the anterior pituitary gland. For instance, following lesioning of the median eminence in rats, PRL increases rapidly in plasma and elevations are maintained, presumably in response to acute release of PRF from lesioning plus chronic removal of PIF (Bishop et al., 1972). Furthermore, rat hypothalamic extracts inhibit pituitary PRL release in vitro (Shaar and Clemens, 1974).

Hypothalamic catecholamines may be responsible for physiological control of PRL release. For example, dopamine (DA) inhibits PRL release in vitro (Shaar and Clemens, 1974; Padmanabhan et al., 1979) while epinephrine (EPI) and norepinephrine (NE) inhibit (Birge et al., 1970; Shaar and Clemens, 1974), increase (Koch et al., 1970) or have no effect (Talwalker et al., 1963) on PRL release. Therefore, it appears that EPI and NE may have some control of PRL release, but the predominant PIF appears to be DA (Leong et al., 1983). In fact, cells of the anterior pituitary gland (presumably lactotrophs) have the capacity to internalize and process DA (Gudelsky et al., 1980).

There are, no doubt, several additional factors such as thyrotropin-releasing hormone (TRH), serotonin, and vasoactive intestinal peptide, that may control PRL secretion (Leong et al., 1983). In fact, Wolinska et al. (1977) have shown in sheep that lesions to the anterior

medial basal hypothalamus (MBH) decrease plasma PRL; whereas, caudal MBH lesions increase PRL. It appears from these data that at least two functional systems are at work in the hypothalamus which stimulate or inhibit PRL release. Perhaps the most prevalent hypothesis concerning PRL release suggests that decreased DA inhibition depletes a releasable pool of PRL from the anterior pituitary gland. Then, a second intracellular pool of PRL becomes releasable by TRH (Grosvenor and Mena, 1980; Lafond and Collu, 1986). Similarly to DA, TRH acts directly on the pituitary gland to regulate PRL release (Clemens et al., 1980).

It is unknown how photoperiod may affect hypothalamic factors which in turn alter PRL secretion. However, a likely candidate for study would be DA, since it appears to be responsible for tonic PRL inhibition. In addition, Naber et al. (1981) have shown that rats possess an endogenous rhythm in DA receptor binding which undergoes seasonal variations. In addition, humans have reduced concentrations of DA in hypothalami during spring/summer compared with fall/winter (Karson et al., 1984). Finally, DA and its metabolites are higher in the retina of rats during periods of light as compared with darkness (Cohen et al., 1983; Melamed et al., 1984), suggesting further that photoperiodic influences on PRL may be partially mediated through DA.

D. SEASONAL AND PITUITARY CONTROL OF GONADAL FUNCTION

Many animals have evolved into seasonal breeders with the primary function being that reproduction occurs at a

time of the year when offspring can be reared in a favorable environment, thus allowing for maximum survival. Although many environmental factors change with the seasons, photoperiod is the primary cue governing reproductive activity or quiescence. Ultimately, the most advantageous time of the year to reproduce is determined by food supply, predation and climate (Baker, 1938); however, photoperiod is used as an advanced announcement of a change in environment. Some species become reproductively active in response to long days (eg. hamsters, ferrets, horses) while reproductive activity is induced in other species by short days (eg. sheep, goats, deer, mink ;Turek and Campbell, 1979; Tucker and Oxender, 1980). Still other animals (eg. cattle, domestic rabbit) are reproductively active without regard to photoperiod. To complicate the situation further, exposing animals to a constantly repetitive photoperiod which induces a characteristic hormonal or reproductive response will not maintain that response indefinitely. For example, PRL concentrations in serum and pituitary glands decrease in hamsters exposed to short days (Bex et al., 1978; Reiter et al., 1975). However, PRL in blood increase spontaneously even when short days are maintained (Goldman et al., 1981). In addition, gonadal regression takes place in hamsters exposed to short days (Steger et al., 1985), but spontaneous gonadal recrudescence occurs within 10 wk of maintained short days (Gaston and Menaker, 1967). When hormone secretion or gonadal responses overcome the applied

photostimulation, the animals are said to have become refractory (Farner and Follett, 1966). Photorefractoriness has also been documented in sheep (Robinson et al., 1985) and several species of birds (Follett, 1978). Therefore, the neuroendocrine mechanism controlling gonadal function in seasonal breeders is not just passively following seasonal photoperiod changes. In actuality, the internal biological clock which animals are purported to have, appears to be running on its own endogenous rhythm and is likely being cued and reset by changing photoperiods.

As pointed out previously, photoperiod-induced changes in gonadal steroid concentrations are fairly well correlated with gonadotropin concentrations. In the classical sense, gonadal steroid concentrations have a negative feedback effect on gonadotropins (Nalbandov, 1976). However, photoperiodic species appear to be an exception to this rule, at least during certain seasons of the year. In fact, exogenous steroids given in conjunction with a stimulatory photoperiod (breeding season) is a less potent inhibitor of gonadotropins than when given during a non-stimulatory photoperiod in sheep (Pelletier and Ortavant, 1975; Legan and Karsch, 1979) and hamsters (Tamarkin et al., 1976; Ellis and Turek, 1979).

In spite of the changes in circulating hormones taking place in response to photoperiod, very little is known about the direct effects of these changes on the gonad. LH and FSH are considered to be essential for maintaining

reproductive function in mammals of both sexes (Sanford et al., 1978), whereas the reproductive role of PRL is less well defined except for its luteotrophic effect in certain rodents (Murphy and Raikumar, 1985) and perhaps sheep (Denamur et al., 1973). In addition, Rhind et al. (1980) established that PRL concentrations in blood during estrus are greater in sheep during March or July (normal anestrus) versus December (normal breeding season). These authors suggest an anti-fertility role for PRL in ewes. Still other evidence suggests that PRL may play a mediating role between photoperiod and feedback sensitivity (Matt et al., 1984). Testosterone is more effective in suppressing LH and FSH in hamsters soon after switching from a short to a long-day photoperiod when compared with suppression observed 30 days after the switch, and this effect is more marked in dopamine-agonist treated hamsters, indicating that decreases in PRL increase feedback sensitivity (Matt et al., 1984). Estrogens and aromatizable androgens administered to castrated rams increase PRL concentrations in serum (Parrott and Hill, 1979) and additional evidence indicates that testosterone inhibits PRL release in wethers exposed to 8L:16D (Pelletier, 1973). Although the evidence is conflicting, suffice it to say that the gonadal steroids may in part modulate their own seasonal secretion via a direct effect on PRL.

In all species in which it has been examined, LH has a direct effect on testosterone production by the gonad

(Bartke et al., 1978). The androgen stimulating action of LH appears to be almost exclusively on the Leydig cells where the mechanism involves synthesis of proteins as well as increased activity of enzymes involved in testosterone production.

E. PUBERTY

1. DEFINITIONS

Defining puberty is often an obscure proposition, particularly in the case of males. However, the following is a list of attempts from a variety of sources.

"The period in both males and females marked by the functional development of the generative system".

Webster's Dictionary (1975).

"Pituitary growth and its hormonal influence on body growth and ovarian activity stimulate growth and development of the uterus and other parts of the reproductive tract. These changes result in the reproductive tract of the heifer becoming functionally operative. Thus puberty is reached."

Salisbury et al. (1978).

"Puberty is the transitional period between the juvenile state and adulthood during which the adolescent growth spurt occurs, secondary sexual characteristics appear, fertility is achieved, and profound psychological changes take place."

Reiter and Grumbach, (1982).

"Puberty might be defined as the point in development when a bull is capable of participating in reproduction."

Amann and Walker, (1983).

There are probably as many variations on the definition of puberty as there are people to give it a definition. However, it is not my concern to add to this list, although for the sake of continuity of this review, I shall refer to puberty as the achievement of sexual maturation and the ability to reproduce. However, my task is to examine the endocrine and neuroendocrine parameters which lead to puberty and how these parameters can be altered. Furthermore, I will limit this discussion primarily to males with some consideration of females where appropriate. The prepubertal sequence is a complex series of events, and in bulls is thought to contain the following order: 1)initiation of spontaneous discharge of LH, 2)hormone induced differentiation of Leydig cells with increased secretion of androstenedione in response to LH stimulation, 3)further differentiation of Leydig cells resulting in LH-stimulated secretion of testosterone, 4)testosterone-induced differentiation of indifferent supporting cells to Sertoli cells concomitant with testosterone-induced differentiation of gonocytes to prespermatogonia and A-spermatogonia, 5)increased sensitivity of the hypothalamus-anterior pituitary to negative feedback of gonadal steroids, 6)diminished frequency and amplitude of LH discharge, 7) formation of functional complexes between Sertoli cells and establishment of the blood-testis barrier, 8)formation of primary spermatocytes and ultimately spermatids and spermatozoa, and 9)continued increase in efficiency of

spermatogensis until sufficient sperm are produced to provide the first ejaculum around 37 to 38 wk of age (Amann, 1983). In the following sections, I will review some of the hormonal changes taking place during the prepubertal period and examine how some of these changes may be altered.

2. ENDOCRINE CHANGES ASSOCIATED WITH ONSET OF PUBERTY

In mammals, secondary sex characteristics as well as the capacity to reproduce are under direct control of gonadal steroids. However, production of gonadal steroids are dependent upon secretions of the anterior pituitary gland (Bartke et al., 1978). For example, several reports indicate that circulating LH concentrations in bulls begin a gradual increase between 1 and 5 mo of age (Rawlings et al., 1978; McCarthy et al., 1979a, 1979b; Amann and Walker, 1983) and may in fact increase further up to 10 mo of age (MacMillan and Hafs, 1968; Rawlings et al., 1978). Testosterone in blood likewise increases gradually from 1 to 10 mo of age but the rate of increase is most pronounced around 4 to 6 mo (Karg et al., 1976; Bedair and Thibier, 1979; McCarthy et al., 1979b). However, highest concentrations of testosterone generally lag behind increased LH concentrations by about 1 mo (McCarthy et al., 1979a). In contrast, age related differences in FSH have not been detected (McCarthy et al., 1979a, 1979b) except in one case in which FSH in blood increased a modest 30% between 1 and 8 mo (Amann and Walker, 1983). Similarly to calves, rams also have a well defined prepubertal period

where LH concentrations increase initially around 5 wk of age, then exhibit a secondary rise by wk 41 before declining (Lee et al., 1976). Likewise, FSH concentrations in sheep are maximal by 5 wk and maintained at those levels while concentrations of testosterone are closely correlated with LH.

Prepubertal hormone changes are relatively well established in rats. In fact, LH concentrations in blood of rats plateau around d 10 while FSH concentrations are maximal around d 30 then decline (Payne et al., 1977). The decline of FSH concentrations coincide with development of mature spermatogenesis (Swerdloff et al., 1971). Whether these gonadotropin concentrations in rats correspond to a prepubertal period similar to that of large domestic animals is in question since spermatogenesis in rats is reported to begin around the time of birth (Lee et al., 1976).

Reports conflict for sheep and cattle in terms of age related changes in circulating PRL, ranging from no age effects (Schams and Reinhardt, 1974) to increased PRL concentrations at 3 mo (Leining et al., 1976; Davis et al., 1977) or 4 to 5 mo of age (McCarthy et al., 1979a). Ravault and Courot (1975) reported a marked increase of PRL secretion in male lambs coincident with rapid growth of testis and establishment of spermatogenesis. At present however, there is no good evidence linking PRL secretion with puberty onset in large domestic animals (i.e. sheep and cattle) at least in terms of spermatogenesis, testis weight

or LH and testosterone secretion. However, PRL deficiency results in reduced weight of seminal vesicles in lambs (Ravault et al., 1977; Ohlson et al., 1981). On the other hand, PRL in rats increases gradually through the time of puberty (Dohler and Wuttler, 1974, 1975) and in male rats PRL is reported to have a synergistic effect with LH on spermiogenesis (Bartke, 1971), and testosterone production (Hafiez et al., 1972). Furthermore, PRL synergizes with testosterone to stimulate growth of accessory sex glands in male rats (Moger et al, 1972; Hostetter and Piacsek, 1977).

3. NEUROENDOCRINE EFFECTS ON PUBERTY

There are three principal components to the control mechanism of puberty onset in mammals (Reiter and Grumbach, 1982). First is the arcuate nucleus of the medial basal hypothalamus along with its projections. Gonadotropin-releasing hormone (GnRH) is synthesized and released by these secretory neurons into the median eminence where GnRH is transported via hypothalamic-hypophyseal portal circulation to the second component of the system, the pituitary gonadotropes. Gonadotropes release LH and FSH in a pulsatile manner in response to the rhythmic GnRH signal. Finally, LH and FSH directly affect the gonads.

Most data suggest that the major control point of the system is at the level of the hypothalamus. For instance, GnRH is detectable in fetal human hypothalami by 10 wk of gestation (Kaplan et al., 1976), and in rats as early as d 17 of gestation (Chiappa and Fink, 1977). Furthermore, in

humans (Groom and Boyns, 1973) and rats (Root et al., 1975) prenatal and neonatal pituitary gland fragments are capable of LH and FSH release in response to GnRH. George et al. (1979) have shown that in rabbit fetuses testosterone secretion becomes gonadotropin dependent beginning in late gestation. Therefore, most controlling influences on puberty onset occur at the hypothalamic level, where initiation of the reproductive process likely begins.

Several studies suggest that the central nervous system (CNS) has a major impact on puberty onset, namely through a mechanism restraining onset of puberty (Reiter and Grumback, 1982). This restraint is removed as puberty approaches (Ramaley, 1979) and has been commonly referred to as the gonadostat theory of puberty onset (Raimirez and McCann, 1965). CNS control of puberty onset probably acts through brain neurotransmitters, such as the catecholamines. For instance, inhibition of catecholamine synthesis in 20-d old rats abolishes hemiovariectomy-induced ovarian hypertrophy (Muller et al., 1972). Those same authors concluded that norepinephrine (NE) was the primary catecholamine involved with prepubertal FSH release. In addition, there is a marked increase in hypothalamic NE turnover preceding prepubertal LH increases in rats (Advis et al., 1978; Wuttke et al., 1980). Likewise, in rats, the usual castration-induced rise in LH and FSH secretion can be inhibited by blocking NE synthesis (Cocchi et al., 1974). Wuttke et al. (1980) reported an increase in hypothalamic NE and DA

turnover between d 21 and puberty in rats while others showed decreased DA and increased turnover of serotonin preceding the first LH surge in rats (Advis et al., 1978). Advis et al. (1978) claimed the changes in DA and serotonin could be responsible for prepubertal increases in circulating PRL of rats.

4. MANIPULATION OF PUBERTY ONSET

Several factors can be used to manipulate onset of sexual maturity in animals and one which certainly has a marked effect is level of feeding. For example, dairy heifers with restricted feed intakes reach sexual maturity later than full-fed controls (Sorensen et al., 1959). These data would suggest that body size is more important than age in determining sexual maturity. However, I will not dwell further on factors which are restrictive in nature, but rather will examine situations where environment and or endocrine manipulation hasten puberty.

Photoperiod has been used extensively to alter sexual maturation in a variety of species. Much of the work on photoperiod-induced puberty onset has been accomplished in rodents. For example, short-day photoperiod exposure from birth delays gonadal development in the mouse, cotton rat, field vole and Djungarian hamster (Lecyk, 1962; Hoffmann, 1978; Johnston and Zucker, 1979; Zucker et al., 1980). Many of the animals in which photoperiod affects puberty onset are seasonal breeders. That is, photoperiod controls reproduction throughout their reproductively active life.

Therefore, one might expect photic influences to be important during reproductive development. Sexual maturation, however, is unaffected by photoperiod in some seasonal breeders such as the Syrian hamster (Gaston and Menaker, 1967; Darrow et al., 1980).

As might be expected, photoperiodic effects on sexual maturation are closely associated with many endocrine changes. For example, Djungarian hamsters grown under long days have increased FSH, LH, PRL and androgen concentrations in serum between 15 and 30 d of age in addition to increased body and testis weights compared with animals receiving short days (Yellon and Goldman, 1984). However, Djungarian hamsters maintained under short days eventually become refractory to this inhibition and attain full gonadal function by 150 d of age (Hoffmann, 1978). In addition, gonadal response to photoperiod in neonatal hamsters may also be dependent on maternal photoperiod exposure, and transfer of that information (Stetson et al., 1986). For example, gonadal maturation rates of offspring are hastened when gestated with 16 h compared with 12 h of daily light.

As stated earlier, both age and body weight are determinates of when puberty occurs, and weight is more important. A long-day photoperiod (18L:6D) will reduce both age and body weight at puberty in female rabbits compared with 6L:18D exposure (Kamwania and Hauser, 1983) Furthermore, gilts born during the autumn and maintained on natural photoperiod (day lengths are decreasing) attain

puberty at a younger age and lower weight than gilts born in the spring (Wiggins et al., 1950; Mavrogenis and Robison, 1976). In contrast, exposing boars (Mahone et al., 1979; Berger et al., 1980) and gilts (Ntunde et al., 1979) to 15 to 18 h of light daily accelerates onset of puberty relative to animals under natural winter photoperiods (9 to 12.7 h of daily light). However, Ntunde et al. (1979) could not attribute the photoperiod effect on puberty to alterations in circulating LH concentrations. The reason natural and artificial photoperiods appear to affect swine differently is unclear.

Similar to swine, in cattle season and photoperiod can also affect timing of puberty onset. For example, heifers born in winter or spring were 52 d younger and 54 kg lighter at puberty than similar heifers born during summer or fall (Roy et al., 1980; Little et al., 1981). In addition, 16L:8D hastens puberty onset and body weight gain in heifers compared with animals receiving 8 h of daily light (Petitclerc et al., 1983a). Hansen et al. (1983) also reduced age at first ovulation in heifers with 18 h of daily light compared with natural autumn day lengths but, as with swine, LH in serum was unaffected by photoperiod. Effects of photoperiod on puberty onset in bulls has not been determined. However, in one of the first experiments to examine photoperiod effects on puberty in rams, Howles et al. (1980) saw no difference in testosterone concentrations or testis growth in response to 8 or 16 h of light per day.

F. LIGHT PERCEPTION AND ENDOCRINE CHANGES

1. FUNCTIONAL ANATOMY

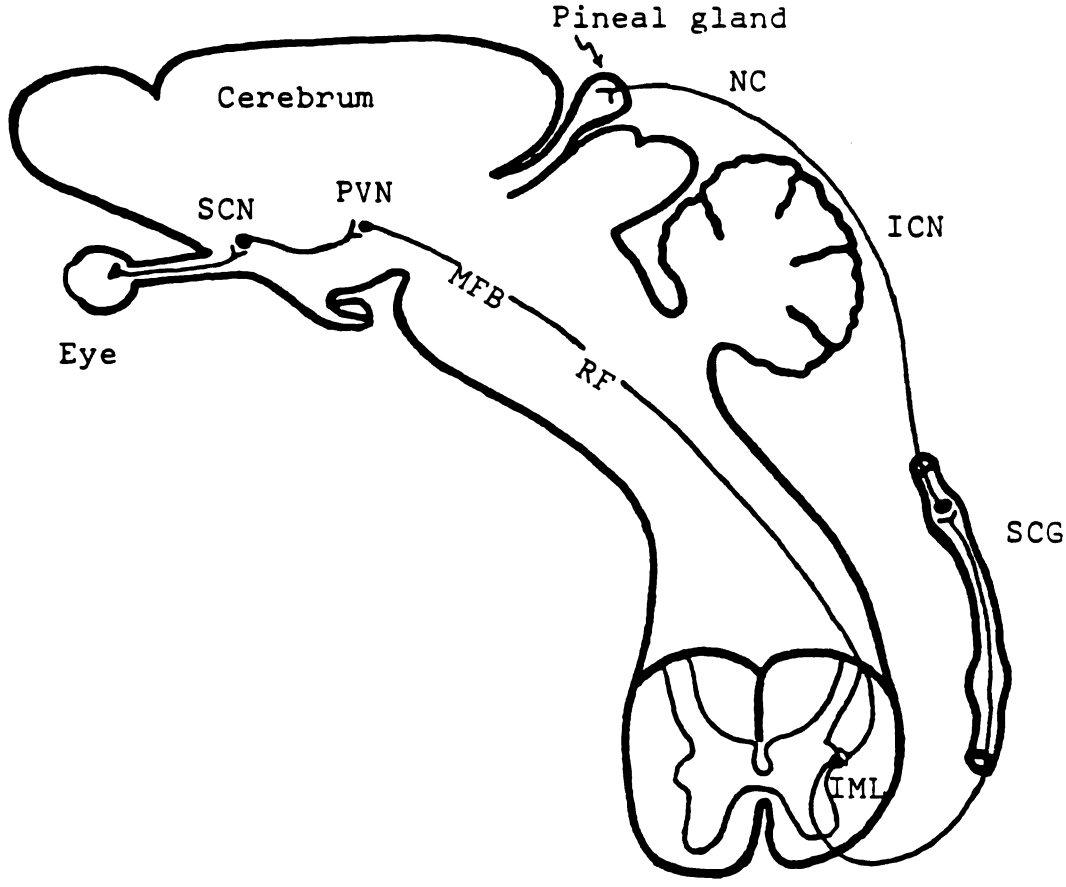
It has long been known that seasonality and photoperiod can alter diurnal as well as circannual activity rhythms of a variety of mammals. However, the mechanism through which light and (or) dark impact upon the animal is generally not understood. Quay (1956, 1963) was one of the first to demonstrate that both structure and function of the mammalian pineal gland could be altered by photoperiod. Around the same time, Lerner et al. (1960) isolated the indolamine, melatonin, from bovine pineal tissue while Wurtman et al. (1963) showed that melatonin synthesis was controlled by light. Thus, the foundation of the photic-endocrine mechanism was in place, but further studies were needed to establish the precise relationship of the pineal gland with rhythmicity in animals. The following is a review describing the neural connections between the eyes and the pineal gland in mammals. Most of the anatomical relationships have been determined in rats.

In terms of photic perception and light measurement, mammals are at a disadvantage compared with non-mammalian vetebrates. Light can pass through the relatively thin skulls of birds and impinge directly upon the pineal gland (Underwood and Groos, 1982). Mammals on the other hand, being thick-skulled creatures, had to develop an alternate pathway to permit light signals to reach the pineal gland. This alternate system involves an external photoreceptor

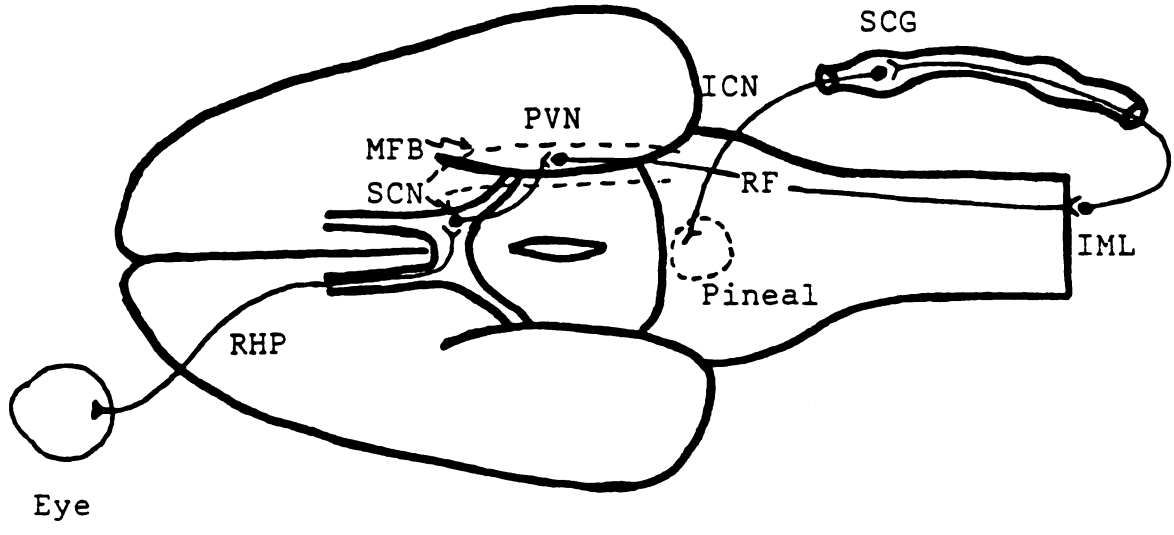
(eye), which transmits light:dark signals via a complex sympathetic network to the pineal gland (figure 1). The first links in the network are retinohypothalamic neural projections from the retina primarily to the contralateral suprachiasmatic nucleus (SCN) within the hypothalamus (Moore, 1983). The SCN is commonly referred to as the master oscillator or pacemaker controlling many circadian rhythms (Klein et al., 1983; Moore, 1983; Turek, 1985). Indeed, it was discovered that destruction of the SCN or its neural connections results in disruption of circadian rhythms in rodents (Turek, 1983). Although the SCN varies in size and shape among mammals, they are always laterally symmetrical and located near the third ventricle and dorsal to the optic chiasm (Turek, 1985). Furthermore, the SCN develops gradually after birth and in the rat does not acquire full adult organization and features until 5 wk of age (Lenn et al., 1977). Axons of SCN neurons project to the paraventricular nucleus (PVN) and perhaps synaptic contacts are made within the PVN (Klein et al., 1983). In fact, complete destruction of either the SCN or PVN is required to block photic stimulation of the pineal gland in rats or monkeys (Klein and Moore, 1979; Reppert et al., 1981; Klein et al., 1983). For instance, as little as 5% of the PVN is sufficient for normal circadian enzyme function in the pineal gland (Klein et al., 1983). Although further neuronal connections to the pineal gland are less well defined, several pathways have been hypothesized (Swanson

Figure 1. Schematic diagram depicting neural connections from the eye to pineal gland of mammals. Legend: RHP = retinohypothalamic projections, SCN = suprachiasmatic nucleus, PVN = paraventricular nucleus, MFB = medial forebrain bundle, RF = reticular formation, IML = intermediolateral cell column, SCG = superior cervical ganglia, ICN = inferior carotid nerve, NC = nervi conarri.

Redrawn from: Moore and Klein, 1974.
Klein et al., 1983.
Reiter, 1983.



Sagittal View



Ventral View

and Kuypers, 1980). The preponderance of information suggests that axons project from the PVN through the medial forebrain bundle (MFB) and reticular formation (RF) where long descending axons carry the signal to the intermediolateral cell column of the upper thoracic spinal cord (Klein et al., 1983; Reiter, 1983). Preganglionic sympathetic neurons then project up the sympathetic trunk and terminate within synapses of the superior cervical ganglia (SCG). The SCG are bilateral, and lie at the junction between the common carotid and the internal and external carotid arteries. Postganglionic axons proceed from the SCG via the inferior carotid nerve (ICN) to the vicinity of the pineal gland where they form distinct nerve bundles (nervi conarri; Kappers, 1960). Neural projections then penetrate the capsule of the pineal gland and terminate primarily in pericapillary spaces (Reiter, 1983). Until recently, it was thought that the mammalian pineal gland acted exclusively as a neuroendocrine organ under orchestration from efferent sympathetic innervation. However, Korf et al. (1986) demonstrated in hamsters that pinealocytes send neural projections to various areas of the brain. Therefore, the mammalian pineal gland may communicate directly via neurons with various areas of the brain in addition to its neuroendocrine function.

