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JOINT HVAC TRANSMISSION EMF ENVIRONMENTAL STUDY: FINAL REPORT ON EXPERIMENT 1

Oregon State University
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TABLE OF CONTENTS

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DISCLAIMER

BPA PERSPECTIVE

Project Description

This report describes an experimental study of the possible effects of electric and magnetic fields (EMF) on the endocrine system of domestic sheep. The study, conducted at BPA's Ostrander Substation near Estacada, Oregon, was conducted by scientists from Oregon State University and their subcontractors from the Oregon Health Sciences University, Portland State University, and the Oregon Regional Primate Research Center, through an intergovernmental agreement with BPA. BPA environmental specialists assisted in the study, and BPA engineers conducted an extensive program to measure the EMF levels to which the sheep were exposed.

There is much interest among utilities, scientists, and the public about possible effects of EMF on people and animals. Because of this wide concern, BPA solicited assistance in sponsoring this research. The following organizations co-sponsored the study:

American Electric Power Service Corp. - Columbus, OH
Houston Lighting and Power Co. - Houston, TX
Hydro-Quebec - Montreal, Quebec, Canada
Salt River Project - Phoenix, AZ
Western Area Power Administration - Golden, CO

The Electric Power Research Institute supported the study by sponsoring the study's science advisors. The U.S. Department of Energy, Office of Energy Storage and Distribution, provided additional technical review through the services of W/L Associates.

Project Objectives

Studies of laboratory animals have reported that EMF exposures can cause significant reductions in levels of the