2. PINEAL GLAND FUNCTION

Photoperiodic information from an animals' environment is translated into a neural signal which travels the

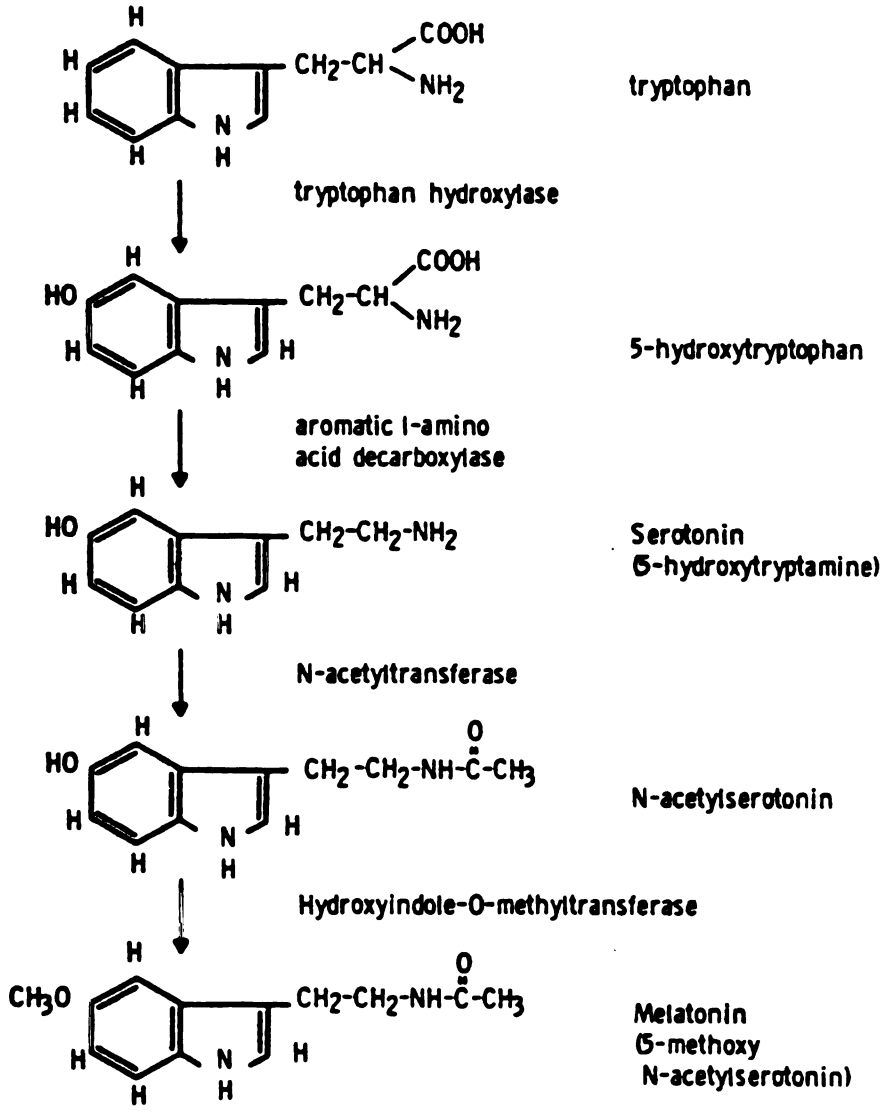
circuitous pathway previously described between the eyes and pineal gland. The pineal gland in turn functions as an endocrine organ and converts the neural signal into a hormonal signal. The primary secretory product of the pineal gland is melatonin. Melatonin is a hormone which is secreted in a circadian pattern under regulation of the CNS (Wurtman et al., 1964a).

3. MELATONIN SYNTHESIS AND SECRETION

Melatonin is synthesized from the amino acid, tryptophan, by the synthetic indole pathway diagramed in figure 2. There are three important points concerning this pathway. First, of the two enzymes responsible for converting serotonin to melatonin (N-acetyltransferase, NAT; and hydroxyindole-O-methyltransferase, HIOMT), NAT is rate-limiting for melatonin formation (Klein, 1973) and is frequently described in the literature as an index of melatonin synthesis. HIOMT serves as a marker for specific sites of melatonin formation. Second, essentially all of the indoles are found in relatively high concentrations in the pineal gland with serotonin attaining greatest concentrations. Furthermore, serotonin turnover is greater in pineal tissue than any other area of the CNS (Cardinali, 1981). The final point I would like to make is that the pathway for indole synthesis occurs in tissues other than the pineal gland including Harderian gland (Cardinaili and Wurtman, 1972), retina (Cardinali and Resner, 1971) and intenstine (Bubenik et al., 1977). However, excent for

Figure 2. Metabolic pathway for melatonin synthesis.

Source: Martin et al. 1976.


tryptophan
tryptophan hydroxylase
5-hydroxytryptophan
aromatic l-amino
acid decarboxylase
Serotonin
(5-hydroxytryptamine)
N-acetyltransferase
N-acetylserotonin
Hydroxyindole-O-methyltransferase
Melatonin
(5-methoxy
N-acetylserotonin)

minor contributions from the retina (Hamm and Menaker, 1980; Pang et al., 1980), diurnal variation of melatonin secretion is attributed primarily to pineal tissue. Extra-pineal melatonin sources are probably responsible for persistent low concentrations of circulating melatonin after pineal gland removal (Ozaki and Lynch, 1976; Kennaway et al., 1977) but function of this melatonin is unknown.

4. CHARACTERITICS OF MELATONIN

Most studies confirm that the amount of melatonin present in pineal tissue is a direct reflection of melatonin concentrations in blood, urine (Ozaki and Lynch, 1976) and usually CSF (Minneman and Wurtman, 1976; Wurtman and Hedlund et al., 1977; Moskowitz, 1977). In addition, melatonin is not stored for extended lengths of time within the pineal gland, but is secreted as it is produced (Reiter, 1983). The half-life of melatonin in blood of rats and sheep is approximately 20-min (Cardinali et al., 1983) but may be longer in neonatal rats due to lower catabolism of melatonin by liver (Weinberg et al., 1981).

Radioactive melatonin injected systemically is taken up by virtually every tissue of the body (Kopin et al., 1961). Specific tissues such as the pineal gland, eye, gonads, adrenal and hypothalamus appear to take up relatively high concentrations of the hormone (Wurtman et al., 1964b; Anton-Tay and Wurtman, 1969). Specific melatonin receptors have since been found in the medial basal hypothalamus (MBH) (Cardinali et al., 1978), pituitary gland (Lang and

Sizonenko, 1980), and in peripheral tissues such as gonads, uterus, liver and eyes (Cohen et al., 1978). Highest affinity binding sites for melatonin seem to be in the MBH. Actions of melatonin on peripheral tissues have not been determined.

Several reports indicate that melatonin secretion decreases with age (Reiter, 1983). Basal melatonin concentrations (with lights on) do not appear to be affected by age, but nocturnally produced melatonin is approximately 3-fold higher in 2-mo old than in 18-mo old hamsters (Reiter et al., 1980). In addition, diminished nocturnal production of melatonin with age is a gradual process in rodents (Reiter et al., 1981a). In humans, peak nocturnal melatonin concentrations increase from birth to about 5 yr of age, but thereafter melatonin declines through puberty, plateaus, and then declines further with old age (Reiter, 1986). The prepubertal melatonin decline is suggested to be a trigger for commencement of prepubertal events in children (Kolata, 1984).

5. BIOLOGICAL EFFECTS OF MELATONIN

More melatonin is secreted from the pineal gland during darkness than during periods of light in virtually all species examined (Wurtman et al., 1983). However, the pattern of nocturnal melatonin varies among species (Reiter, 1983). For example, Syrian hamsters exhibited peak melatonin secretion late in the dark period; whereas, albino rats and Turkish hamsters show peak melatonin activity

during the middle of the dark period. In contrast, peak melatonin concentrations appear in Richardson's ground squirrel early in the dark period (Reiter et al., 1981b). Another variation in melatonin secretory patterns appears in Djungarian hamsters, cotton rats, white-footed mice (Reiter, 1983) and Suffolk sheep (Bittman et al., 1983) where nocturnal pattern of melatonin secretion closely corresponds to the total duration of darkness provided.

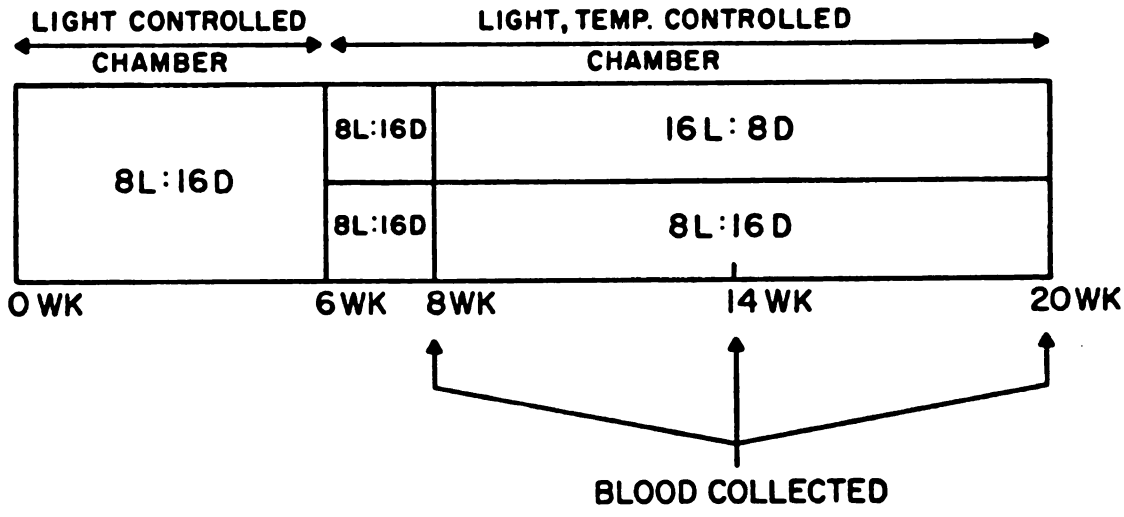
The means by which melatonin conveys photic information so that an animal can adjust its' endocrine profile in accordance with season has only recently been examined. Two major hypotheses exist, which are not necessarily exclusive (Reiter, 1986). The first hypothesis builds on the fact that in some species, the longer the night, the more prolonged is the daily melatonin rise. This is the duration hypothesis, which stated simply, relays photic information to an animal through duration of increased daily melatonin secretion from the pineal gland. In fact, it is duration of elevated melatonin concentrations which control reproductive and hormonal responses to melatonin in sheep (Bittman and Karsch, 1984) and Djungarian hamsters (Goldman et al., 1984). The second hypothesis, known as the internal coincidence hypothesis, predicts the existence of two rhythms. One rhythm is provided by diurnal melatonin secretion and the second is an endogenous rhythm of sensitivity to melatonin. The animal's physiological response is based on the synchrony and coincidence of these

two rhythms (Reiter, 1986). Predictably, the internal coincidence hypothesis is much more difficult to test, particularly in terms of melatonin sensitivity.

G. OBJECTIVES

The overall objective of the following series of experiments is to study the effect of photoperiod on hormones which may be important in photoperiod-regulated growth and onset of puberty responses, and to examine the mechanism through which photoperiod affects circulating hormone concentrations in bull calves. The following general design was utilized in five different series (chapters) of experiments.

EXPERIMENTAL DESIGN:



Modifications of this design are explained within the text of each chapter and consist primarily of differences in photoperiod treatments and blood collection intervals. Eight bull calves were placed into the light controlled chamber within 3 d of age and assigned to one of two light- and temperature-controlled chambers (four calves per chamber) at approximately 6 wk of age.

Chapter 1

Effect of Continuous Light on Prolactin Secretion in Prepubertal Holstein Bulls

Introduction

A light-dark cycle of 16 h light:8 h dark (16L:8D) increases milk yield (Peters et al., 1978), growth rates (Peters et al., 1978) and hastens puberty onset (Petitclerc et al., 1983a) in cattle compared with 9 to 12 h daily light. Prolactin (PRL) concentrations in blood of cattle are consistently modulated by photoperiod. For example, after abruptly switching from 8L:16D (short-day) to 16L:8D (long-day), at least 1 wk elapses before concentrations of PRL in serum begin to increase (Leining et al., 1979; Stanisiewski et al., 1984) and within 4 to 6 wk, concentrations of PRL increase about 5-fold in serum of bull calves (Leining et al., 1979). Conversely, decreasing daily duration of light exposure from 16 to 8 h decrease PRL concentrations in serum (Bourne and Tucker, 1975). Similar PRL changes in response to photoperiod have been reported for sheep (Lincoln et al., 1978; Sanford et al., 1978).

Rats exposed to 24L:0D have greater PRL concentrations in blood than animals receiving 10 to 12 h of darkness daily (Piacsek et al., 1978; Vaticon et al., 1980). In calves, when daily duration of light is increased gradually (.38 h per day) from 8 to 24 h, PRL increases in parallel with duration of light until 24L:0D is reached, after which PRL decreases within 1 wk to concentrations that are not different from concentrations in calves exposed to 8L:16D (Bourne and Tucker, 1975). Whether this decrease in PRL is

due to abrupt loss of darkness (scotoperiod) or duration below a critical scotoperiod is unknown. In addition, it is not known whether a period of absolute darkness is required to obtain the characteristic PRL response to 8 or 16 h of light per day. Therefore, experiments were conducted to quantify PRL concentrations in bull calves after switching from 8L:16D to either 16L:8D or to continuous light.

Materials and Methods

Animals and Housing. Eight Holstein bulls were used in each of three experiments as described below. Beginning at approximately 3 d of age bulls were individually penned in 1.1 by 1.8 m stalls within a light-controlled room. Calves received 8L:16D for 6 to 10 wk from cool-white fluorescent lamps (General Electric F40CW/RS/WM). All lamps came on at 0700 h and switched off at 1500 h. An 8L:16D photoperiod was used to establish low baseline concentrations of PRL in serum of calves (Leining et al., 1979). Following this pretreatment conditioning, calves were assigned by weight to one of two light and temperature controlled chambers (four calves per chamber). Calves were unrestrained within each chamber and received a grain concentrate ration (BIR Milking Chow, Ralston Purina Co., St. Louis, MO), alfalfa hay, mineral supplement block and water ad libitum. Ambient temperature in each chamber was maintained at 21 $\pm$ 2 C. After 2 wk additional pretreatment exposure to 8L:16D in the light and temperature controlled chambers, calves were fitted with an indwelling jugular polyvinyl cannula (Ico-

Rally Corp. SLV 105 18 clr) as reported (Leining et al., 1979). On the following day (day 0 of experiment) at 0700 h animals were restrained loosely with halters, and blood was sampled (10 ml) and discarded at 15-min intervals for 1 h to acclimate the animals to the sampling procedure. Starting at 0800 h, blood was collected at 30-min intervals for 26 (Exp. 1) or 6 h (Exp. 2). Between collection of samples cannulas were filled with 3.5% sodium citrate to maintain cannula patency.

Exp. 1. The objective of this experiment was to compare concentrations of PRL in serum of calves abruptly switched after the pretreatment period of 8L:16D to 16L:8D or 24L:0D . At 6 wk of age, mean body weights of four bulls in each chamber averaged 76.6 and 77.1 $\pm$ 7.6 kg (means $\pm$ S.E.D.). Median light intensities at eye level of standing calves were 364 and 395 lx.

The day after collection of initial blood samples, photoperiod was switched from 8L:16D to 16L:8D (0300-1900 h) in one chamber and to 24L:0D in the other for 9 wk. Calves were bled three additional times at 3-wk intervals as described.

Exp. 2. The design of Exp. 1 precluded direct comparisons between calves of the same age maintained on 8L:16D and calves abruptly switched to continuous light. Thus, Exp. 2 was conducted to examine the PRL response in calves abruptly switched from 8L:16D to 24L:0D vs calves continuously maintained under 8L:16D.

Bulls were preconditioned to 8L:16D as previously described. Mean body weight of four bulls in each chamber at initiation of the experiment was 111.5 and 111.7 ± 8.6 kg. Following preconditioning under 8L:16D, photoperiod was maintained at 8L:16D in one chamber but switched to 24L:0D in the other for 8 wk. Median light intensities were 460 and 471 lx. Bulls were bled for 6 h at 30-min intervals on wk 0, 4 and 8.

Blood Handling and Assay. Blood samples were allowed to clot for 6 to 8 h at room temperature, stored for 24 h at 5 C, then centrifuged at approximately 1000 x g for 20 min. Serum was decanted and frozen at -20 C until assayed for PRL as previously described (Koprowski and Tucker, 1973).

Statistical Analysis. For each experiment data were analyzed using split-plot analysis of variance (Gill, 1978b) to test for differences in concentrations of serum PRL between and within bleeding periods. For a given treatment within a 26 or 6 h bleeding period PRL did not differ over time; therefore, a mean PRL value was calculated for each animal during each 26 (Exp. 1) or 6 (Exp. 2) h bleeding period. Within each photoperiod treatment, contrasts between bleeds were made using Bonferroni-t tests (Gill, 1978b). For the purpose of treatment and bleed comparisons, variances were expressed as standard errors of differences (Gill, 1981). See appendix for a listing of sources of variation and specific contrasts.

Results

Exp. 1. After 8 wk pretreatment exposure to 8 h of light per day PRL averaged 18.6 and 18.5 ng/ml of serum in the two pretreatment groups ($P > .05$; figure 3). After subjecting bulls to 24L:0D or 16L:8D for 3 wk, PRL was unchanged in bulls receiving 24L:0D but was increased 3.6-fold in bulls exposed to 16L:8D ($P < .01$). After 6 wk, concentrations of PRL in serum increased 2.4- to 3.5-fold in bulls subjected to 24L:0D or 16L:8D relative to previous exposure of 8L:16D. Overall, concentrations of PRL in calves receiving 24L:0D did not differ from concentrations in calves exposed to 16L:8D over the 9-wk duration of the experiment ($P > .05$).

Exp. 2. After 10 wk pretreatment exposure to 8L:16D, PRL averaged 22.0 and 17.0 ng/ml of serum in the two groups of four animals ($P > .05$; figure 4). Four wk after abruptly switching to 24L:0D, PRL was unchanged and did not differ from concentrations in calves maintained under 8L:16D. However, 8 wk after the switch, PRL had increased 1.9-fold in bulls exposed to 24L:0D compared with bulls maintained under 8L:16D ($P < .05$).

Discussion

The 3.6-fold increase in concentrations of PRL in serum of bull calves (Figure 1) following an abrupt shift from 8L:16D to 16L:8D observed at wk 3 in Exp. 1 confirmed previous observations using similar protocols (Leining et al., 1979; Stanisiewski et al., 1984). Furthermore, it was previously shown that after a lag of 2 to 3 wk, concentrations of PRL

Figure 3. Prolactin in serum from prepubertal bulls switched from 8 h of light per day (at wk 0) to continuous (24 L) or 16 h of light per day. Each observation represents the mean of four bulls where blood samples were collected for 26 h through a jugular cannula. Standard error of the difference between treatments was 27.9 ng/ml of serum and for wk was 10.6 ng/ml of serum.

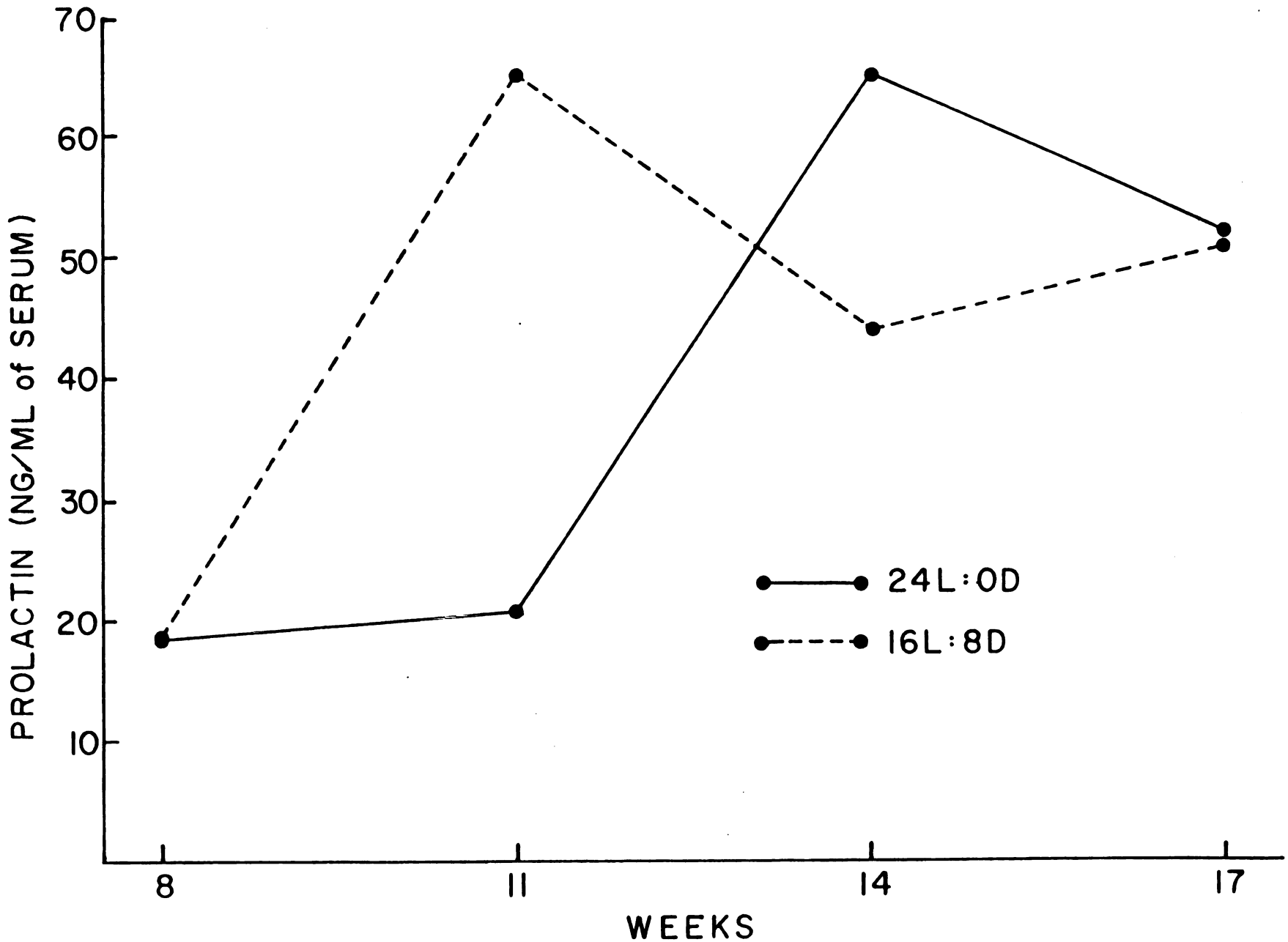
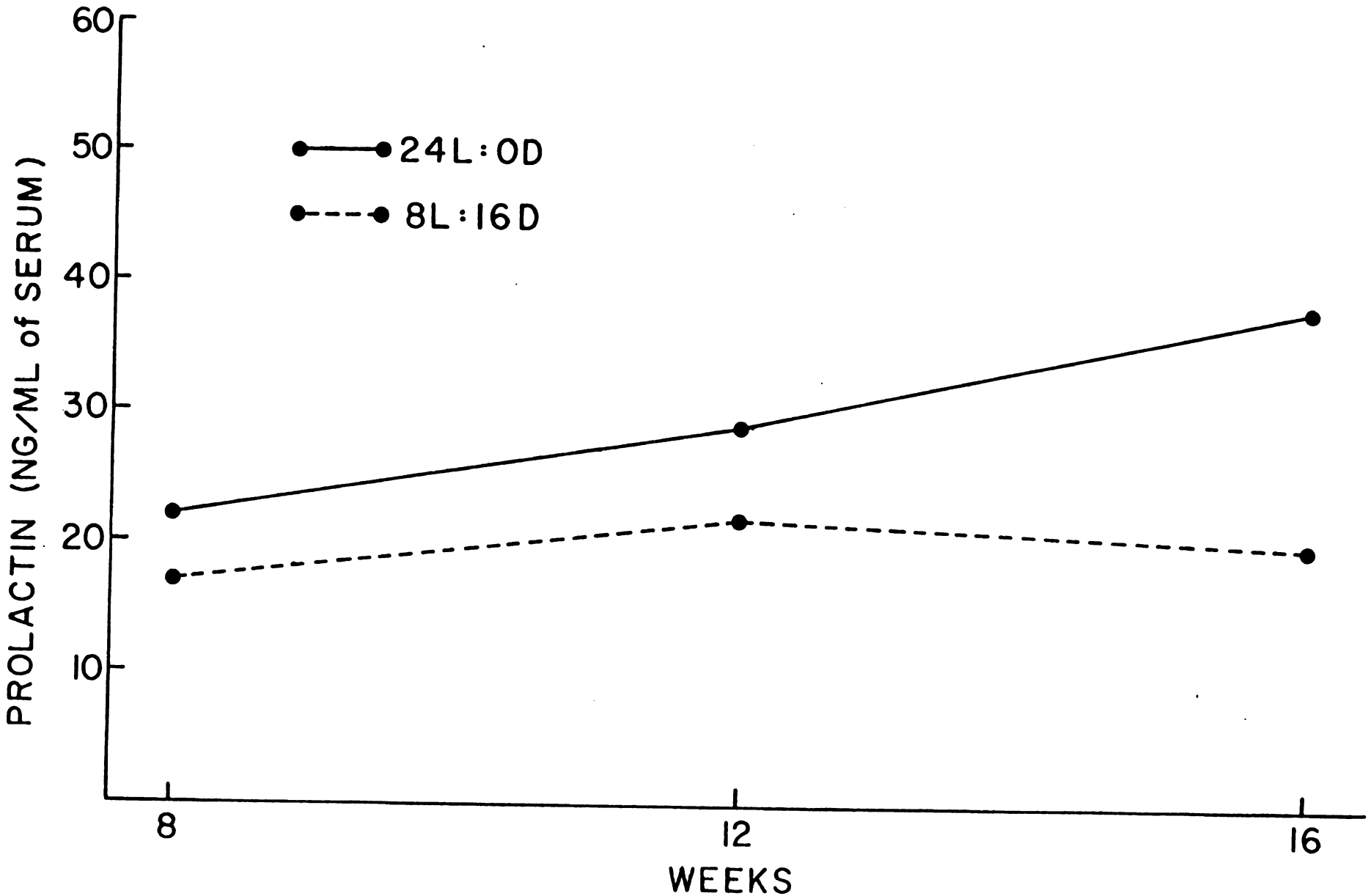

70
60
50
40
30
20
10
PROLACTIN (NG/ML of SERUM)
8
11
14
17
WEEKS
24L:0D
16L:8D

Figure 4. Prolactin in serum from prepubertal bulls switched from 8 h of light per day (at wk 0) to continuous (24 L) or maintained on 8L:16D. Each observation represents the mean of four bulls where blood samples were collected for 6 h through a jugular cannula. Standard error of the difference between treatments was 5.5 ng/ml of serum and for wk was 4.6 ng/ml of serum.


60
50
40
30
20
10
24L:0D
8L:16D
PROLACTIN (NG/ML of SERUM)
8
12
16
WEEKS

in serum of bull calves gradually increased in parallel with gradual increases in duration of light between 8L and 24L only to be followed by a decrease as 24 L continued (Leining et al., 1979). In the present series of experiments, concentrations of PRL in serum increased when photoperiod was abruptly switched from 8L:16D to continuous light. However, in Exp. 1, calves abruptly switched from 8L:16D to 16L:8D achieved maximum concentrations of PRL after 3 wk, whereas switching to continuous light required 6 wk before a peak in serum PRL was seen. A similar observation was made in Exp. 2 in which a total of 8 wk were required after the switch from 8 to 24L before a significant increase in PRL was detected. Absolute increments of PRL in response to 24 L were not the same in Exp. 1 and 2; however, responses of different groups of calves to identical photoperiods are rarely similar in terms of magnitude of PRL change (Stanisiewski et al., 1984). The data are consistent with the hypothesis that daily dawn-dusk (light-dark) transitions provide an external cue used to mediate seasonal changes in the physiology of mammals (Pelletier et al., 1985). The lack of a daily dawn-dusk transition in bulls abruptly switched to continuous light may explain the slower responsiveness in terms of increased serum concentrations of PRL. The mechanism whereby continuous light causes an eventual increase in concentrations of PRL in bulls is not known, but I speculate that it may be associated with a circannual rhythm analogous to that observed in circadian

rhythms (Simpson and Follett, 1982). Rats receiving 24L:0D demonstrate a rhythm of PRL secretion with a periodicity of 6 to 8 h (Bethea and Neill, 1979) but no such rhythm was detected in my calves, as differences at 30 min intervals were small. In the absence of photoperiodic and seasonal cues, Petitclerc et al. (1983b) observed an endogenous circannual rhythm of PRL secretion in cattle. However, the duration of present experiments were not sufficient to detect a complete cycle of hormone activity. Therefore, I do not know whether the animals were exhibiting a circannual rhythm.

When compared with 12 to 14 h daily dark exposure, male rats receiving 24L:0D for 7 d have lower PRL concentrations in blood (Relkin, 1972), whereas male and female rats receiving 24L:0D for 41 to 60 d have higher PRL in blood (Piacsek et al., 1978; Vaticon et al., 1980). Likewise, in my calves, continuous light must be applied for 6 to 8 wk before PRL increases. Furthermore, PRL enhances reproductive development in male rats (Moger et al., 1972). In fact, male rats exposed to 24L:0D from birth have larger testis by d 55 than rats exposed to periods of daily darkness (Hoffmann, 1973). But whether the ability of photoperiod to hasten sexual maturation in cattle (Petitclerc et al., 1983a; Hansen et al., 1983) is mediated in part through PRL remains to be determined.

The tendency ($P < .1$) for concentrations of PRL to decline in Exp. 1 despite maintenance of 16L:8D or

continuous light is consistent with the hypothesis that animals may become refractory to a given repetitive photoperiodic signal (Sharp and Moss, 1977; Follett et al., 1975) Decline in concentrations of serum PRL in response to continuous lighting has been reported previously (Leining et al., 1979).

In summary, calves receiving constant light for more than 3 wk have greater concentrations of PRL in serum than calves exposed to 8 h of daily light. However, maximal PRL concentrations can be achieved sooner if a 16L:8D photoperiod is provided. I found that continuous light photoperiods stimulate PRL secretion in bull calves relative to 8L:16D, but the time course to achieve maximum PRL concentrations in serum is slower with continuous light vs 16L:8D.

Chapter 2

Effect of Photoperiod and Castration on Prolactin, Testosterone and Luteinizing Hormone Concentrations in Male Calves.

Introduction

Switching photoperiod from 8L:16D to 16L:8D increases serum PRL concentrations in prepubertal bulls 2- to 8-fold (Bourne and Tucker, 1975; Stanisiewski et al., 1984). In previous experiments with cattle comparing 8L:16D with 16L:8D, maximum duration of exposure to a single photoperiod was 6 wk (Stanisiewski et al., 1984). The effect of photoperiods maintained longer than 6 wk on PRL is unknown. In addition, rams exposed to decreasing daily light decreased PRL but increased LH and testosterone concentrations in plasma (Lincoln et al., 1977; Sanford et al., 1978). Previous researchers failed to detect a photoperiod effect on LH concentrations in cattle (Bourne and Tucker, 1975; Rzepkowski et al., 1982). However, progesterone concentrations in serum of young heifers exposed to long days increase at an earlier age relative to heifers receiving short-day photoperiods (Petitclerc et al., 1983a), which suggests that long-day photoperiods may hasten onset of puberty in heifers.

My objective was to examine long term effects of long- vs short-day photoperiods on PRL, LH and testosterone secretion in bull calves. A second objective was to determine if PRL response to photoperiod in prepubertal bulls was dependent upon the testes.

Materials and Methods

Pre-treatment. In each of two experiments (1 and 2) eight Holstein bulls (approximately 3 d of age) were placed

into a light-controlled room as previously described (Stanisiewski et al., 1984). Fluorescent lights were programmed on at 0700 h and off at 1500 h (8L:16D). After weaning at approximately 6 wk of age, calves were moved to one of two light-and temperature-controlled chambers described previously (Stanisiewski et al., 1984), where ambient temperatures were maintained at 21 ± 2C during Exp. 1 and at 15 ± 1C during Exp. 2. Photoperiod was then maintained at 8L:16D for 2 additional wk. Thus, pretreatment photoperiod lasted a total of 8 wk. Calves were fed a concentrate mixture plus alfalfa hay and water ad libitum.

In Exp. 2, testes were removed surgically from four calves at 2 wk of age. Four additional calves were maintained as gonadally intact controls.

Treatments. In Exp. 1, four calves were maintained on 8L:16D, whereas four calves were switched to 16L:8D (lights on from 0300 to 1900 h) for 12 additional wk. In Exp. 2, photoperiod was switched from 8L:16D to 16L:8D on all calves (four castrates, four gonadally intact controls) for 6 wk. Median light intensities measured 1 m above the floor were equalized between chambers within each experiment (546 lx, Exp. 1; 517 lx, Exp. 2).

Blood Sampling. Two d prior to start of treatments, calves were fitted with an indwelling jugular cannula (SLV 105 18 clr)[3] and bled the following day. On day of sampling, animals were loosely restrained by halter at 0700

h and blood samples were discarded at 15-min intervals for 1 h to accustom animals to the sampling procedure. Beginning at 0800 h, 8 to 10 ml of blood was collected for 6 h at 30-min intervals. All calves had been exposed to a total of 8 wk of 8L:16D at the time of this first blood collection. In Exp. 1, blood was sampled again at wk 14 and 20 of age; in Exp. 2, blood was sampled again at wk 14.

Sodium citrate (3.5%) was used to prevent coagulation of blood in cannulas between collection of samples. Blood samples were allowed to clot for 6 to 8 h at room temperature, stored for 24 h at 5C, then centrifuged at approximately 1000 x g for 20 min. Serum was decanted and frozen at -20C until assayed.

Hormone Assays. Previously described radioimmunoassays were used to quantify PRL (Koprowski and Tucker, 1971), LH (Convey et al., 1976) and testosterone (Haynes et al., 1977) in serum.

Statistical Analysis. Data from each experiment were analyzed using split-plot analysis of variance (Gill, 1978b) to test for differences in concentrations of serum PRL, LH, and testosterone between and within bleeding periods. Main effects were photoperiod treatment and day of bleeding. Hormone values were averaged over each 6-h sampling period to obtain a representative mean. Bonferroni t test (Gill, 1978a) was used to test for differences between means of treatments and bleeding days. Frequency of LH and testosterone pulses per 6-h were determined using a pulse

analysis program (PULSAR; Merriam and Wachter, 1982). See appendix for a listing of sources of variation and specific contrasts.

Results

Exp. 1. After 8 wk pretreatment with 8L:16D, PRL averaged 17.0 and 19.6 (± 4.2, pooled SE) ng/ml (P > .05) of serum for both groups of four animals (figure 5). PRL in serum was greater (P < .02) in calves exposed to 6 wk of 16L:8D than in calves maintained under 8L:16D [93.8 vs 36.9 (± 16.1) ng/ml; figure 5]. However, after 12 wk exposure to 16L:8D (20 wk of age), PRL in serum declined and was not different (P > .05) from PRL in bulls exposed to 8L:16D [34.7 and 17.2 (± 6.4) ng/ml; figure 5]. In calves maintained under short days, PRL did not change (P > .05) throughout the experiment.

Concentrations of LH were not different (P > .05) between photoperiod treatments nor between bleeding periods (figure 6). Pooled among treatments, LH averaged .80 ± .16, 1.06 ± .13 and .73 ± .20 ng/ml of serum for the eight calves at 8, 14 and 20 wk of age (figure 6). Frequency of LH pulses per 6-h were unaffected (P > .25) by photoperiod treatment and did not change with age, averaging 2.9, 2.9 and 2.1.

Testosterone in each group of four bulls after pretreatment with 8L:16D for 8 wk averaged .39 and .47 (± .03) ng/ml of serum (P > .05, figure 7). Six wk later (age 14 wk), testosterone in serum was greater (P < .05) in

Figure 5. Prolactin in serum from bull calves exposed to 8 h of light per day from 3 d to 8 wk of age and subsequently switched to 16 h of light per d or maintained on 8L:16D. Each observation represents the mean of four bulls where blood samples were collected at 30-min intervals for 6 h through a jugular cannula. Standard error of the difference for treatments was 23.9 ng/ml of serum and for wk of age was 9.8 ng/ml of serum.

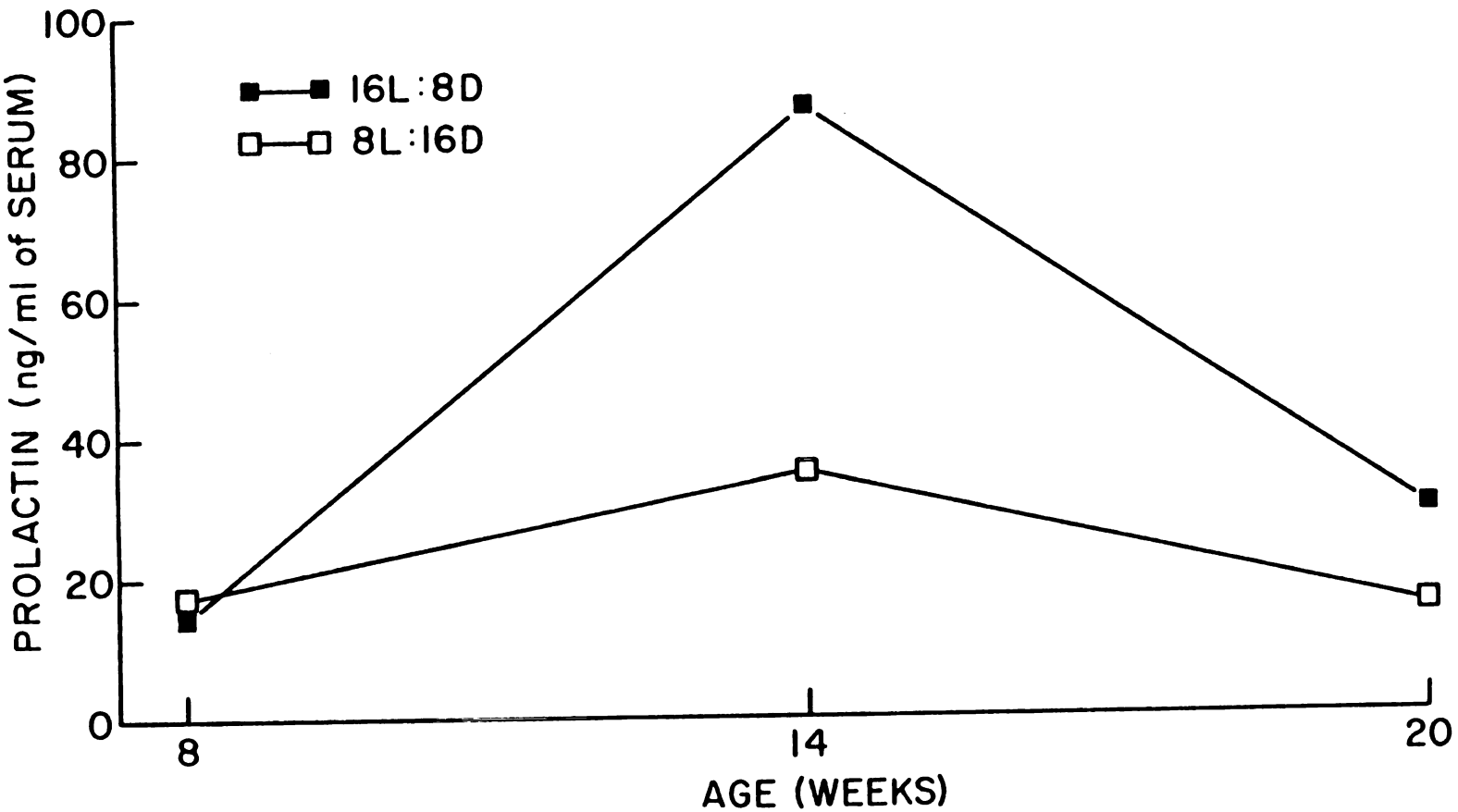
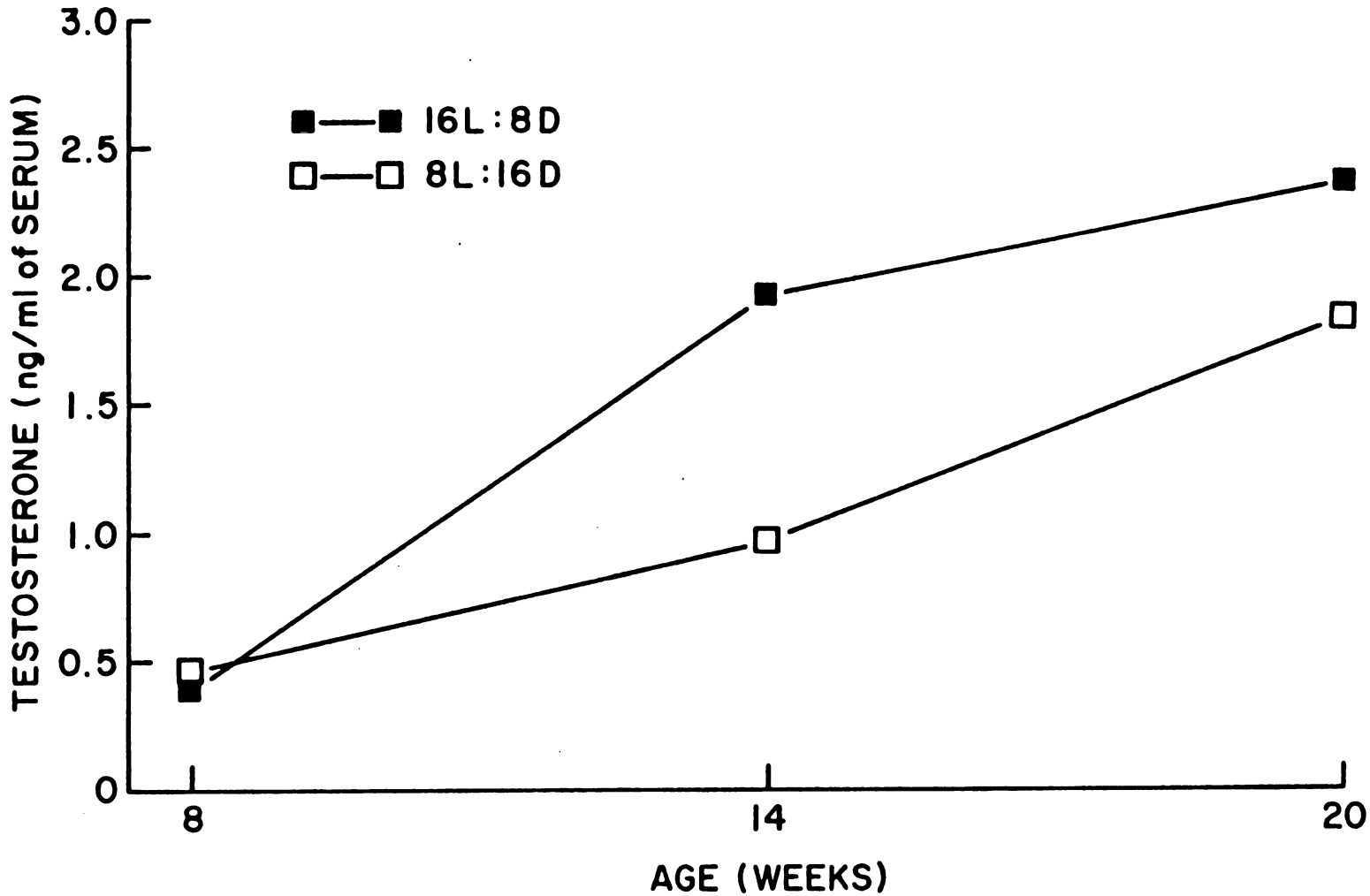

100
80
60
40
20
0
PROLACTIN (ng/ml of SERUM)
16L:8D
8L:16D
8
14
20
AGE (WEEKS)

Figure 6. Luteinizing hormone in serum from bull calves exposed to 8 h of light per day from 3 d to 8 wk of age and subsequently switched to 16 h of light per d or maintained on 8L:16D. Each observation represents the mean of four bulls where blood samples were collected at 30-min intervals for 6 h through a jugular cannula. Standard error of the difference for treatments was .23 ng/ml of serum and for wk of age was .19 ng/ml of serum.


2.0
1.5
1.0
0.5
0
16L:8D
8L:16D
LH (ng/ml of SERUM)
8
14
20
AGE (WEEKS)

Figure 7. Testosterone in serum from bull calves exposed to 8 h of light per day from 3 d to 8 wk of age and subsequently switched to 16 h of light per d or maintained on 8L:16D. Each observation represents the mean of four bulls where blood samples were collected at 30-min intervals for 6 h through a jugular cannula. Standard error of the difference for treatments was .23 ng/ml of serum and for wk of age was .29 ng/ml of serum.


3.0
2.5
2.0
1.5
1.0
0.5
0
TESTOSTERONE (ng/ml of SERUM)
16L:8D
8L:16D
8
14
20
AGE (WEEKS)

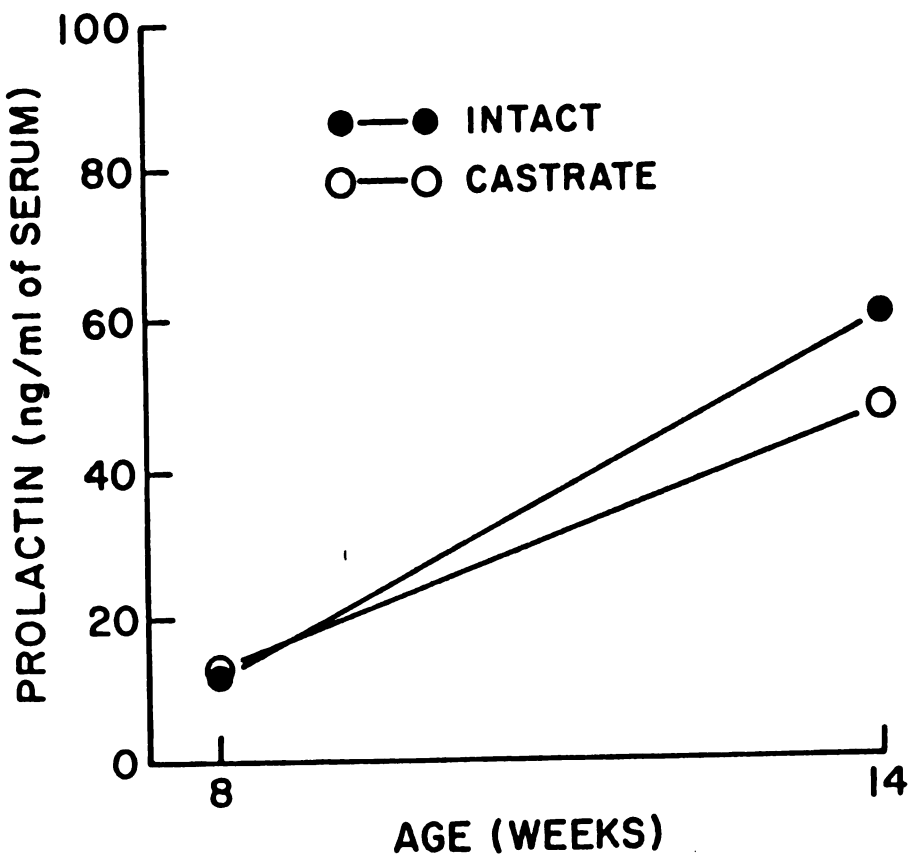
calves receiving 16L:8D (1.92 ± .19 ng/ml) than in calves maintained under 8L:16D (.97 ± .19 ng/ml, figure 7). By wk 20 of age, testosterone in serum was numerically greater in 16L:8D exposed bulls but not statistically different ($P > .05$) from testosterone in bulls given 8L:16D (2.36 and 1.83 ± .21 ng/ml). Frequency of testosterone pulses per 6-h were unaffected ($P > .25$) by photoperiod treatment and did not change ($P > .25$) with age, averaging 2.0, 2.5 and 2.0 at wk 8, 14 and 20 of age.

Exp. 2. PRL in serum was not different ($P > .05$) between castrated (12.5 ng/ml) and intact (12.1 ng/ml) calves pretreated with 8L:16D through 8 wk of age (figure 8). Subsequently, PRL concentrations in serum after 6 wk of 16L:8D increased ($P > .01$) similarly in castrated and intact calves to (48.0 and 59.8 (± 12.7) ng/ml, ($P > .05$) respectively. LH in serum was greater ($P < .01$) in castrated (3.0 ± .35 ng/ml) than in intact (1.4 ± .35 ng/ml) calves 6 wk after castration. LH in serum was unchanged ($P < .19$) after 6 wk of 16L:8D in castrated (3.6 ± .27 ng/ml) and intact (1.7 ± .27 ng/ml) calves.

Discussion

I previously showed that abruptly switching photoperiods of calves from short-to long-days linearly increased serum PRL concentrations with the maximum occurring within 6 wk after the switch (Stanisiewski et al., 1984). Data in the present study confirm this response. In addition, I have now demonstrated that long-day induced

Figure 8. Prolactin in serum from calves exposed to 8 h of light per day from 3 d to 8 wk of age and subsequently switched to 16 h of light per day. Each observation represents the mean of four bulls where blood samples were collected at 30-min intervals through a jugular cannula for 6 h. Castrations were performed at 1.5 to 2 wk of age. Standard error of the difference for treatments was 14.1 ng/ml of serum and for wk of age was 11.1 ng/ml of serum.


100
80
60
40
20
0
PROLACTIN (ng/ml of SERUM)
INTACT
CASTRATE
8
14
AGE (WEEKS)

increases in PRL concentrations (at 6 wk after switch) are not maintained indefinitely, but decline to concentrations that are not significantly different from those in calves maintained under short-days. Thus, elevated PRL concentrations in bull calves become refractory to a once stimulatory photoperiod. Photorefractoriness as reflected by PRL secretion and gonadal function has been documented in sheep (Almeida and Lincoln, 1984) and hamsters (Bittman, 1978). In seasonal breeding species such as sheep and hamsters, photorefractoriness serves as an inherent control mechanism whereby reproduction is limited to specific seasons (Robinson et al., 1985). On the other hand, the reason bull calves become refractory to long-days in terms of PRL secretion and what function this serves is unclear.

Circulating concentrations of testosterone normally increase linearly between 8 and 24 wk of age in bulls (Bedair and Thibier 1979; Amann and Walker, 1983). My results are in agreement with this pattern. In addition, I discovered that long-days increase the rate at which serum testosterone increases. Therefore, I speculate that puberty onset may be achieved at an earlier age in bulls exposed to long-days when compared with bulls exposed to short-day photoperiods. Duration of photoperiod hastens onset of puberty in heifers (Petitclerc et al., 1983a), boars (Mahone et al., 1979), rams (Howles et al., 1980), deer (Budde, 1983) and hamsters (Hoffmann, 1978). Photoperiod control of sexual development at puberty plays an important role in

seasonal breeders in which reproductive activity is limited to certain times of the year, but the function of this maturational response in nonseasonal breeders such as cattle is not known.

Long-day photoperiods stimulate growth rates and protein deposition in cattle (Tucker et al., 1984) and sheep (Forbes et al., 1979). In cattle, these effects require the gonad (Tucker et al., 1984). Since, in the present study, long days induced an early increase in serum concentrations of testosterone and testosterone stimulates growth in ruminants (Hafs et al., 1971; Schanbacher et al., 1980) I suggest that testosterone may at least be a partial mediator of photoperiod effects on growth in bulls. Because of few animal numbers in the present study, photoperiod treatment effects on body growth were not measured.

As reported previously in cattle (Bourne and Tucker, 1975; Rzepkowski et al., 1982), LH concentrations in castrated and intact bulls were unaffected by photoperiod. In adult bulls, LH controls testosterone secretion (Hafs and McCarthy, 1980); however, in bulls younger than 20 wk of age, serum LH is not closely coupled to testosterone secretion (Karg et al., 1976; Lacroix and Pelletier, 1979). Therefore, it is not surprising that concentrations of LH were not associated with long-day stimulation of testosterone secretion in my experiment. In fact, frequency of LH and testosterone pulses did not change throughout Exp. 1, and pulses between the two hormones were usually

asynchrouous. Furthermore, PRL is capable of increasing LH receptors in rat (Morris and Saxena, 1980) and hamster (Rex et al., 1978) testes, but similar effects are not seen in rams (Barenton, 1981). In rats, hyperprolactinemia transiently increases circulating testosterone (Waeber et al., 1983) while LH concentrations are unchanged. Perhaps long-day induced PRL concentrations increase LH receptors in prepubertal calf testes, and thus may potentiate the effects of LH on testosterone secretion.

In the present study I showed that long-days increased concentrations of PRL in serum 4- to 5-fold compared with previous short-day exposure, and this response occurred independent of the testes. The observed increase in PRL was well within the 2- to 8-fold range previously reported (Bourne and Tucker, 1975; Stanisiewski et al., 1984). However, I do not know whether long-day induced increases in PRL are causally associated with increased secretion of testosterone. To test this possibility in a preliminary study, four bull calves received a long-day photoperiod while four similar calves received a short-day photoperiod. Two long-day exposed bulls were given 2-Br- ergocryptine (CB-154) to reduce PRL secretion. After 4 wk of treatment, PRL averaged 2.8, 43.1 and 23.0 ng/ml of serum in long-day plus CB-154, long-day and short-day treated animals, respectively. Concurrently, testosterone in serum averaged .51, .95 and .85 ng/ml for the same respective treatments. Testosterone was lowest in calves with lowest PRL

concentrations. However, animal numbers were too few and animals may have been too young to detect significant differences. The trend supports the hypothesis that photoperiod-induced testosterone increases were associated with increased secretion of PRL, however, more animals are needed to substantiate the trend.

In summary, long-days stimulated PRL secretion relative to short-days in bull calves, but the stimulation was not maintained indefinitely. This response occurred independent of the gonad. I speculate that onset of puberty may also be hastened in bulls exposed to 16L:8D because testosterone in serum of these bulls was elevated at an earlier age than in bulls given 8L:16D. This early increase in testosterone was associated with PRL but not LH.

Chapter 3

Prolactin and Catecholamine Concentrations in Blood and Pituitaries of Prepubertal Bulls Exposed to 16 or 8 Hours of Light per Day

Introduction

Concentrations of PRL in serum of bull calves are increased about 5-fold when photoperiod is switched from 8L:16D to 16L:8D compared with calves maintained on 8L:16D (Stanisiewski et al., 1984). The neuroendocrine mechanism controlling photoperiod-induced PRL secretion is unknown. However, evidence indicates that PRL release from the anterior pituitary gland is tonically inhibited by dopamine (DA) (Leong et al., 1983), and possibly other catecholamines (Shaar and Clemens, 1974). Both DA receptor binding and hypothalamic DA concentration vary with season in rats (Naber et al., 1981) and humans (Karson et al., 1984). Thyrotropin-releasing hormone (TRH) stimulates PRL release in vitro and in vivo (Beck et al., 1979, Smith and Convey, 1975) and is considered a principal physiological regulator of PRL. Furthermore, TRH-induced PRL release is greater in sheep (Howland et al., 1983) and calves (Leining et al., 1979) exposed to long days compared with short days. Therefore, our objective was to determine effects of 16L:8D and 8L:16D on concentrations of DA, norepinephrine (NE) and epinephrine (EPI) in the pituitary and on release of PRL from pituitaries of prepubertal bulls.

Materials and Methods

Animals and Housing. Beginning at approximately 3 d of age, eight bull calves were penned individually in a light-controlled (8L:16D) room. This pretreatment period was used to establish low baseline PRL concentrations in serum

(Stanisiewski et al., 1984). Specific housing conditions and diets were previously described (Leining et al., 1979). At about 7 wk of age, calves were assigned by weight (average 71.4 ± 2.2 kg) in to one of two light and temperature controlled chambers (four calves per chamber). Two wk later (wk 0 of experiment) calves were fitted with an indwelling jugular cannula as described (Leining et al., 1979) and bled (10-ml per sample) the following d from 0700 h to 0800 h (15-min interval presampling) and thereafter at 30-min intervals for 6 h. Ambient temperatures were 21.3 ± .8 C. Four calves then received 16L:8D for 6 additional wk while the other four calves were maintained on 8L:16D. At the completion of the experiment (wk 6), calves were bled again as described and slaughtered the following d at a local abattoir, where pituitaries were removed from each calf within 10 min of death.

Blood Handling and Assay. At the time of collection of each blood sample , approximately 2 ml of blood was centrifuged at 1000 x g for 10 min and 500 ul of resulting plasma was added to 50 ul .2N perchloric acid and frozen immediately on dry ice until assayed (radioenzymatic) for total DA, NE and EPI (Peuler and Johnson, 1977: Umezu and Moore, 1978). Remaining portions of samples were allowed to clot for 6 to 8 h at room temperature, stored for 24 h at 5 C, then centrifuged at 1000 x g for 20 min. Serum was decanted and frozen at -20 C until assayed for PRL as previously described (Koprowski and Tucker, 1973).

Tissue Handling and Incubation. Pituitaries were removed from calves and placed on ice. Approximately 20 min later, a medial slice of posterior and anterior pituitaries was frozen (-70 C) until assayed for DA, EPI and NE content as described above. Remaining anterior pituitary glands from each calf were sliced into 1 mm^2 explants and about 20 mg (weights recorded) of tissue was transferred to 3 ml Medium 199. Tissues were washed at 15-min intervals for 1 h then incubated for 1.5 h in a Metabolic Shaking Incubator (Precision Scientific, Chicago, IL) at 37 C under oxygen. Media were harvested (pre-treatment) for quantification of basal PRL concentrations released. In a 2 by 3 factorial experimental design, tissue from calves exposed to each photoperiod was incubated for an additional 2 h with TRH (12 ng/ml of media), DA (60 ng/ml of media) or vehicle (medium 199). Concentrations of TRH and DA are within reported ranges to satisfactorily stimulate or inhibit PRL release from pituitaries in vitro (Smith and Convey, 1975; Fagin and Neill, 1981). Treated media were decanted (post-treatment) and stored (-20 C) until assayed for PRL.

Statistical Analysis. Split-plot analysis of variance (Gill, 1978b) was used to compare hormone concentrations in blood, as well as PRL concentrations in media. PRL release into media was expressed as pre-to post-treatment difference. Catecholamine contents in pituitaries were analyzed by Chi Square analysis (Gill, 1978a). See appendix for a listing of sources of variation and specific contrasts.

Results

Prolactin in Blood. At wk 0 of the experiment, PRL averaged 43 ng/ml of serum over 6 h in each group of calves receiving 8L:16D. PRL was unchanged ($P > .1$) 6 wk later in calves maintained under 8L:16D (32 ng/ml of serum) but was increased ($P < .01$) after 16L:8D treatment (75 ng/ml of serum).

Catecholamines in Blood. Concentrations of catecholamines in blood were quantified at the end of 6 wk treatment with 8L:16D or 16L:8D. DA declined ($P < .01$) throughout the 6 h sampling period in all calves averaging .33 and .36 ng/ml of plasma ($P > .25$) in 8L:16D and 16L:8D exposed calves (figure 9). NE averaged .41 and .44 ng/ml over the 6 h and were not significantly different ($P > .25$), and EPI averaged .26 and.25 ng/ml ($P > .25$) in 8L:16D- and 16L:8D-exposed calves, respectively, (figure 10, 11).

Catecholamines in Pituitaries. Anterior pituitary DA tended to be greater ($P < .16$) in calves exposed to 8L:16D (.35 ng/mg of protein) than in 16L:8D-exposed calves (.21 ng/mg of protein; table 1). NE was not detectable and EPI in anterior pituitaries was not different ($P > .25$) between photoperiod treatments (table 1). Posterior pituitary concentrations of DA, NE and EPI concentrations did not differ ($P > .25$) between photoperiod treatments.

In Vitro PRL Release. Pre-treatment PRL concentrations averaged 440 and 326 ng/ml medium·mg^{-1} pituitary ($P > .25$) in 8L:16D- and 16L:8D-exposed calves, respectively, (table

Figure 9. Dopamine in plasma from prepubertal bulls receiving 8 or 16 h of light per day. Each observation represents the mean of four bulls where blood samples were collected for 6 h through a jugular cannula.

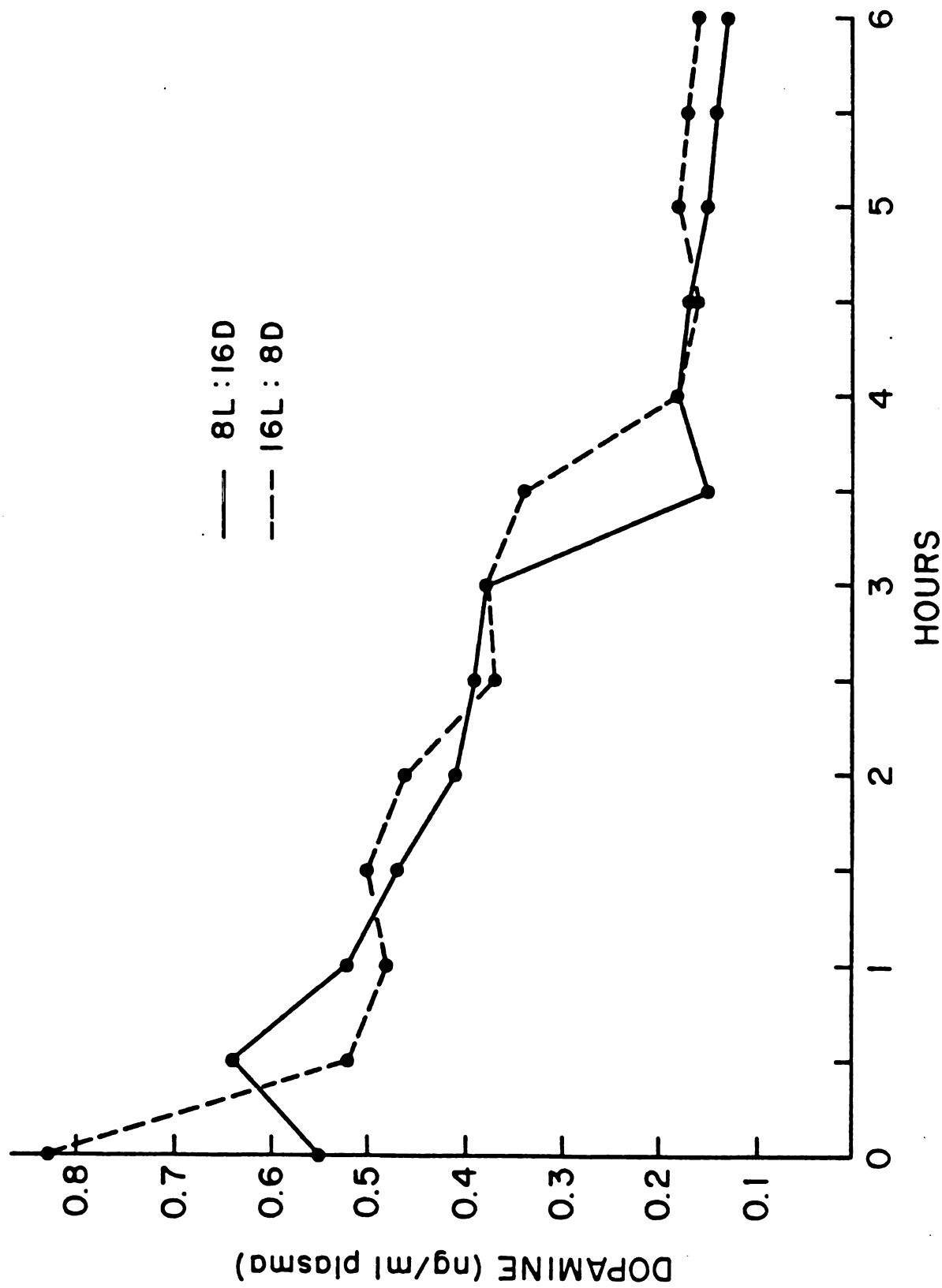

8L:16D
16L:8D
DOPAMINE (ng/ml plasma)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
HOURS

Figure 10. Norepinephrine in plasma from prepubertal bulls receiving 8 or 16 h of light per day. Each observation represents the mean of four bulls where blood samples were collected for 6 h through a jugular cannula.

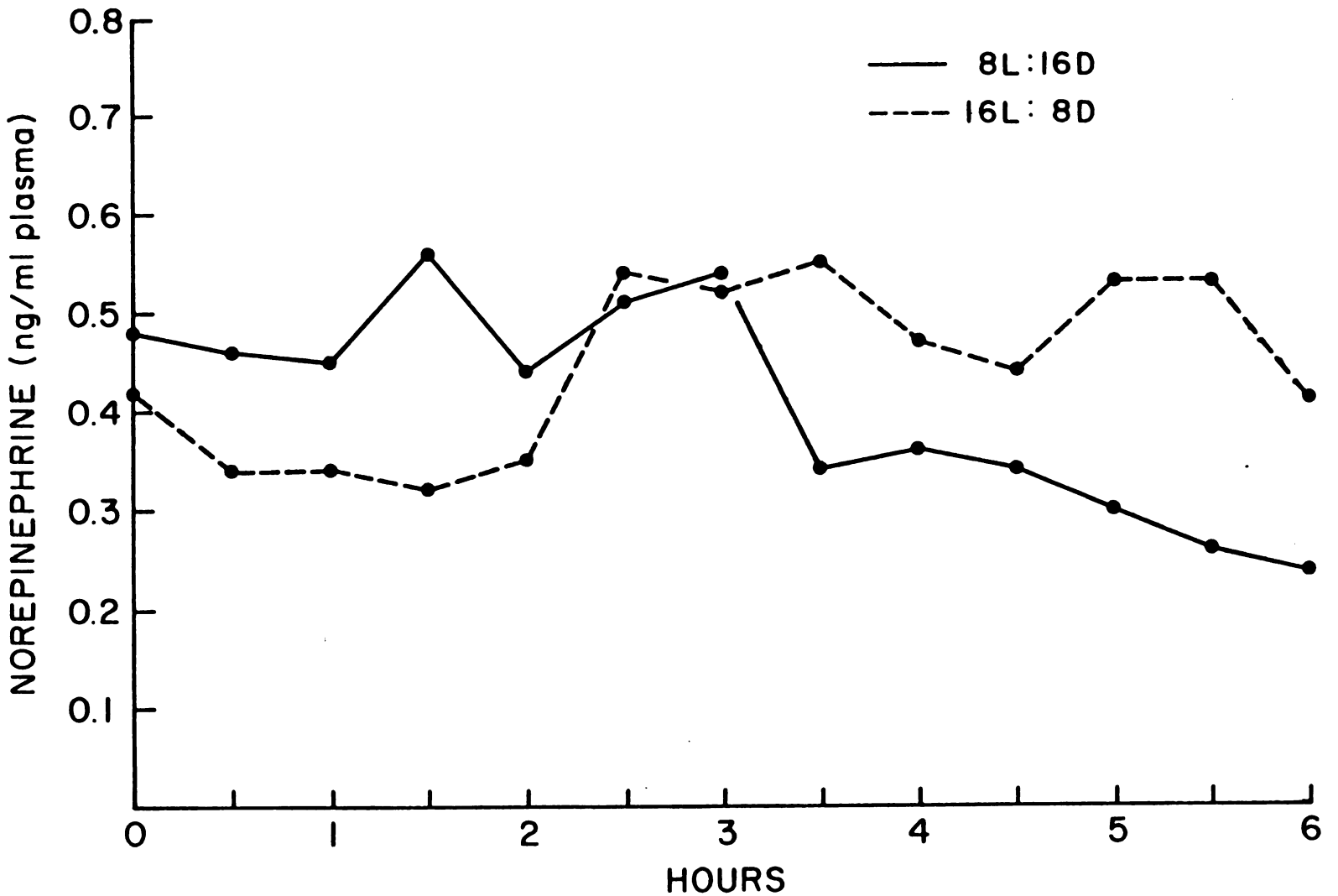

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
NOREPINEPHRINE (ng/ml plasma)
8L:16D
16L: 8D
0
1
2
3
4
5
6
HOURS

Figure 11. Epinephrine in plasma from prepubertal bulls receiving 8 or 16 h of light per day. Each observation represents the mean of four bulls where blood samples were collected for 6 h through a jugular cannula.

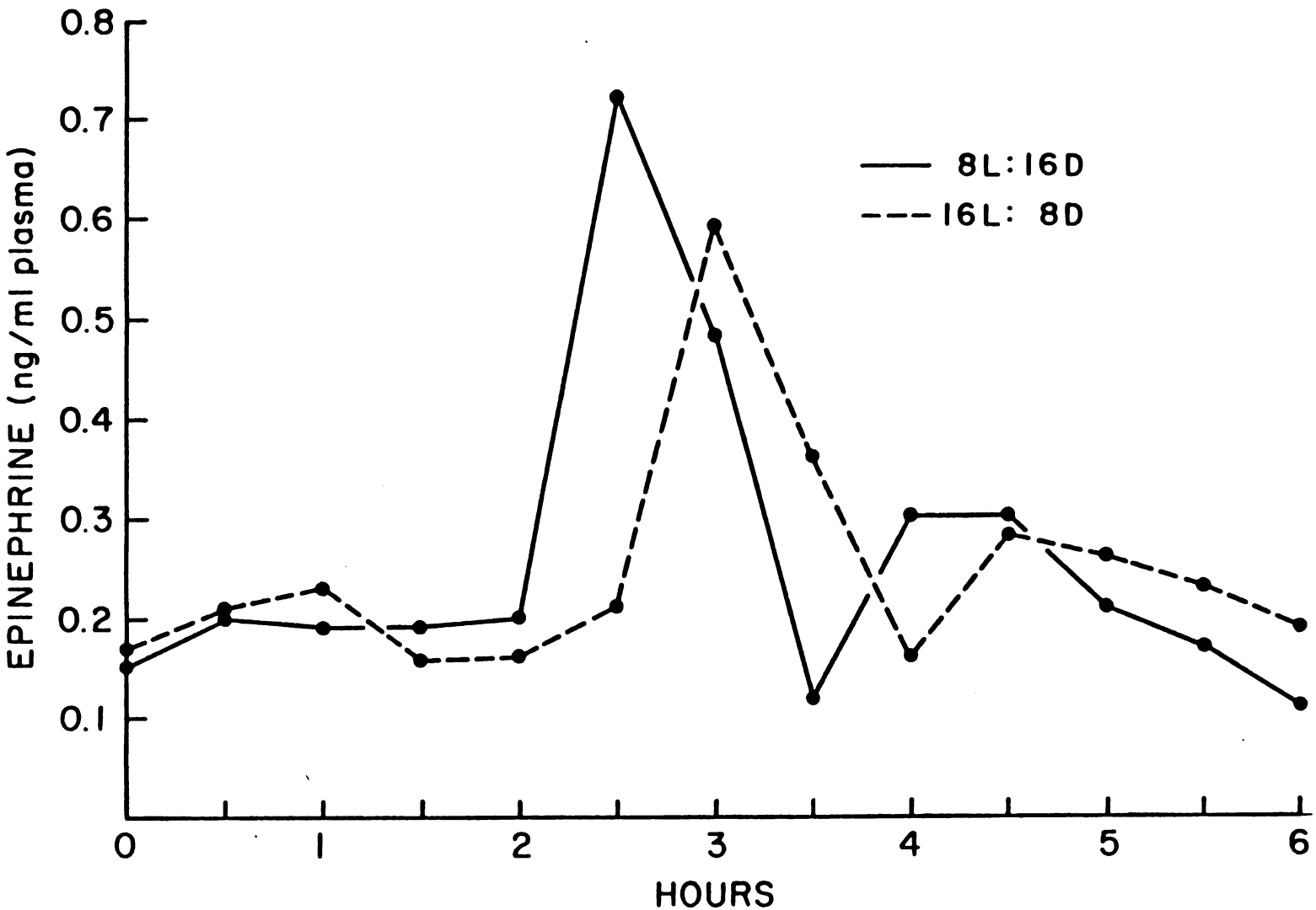

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
EPINEPHRINE (ng/ml plasma)
8L:16D
16L: 8D
0
1
2
3
4
5
6
HOURS

Table 1. PITUITARY CATECHOLAMINE CONCENTRATIONS[a]

ITEM		16L:8D	8L:16D	P
Anterior pituitary	DA	.21	.35	<.16
	NE	-	-	
	EPI	.06	.05	NS
Posterior pituitary	DA	2.58	2.52	NS
	NE	.26	.14	NS
	EPI	.13	.16	NS

[a] ng/mg protein.

Table 2. PRL RELEASE FROM ANTERIOR PITUITARY EXPLANTS[a]

	16L:8D	8L:16D
Basal release	326	440
Treatment challenge[b]		
Control	0[c]	0[c]
TRH	+141[d]	+90[d]
DA	-55[e]	-105[e]

[a] ng/ml medium·mg^{-1} pituitary.

[b] Data expressed as change from control.

c,d,e, Values with different superscripts differ ($P<.01$).

2). PRL concentrations were unchanged (P > .25) in media from pituitaries challenged with vehicle, but relative to controls TRH increased (P < .01) PRL release 90 and 141 ng/ml medium.mg^{-1} pituitary (P > .2) in explants from 8L:16D and 16L:8D exposed calves, respectively, (table 2). Conversely, addition of DA to media decreased (P < .01) PRL release compared with controls [-105 ng/ml.mg^{-1}, 8L:16D and -55 ng/ml.mg^{-1}, 16L:8D (P > .2)].

Discussion

In agreement with previous studies (Leining et al., 1979; Stanisiewski et al., 1984), PRL concentrations in serum were increased by 16L:8D exposure relative to 8L:16D. Photoperiod is a major contributor to seasonal variability of PRL concentrations in cattle (Koprowski and Tucker, 1973; Petitclerc et al., 1983b). Consequently, in vitro release and pituitary gland content of PRL correspond to seasonal changes in circulating concentrations of the hormone in cattle (Dular and LaBella, 1977). I now have evidence that DA may be involved in photoperiod-modulated PRL release since DA concentrations were greatest in anterior pituitary glands of calves with lowest circulating PRL concentrations.

Van Maanen and Smelik (1968) first proposed the concept that the monaminergic tubero-infundibular neural system was responsible for direct control of PRL secretion. Measurement of DA in hypophyseal stalk plasma in quantities sufficient to inhibit PRL release substantiated this hypothesis (De Greef and Neill, 1979). Furthermore, Gudelsky et al. (1980) suggested

that 75 to 85% of DA in the anterior pituitary gland is associated with secretory granules (lactotrophs) containing PRL. I propose that the difference in pituitary gland DA concentrations is a chronic response to several weeks of photoperiod treatment. Further experiments are needed to determine whether changes in anterior pituitary DA concentrations are closely coupled with change in serum concentrations of PRL in response to photoperiod.

Others have shown that TRH-induced PRL release is greater in bulls (Leining et al. 1979) and ewes (Howland et al., 1983) exposed to long as compared with short days. Furthermore, I found that in vitro TRH-induced PRL release was numerically greater (57%) from pituitary glands previously exposed to 16L:8D compared with 8L:16D exposure although this preliminary result needs to be confirmed. However, if TRH increases PRL secretion in vitro more in 16L:8D- than in 8L:16D-exposed calves, then perhaps a chronic photoperiod-induced change in the lactotrophs has occurred. It is unknown whether this change is associated with change in number of lactotrophs or sensitivity of lactotrophs to PRL releasing or inhibiting factors. Since TRH and DA probably mediate PRL secretion through opposing effects on intracellular signals within lactotrophs (Ray and Wallis, 1984), higher DA concentrations in 8L:16D exposed pituitary glands may partially explain photoperiodic differences in TRH-induced PRL release.

Chapter 4

Effect of Pinealectomy on Prolactin, Testosterone and Luteinizing Hormone Concentrations in Plasma of Bull Calves Exposed to 8 or 16 Hours of Light per Day

Introduction

PRL concentrations in serum are greater in calves exposed to 16L:8D than in calves exposed to 8L:16D (Bourne and Tucker, 1975; Stanisiewski et al., 1984). In addition, testosterone concentrations in peripubertal bulls increase at an earlier age with 16L:8D exposure than with 8L:16D (Stanisiewski et al., 1987b). Concentrations of LH in serum of cattle are unaffected by photoperiod (Bourne and Tucker, 1975; Rzepkowski et al., 1982).

The mechanism whereby photoperiod affects hormone concentrations in cattle is not understood, but in sheep, removal of the pineal gland abolishes normal photoperiod-induced changes in PRL (Brown and Forbes, 1980; Brinklow and Forbes, 1984) and testosterone (Barrell and Lapwood, 1979). Pinealectomy of young steers reduces 16L:8D-induced increments in PRL concentrations in serum, whereas blinding completely abolishes the response (Petitclerc et al., 1983b). The pathway for signal transmission between the pineal gland and anterior pituitary gland is less clear; however, others have suggested that the indolamine, melatonin, likely acts as a hormonal transducer (Goldman and Darrow, 1983). Melatonin is synthesized primarily in the pineal gland, and serum concentrations are greatest during darkness (Goldman and Darrow, 1983).

My objective was to examine the effect of pinealectomy on PRL, testosterone and LH in peripubertal bulls exposed to 8 or 16 h of light per day.

Materials and Methods

Animals and Housing. The following housing conditions and treatments were followed using two replicates of eight Holstein bulls. Eight bulls approximately 3 d of age were placed into a light-controlled room as previously described (Stanisiewski, et al., 1984). All calves received 8L:16D daily (lights on 0700 h) through weaning at approximately 6 wk of age. Within replicate, four calves were pinealectomized (PX) on average at 5.6 wk of age while four additional calves were sham pinealectomized (SPX) using surgical procedures previously reported (Braun et al., 1977). Excised tissue was examined histologically to confirm removal of pineal tissue. Surgeries were performed at the Michigan State University Veterinary Clinical Center where calves remained under constant light for approximately 1.5 wk while recovering from surgery. Calves were then returned to 8L:16D light exposure. At approximately 18 wk of age, calves were assigned to one of two light-and temperature-controlled chambers as described (Stanisiewski et al., 1984) and fed a commercial calf grain diet plus alfalfa hay ad libitum. Body weights were 116.1 $\pm$ 6.4 kg (mean $\pm$ S.E.) at the time of chamber assignment. Within replicate there were two PX and two SPX calves per chamber and photoperiod remained at 8L:16D for 2 additional wk. Ambient temperatures were 22.1 $\pm$ 1.8C for the first replicate of eight animals and at 20.9 $\pm$ .6C for the second replicate. Median light intensities in chambers averaged 411 lx 1 m above the floor.

Photoperiod Treatment. Within replicate beginning at approximately 20 wk of age (wk 0 of experiment), two PX and two SPX calves received 16L:8D daily for 12 wk while at the same time two PX and two SPX calves were maintained on 8L:16D.

Blood Sampling. Two d prior to initiation of photoperiod treatments at wk 0, calves were fitted with an indwelling jugular catheter connected to a 3 m cannula and the cannula was passed into an adjoining room. Thus, blood could be sampled remotely without disruption of calves' ambient photoperiod/scotoperiod. On the day prior to initiation of photoperiod treatment at wk 0, calves were pre-sampled (blood was discarded) beginning at 1300 h for 1 h and thereafter bled for 26 h at 30-min intervals. All calves had been exposed to approximately 14 wk of 8L:16D at the time of this first collection of blood. Calves were recannulated blood was sampled again at wk 4, 8 and 12. Ambient temperatures were recorded at the time of each sample collection.

Sodium citrate (3.5%) was used to prevent coagulation of blood in cannulas between sample collections. Blood plasma was collected using .25 ml of a 30% sodium citrate solution per 10 ml of blood and stored at 5C. Samples were centrifuged at approximately 1000 x g for 20 min and plasma was frozen at -20C until assayed.

Hormone Assays. Previously described radioimmunoassays were used to quantify PRL (Koprowski and Tucker, 1971),

testosterone (Haynes et al., 1977) and LH (Convey et al., 1976) concentrations in plasma. Direct assay of plasma concentrations of melatonin were performed using the procedure and antibody of Fraser et al. (1983). To validate this assay in our laboratory, melatonin was quantitatively recovered (100 pg: 91.0 ± 4.7%, 500 pg: 94.7 ± 3.8%, 1000 pg: 90.6 ± 7.3%; mean ± S.E., n = 6). In addition, assay parallelism was tested using three serum samples at dilutions compatable with the usable range of the standard curve (table 3).

Statistical analysis. Main effects (replicates, surgical treatment, photoperiod treatment and week on experiment) on hormone concentrations were analyzed using split-plot analysis of variance (Gill, 1978b). Subsequent analyses were performed using Bonferroni t test (Gill, 1986) to examine differences between treatments and week on experiment. To account for the range of ages among calves and ambient temperature differences between replicates (see p. 83), age and ambient temperature were used as covariates to adjust testosterone and PRL concentrations, respectively. See appendix for a listing of sources of variation and specific contrasts.

Results

At wk 12 of experiment, melatonin in plasma of PX calves receiving 16L:8D averaged 18.9 pg/ml with lights on and 21.9 pg/ml with lights off ($P > .25$; figure 12). In SPX calves receiving 16L:8D, melatonin averaged 15.8 pg/ml when

Table 3. CONCENTRATIONS OF MELATONIN IN DIFFERENT VOLUMES OF BOVINE SERUM

Sample no.[a]	Volume of serum (ul)	Calculated melatonin (pg/ml)
1	100	56
	200	49
2	200	84
	300	86
	400	93
3	100	192
	200	248
	300	225

[a]There were six replicates per sample.

lights were on and 92.0 pg/ml when lights were off (figure 12). One PX calf receiving 8L:16D had average light:dark melatonin values of 6.5 and 5.4 pg/ml of plasma while the remaining three PX calves in this treatment demonstrated a dark-induced rise in melatonin from 16.7 during lights on to 60.1 pg/ml of plasma during the dark period (figure 13). Since these three calves were apparently incompletely pinealectomized, data from these calves were deleted from calculations. With lights on, melatonin averaged 16.6 pg/ml of plasma in SPX calves on 8L:16D and increased ($P < .05$) to 71.2 pg/ml when lights were off (figure 13).

Concentrations of PRL in plasma differed ($P < .05$) between the two replicates of eight animals, but this difference was eliminated when data were adjusted for differences in ambient temperatures. Therefore, replicate was eliminated from the statistical model.

After 14 wk of 8L:16D treatment (wk 0 of the experiment), PRL averaged 47.1 ng/ml of plasma among calves (figure 14). Concentration of PRL in plasma was unaffected ($P > .25$) by surgical treatment. At wk 4 of experiment, PRL increased ($P < .01$) in plasma of PX (79.8 ng/ml) and SPX (96.3 ng/ml) calves receiving 16L:8D while remaining unchanged ($P > .25$) in PX (52.8 ng/ml) and SPX (47.8 ng/ml) calves maintained on 8L:16D (figure 14). Prolactin concentrations in plasma remained higher ($P < .05$) in calves receiving 16L:8D compared with calves maintained on 8L:16D through wk 12 of experiment.

Figure 12. Twenty-six h profiles of melatonin in plasma of bull calves receiving 16L:8D after 12 wk on experiment. Each observation represents the mean of four bulls. Samples were collected at 1-h intervals through a jugular cannula. The hatched bar along the x-axis represents darkness. Standard errors of means during light and darkness; respectively, were 6.8 and 21.8 pg/ml of plasma for SPX calves and 6.3 and 6.0 for PX calves.

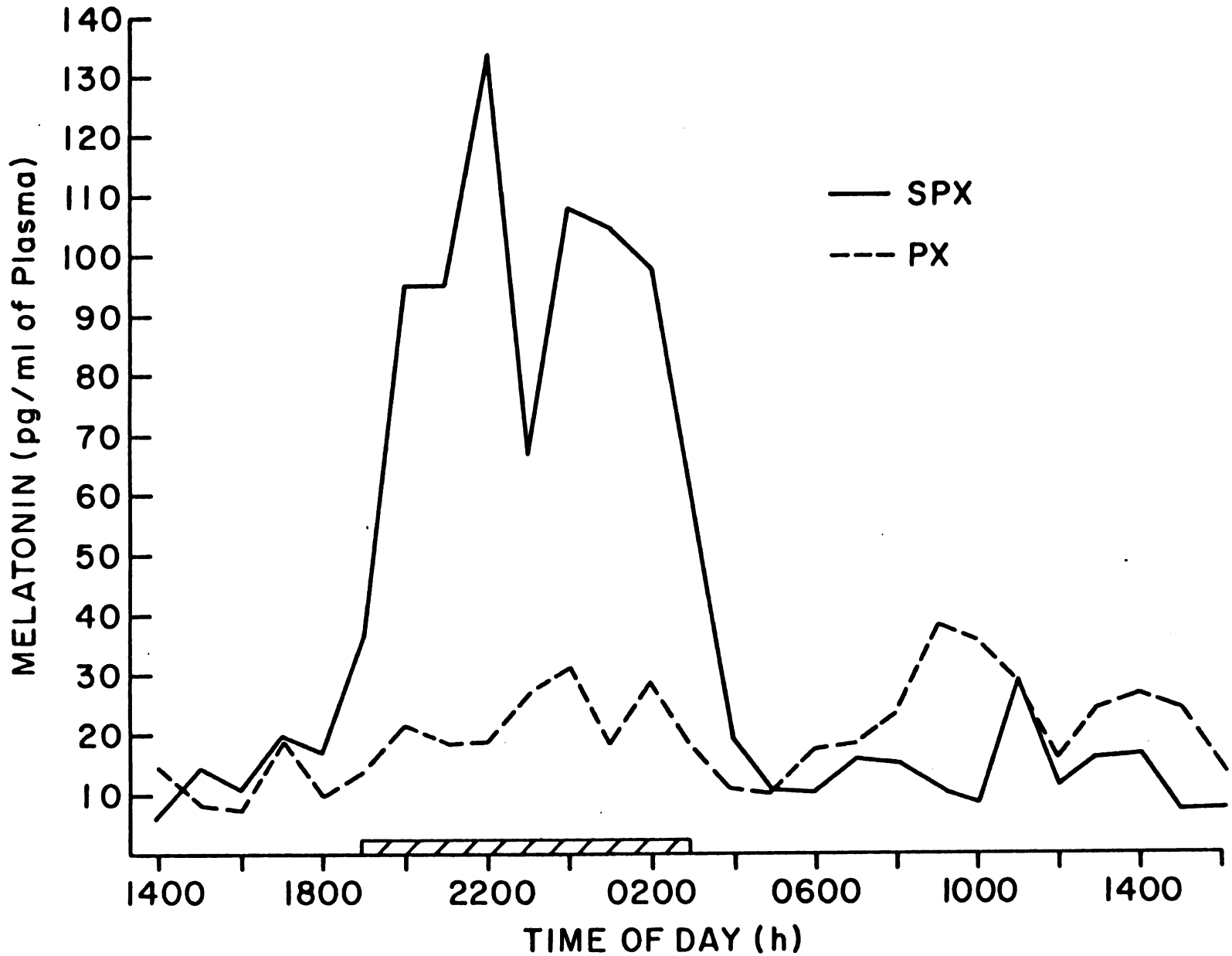

MELATONIN (pg/ml of Plasma)
140
130
120
110
100
90
80
70
60
50
40
30
20
10
SPX
PX
1400
1800
2200
0200
0600
1000
1400
TIME OF DAY (h)

Figure 13. Twenty-six h profiles of melatonin in plasma of bull calves receiving 8L:16D after 12 wk on experiment. Each observation represents the mean of four (SPX), three (Incomplete PX) or one (PX) bulls. Samples were collected at 1-h intervals through a jugular cannula. Hatched bars along the x-axis represent darkness. Standard errors of means during light and darkness; respectively, were 8.3 and 35.6 pg/ml of plasma for SPX calves, 6.9 and 27.2 pg/ml of plasma for incomplete PX calves and 6.4 and 5.4 pg/ml of plasma for the PX calf.

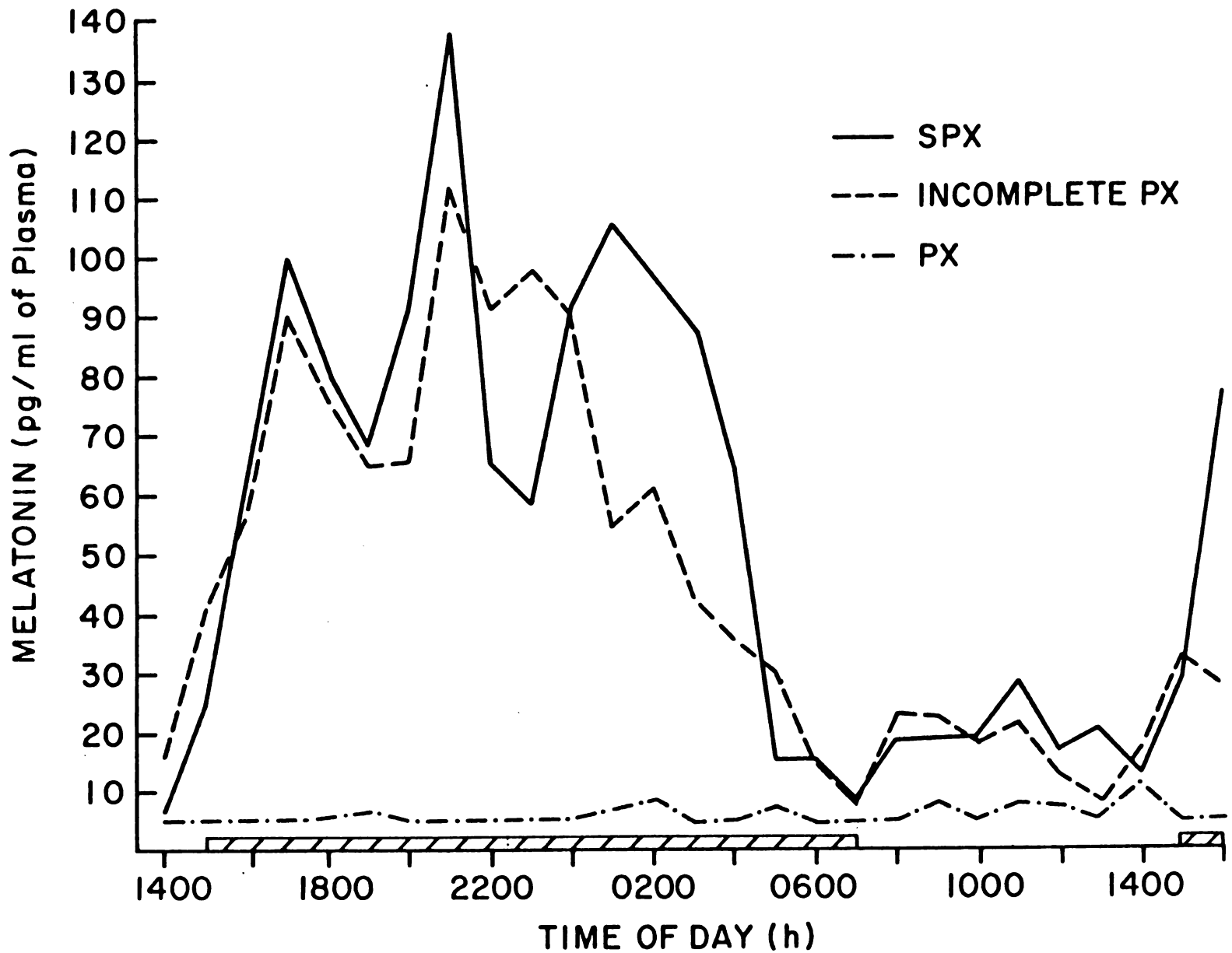
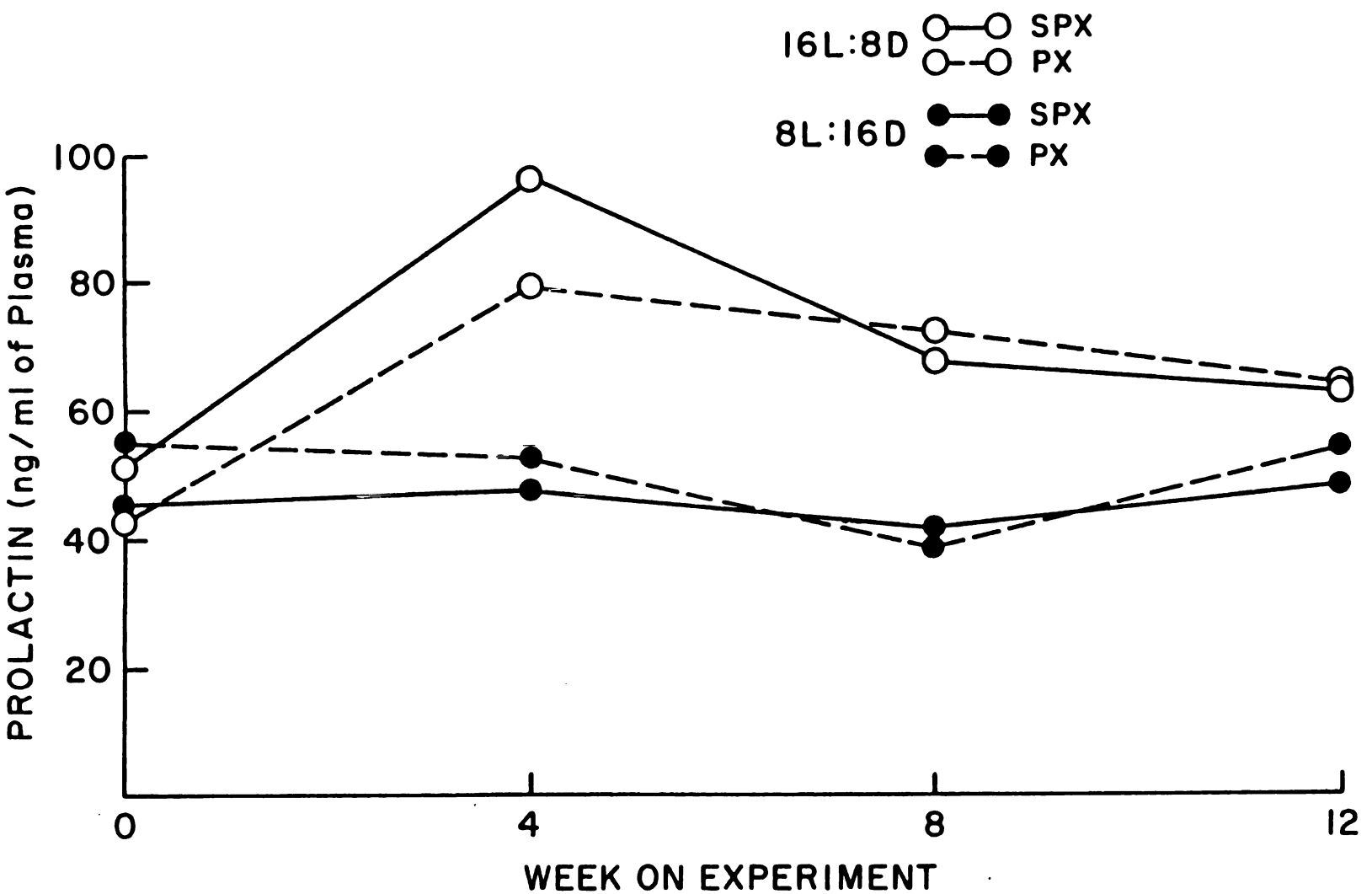

MELATONIN (pg/ml of Plasma)
140
130
120
110
100
90
80
70
60
50
40
30
20
10
1400
1800
2200
0200
0600
1000
1400
TIME OF DAY (h)
SPX
INCOMPLETE PX
PX

Figure 14. Prolactin in plasma from PX and SPX bull calves exposed to 8 h of light per day (at wk 0) and subsequently switched to 16 h of light per day or maintained on 8L:16D. Each observation represents the mean of four bulls except the treatment combination of 8L:16D-PX which represents one bull. Samples were collected at 30-min intervals for 26 h through a jugular cannula. Standard error difference for photoperiod treatment was 44.0 ng/ml of serum and for age was 27.8 ng/ml of serum.


16L:8D
SPX
PX
8L:16D
SPX
PX
100
80
60
40
20
PROLACTIN (ng/ml of Plasma)
0
4
8
12
WEEK ON EXPERIMENT

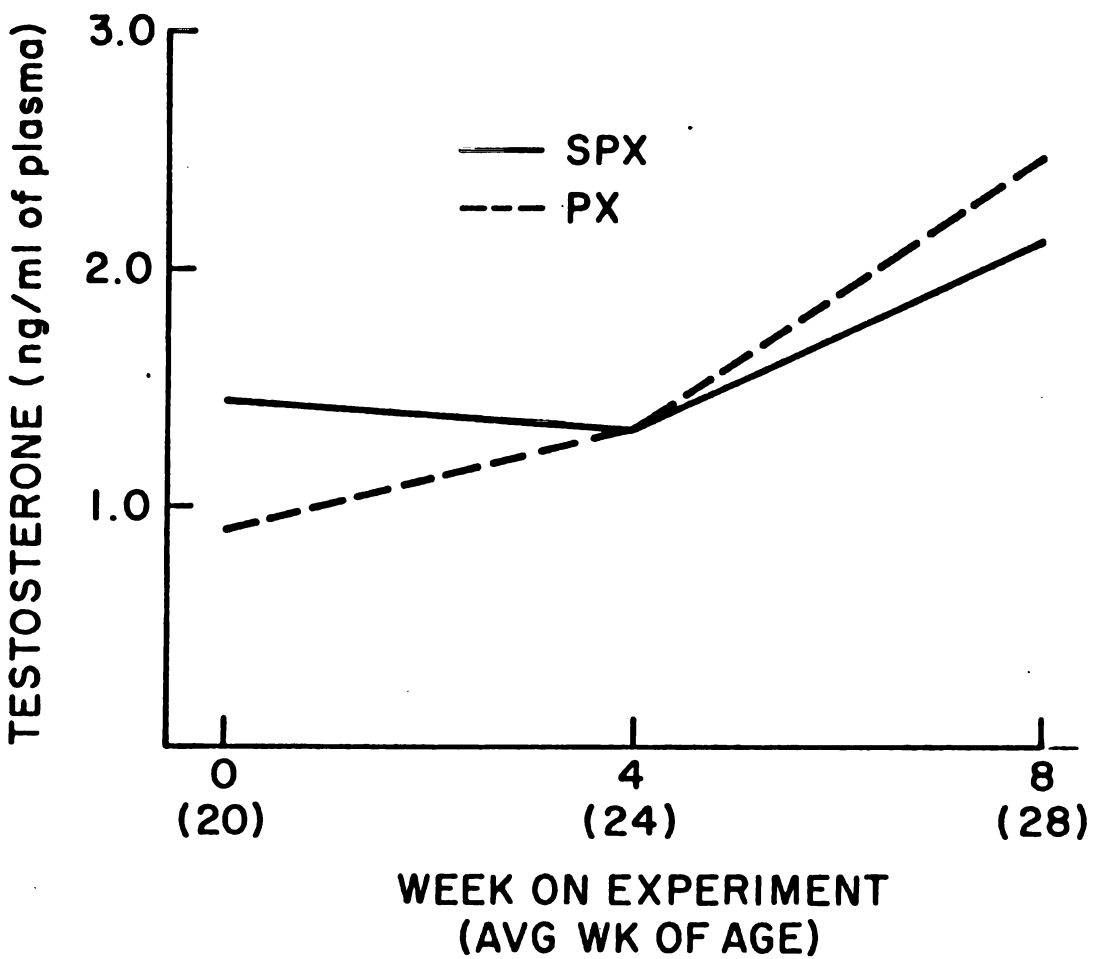
Testosterone in plasma was unaffected (P > .25) by photoperiod but at wk 0 (20 wk of age) tended to be greater (P = .14) in SPX (1.57 ng/ml) than in PX (.67 ng/ml) calves (figure 15). However, this divergence was not maintained and testosterone concentrations were not different (P > .25) between surgical treatments at wk 4 and 8.

At wk 0, LH in plasma was numerically greater in PX (6.1 ng/ml) vs SPX (4.1 ng/ml) calves but the difference was not significant (P > .25). By wk 4, LH converged (P < .1) to 2.9 and 3.2 ng/ml of plasma in PX and SPX calves. Photoperiod treatment did not affect plasma concentrations of LH.

Discussion

In cattle, increasing day length from 8 to 16 h per day is positively correlated with PRL concentrations in blood (Stanisiewski et al., 1984). Furthermore, the eye is essential for mediating the photoperiod-induced PRL response in cattle (Petitclerc et al., 1983b). As previously described, photic signals reaching the retina in most mammals are transduced into neural signals which follow a path through the suprachiasmatic nuclei (SCN) to the lateral hypothalamus where sympathetic axons project to the upper thoracic cord, through the superior cervical ganglia (SCG) and finally terminate at the pineal gland (Reiter, 1983). Following SCG removal (Lincoln et al., 1982) or pinealectomy (Barrell and Lapwood, 1979) serum concentrations of PRL and testosterone do not change in response to changes in

Figure 15. Age-adjusted testosterone in plasma from PX and SPX bull calves. Each observation represents the mean of eight (SPX) or five (PX) bulls where samples were collected at 30-min intervals for 26 h through a jugular cannula . Standard error difference for surgical treatment was .27 ng/ml of serum and for age was .13 ng/ml of serum.


TESTOSTERONE (ng/ml of plasma)
3.0
2.0
1.0
SPX
PX
0
(20)
4
(24)
8
(28)
WEEK ON EXPERIMENT
(AVG WK OF AGE)

photoperiod in rams. In contrast, I demonstrated that 16L:8D increased PRL concentrations in PX as well as SPX calves, although the response tended to be less in PX calves. These results confirm those of Petitclerc et al. (1983b) using more animals. Furthermore, both PX and SPX calves receiving 16L:8D appeared to become refractory to this photoperiod since PRL concentrations numerically declined between 4 and 12 wk. This phenomenon has been shown previously in calves (Stanisiewski et al., 1987). In cattle, except for the eyes, the essential components of the pathway for photoperiod controlled PRL secretion are unknown. However, based on current evidence, the pineal gland in cattle may not be essential for transduction of photic signals to the anterior pituitary gland.

Photoperiod can modulate puberty onset in a variety of species including heifers (Petitclerc et al., 1983a), boars (Mahone et al., 1979), rams (Howles et al., 1980) and deer (Budde, 1983). For example, 14 wk-old peripubertal bulls exposed to 16L:8D have higher testosterone concentrations in serum than controls receiving 8L:16D (Stanisiewski et al., 1987b) suggesting a photoperiod effect on puberty. In the present study, however, photoperiod did not affect testosterone concentrations. The reason for this discrepancy is unknown, although calves in the present study were approximately 12 wk older at the start of photoperiod treatment than in the previous study. This age difference may be important if bull calves are only photoreceptive, in terms of puberty onset, prior to 20 wk of age.

Testosterone concentrations normally increase in bulls between 8 and 24 wk of age (Bedair and Thibier, 1979; Amann and Walker, 1983). Relative to testosterone concentrations in SPX calves in the present study, pinealectomy tended to delay the increase. Based on concentrations of testosterone in blood of 14-wk old calves (Stanisiewski et al., 1987b), it appears that photoperiod affects puberty onset in bulls, and at least during part of the peripubertal cascade of hormonal events (McCarthy et al., 1979b) the pineal gland may be involved.

In agreement with previous reports in cattle (Bourne and Tucker, 1979; Rzepkowski et al., 1982), concentrations of LH were not affected by photoperiod and therefore are not a likely mediator of photoperiod or pineal-regulated testosterone secretion. Species such as hamsters, sheep and goats are seasonal breeders and rely heavily on an accurate photoperiod evaluation system so that reproductive capabilities are maximized during optimal times of the year. In seasonal breeders, the photoperiod evaluation system appears to consist of neural connections between the retina, hypothalamus and pineal gland. Cattle, on the other hand, are non-seasonal breeders and may have different requirements for photoperiodic regulation of hormone secretion, and the pineal gland is not involved in the mechanism with photoperiod. Perhaps this explains why the pineal gland of cattle is not essential for mediation of photoperiod-induced changes in secretion of PRL and why LH is unaffected by photoperiod.

In cattle, as is the case for all mammals examined so far (Vollrath, 1981), melatonin concentrations in blood are greatest in response to darkness as compared with exposure to light. Pinealectomy abolishes nocturnal increases of melatonin but does not completely eliminate the hormone from blood (Cardinali, 1981; Bittman et al., 1983) and my study confirms these results. Although the pineal gland is the predominant source of melatonin, and the source of essentially all of the nocturnally-secreted increment in melatonin (Goldman and Darrow, 1983), other tissues can synthesize melatonin (Binkley et al., 1979; Rubenik, 1980). Extrapineal sources could account for the chronic presence of melatonin in our PX calves. In addition, other evidence suggests that baseline melatonin in blood of sheep increases with time after pinealectomy (Kennaway et al., 1977) and perhaps in the absence of the pineal gland, other tissues increase production of melatonin. It is also interesting to note that partial removal of pineal tissue in three of my calves did not appear to diminish nocturnal melatonin responses, which suggests pineal regeneration or compensatory secretion by the remaining tissue.

In several species in which photoperiod regulates anterior pituitary hormone secretion via melatonin output from the pineal gland, duration of elevated melatonin is the major regulator (Goldman et al., 1984; Yellon et al., 1985) rather than amplitude or total output of melatonin. In fact, a 16 h elevation of melatonin in blood of sheep,

induced by feeding or injecting melatonin, decreases PRL in blood relative to 16L:8D photoperiod exposure (Kennaway et al., 1982, 1982/83). However, because 16L:8D stimulated PRL concentrations in my PX calves without a concomitant nocturnal melatonin rise, I conclude that diurnal melatonin changes are not important in the regulation of photoperiod-induced PRL release in cattle.

Bovine pineal tissue also contains specific PRL releasing and PRL inhibiting factors (Blask et al., 1976; Vriend et al., 1980). Whether these pineal factors play a physiological role in photoperiod-induced PRL control is unknown.

I conclude that the pineal gland is not an essential mediator of changes in photoperiod-induced PRL concentrations but may be involved with prepubertal testosterone changes in bull calves.

Chapter 5

Melatonin and Prolactin Concentrations in Serum of Bull Calves Exposed to 8, 16 or 24 Hours of Daily Light

Introduction

Melatonin is synthesized by the pineal gland of vertebrate species in a circadian pattern with concentrations being greatest during darkness and lowest during periods of light (Wurtman et al., 1969; Cardinali , 1981). Melatonin is also produced by extrapineal gland sources, including gut (Bubenik, 1980), retina (Ralph, 1981) and Harderian gland (Cardinali and Wurtman, 1972), and this may account for the inability of pinealectomy to abolish completely circulating melatonin concentrations (Bittman et al., 1983; Cardinali, 1981). Nevertheless, the pineal gland remains the primary source of nocturnal increments in serum melatonin (Arendt et al., 1980; Bittman et al., 1983).

In seasonal breeding species such as sheep and hamsters, the secretory pattern of melatonin acts as a transducer between photoperiod and secretion of gonadotropins and PRL (Bittman and Karsch, 1984; Goldman and Darrow, 1983; Kennaway et al., 1982/83). In cattle, a non-seasonal breeding species, long-day photoperiods (16L:8D) consistently increase PRL concentrations in serum relative to those in animals exposed to short days (8L:16D) (Stanisiewski et al., 1984; Tucker et al., 1984). However, pinealectomy does not abolish the ability of photoperiod to alter serum concentrations of PRL in cattle (Petitclerc et al., 1983b; Stanisiewski et al., 1987a). My objective was to characterize melatonin concentrations in blood collected in calves exposed to 8, 16 or 24-h of daily light and to

determine whether daily administration of melatonin for 8 or 16 h affects PRL secretion in calves with or without pineal glands.

Materials and Methods

Pretreatment. Holstein bull calves were used in three experiments (eight per experiment) and were initially housed in a light-controlled room from approximately 3 d of age and received 8L:16D daily. For Exp. 1 and 2, calves were subsequently moved into one of two light- and temperature-controlled (16.6 ± 1.3 C) chambers (Stanisiewski et al., 1984) at 14 wk of age and maintained under 8L:16D for 3 additional wk. There were four calves per chamber.

In Exp. 3, two calves were pinealectomized (PX) and four sham pinealectomized (SPX) between 6 and 7 wk of age. The PX surgical procedure followed was that of Braun et al. (1977). PX and SPX surgeries were performed identically except in SPX calves the pineal gland was identified but left intact. After surgery calves were moved into the light- and temperature-controlled (23.4 ± 1.5 C) chambers at 18 ± 3 (X ± S.E.) wk of age. All calves in Exp. 3 received continuous light (24L:0D) from 13 wk of age.

Treatments and Blood Collection Exp. 1. On the last day of pretreatment (d 1 of treatment), calves were fitted with indwelling jugular catheters coupled to a 3 m cannula which was passed into an adjourning room where blood was collected remotely without disturbing the animals. Blood was collected and discarded for 1 h to establish proper

cannula function. Beginning at 1200 h, blood was collected (initial bleeding) for 24 h (d 1) at 30-min intervals and at 2-h intervals for an additional 24 h (d 2). On the day after initial blood collection, photoperiod was switched to 16L:8D in one chamber while the remaining four calves in the other chamber were maintained on 8L:16D. After 4 wk of photoperiod treatment calves were catheterized and bled again (final bleeding) as described. One calf receiving 8L:16D died during the experiment, and all data from this calf were discarded.

Exp. 2. Housing, duration of treatments and blood sampling procedures were identical to those of Exp. 1. However, photoperiod treatments were 24L:0D (four calves) and 8L:16D (four calves).

Exp. 3. All calves were maintained under 24L:0D for the duration of treatment. Approximately 3 wk after being moved into the light- and temperature-controlled chambers, each calf was fitted with a jugular cannula connected to an infusion pump. Melatonin in saline was infused at a rate of 60.7 ug/h·50 kg^{-1} body weight for 16 h (pumps on at 1700 h). The dose of melatonin was similar to that used in sheep to mimic nocturnal concentrations in serum (Bittman et al., 1983). After 4 wk of 16 h daily melatonin infusion, the contralateral jugular of each calf was catheterized and blood was collected (initial bleeding) starting at 0800 h for 25 h at 30-min intervals. On the following day, one PX and two SPX calves were switched to an 8 h infusion regimen

(pumps on at 1700 h) while the remaining PX and SPX calves were maintained on 16 h daily infusion for 5 additional wk. On the final day of the experiment, blood was collected again (final bleeding) for 25 h as described.

Blood Handling and Radioimmunoassays. Blood samples were allowed to clot for 1 to 2 h at room temperature, then stored for 24 h at 5 C. Samples were centrifuged for 20 min at 1000 x g, decanted, and serum stored at -20 C until assayed.

Prolactin was quantified in serum as reported previously (Koprowski and Tucker, 1971). Direct assay of serum concentrations of melatonin were performed using the procedures and antibody of Fraser et al. (1983). Validation of the melatonin assay has been reported (Stanisiewski et al., 1987a).

Statistical Analysis. Split-plot analysis of variance (Gill, 1978b) was used to test for hormone concentration differences within and among treatments in Exp. 1 and 2. A 2 X 2 factorial design with repeat measure was used in Exp. 3. See appendix for a listing of sources of variation and specific contrasts.

Results

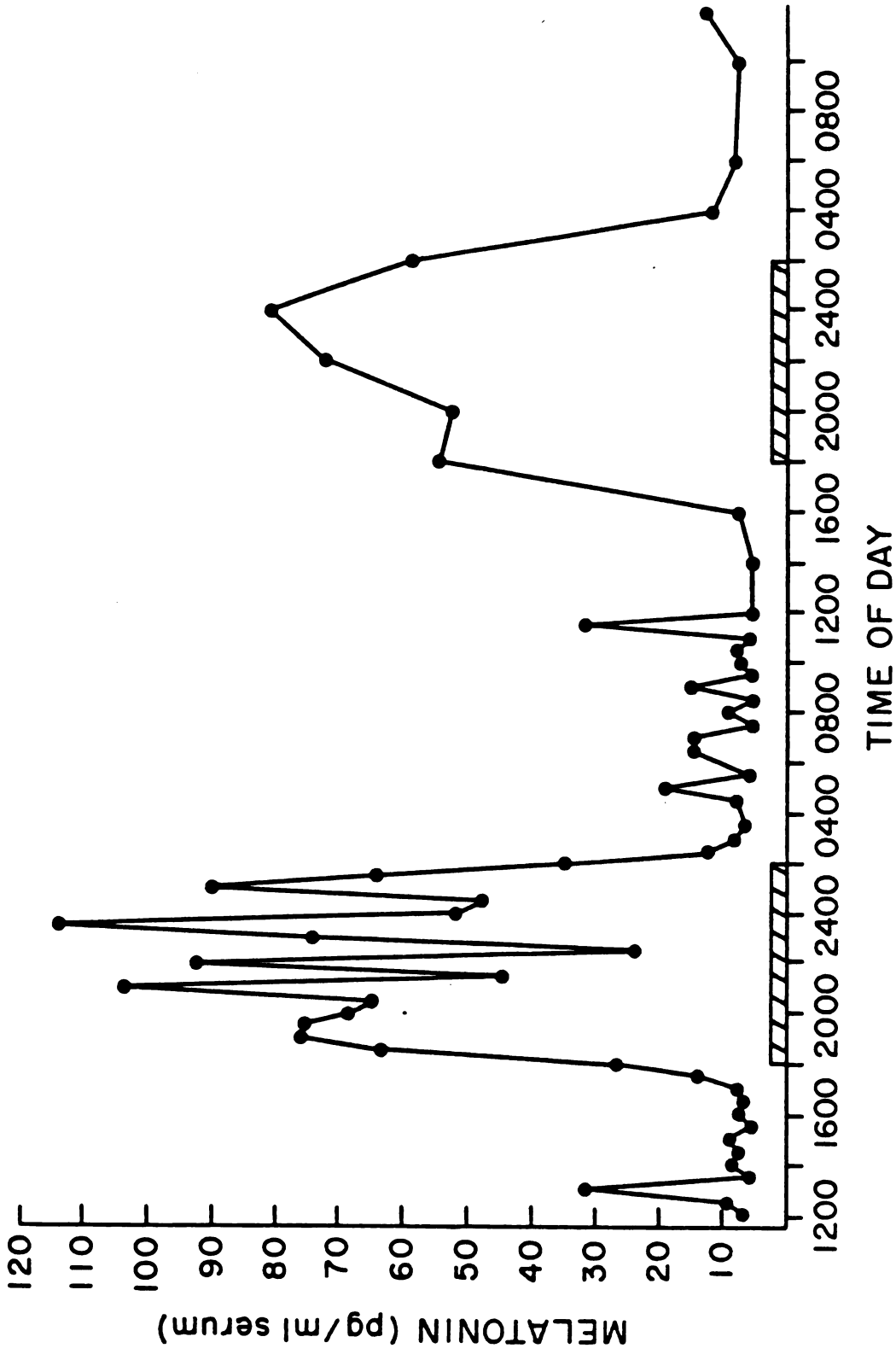
Exp. 1, Prolactin. After 17 wk exposure to 8L:16D, PRL averaged 6.3 and 3.8 ng/ml of serum ($P > .25$) on d 1 of treatment for the two groups of calves. In calves subsequently switched to 16L:8D for 4 wk, PRL increased ($P < .05$) to 30.9 ng/ml of serum compared with 7.0 ng/ml in

calves maintained on 8L:16D. Concentrations of PRL did not vary diurnally.

Melatonin. Serum concentrations of melatonin on d 2 were similar to those on d 1, and since blood was collected more frequently on d 1, statistical analysis of d 1 values will be described. Melatonin during darkness averaged 41.8 and 32.4 pg/ml of serum ($P < .25$) in the two groups of calves at initial bleeding (8L:16D). With lights on, melatonin decreased ($P < .01$) to an average of 13.9 and 20.2 pg/ml of serum ($P > .25$) in each group. One calf had persistently high melatonin in blood throughout d 1 and 2 and was eliminated from initial bleed calculations after determination (procedure of Grubbs and Beck) that he was an outlier ($P < .05$; Gill, 1978a). At final bleeding, nocturnal melatonin averaged 60.1 and 35.0 ng/ml of serum ($P < .05$) in 16L:8D and 8L:16D treatments, respectively (figure 16, 17). With lights on, melatonin decreased ($P < .05$) to an average of 11.9 (16L:8D) and 9.6 (8L:16D) pg/ml of serum ($P > .25$; figure 16, 17).

During initial bleeding, melatonin expressed as total area under the curve was greater ($P < .01$) during darkness (40.9 and 31.3 $ng \cdot min \cdot ml^{-1}$; $P > .25$) in each group of calves compared with values during the light period (5.8 and 9.2 $ng \cdot min \cdot ml^{-1}$; $P > .25$) At final bleeding, nocturnal melatonin areas (27.0 and 34.0 $ng \cdot min \cdot ml^{-1}$; $P < .1$) remained greater ($P < .01$) than areas during light exposure (10.3 and 4.2 $ng \cdot min \cdot ml^{-1}$; $P < .05$) in 16L:8D and 8L:16D treatments,

Figure 16. Forty-eight h profiles of melatonin in serum of four bull calves receiving 16L:8D after 4 wk on experiment. Samples were collected through a jugular cannula at 30-min intervals for the first 24 h and at 2 h intervals thereafter. The hatched bar along the x-axis represents darkness. Standard errors of means during light and darkness; respectively, were 8.2 and 27.3 pg/ml of serum.



in ser
after
cted
nterva
ls
e x-
rrors
espec
um.

Figure 17. Forty-eight h profiles of melatonin in serum of three bull calves receiving 8L:16D after 4 wk on experiment. Samples were collected through a jugular cannula at 30-min intervals for the first 24 h and at 2 h intervals thereafter. The hatched bar along the x-axis represents darkness. Standard errors of means during light and darkness; respectively, were 6.6 and 18.0 pg/ml of serum.

MELATONIN (pg/ml serum)
100
90
80
70
60
50
40
30
20
10
1200 1600 2000 2400 0400 0800 1200 1800 2000 2400 0400 0800 1200
TIME OF DAY

respectively. Total melatonin areas per 24 h were not different between 8L:16D vs 16L:8D ($P > .25$)

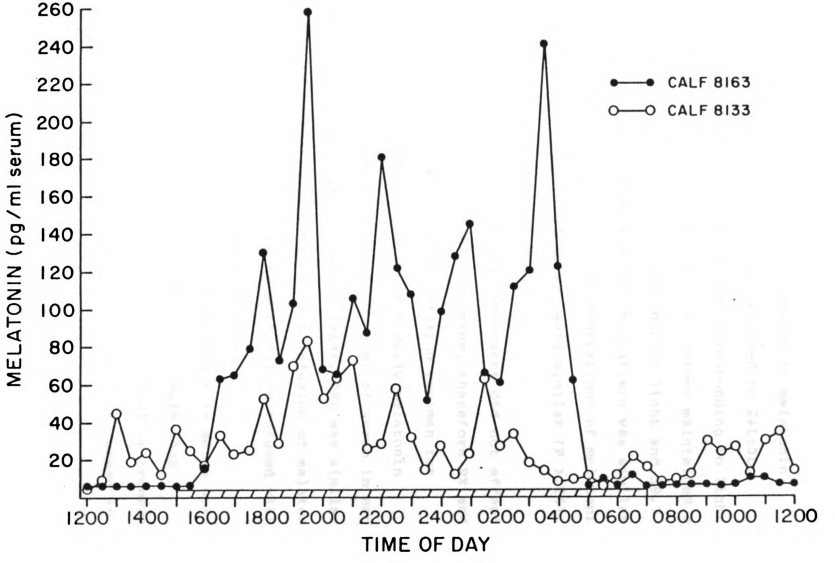
When darkness was administered for 16 h at initial and final (figure 17) bleedings, melatonin declined to baseline concentrations 2 to 4 h prior to lights on; in other words, the nocturnal melatonin increment lasted 12 to 14 h. In calves receiving 8 h of darkness at final bleeding, melatonin concentrations were elevated for the entire duration of dark exposure; i.e. 8 h (figure 16), but was suppressed with onset of lighting.

Great variability existed among individual calves both in terms of pulsatile activity and magnitude of the nocturnally-induced melatonin increment (figure 18). Highest daily concentrations of melatonin for individual calves always occurred in response to darkness and ranged from 34.0 to 291.0 pg/ml of serum. In contrast, highest melatonin values with lights on were 9.6 to 57.2 pg/ml of serum.

Exp. 2, Prolactin. After 17 wk exposure to 8L:16D, PRL averaged 11.0 and 11.3 ng/ml of serum ($P > .25$) on d 1 for each group of calves. Prolactin in serum was unchanged ($P > .25$) 4 wk later in response to either 24L:0D or to continuation of 8L:16D (12.9 vs 15.6 ng/ml; $P > .25$).

Melatonin. At initial bleeding, mean melatonin was 265% greater ($P < .01$) during the dark (30.7 pg/ml of serum) period as compared with the light (8.4 pg/ml) period in each group of calves exposed to 8L:16D. In three calves

Figure 18. Twenty-four h profiles of melatonin in serum of two bull calves receiving 8L:16D. Samples were collected at 30-min intervals through a jugular cannula. The hatched bar along the x-axis represents darkness. Standard errors of means during light and darkness; respectively, were 2.5 and 42.0 pg/ml of serum for calf 8163 and 7.4 and 19.6 pg/ml for calf 8133.


MELATONIN (pg/ml serum)
260
240
220
200
180
160
140
120
100
80
60
40
20
CALF 8163
CALF 8133
1200 1400 1600 1800 2000 2200 2400 0200 0400 0600 0800 1000 1200
TIME OF DAY

receiving 24L:0D, melatonin averaged 9.3 pg/ml of serum over 24 h and there was no nocturnal increase in melatonin. However, in one calf subsequently switched to 24L:0D, melatonin increased during a period corresponding to light exposure 4 wk earlier (figure 19). In calves maintained on 8L:16D melatonin averaged 8.0 pg/ml during light and 39.7 pg/ml of serum during darkness and the pattern was similar to that in Exp. 1 (figure 17). Concentrations of melatonin in all treatments and in all calves were similar ($P > .25$) between bleedings on d 1 and d 2.

Exp. 3, Prolactin. Surgical treatment did not affect ($P > .25$) concentrations of PRL in serum, therefore PX and SPX data were pooled within each infusion regimen for further analysis. After 4 wk of 16 h daily melatonin infusion, PRL averaged 22.7 and 20.0 ng/ml of serum in each group of calves ($P > .25$). Five wk later, PRL was similar ($P > .25$) in calves switched to an 8 h infusion of melatonin (21.0 ng/ml of serum) as compared with calves infused with melatonin for 16 h per day (20.8 ng/ml).

Melatonin. Concentrations of melatonin in serum were quantified only during final bleeding to ensure that indwelling infusion cannulas continued to function properly. Within 1 to 3 h of infusion onset, melatonin increased in serum (figure 20, 21). In serum melatonin averaged 637.8 pg/ml during the 8 h infusion and decreased ($P < .01$) to an average of 88.9 pg/ml during the 16-h interval when bulls were not infused. In calves infused for 16 h, melatonin

Figure 19. Twenty-four h profiles of melatonin in serum of three (8164, 8170, 8171) or one (8166) bull calves receiving 24L:0D. Samples were collected at 30-min intervals through a jugular cannula. Standard errors of means were 1.6 pg/ml of serum for calves 8164, 8170 and 8171 and 12.7 pg/ml for calf 8166.

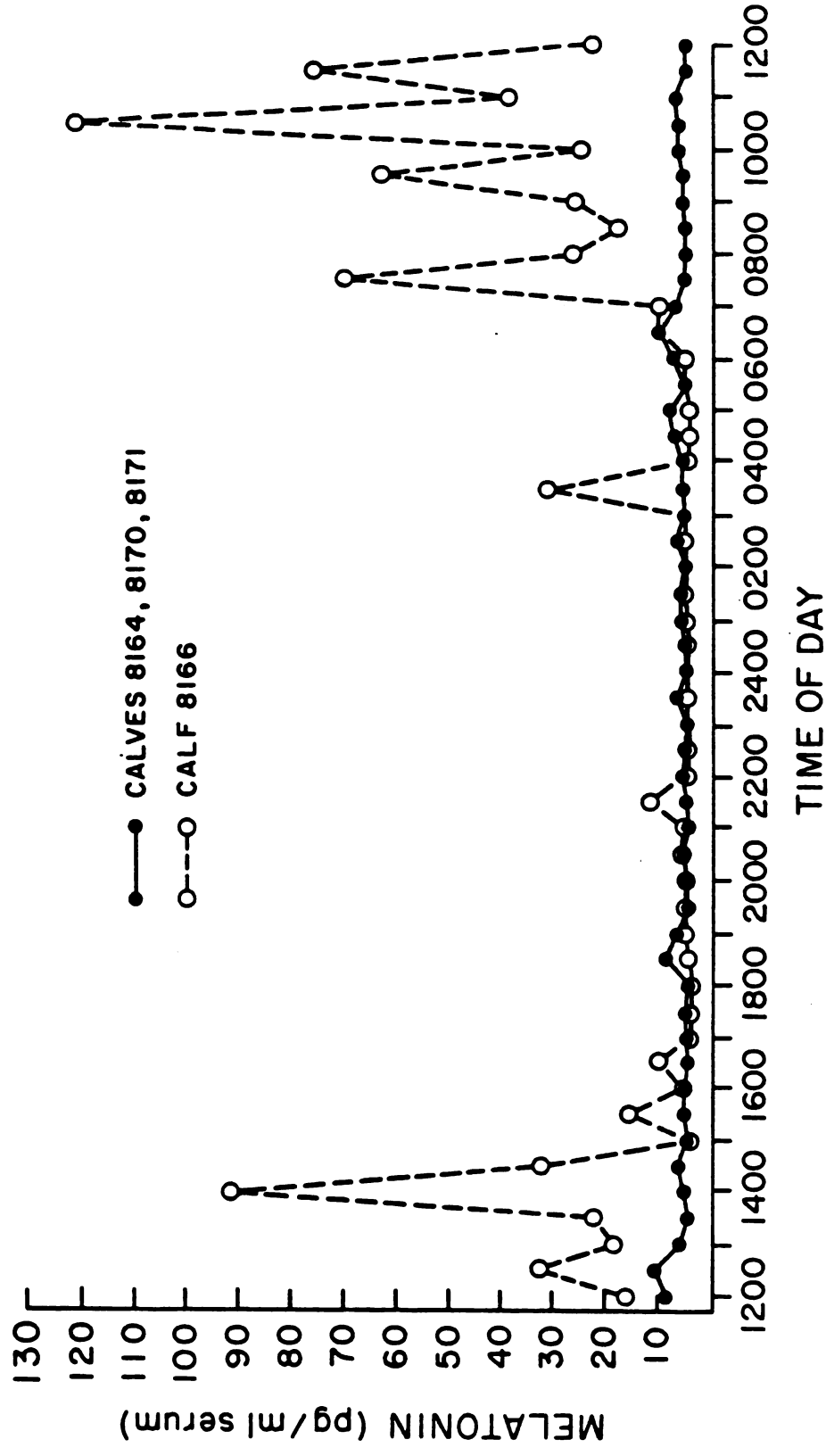

MELATONIN (pg/ml serum)
130
120
110
100
90
80
70
60
50
40
30
20
10
1200
1400
1600
1800
2000
2200
2400
0200
0400
0600
0800
1000
1200
TIME OF DAY
CALVES 8164, 8170, 8171
CALF 8166

Figure 20. Twenty-four h profiles of melatonin in serum of three bull calves receiving 24L:0D and infused intravenously with melatonin for 8 h (hatched bar). Standard errors of means during non-infusion were 26.6 pg/ml of serum and 318.9 pg/ml during infusion.

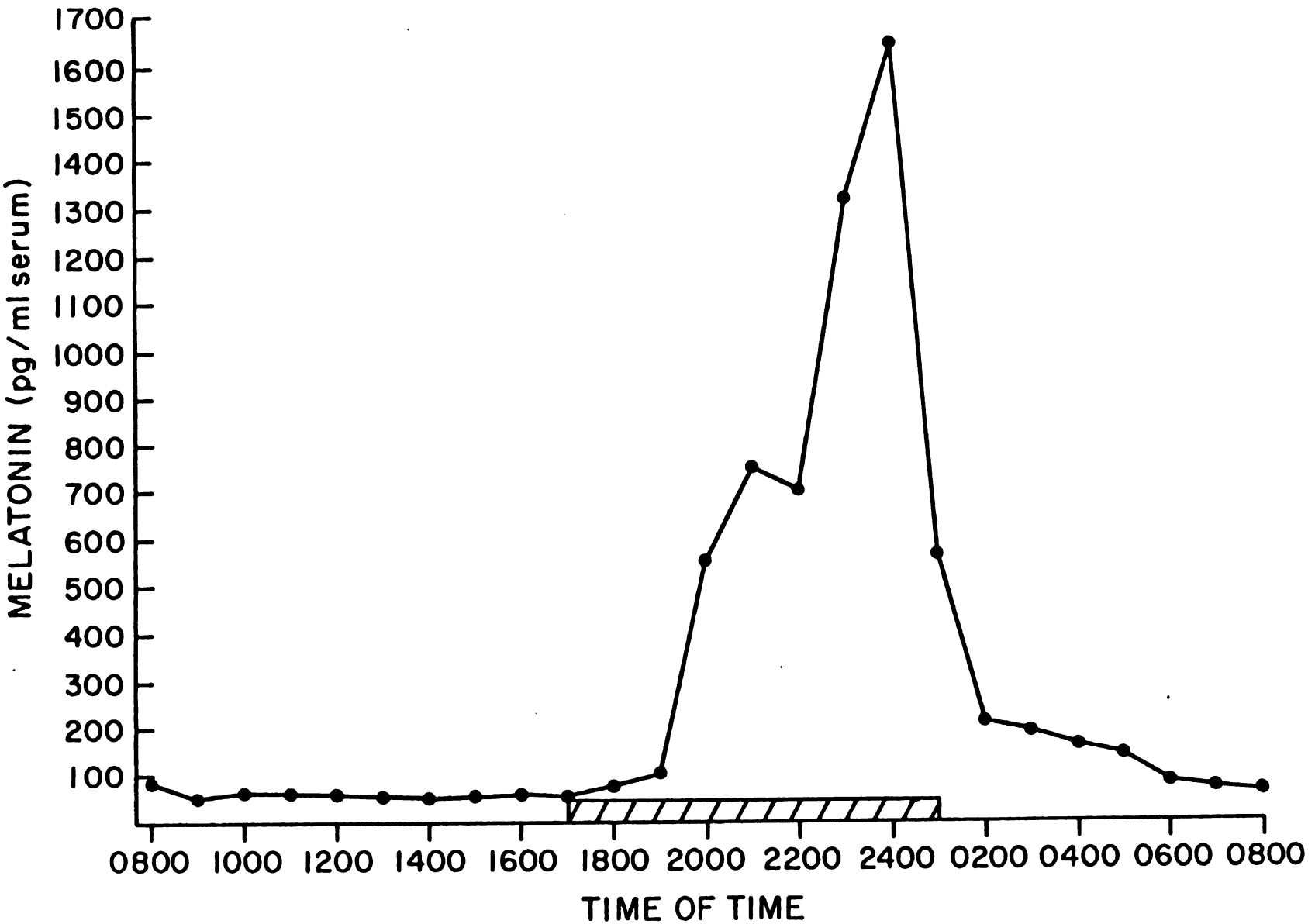
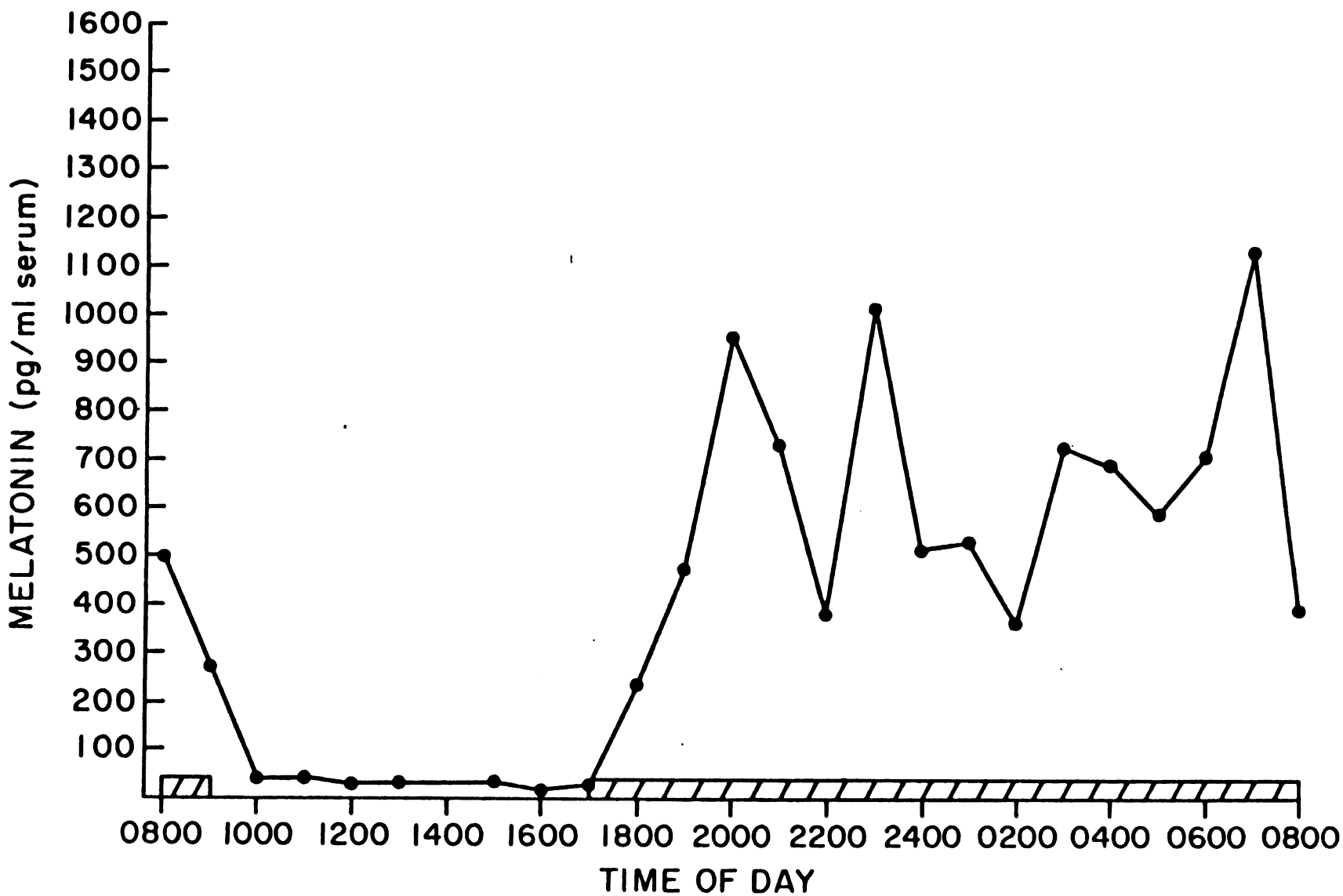

MELATONIN (pg/ml serum)
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0800 1000 1200 1400 1600 1800 2000 2200 2400 0200 0400 0600 0800
TIME OF TIME

Figure 21. Twenty-four h profiles of melatonin in serum of three bull calves receiving 24L:0D and infused intravenously with melatonin for 16 h (hatched bar). Standard errors of means during non-infusion were 6.9 pg/ml of serum and 141.8 pg/ml during infusion.


MELATONIN (pg/ml serum)
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0800 1000 1200 1400 1600 1800 2000 2200 2400 0200 0400 0600 0800
TIME OF DAY

averaged 565.0 pg/ml of serum and decreased ($P < .01$) to an average of 31.4 pg/ml during the 8-h interval when not infused.

Discussion

In agreement with results in other vertebrates (Cardinali, 1981), I found that circulating melatonin increases in response to darkness in calves. I also expanded on the results of Hedlund et al. (1977) and Martin et al. (1983), who demonstrated a 6- to 7-fold nocturnal rise of serum melatonin in cattle, to show that duration of the nocturnal increment is altered with duration of darkness. Through frequent (30-min interval) sampling, marked pulsatile changes were observed in concentrations of melatonin in calf serum. Bittman et al. (1983) also suggested that melatonin is released episodically in sheep.

Stanisiewski et al. (1984) previously demonstrated that relative to 8L:16D, PRL concentrations in serum of bull calves increase in response to 16L:8D exposure. In addition, previous results were confirmed (Stanisiewski et al., 1987c) that calves receiving continuous light for less than 6 wk have serum PRL concentrations that do not differ from those in calves exposed to 8L:16D. Similar to results in cattle, PRL concentrations in sheep are greater in response to 16L:8D exposure than to 8L:16D (Pelletier, 1973). Pinealectomy of rams (Barrell and Lapwood, 1979), ewes (Munro et al., 1980) or white-tailed deer bucks (Schulte et al., 1981), abolishes or greatly reduces

photoperiod-induced PRL rhythms, thus implicating melatonin or some other pineal gland product as a regulator of photoperiod-induced PRL secretion. In fact, afternoon feeding of melatonin to sheep or deer (Kennaway et al., 1982; Webster and Barrell, 1985) or injecting ewes (Kennaway et al., 1982/83) with melatonin reduces concentrations of PRL in blood similar to that in animals receiving short-day photoperiods. In contrast, pinealectomy of cattle has little if any effect on photoperiod-induced PRL concentrations (Petitclerc et al., 1983b; Stanisiewski et al., 1987a). When comparing 16L:8D vs 8L:16D treatments in my present study, daily mean serum concentrations of melatonin as well as area under the curve per 24 h were equivalent yet mean PRL concentrations were different. On the other hand, daily mean concentrations of melatonin were greater in calves exposed to 8L:16D than in calves receiving 24L:0D, yet, PRL concentrations were the same. It appears from my results that mean daily concentrations of melatonin have no correlation with mean daily concentrations of PRL in prepubertal bull calves.

As stated, duration of increased nocturnal melatonin concentrations in serum coincided with the duration of darkness that calves received, and mean nocturnal melatonin concentrations tended to be greater with shorter durations of dark exposure. This was similar to data in rams where mean nocturnal melatonin concentrations are greater in animals exposed to long days compared with short days

(Lincoln et al., 1982). I successfully mimicked this pattern in our melatonin-infused calves (Exp. 3). Although melatonin concentrations in serum of our infused animals were approximately 15-fold greater than average nocturnal concentrations in non-infused calves in Exp. 1 and 2, these infusion-induced concentrations achieved our goal of matching reported values in melatonin-infused sheep (Bittman et al., 1983). In 2-yr old steers, we observed nocturnal melatonin above 400 pg/ml of serum (Stanisiewski and Tucker, unpublished data). Therefore, I believe that infused concentrations in the present study approximate those which cattle may normally achieve. Since altering duration of melatonin exposure by infusion (Exp. 3) did not affect circulating PRL concentrations, it appears that duration of elevated melatonin in cattle, unlike sheep (Bittman and Karsch, 1984), is not a major controlling factor of photoperiod-induced PRL release. In sheep (Bittman and Karsch, 1984) and hamsters (Goldman et al., 1984), duration of elevated melatonin concentrations control reproductive and hormonal responses to melatonin. However, melatonin secretion in cattle has not been definitively linked with reproductive function or secretion of other hormones such as PRL. Therefore, I cannot yet determine the functional importance of factors such as duration, episodic release, amplitude, total amount secreted or temporal phase (relative to ambient light-dark exposure) of melatonin secretion in cattle. I speculate that lack of control of the pineal

gland over PRL release in cattle may represent a divergence of non-photoperiodic from photoperiod-regulated breeders.

This is also the first report in cattle demonstrating that continuous light suppresses concentrations of melatonin throughout a day. Similar results were reported in sheep (Kennaway et al., 1983; Rollag and Niswender, 1976), rhesus monkeys (Perlow et al., 1980) and rats (Ralph et al., 1971). However, I cannot explain the diurnal melatonin pattern in one calf receiving 24L:0D. This pattern (detected on consecutive days) was 180 degrees out of phase with the 8L:16D-induced pattern of 4 wk earlier. Humans are the only species known to have melatonin rhythms which persist in constant illumination (Vaughan et al., 1976; Weitzman et al., 1978). However, this phenomenon in humans may be a result of exposure to insufficient light intensity to suppress endogenous melatonin rhythms. For example, in Syrian hamsters, 1 lx is sufficient to suppress melatonin (Reiter, 1983) while in ewes (Kennaway et al., 1983), 50 lx is sufficient. Humans, on the other hand, require 1500 to 2500 lx (Lewy et al., 1980). Whether the light intensity used in the present study was only marginally adequate to suppress melatonin in calves is unknown. Another possibility is that calves may go through a natural progression in which diurnal melatonin release continues upon removal of darkness, and this diurnal pattern eventually decreases and (or) phase shifts. Perhaps this had not yet happened in the one calf.

In summary, blood collection intervals of 2 h are adequate for characterization of nocturnal melatonin duration. Elevated melatonin concentrations follow duration of darkness and constant illumination eliminates the diurnal pattern. Prolactin in serum is greater in calves receiving 16L:8D than in 8L:16D exposed animals, however, altering increments of melatonin by infusion of melatonin does not affect PRL. I conclude that diurnal melatonin secretion probably does not mediate photoperiod-induced changes in serum PRL concentrations in prepubertal bulls.

SUMMARY AND CONCLUSIONS

Photoperiod has marked effects on PRL concentrations in serum of cattle. Namely, relative to 8L:16D, 16L:8D increases PRL concentrations in blood but the increased level is not maintained indefinitely. On the other hand, 8L:16D maintains relatively constant PRL concentrations in calves for at least 12 wk, provided ambient temperature is constant. Abruptly switching from 8L:16D to continuous lighting will also increase PRL in blood, but the time course of the response is delayed with respect to 16L:8D.

In addition, serum testosterone concentrations increase at an earlier age in young bulls receiving 16L:8D compared with bulls receiving 8L:16D. Furthermore, this response is not synchronous with changes in mean concentrations or pulses of LH. Also, concentrations of PRL are increased equally in castrated and intact male calves switched from 8 to 16 h of light per day. Therefore, the gonads are not required for regulation of photoperiod-induced PRL concentrations. However, since LH concentrations are unaffected by photoperiod, the photic response on the gonad may be a result of PRL action alone or in synergy with basal LH concentrations.

The neuroendocrine mechanism whereby light affects PRL release in cattle remains a mystery, although the PRL inhibitor, DA, may be involved. For example, concentrations

of DA were higher in pituitary glands from calves receiving 8L:16D as compared with concentrations in glands from 16L:8D-exposed calves. In addition, since PX does not abolish 16L:8D-induced PRL concentrations, the pineal gland is not necessary for photic signal transduction in cattle. The lack of requirement for the pineal gland to relay photoperiod-controlled PRL secretion in cattle is in contrast with other species such as hamsters and sheep, and may represent an evolutionary divergence of non-photoperiodic and photoperiod-regulated breeders. One function the pineal gland may serve in cattle is on regulation of puberty onset, since concentrations of testosterone were lower at 20 wk of age in PX bulls compared with SPX bulls, but again, LH concentrations were not different between the two groups.

Mean concentrations and diurnal rhythmicity of melatonin are altered by photoperiod in calves. Melatonin is secreted in pulses, and circulating concentrations are greatest during darkness. Furthermore, providing 8 h of darkness results in melatonin concentrations being elevated for 8 h whereas melatonin is high for only the first 12 to 14 h of a 16 h dark period. In addition, melatonin is suppressed to basal (daylight) concentrations when continuous daily light is provided. I showed that concentrations of PRL in serum of calves can be the same in spite of markedly different patterns and concentrations of melatonin. Infusion of melatonin for 8 or 16 h does not

affect PRL concentrations in calves. Since melatonin is a primary hormone secreted from the pineal gland, the lack of melatonin effect on PRL is further evidence of the dissociation of the pineal gland from photoperiod-induced PRL regulation in cattle.

It is concluded that although long-day photoperiod exposure increases serum concentrations of PRL in bull calves, the mechanism does not appear to involve the pineal gland or melatonin secretion. However, DA appears to be a photoperiod-regulated neurohormone which may at least partially be responsible for photoperiod-induced PRL release. Furthermore, PRL concentrations in bull calves become refractory to prolonged long-day exposures.

The mechanism through which long-days increase testosterone concentrations at an earlier age in bulls appears to be dependent upon the pineal gland, and may be a result of synergism of PRL and (or) pineal secretions on basal concentrations of LH.

APPENDIX

APPENDIX

Experimental models depicting sources of variation and degrees of freedom for experiments contained within each chapter. Animals excluded from analysis were treated as missing data.

Chapter 1. Exp. 1:

Source of Variation	df
Photoperiod Treatment(P)	1
Calves/P	6
Day of Bleeding (B)	3
P x B	3
Calves/P x B	18
Time of Blood Collection(T)[1]	52
P x T	52
B x T	156
P x B x T	156
Calves/P x B x T	1009
Total =	1456

Exp. 2:

Source of Variation	df
P	1
Calves/P	6
B	2
P x B	2
Calves/P x B	12
T	12
P x T	12
B x T	24
P x B x T	24
Calves/P x B x T	143
Total =	238

Chapter 2. Exp. 1:

Source of Variation	df
P	1
Calves/P	6
B	2
P x B	2

[1]When not significant (see text), T was deleted from models.

Calves/P x B		12
T		12
P x T		12
B x T		24
P x B x T		24
Calves/P x B x T		204
	Total =	299

Exp. 2

Source of Variation		df
Effect of Castration(C)		1
Calves/C		6
B		1
C x B		1
Calves/C x B		6
T		12
C x T		12
B x T		12
C x B x T		12
Calves/C x B x T		144
	Total =	207

Chapter 3.

Sources of Variation		df
P		1
Calves/P		6
B		1
P x B		1
Calves/P x B		6
T		12
P x T		12
B x T		12
P x B x T		12
Calves/P x B x T		103
	Total =	166

Chapter 4.

Source of Variation	df
Replicate (R)[2]	1
Effect of Pinealectomy(S)	1
P	1
S x P	1
R x S	1
R x P	1
R x S x P	1
Covariate[3]	1
Calves/R x S x P	7
B	3

[2]Not significant for testosterone, LH or PRL (when adjusted for ambient temperatures).
[3]Covariate = Age(testosterone, LH) or Ambient temperature (PRL).

	S x B	3
	P x B	3
	S x P x B	3
	R x B	3
	R x S x B	3
	R x P x B	3
	R x S x P x B	3
	Covariate	1
	Calves/R x S x P x B	23
	Total =	63

Chapter 5. Exp. 1, 2:

Source of Variation	df
P	1
Calves/P	6
B	1
P x B	1
Calves/P x B	6
Presence/Absence of Light(L)	1
P x L	1
Calves/P x L	6
B x L	1
P x B x L	1
Calves/P x B x L	27
Total =	52

Exp. 3

Source of Variation	df
S	1
Melatonin Infusion (I)	1
S x I	1
Calves/S x I	2
B	1
S x B	1
I x B	1
S x I x B	1
Calves/S x I x B	357
Total =	366

BIBLIOGRAPHY

BIBLIOGRAPHY

Advis, J. P., J. W. Simpkins, H. T. Chen and J. Meites. 1978. Relation of biogenic amines to onset of puberty in the female rat. Endocrinology.103:11.

Almeida, O. F. X. and G. A. Lincoln. 1984. Reproductive photorefractoriness in rams and accompanying changes in the patterns of melatonin and prolactin secretion. Biol. Reprod. 30:143.

Amann, R. P. 1983. Endocrine changes associated with onset of spermatogenesis in Holstein bulls. J. Dairy Sci. 66:2606.

Amann, R. P. and D. A. Walker. 1983. Changes in the pituitary-gonadal axis associated with puberty in Holstein bulls. J. Anim. Sci. 57:433.

Anton-Tay, F. and R. J. Wurtman. 1969. Regional uptake of ^{3}H-melatonin from blood or cerebrospinal fluid by rat brain. Nature 221:474.

Arendt, J., J. M. Forbes, W. B. Brown and A. Marston. 1980. Effect of pinealectomy on immunoassayable melatonin in sheep. J. Endocrinol. 85:1P.

Baker, J. R. 1938. The Evolution of breeding season. In: Evolution. (Essays presented to E.S. Goodrich) p. 161.

Barenton, B. 1981. Failure of short-term hyper-prolactinaemia to increase the number of gonadotropin receptors in the ram testis. J. Endocrinol. 88:451.

Barrell, G. K. and K. R. Lapwood. 1979. Effects of pinealectomy on the secretion of luteinizing hormone, testosterone and prolactin in rams exposed to various lighting regimes. J. Endocrinol. 80:397.

Bartke, A. J. 1971. Effects of prolactin on spermatogenesis in hypophysectomized mice. J. Endocrinol. 49:311.

Bartke, A., A. A. Hafiez, F. J. Bex, and S. Dalterio. 1978. Hormonal interactions in regulation of androgen secretion. Biol. Reprod. 18:44.

Beck, N. F. G., H. A. Tucker and W. D. Oxender. 1979. Mammary arterial and venous concentrations of prolactin in lactating cows after milking or administration of thyrotropin-releasing hormone or ergocryptine. Endocrinology 104:111.

Bedair, G. A. M. and M. Thiber. 1979. Peripheral plasma androstenedione and testosterone concentrations in bulls before and during puberty. J. Reprod. Fertil. 56:7.

Ben-Johnson, N. and J. C. Porter. 1976. A sensitive radioenzymatic assay for dopamine, norepinephrine, and epinephrine in plasma and tissue. Endocrinology 98:1497.

Berger, T., J. P. Mahone, G. S. Svoboda, K. W. Metz and E. D. Clegg. 1980. Sexual maturation of boars and growth of swine exposed to extended photoperiod during decreasing natural photoperiod. J. Anim. Sci. 51:672.

Berndtson, W. E., C. Desjardins. 1974. Circulating LH and FSH levels and testicular function in hamsters during light deprivation and subsequent photoperiodic stimulation. Endocrinology 95:195.

Berndtson, W. E., B. W. Pickett and T. M. Nett. 1974. Reproductive physiology of the stallion IV. Seasonal changes in the testosterone concentration of peripheral plasma. J.Reprod. Fertil. 39:115.

Bethea, C. L. and J. D. Neill. 1979. Prolactin secretion after cervical stimulation of rats maintained in constant dark or constant light. Endocrinology 194:870.

Bex, F. J., A. Bartke, B. D. Goldman and S. Dalterio. 1978. Prolactin, growth hormone, luteinizing hormone receptors and seasonal changes in testicular activity in the golden hamster. Endocrinology 103:2069.

Binkley, S., M. Hryshchyshyn and K. Reilly. 1979. N-actetyltransferase activity responds to environmental lighting in the eye as well as in the pineal gland. Nature 281:479.

Birge, C. A., L. S. Jacobs, C. T. Hammer and W. H. Danghaday. 1970. Catecholamine inhibition of prolactin secretion by isolated rat adenohypophyses. Endocrinology 86:120.

Bishop W., C. P. Fawcett, L. Krulich and S. M. McCann. 1972. Acute and chronic effects of hypothalamic lesions on the release of FSH, LH and PRL in intact and castrated rats. Endocrinology 91:643.

Bittman, E. L. 1978. Hamster refractoriness: The role of insensitivity of pineal target tissues. Science 202:648

Bittman, E. L., R. J. Dempsey and F. J. Karsch. 1983. Pineal melatonin secretion drives the reproduction response to daylength in the ewe. Endocrinology 113:2276.

Bittman, E. L. and F. J. Karsch. 1984. Nightly duration of pineal melatonin secretion determines the reproductive response to inhibiting day length in the ewe. Biol. Reprod. 30:585.

Bivens, C. H., H. E. Lebovitz and J. M. Feldman. 1973. Inhibition of hypoglycemia-induced growth hormone secretion by the serotonin antagonists cyproheptadine and methysergide. N. Eng. J. Med. 289:236.

Blask, D. E., M. K. Vaughan, R. J. Reiter, L. Y. Johnson and G. M. Vaughan. 1976. Prolactin-releasing and release-inhibiting factor activities in the bovine, rat, and human pineal gland: in vitro and in vivo studies. Endocrinology 99:152.

Bourne, R. A. and H. A. Tucker. 1975. Serum prolactin and LH response to photoperiod in bull calves. Endocrinology 97:473.

Boyer, R., J. Finkelstein, H. Roffwarg, S. Kapen, E. D. Weitzman and L. Hellman. 1972. Synchronization of augmented luteinizing hormone secretion with sleep during puberty. New Eng. J. Med. 287:582.

Braun, R. K., L. A. Gilden, T. W. Riebold and R. R. Peters. 1977. Surgical approach for pinealectomy in the calf. Amer. J. Vet. Res. 38:1973.

Brinkow, B. R. and J. M. Forbes. 1984. Effect of pinealectomy on the plasma concentrations of prolactin, cortisol and testosterone in sheep in

short and skeleton long photoperiod. J. Endocrinol. 100:287.

Brown, W. B. and J. M. Forbes. 1980. Diurnal variations of plasma prolactin in growing sheep under two lighting regimes and the effect of pinealectomy. J. Endocrinol. 84:91.

Bubenik, G. A. 1980. Localization of melatonin in the digestive tract of the rat: effect of maturation, diurnal variation, melatonin treatment and pinealectomy. Horm. Res. 12:313.

Bubenik, G. A., G. M. Brown and L. J. Grota. 1977. Immuno-histological localization of melatonin in the rat digestive system. Experientia 33:662.

Bubenik, G. A., A. B. Bubenik, G. M. Brown, A. Trenkle and D. I. Wilson. 1975. Growth hormone and cortisol levels in the annual cycle of white-tailed deer (Odocoileus virgeneanus). Can. J. Physiol. Pharmacol. 53:787.

Bubenik, G. A. and D. Schams. 1986. Relationship of age to seasonal levels of LH, FSH, prolactin and testosterone in male, white-tailed deer. Comp. Biochem. Physiol. 83A:179.

Budde, W. S. 1983. Effects of photoperiod on puberty attainment of female white-tailed deer. J. Wildlife Manage. 47:595.

Buttle, H. L. 1974. Seasonal variation of prolactin in plasma of male goats. J. Reprod. Fertil. 37:95.

Cardinali, D. P. 1981. Melatonin. A mammalian pineal hormone. Endocrine Rev. 2:327.

Cardinali, D. P. and J. M. Rosner. 1971. Metabolism of serotonin by the rat retina in vitro. J. Neurochem. 18:1769.

Cardinali, D. P., M. I. Vacas and E. Estevez Boyer. 1978. High affinity binding of melatonin in bovine medial basal hypothalamus. IRCS Med. Sci. 6:357.

Cardinali, D. P., M. I. Vacas, M. I. Keller Sarmiento and E. Morguerstern. 1983. Melatonin action: sites and possible mechanisms in brain. In: J. Axelrod, F. Fraschini and G.P. Velo (Ed.) The Pineal Gland and its Endocrine Role. pp. 277-299. Plenum Press, New York.

Cardinali, D. P. and R. J. Wurtman. 1972. Hydroxyindole-O-methyl transferase in rat pineal, retina and Harderian gland. Endocrinology 91:247.

Chippa, S. A. and G. Fink. 1977. Releasing factor and hormonal changes in the hypothalamic-pituitary gonadotrophin and adrenocorticotropin systems before and after birth and puberty in male, female and androgenized female rats. J. Endocrinol. 72:211.

Clemens, J. A., C. J. Shaar and E. B. Smalstig. 1980. Dopamine, PIF, and other regulators of prolactin secretion. Fed. Proc. 39:2907.

Cocchi, D., F. Fraschini, H. Jalanbo and E. E. Muller. 1974. Role of brain catecholamines in postcastration rise in plasma LH of prepubertal rats. Endocrinology 95:1649.

Cohen, J., M. Hadjiconstantinou and N. H. Neff. 1983. Activation of dopamine-containing amacrine cells of retina: Light-induced increase of acidic dopamine metabolites. Brain Res. 260:125.

Cohen, M., D. Rossele, B. Chabner, T. J. Schmidt and M. Lippman. 1978. Evidence for a cytoplasmic melatonin receptor. Nature 274:894.

Convey, E. M., W. E. Beal, B. E. Sequin, K. J. Tannen and Y. C. Lin. 1976. Gonadotropin releasing hormone luteinizing hormone release after prostaglandin F_2 in heifers. Proc. Soc. Exp. Biol. Med. 151:84.

Darrow, J. M., F. C. Davis, J. A. Elliott, M. H. Stetson, F. W. Turek and M. Menaker. 1980. Influence of photoperiod on reproductive development in the golden hamster. Biol. Reprod. 22:443.

Davis, S. L., R. G. Sasser, D. L. Thacker and R. H. Ross. 1977. Growth rate and secretion of pituitary hormones in relation to age and chronic treatment with thyrotropin-releasing hormone in prepubertal dairy heifers. Endocrinology 100:1394.

de Champlain, J., L. Farley, D. Cousineau and M. R. van Ameinger. 1976. Circulating catecholamine levels in human and experimental hypertension. Circ. Res. 38:109.

Denamur, R., J. Martinet and R. V. Short. 1973. Pituitary control of the ovine corpus luteum. J. Reprod. Fertil. 32:207.

De Greef, W. J. and J. D. Neill. 1979. Dopamine levels in hypophyseal stalk plasma of the rat during surges of prolactin secretion induced by cervical stimulation. Endocrinology 105:1093.

Dohler, K. D. and W. Wuttke. 1974. Serum LH, FSH, prolactin and progesterone from birth to puberty in female and male rats. Endocrinology 94:1003.

Dohler, K. D. and W. Wuttke. 1975. Changes with age in levels of serum gonadotropins, prolactin, and gonadal steroids in prepubertal male and female rats. Endocrinology 97:898.

Dular, R. and F. LaBella. 1977. Isolated prolactin and growth hormone granules from bovine pituitary: Content and stability are seasonally dependent. Endocr. Res Comm. 4:195.

Dunn, J. D., A. Arimura and L. E. Scheving. 1972. Effect of stress on circadian periodicity in serum LH and prolactin concentration. Endocrinology 90:29.

Ellis, G. B. and F. W. Turek. 1979. Time course of the photoperiod-induced change in sensitivity of the hypothalamic-pituitary axis to testosterone feedback in castrated male hamsters. Endocrinology 104:625.

Fagin, K. D. and J. D Neill. 1981. The effect of dopamine on thyrotropin-releasing hormone-induced prolactin secretion in vitro. Endocrinology 106:1835.

Farner, D. S. and B. K. Follett. 1966. Light and other environmental factors affecting avian reproduction. J. Anim. Sci. 25:90.

Follett, B. K. 1978. Photoperiodism and seasonal breeding in birds and mammals. In: Control of Ovulation. Butterworth Press. London.

Follett, B. K. 1982. Photoperiodic physiology in animals. In: J.Brady (Ed.) Biological Timekeeping. Soc. Exp. Biol. Sem. Series 14.

Follett, B. K., D. S. Farner and P. W. Mattocks. 1975. Luteinizing hormone in the plasma of White-crowned sparrows (Zonotrichia leucophrys gambelii) during artifical photostimulation. Gen. Compar. Endocrinol. 26:126.

Forbes, J. M. 1982. Effects of lighting pattern on growth, lactation and food intake of sheep, cattle and deer. Livestock Prod. Sci. 9:361.

Forbes, J. M., A. A. El Shahat, R. Jones, J. G. S. Duncan and T. G. Boaz. 1979. The effect of daylength on the growth of lambs. I. Comparisons of sex, level of feeding and shearing and breed of sire. Anim. Prod. 29:33.

Fraser, S. P. Cowen, M. Franklin, C. Franey and J. Arendt. 1983. Direct radioimmunoassay for melatonin in plasma. Clin. Chem. 29:396.

Gaston, S. and M. Menaker. 1967. Photoperiodic control of hamster testis. Science 158:925.

George, F. W., K. J. Catt and J. D. Wilson. 1979. Regulation of the onset of steriod hormone synthesis in fetal gonads. In: T. H. Hamilton, J. H. Clark and W. A. Sadler (Ed.) Ontogeny of Receptors and Reproductive Hormone Action. p. 411. Raven Press, New York.

Gill, J. L. 1978a. Design and Analysis of Experiments in the Animal and Medical Science, Vol 1. The Iowa State Univ. Press, Ames.

Gill, J. L. 1978b. Design and Analysis of Experiments in the Animal and Medical Sciences, Vol 2. The Iowa State Univ. Press, Ames.

Gill, J. L. 1981. Evolution of statistical design and analysis of experiments. J. Dairy Sci. 64:1494.

Gill, J. L. 1986. Repeated measurement: Sensitive tests for experiments with few animals. J. Anim. Sci. 63:943.

Goldman, B. D. and J. M. Darrow. 1983. The pineal gland and mammalian photoperiodism. Neuroendocrinology 37:386.

Goldman, B. D., J. M. Darrow and L. Yogev. 1984. Effects of timed melatonin infusions on reproductive development in the Djungarian hamster (Phodopus sungorus). Endocrinology 114:2074.

Goldman, B. D., K. S. Matt, P. Roychoudhury and M. H. Stetson. 1981. Prolactin release in golden hamsters: Photoperiod and gonadal influences. Biol. Reprod. 24:287.

Groem, G. V. and A. R. Boyns. 1973. Effect of hypothalamic releasing factors and steroids on release of gonadotropins by organ cultures of human fetal pituitaries. J. Endocrinol. 59:511.

Grosvenor, C. E. and I. Mena. 1980. Evidence that thyrotropin-releasing hormone and a hypothalamic prolactin-releasing factor may function in the release of prolactin in the lactating rat. Endocrinology 107:863.

Gudelsky, G. A., D. D. Nansel and J. C. Porter. 1980. Uptake and processing of dopamine by cells of the anterior pituitary gland. Endocrinology 107:30

terHaar, M. B., P. C. B. Mackinnen and M. G. Bulmer. 1974. Sexual differentiation in the phase of the circadian rhythm of methionine incorporation into cerebral proteins, and of serum gonadotrophin levels. J. Endocrinol. 62:257.

Hafiez, A. A., C. W. Lloyd and A. J. Bartke. 1972. The role of prolactin in the regulation of testis function: the effects of prolactin and luteinizing hormone on the plasma levels of testosterone and androstenedione in hypophysectionized rats. J. Endocrinol. 52:327.

Hafs, H. D. and M. S. McCarthy. 1980. Endocrine control of testicular function, In: H. Hawk (Ed.) Animal Reproduction (BARC Symp no 3). pp. 345-364. Allanheld, Osmun, Montclair.

Hafs, H. D., R. W. Purchas and A. M. Pearson. 1971. A review: Relationships of some hormones to growth and carcass quality of ruminants. J. Anim. Sci. 33:64.

Hamm, H. E. and M. Menaker. 1980. Retinal rhythms in chicks: Circadian variation in melatonin and serotonin N-acetyltransferase activity. Proc. Natl. Acad. Sci. USA. 77:4998.

Hansen, P. J., L. A. Kamwanja and E. R. Hauser. 1983. Photoperiod influences age at puberty of heifers. J. Anim. Sci. 57:985.

Haynes, N. B., T. E. Kiser, H. D. Hafs, T. D. Carruthers, W. D. Oxender and M. S. McCarthy. 1977. Effect of intracarotid infusion of prostaglandin F_2 on plasma luteinizing hormone, testosterone and glucocorticoid concentrations in bulls. J. Anim. Sci. 45:1102.

Hedlund, L., M. M. Lischko, M. D. Rollag and G. D. Niswender. 1977. Melatonin: Daily cycle in plasma and cerebrospinal fluid of calves. Science 195:686.

Hoffmann, J. C. 1973. Effects of photoperiod and age on reproductive organs and serum LH in the male rat. Amer. J. Physiol. 224:245.

Hoffmann, K. 1978. Effect of short photoperiods on puberty, growth and moult in the Djungarian hamster (Phodopus sungorns) J. Reprod. Fertil. 54:29.

Hostetter, M. W. and B. E. Piacsek. 1977. The effect of prolactin deficiency during sexual maturation in the male rat. Biol. Reprod. 17:574.

Howland, B. E., L. M. Sanford and W. M. Palmer. 1985. Changes in serum levels of LH, FSH, prolactin, testosterone, and cortisol associated with season and mating in male Pygmy goats. J. Androl. 6:89.

Howland, B. E., D. Sonya, L. M. Sanford and W. M. Palmer. 1983. Influence of photoperiod on thyrotropin releasing hormone-induced prolactin release in ewes. Can. J. Anim. Sci. 63:67.

Howles, C. M., G. M. Webster and N. B. Haynes. 1980. The effect of rearing under a long or short photoperiod on testis growth, plasma, testosterone and prolactin concentrations and the development of sexual behavior in rams. J. Reprod. Fertil. 60:437.

Johke, T. 1970. Factors affecting the plasma prolactin level in the cow and the goat as determined by radioimmoassay. Endocrinol. Japon. 17:393.

Johnston, P. G. and I. Zucker. 1979. Photoperiodic influences on gonadal development and maintenance in the cotton rat, Sigmorden hispidus. Biol. Reprod. 21:1.

Kamwanja, L. A. and E. R. Hauser. 1983. The influence of photoperiod on the onset of puberty in the female rabbit. J. Anim. Sci. 56:1370.

Kaplan, S. L., M. M. Grumbach and M. L. Aubert. 1976. The ontogenesis of pituitary hormones and hypothalamic factors in the human fetus: Maturation of central nervous system regulation of anterior pituitary function. Recent Prog. Horm. Res. 32:161.

Kappers, J. A. 1960. The development, topographical relations and innervation of the epiphysis cerebri in the albino rat. Z. Zellforsch. 52:163.

Karg, H., T. Gimenez, M. Hartl, B. Hoffmann, E. Schallenberger and D. Schams. 1976. Testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) in peripheral plasma of bulls: Levels from birth through puberty and short-term variations. Zbl. Vet. Med. 23:793.

Karson, C. N., K. F. Berman, J. Kleinman and F. Karoum. 1984. Seasonal variation in human central dopamine activity. Psychiat. Res. 11:111.

Kennaway, D. J., E. A. Dunstan, T. A. Gilmore and R. F. Seamark. 1982/83. Effects of shortened daylength and melatonin treatment on plasma prolactin and melatonin levels in pinealectomized and sham-operated ewes. Anim. Reprod. Sci. 5:287.

Kennaway, D. J., R. G. Frith, G. Phillipou, C. D. Matthews and R. F. Seamark. 1977. A specific radioimmunoassay for melatonin in biological tissue and fluids and its validation by gas chromatography-mass spectrometry. Endocrinology 101:119.

Kennaway, D. J., T. A. Gilmore and R. F. Seamark. 1982. Effect of melatonin feeding on serum prolactin and gonadotropin levels and the onset of seasonal estrous cyclicity in sheep. Endocrinology 110:1766.

Kennaway, D. J., J. M. Obst, E. A. Dunstan and H. G. Friesen. 1981. Ultradian and seasonal rhythms in plasma gonadotropins, prolactin, cortisol and testosterone in pinealectomized rams. Endocrinology 108:639.

Kennaway, D. J., L. M. Sanford, B. Godfrey and H. G. Friesen. 1983. Patterns of progesterone, melatonin and prolactin secretion in ewes maintained in four different photoperiods. J. Endocrinol. 97:229.

Kensinger, R. S., D. E. Bauman and R. J. Collier. 1979. Season and treatment effects on serum prolactin and milk yield during induced lactation. J. Dairy Sci. 62:1880.

Klein, D. C. 1973. The role of serotonin N-acetyltransferase in the adrenergic regulation of indole metabolism in the pineal gland, In: J. Banchas and E. Usdir (Ed.). Serotonin and Behavior. p. 109. Academic Press, New York.

Klein, D. C. and R. Y. Moore. 1979. Pineal N-acetyltransferase and hydroxyindole-O-methyltransferase: Control by the retino-hypothalamic tract and the suprachiasmatic nucleus. Brain Res. 174:245.

Klein, D. C., R. Smoot, J. L. Weller, S. Higa, S. P. Markey, G. J. Creed and D. M. Jacobowitz. 1983. Lesions of the paraventricular nucleus area of the hypothalamus disrupt the suprachiasmatic-spinal cord circuit in the melatonin rhythm generating system. Brain Res. Bull. 10:647.

Koch, Y., K. H. Lu and J. Meites. 1970. Biphasic effects of catecholamines on pituitary prolatin release in vitro. Endocrinology 87:673.

Kolata, G. 1984. Puberty mystery solved pineal..puberty..melatonin. Science 223:272.

Kopin, I. J., C. M. B. Pare, J. Axelrod and H. Weissbach. 1961. The fate of melatonin in mammals. J. Biol. Chem. 236:3072.

Koprowski, J. A. and H. A. Tucker. 1971. Failure of oxytocin to initiate prolactin or luteinizing hormone release in lactating dairy cows. J. Dairy Sci. 54:1675.

Koprowski, J. A. and H. A. Tucker. 1973. Serum prolactin during various physiological states and its relationship to milk production in the bovine. Endocrinology 92:1480.

Korf, H. W., A. Oksche, P. Ekstrom, I. Gery, J. S. Ziqler, Jr. and D. C. Klein. 1986. Pinealocyte projections into the mammalian brain revealed with S-antigen antiserum. Science 236:735.

Lacroix, A. and J. Pelletier. 1979. Short-term variations in plasma LH and testosterone in bull calves from birth to 1 year of age. J. Reprod. Fertil. 55:81.

Lafond, J. and R. Collu. 1986. Effects of repeated stimuli on prolactin release in vitro from normal and adenomatous rat lactotrophs. Mol. Cell. Endocrinol. 47:175.

Lang, U. and P. C. Sizenenko. 1980. Tissue and cellular location of melatonin receptors. Internat. Symp. on Melatonin, Bremen, F. R. G. (Abstr. 143).

Lecyk, M. 1962. The effect of the length of daylight on reproduction in the field vole (Microtus arvalis Pall.) Zool. Polon. 112:189.

Lee, V. M. K., I. A. Cumming, D. M. deKretser, J. K. Findlay, B. Hudson and E. J. Keogh. 1976. Regulation of gonadotropin secretion in rams from birth to sexual maturity I. Plasma LH, FSH and testosterone levels. J. Reprod. Fertil. 46:1.

Legan, S. J. and F. J. Karsch. 1979. Neuroendocrine regulation of the estrous cycle and seasonal breeding in the ewe. Biol. Reprod. 20:74.

Leining, K. B., R. A. Bourne and H. A. Tucker. 1979. Prolactin response to duration and wavelength of light in prepubertal bulls. Endocrinology 104:289.

Leining, K. B., H. A. Tucker, J. H. Britt and D. T. Vines. 1976. Prolactin and growth hormone in calves selected for milk yield. J. Anim. Sci. 43:293 (Abstr).

Leining, K. B., H. A. Tucker and J. S. Kesner. 1980. Growth hormone, glucocorticoid and thyroxine response to duration, intensity and wavelength of light in prepubertal bulls. J. Anim. Sci. 51:932.

Lenn, N. J., B. Beebe and R. Y. Moore. 1977. Postnatal development of the suprachiasmatic hypothalamic nucleus of the rat. Cell Tissue Res. 178:463.

Leong, D. A., L. S. Frawley and J. D. Neill. 1983. Neuroendocrine control of prolactin secretion. Ann. Rev. Physiol. 45:109.

Lerner, A. B., J. D. Case and Y. Takahashi. 1960. Isolation of melatonin and 5-methoxyindole-3-acetic acid from bovine pineal glands. J. Biol. Chem. 235:1992.

Lewy, A. J., T. A. Wehr, F. K. Goodwin, D. A. Newsome and S P. Markey. 1980. Light suppresses melatonin secretion in humans. Science 210:1267.

Lincoln, G. A., O. F. X. Almeida, H. Klandorf and R. A. Cunningham. 1982. Hourly fluctuations in the

blood levels of melatonin, PRL, LH, FSH, testosterone, triiodothyronine, thyroxine and cortisol in rams under artificial photoperiods, and the effect of cranial sympathectomy. J. Endocrinol. 92:237.

Lincoln, G. A. and W. Davidson. 1977. The relationship between sexual and aggressive behavior and pituitary and testicular activity during the seasonal sexual cycle of rams, and the influence of photoperiod. J. Reprod. Fertil. 49:267.

Lincoln, G. A., A. S. McNeilly and C.L. Cameron. 1978. The effects of a sudden decrease or increase in daylength on prolactin secretion in the ram. J. Reprod. Fertil. 52:305.

Lincoln, G. A., M. J. Peet and R. A. Cunningham. 1977. Seasonal and circadian changes in the episodic release of FSH, LH, and T in rams exposed to artificial photoperiods. J. Endocrinol. 72:337.

Little, W., C. B. Mallinson, D. N. Gibbons and G. J. Rowlands. 1981. Effects of plane of nutrition and season of birth on the age and body weight at puberty of British Friesian heifers. Anim. Prod. 33:273.

MacMillan, K. L. and H. D. Hafs. 1968. Pituitary and hypothalamic endocrine changes associated with reproductive development of Holstein bulls. J. Anim. Sci. 27:1614.

Mahone, J. P., T. Berger, E. D. Clegg and W. L. Singleton. 1979. Photoinduction of puberty in boars during naturally occurring short daylengths. J. Anim. Sci. 48:1159.

Marshall, F. H. A. 1937. On the change over in the oestrous cycle in animals after transference across the equator, with further observations on the incidence of the breeding seasons and the factor controlling sexual periodicity. Proc. R. Soc. B. 122:413.

Martin, J. B., S. Reichlin and G. M. Brown. 1978. Clinical Neuroendocrinology. p.232. F. A. Davis Co., Philadelphia.

Martin, T. C., N. F. Cunningham and N. Saba. 1983. Estimation of melatonin in bovine plasma: Patterns in Jersey heifers treated with cloprostenol at two times of day. J. Endocrinol. 98:189.

Matt, K. S., A. Bartke, M. J. Soares, F. Talamantes, A. Hebert and M. P. Hogan. 1984. Does prolactin modify testosterone feedback in the hamster? Suppression of plasma prolactin inhibits photoperiod-induced decreases in testosterone feedback sensitivity. Endocrinology 115:2098.

Mavrogenis, A. P. and O. W. Robison. 1976. Factors affecting puberty in swine. J. Anim. Sci. 42:1251.

McCarthy, M. S., E. M. Convey and H. D. Hafs. 1979a. Serum hormonal changes and testicular response to LH during puberty in bulls. Biol. Reprod. 20:1221.

McCarthy, M. S., H. D. Hafs and E. M. Convey. 1979b. Serum hormone patterns associated with growth and sexual development in bulls. J. Anim. Sci. 49:1012.

Meites, J., K. H. Lu, W. Wuttke, C. W. Welsch, H. Nagasawa and S. K. Quadri. 1972. Recent studies on functions and control of prolactin secretion in rats. Recent Prog. Horm. Res. 28:471.

Melamed, E., Y. Frucht, M. Lemor, A Uzzan and Y. Rosenthal. 1984. Dopamine turnover in rat retina: A 24-hour light-dependent rhythm. Brain Res. 305:148.

Merriam, G. R. and K. W. Wachter. 1982. Algorithms for the study of episodic hormone secretion. Amer. J. Physiol. 243:E310.

Minneman, K. P. and R. J. Wurtman. 1976. The pharmacology of the pineal gland. Ann. Rev. Pharmacol. Toxicol. 16:33

Moger, W. H., I. I. Geschwind and H. H. Cole. 1972. The action of prolactin on the sex accessory glands of the male rat. Proc. Soc. Exp. Biol. Med. 141:1017.

Moore, R. Y. 1983. Organization and function of a central nervous system circadian oscillator: The suprachiasmatic hypothalamic nucleus. Fed. Proc. 42:2783.

Moore, R. Y. and D. C. Klein. 1974. Visual pathways and the central neural control of a circadian rhythm in pineal serotonin N-acetytransferase activity. Brain Res. 17.17.

Morris, P. L. and R. B. Saxena. 1980. Dose-and age-dependent effects of prolactin (PRL) or luteinizing hormone-and PRL-binding sites in rat Leydig cell homogenates. Endocrinology 107:1639.

Muduuli, D. S., L. M. Sanford, W. M. Palmer and B. E. Howland. 1979. Secretory patterns and circadian and seasonal changes in luteinizing hormone, follicle stimulating hormone, prolactin and testosterone in the male Pygmy goat. J. Anim. Sci. 49:543.

Muller, E. E., D. Cocchi, A. Villa, F. Zambotti and F. Fraschini. 1972. Involvement of brain catecholamines in the gonadotropin-releasing mechanism(s) before puberty. Endocrinology 90:1267.

Munro, C. J., K. P. McNatty and L. Renshaw. 1980. Circa-annual rhythms of prolactin secretion in ewes and the effect of pinealectomy. J. Endocrinol. 84:83

Murphy, B. D. and K. Rajkumar. 1985. Prolactin as a luteotrophin. Can. J. Physiol. Pharmacol. 63:257.

Naber, D., A. Wirz-Justice, M. S. Kafka, I. Tobler and A. A. Borbeily. 1981. Seasonal variations in the endogenous rhythm of dopamine receptor binding in rat striatum. Biol. Psychiat. 16:831.

Nalbandov, A. V. 1976. Reproductive Physiology of Mammals and Birds. 3rd ed. p. 230. W. H. Freeman and Co. San Francisco.

Ntunde, B. N., R. R. Hacker and G. J. King. 1979. Influence of photoperiod on growth, puberty and plasma LH levels in gilts. J. Anim. Sci. 48:1401.

Ohlson, D. L., L. J. Spicer and S. L. Davis. 1981. Use of active immunization against prolactin to study the influence of prolactin on growth and reproduction in the ram. J. Anim. Sci. 52:1350.

Oxender, W. D., P. A. Noden and H. D. Hafs. 1977. Estrus, ovulation and serum progesterone, estradiol and LH concentrations in mares after an increased photoperiod during winter. Amer. J. Vet. Res. 38:203.

Ozaki, Y. and H. J. Lynch. 1976. Presence of melatonin in plasma and urine of pinealectomized rats. Endocrinology 99:641.

Padmanabhan, V., E. M. Convey and H. A. Tucker. 1979. Pineal compounds alter prolactin release from bovine pituitary cells. Proc. Soc. Exp. Biol. Med. 160:340.

Pang, S. F., H. S. Yu, H. C. Suen and G. M. Brown. 1980. Melatonin in the retina of rats: a diurnal rhythm. J. Endocrinol. 87:89.

Parrott, R. F. and F. Hill. 1979. Serum prolactin levels in castrated rams at various times of the year and during treatment with androgens or oestrogen. J. Endocrinol. 83:27.

Payne, A. H., R. P. Kelch, E. P. Murono and J. T. Kerlan. 1977. Hypothalamic, pituitary and testicular function during sexual maturation of the male rat. J. Endocrinol. 72:17.

Pelletier, J. 1973. Evidence for photoperiodic control of prolactin release in rams. J. Reprod. Fertil. 35:143.

Pelletier, J., V. Brieu and J. P. Ravault. 1985. Light entrainment and stimulation of plasma prolactin in rams. C. R. Acad. Sc. Paris 301:323.

Pelletier, J. and R. Ortavant. 1975. Photoperiodic control of LH Release in the Ram. II. Light - androgens interaction. Acta Endocrinol. 78:442.

Perlow, M. J., S. M. Reppert, L. Tamarkin, R. J. Wyatt and D. C. Klein. 1980. Photic regulation of the melatonin rhythm: Monkey and man are not the same. Brain Res. 182:211.

Peters, R. R., L. T. Chapin, R. S. Emery and H. A. Tucker. 1981. Milk yield, feed intake, prolactin, growth hormone and glucocorticoid response of cows to supplemental light. J. Dairy. Sci. 64:1671.

Peters, R. R., L. T. Chapin, K. B. Leining and H. A. Tucker. 1978. Supplemental lighting stimulates growth and lactation in cattle. Science 199:911.

Petitclerc, D., L. T. Chapin, R. S. Emery and H. A. Tucker. 1983a. Body growth, growth hormone, prolactin and puberty response to photoperiod and plane of nutrition in Holstein heifers. J. Anim. Sci. 57:892.

Petitclerc, D., R. R. Peters, L. T. Chapin, W. D. Oxender, K. R. Refsal, R. K. Braun and H. A. Tucker.

1983b. Effect of blinding and pinealectomy on photoperiod and seasonal variations in secretion of prolactin in cattle. Proc. Soc. Exp. Biol. Med. 174:205.

Peular, J. D. and G. A. Johnson. 1977. Simultaneous single isotope radioenzymatic assay of plasma norepinephrine, epinephrine and dopamine. Life Sci. 21:625.

Piacsek, B. E., N. J. Statham and M. P. Goodspeed. 1978. Sexual maturation of male rats in continuous light. Amer. J. Physiol. 234:E262.

Quay, W. B. 1956. Volumetric and cytologic variation in the pineal body of Peromyscus leucopus (Rodentia) with respect to sex, captivity and daylength. J. Morphol. 98:471.

Quay, W. B. 1963. Circadian rhythm in rat pineal serotonin and its modifications by estrous cycle and photoperiod. Gen. Comp. Endocrinol. 3:473.

Raimirez, V. D. and S. M. McCann. 1965. Inhibitory effect of testosterone on luteinizing hormone secretion in immature and adult rats. Endocrinology 76:412.

Ralph, C. L. 1981. Melatonin production by extra-pineal tissues. Adv. Biosci. 29:35.

Ralph, C. L., D. Mull, H. J. Lynch and L. Hedlund. 1971. A melatonin rhythm persists in rat pineals in darkness. Endocrinology 89:1361.

Ramaley, J. A. 1979. Development of gonadotropin regulation in the prepubertal mammal. Biol. Reprod. 20:1.

Ravault, J. P. and M. Courot. 1975. Blood prolactin in the male lamb from birth to puberty. J. Reprod. Fertil. 42:563.

Ravault, J. P., M. Courot, D. Garnier, J. Pelletier and M. Terqui. 1977. Effect of 2-Bromo- -Ergocryptine (CB 154) on plasma prolactin, LH and testosterone levels, accessory reproductive glands and spermatogenesis in lambs during puberty. Biol. Reprod. 17:192.

Rawlings, N. C., P. W. Fletcher, D. M. Henricks and J. R. Hill. 1978. Plasma luteinizing hormone (LH) and testosterone levels during sexual maturation in beef bull calves. Biol. Reprod. 19:1108.

Ray, K. P. and M. Wallis. 1984 Studies of TRH-induced prolactin secretion and its inhibition by dopamine, using ovine pituitary cells. Mol. Cell. Endocrinol. 36:131.

Reiter, E. O. and M. M. Grumbach. 1982. Neuroendocrine control mechanisms and the onset of puberty. Ann. Rev. Physiol. 44:595.

Reiter, R. J. 1983. The role of light and age in determining melatonin production in the pineal gland. In: J. Axelrod, F. Fraschini and G. P. Velo (Ed.) The Pineal Gland and its Endocrine Role. p. 227. Plenum Press. New York.

Reiter, R. J. 1986. The pineal gland: An important link to the environment. News Physiol. Sci. 1:202.

Reiter, R. J., C. M. Craft, J. E. Johnson, Jr., T. S. King, B. A. Richardson, G. M. Vaughan and M. K. Vaughan. 1981. Age-associated reduction in nocturnal pineal melatonin levels in female rats. Endocrinology 109:1295.

Reiter, R. J., B. A. Richardson and E. C. Hurlbut. 1981b. Pineal, retinal and Harderian gland melatonin in a diurnal species, The Richardson's ground squirrel (Spermophilus richardsonii). Neurosci. Lett. 22:285.

Reiter, R. J., B. A. Richardson, L. Y. Johnson, B. N. Ferguson and D. T. Dinh. 1980. Pineal melatonin rhythm: Reduction in aging Syrian hamsters. Science 210:1372.

Reiter, R. J., M. K. Vaughan, P. K. Rudeen, G. M. Vaughan and P. J. Waring. 1975. Melatonin - pineal relationships in female Golden hamster. Proc. Soc. Exp. Biol. Med. 149:290.

Relkin, R. 1972. Effects of variations in environmental lighting on pituitary and plasma prolactin levels in rats. Neuroendocrinology 9:278.

Reppert, S. M., M. J. Perlow, L. Ungerleider, M. Mishkin, L. Tamarkin, D. G. Orloff, H. Hoffman and D. C. Klein. 1981. Effects of damage to the suprachiasmatic area of the anterior hypothalamus on the daily melatonin and cortisol rhythms in the rhesus monkey. J. Neurosci. 1:1414.

Rhind, S. M., J. J. Robinson, J. M. Chesworth and R. M. J. Crofts. 1980. Effects of season, lactation and

the role of prolactin in the control of ewe fertility. J. Reprod. Fertil. 58:145.

Robinson, J. E., N. L. Wayne and F. J. Karsch. 1985. Refractoriness to inhibitory daylengths initiates the breeding season of the Suffolk ewe. Biol. Reprod. 32:1024.

Rollag, M. D. and G. D. Niswender. 1976. Radio-immunoassay of serum concentrations of melatonin in sheep exposed to different lighting regimens. Endocrinology 98:482.

Root, A. W., B. H. Shaprio, G. E. Duckett and A. S. Goldman. 1975. Effect of synthetic luteinizing hormone-releasing hormone in newborn rats. Proc. Soc. Exp. Biol. Med. 148:631.

Roy, J. H. B., C. M. Gillies, M. W. Perfitt and I. J. F. Stobo. 1980. Effect of season of the year and phase of the moon on puberty and on the occurrence of oestrus and conception in dairy heifers reared on high planes of nutrition. Anim. Prod. 31:13.

Rzepkowski, R. A., J. J. Ireland, R. L. Fogwell, L. T. Chapin and H. A. Tucker. 1982. Serum luteinizing hormone, follicle stimulating hormone and prolactin response to photoperiod during the estrous cycle of Holstein heifers. J. Anin. Sci. 55:1125.

Salisbury, G. W., N. L. VanDemark and J. R. Lodge. 1978. Physiology of Reproduction and Artificial Insemination on Cattle (2nd Ed.) p. 57. W. H. Freeman Co. San Francisco.

Sanford, L. M., D. B. Beaton, B. E. Howland and W. M. Palmer. 1978. Photoperiod - induced changes in LH, FSH, prolactin and testosterone secretion in the ram. Can. J. Anim. Sci. 58:123.

Schams, D. 1972. Prolactin levels in bovine blood, influenced by milking manipulation, genital stimulation and oxytocin administration with specific consideration of the seasonal variations. Act. Endocrinol. 71:684.

Schams, D. and V. Reinhardt. 1974. Influence of the season on plasma prolactin level in cattle from birth to maturity. Horm. Res. 5:217.

Schanbacher, B. D. and J. D. Crouse. 1981. Photoperiodic regulation of growth: A photosensitive phase

during light-dark cycle. Amer. J. Physiol. 241:E1.

Schanbacher, B. D., J. D. Crouse and C. L. Ferrell. 1980. Testosterone influences on growth, performance, carcass characteristics and composition of young market lambs. J. Anim. Sci. 51:685.

Schanbacher, B. D. and J. J. Ford. 1976. Seasonal profiles of plasma LH, T and estradiol in the ram. Endocrinology 99:752.

Schulte, B. A., U. S. Seal, E. D. Plotka, M. A. Letellier, L. Verme, J. J. Ozoga and J. A. Parsons. 1981. The effect of pinealectomy on seasonal changes in prolactin secretion in the white-tailed deer (Odocoileus virginianus borealis). Endocrinology 108:173.

Shaar, C. J. and J. A. Clemens. 1974. The role of catecholamines in the release of anterior pituitary prolactin in vitro. Endocrinology 95:1202.

Sharp, P. J. and R. Moss. 1977. The effects of castration on concentrations of luteinizing hormone in the plasma of photorefractory red grouse (Lagopus lagopus scoticus). Gen. Comp. Endocrinol. 32:289.

Simpson, S. M. and B. K. Follett. 1982. Formal properties of the circadian system underlying photoperiodic time-measurement in Japanese quail. J. Comp. Physiol. 145:381.

Smith, V. G. and E. M. Convey. 1975. TRH-stimulation of prolactin release from bovine pituitary cells. Proc. Soc. Exp. Biol. Med. 149:70.

Smith, V. G., R. R. Hacker and R. G. Brown. 1977. Effect of alterations in ambient temperature on serum prolactin concentration in steers. J. Anim. Sci. 44:645.

Sorensen, A. M. W. Hansel, W. H. Hongh, D. T. Armstrong, K. McEntee and R. W. Bratton. 1959. Causes and prevention of reproductive failures in dairy cattle. I. Influence of underfeeding and overfeeding on growth and development of Holstein heifers. Cornell Univ. Agr. Exp. Sta. Bull. 936.

Stanisiewski, E. P., N. K. Ames, L. T. Chapin, C. A. Blaze and H. A. Tucker. 1987a. Effect of pinealectomy

on prolactin, testosterone and luteinizing hormone concentrations in plasma of bull calves exposed to 8 or 16 hours of light per day. J. Anim. Sci. (submitted).

Stanisiewski, E. P., L. T. Chapin, D. Petitclerc and H. A. Tucker. 1987b. Effect of photoperiod and castration on prolactin, testosterone and luteinizing hormone concentrations in male calves. J. Anim. Sci. (accepted).

Stanisiewski, E. P., L. T. Chapin, R. A. Rzepkowski and H. A. Tucker. 1987c. The effect of continuous light on prolactin secretion in prepubertal Holstein bulls. Life Sci. (accepted).

Stanisiewski, E. P., L. T. Chapin and H. A. Tucker. 1984. The effect of lamps with different spectral properties on prolactin release in prepubertal bulls. Proc. Soc. Exp. Biol. Med. 175:226.

Stanisiewski, E. P., R. W. Mellenberger, C. R. Anderson and H. A. Tucker. 1985. Effect of photoperiod on milk yield and milk fat in commercial dairy herds. J. Dairy Sci. 68:1134.

Steger, R. W., K. Matt and A. Bartke. 1985. Neuroendocrine regulation of seasonal reproductive activity in the male Golden hamster. Neurosci. Biobehav. Rev. 9:191.

Stetson, M. H., J. A. Elliott and B. D. Goldman. 1986. Maternal transfer of photoperiodic information influences the photoperiodic response of prepubertal Djungarian hamsters (Phodopus sungorus sungorus) Biol. Reprod. 34:664.

Swanson, L. W. and H. G. J. M. Kuypers. 1980. The paraventricular nucleus of the hypothalamus: cytoarchitectonic subdivisions and organization of projections to the pituitary, dorsal vagal complex, and spinal cord as demonstrated by retrograde fluorescence double-labeling methods. J. Comp. Neurol. 194:555.

Swerdloff, R. S., P. C. Walsh, H. S. Jacobs and W. D. Odell. 1971. Serum LH and FSH during sexual maturation in the male rat: effect of castration and cryptorchidism. Endocrinology 88:120.

Talwalker, P. K., A. Ratner and J. Meites. 1963. In vitro inhibition of pituitary prolactin synthesis and release by hypothalamic extract. Amin. J. Physiol. 205:213.

Tamarkin, L., J. S. Hutchison and B. D. Goldman. 1976. Regulation of serum gonadotropin by photoperiod and testicular hormone in the Syrian hamster. Endocrinology 99:1528.

Tucker, H. A. 1982. Seasonality in cattle. Theriogenology 17:53.

Tucker, H. A. and W. D. Oxender. 1980. Seasonal aspects of reproduction, growth and hormones in cattle and horses. Prog. Reprod. Biol. 5:155.

Tucker, H. A., D. Petitclerc and S. A. Zinn. 1984. The influence of photoperiod on body weight gain, body composition, nutrient intake and hormone secretion. J. Anim. Sci. 59:1610.

Tucker, H. A. and R. P. Wettmann. 1976. Effects of ambient temperature and relative humidity on serum prolactin and growth hormone in heifers. Proc. Soc. Exp. Biol. Med. 151:623.

Turek, F. W. 1983. Neurobiology of circadian rhythms in mammals. Bioscience 33;439.

Turek, F. W. 1985. Circadian neural rhythms in mammals. Ann. Rev. Physiol. 47:49.

Turek, F. W. and C. S. Campbell. 1979. Photoperiodic regulation of neuroendocrine - gonadal activity. Biol. Reprod. 20:32.

Umezu, K. and K. Moore. 1978. Effects of drugs on regional brain concentrations of dopamine and dihydroxyphenylacetic acid. J. Pharmacol. Exp. Therapeut. 208:49.

Underwood, H. and G. Groos. 1982. Vertebrate circadian rhythms: retinal and extraretinal photoreception. Experientia 38:1013.

Van Maanen, J. H. and P. G. Smelik. 1968. Induction of pseudopregnancy in rats following local depeletion of monoamines in the median eminence of the hypothalamus. Neuroendocrinology 3:177.

VanMourik, S. and T. Stelmasiak. 1985. Seasonal variation in plasma prolactin concentrations in adult, male Rusa deer (cervus rusa timorensis). Comp. Biochem. Physiol. 82A:323.

Vaticon, M. D., C. Fernandez-Galaz, A. Esquifino, A. Tejero and E. Aquilar. 1980. Effects of constant light on prolactin secretion in adult female rats. Hormone Res. 12:277.

Vaughan, G. M., R. W. Pelham, S. F. Pond, L. Longhlin, K. M. Wilson, K. L. Sandock, M. K. Vaughan, S. H. Koslow, and R. J. Reiter. 1976. Nocturnal elevation of plasma melatonin and 5-hydroxyindoleacetic acid in young men: Attempts at modification by brief changes in environmental lighting and sleep by autonomic drugs. J. Clin. Endocrinol. 42:752.

Vollrath, L. 1981. The pineal organ. In: A. Oksche, L. Vollrath (Ed.) Handbuch der Mikroskopischen Anatomie des Menschen. Springer-Verlag, New York.

Vriend, J., P. M. Hinkle and K. M. Knigge. 1980. Evidence for a thyrotropin-releasing hormone inhibitor in the pineal gland. Endocrinology 107:1791.

Waeber, C., O. Reymond, M. Reymond and T. Lemarchand-Beraud. 1983. Effects of hyper-and hypoprolactinemia on gonadotropin secretion, rat testicular luteinizing hormone/human chorionic gonadotropin receptors and testosterone production by isolated Leydig cells. Biol. Reprod. 28:167.

Webster J. R. and G. K. Barrell. 1985. Advancement of reproductive activity, seasonal reduction in prolactin secretion and seasonal pelage changes in pubertal and deer kinds (Cervus elaphus) subjected to artificially shortened daily photoperiod or daily melatonin treatments. J. Reprod. Fertil. 73:255.

Weinberg, U., F. J. Gasparini and E. D. Weitzman. 1981. The developmental pattern of in vitro rat liver melatonin degrading activity. Endocrinology 108:1081.

Weitzman, E. D., U. Weinberg, R. D'Elette, H. Lynch, R. J. Wurtman, C. Czeisler and S. Erlich. 1978. Studies of the 24-hour rhythm of melatonin in man. J. Neurol. Trans. 13:325.

Wettemann, R. P. and H. A. Tucker. 1974. Relationship of ambient temperature to serum prolactin in heifers. Proc. Soc. Exp. Biol. Med. 146:908.

Wiggins, E. L., L. E. Casida and R. H. Grummer. 1950. The effects of season of birth on sexual development of gilts. J. Anim. Sci. 9:277.

Wolinska, E., J. Polkowska and E. Domanski. 1977. The hypothalamic centres involved in the control of production and release of prolactin in sheep. J. Endocrinol. 73:21.

Wurtman, R. J. and F. Anton-Tay. 1969. The mammalian pineal gland as a neuroendocrine transducer. Rec. Prog. Horm. Res. 25:493.

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 143:1328.

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

Wurtman, R. J., J. Axelrod and L. T. Potter. 1964b. The uptake of H^3-melatonin in endocrine and nervous tissues and the effects of constant light exposure. J. Pharmacol. Exp. Theriogenol. 143:314.

Wurtman, R. J., M. H. Deng and P. Ronsheim. 1983. The responses of melatonin rhythms to environmental lighting. In: J. Axelrod, F. Fraschini, G. P. Velo (Ed.) The Pineal Gland and its Endocrine Role. p. 221. Plenum Press, New York.

Wurtman, R. J. and M. A. Moskowity. 1977. The pineal organ, (Part 1). New Engl. J. Med. 296:1329.

Wuttke, W., K. Homma, R. Lamberts and K. G. Hohn. 1980. The role of monoamines in female puberty. Fed. Proc. 39:2378.

Wylie, C. E. 1925. The effects of season on the milk and fat production of Jersey cows. J. Dairy Sci. 8:127.

Yellon, S. M., E. L. Bittman, M. N. Lehman, D. H. Olster, J. E. Robinson and F. J. Karsch. 1985. Importance of duration of nocturnal melatonin secretion in determining the reproductive response to inductive photoperiod in the ewe. Biol. Reprod. 32:523.

Yellon, S. M. and B. D. Goldman. 1984. Photoperiod control of reproductive development in the male Djungarian hamster (Phodopus sungorus). Endocrinology 114:664.

Zinn, S. A., L. T. Chapin, W. J. Enright, A. L. Schroeder, E. P. Stanisiewski and H. A. Tucker, 1987. Growth, carcass composition and plasma melatonin in postpubertal beef heifers fed melatonin. J. Anim. Sci. (submitted).

Zucker, I., P. G. Johnston and D. Frost. 1980. Comparative physiological, and biochronometric analyses of rodent seasonal reproductive cycles. Prog. Reprod. Biol. 5:102.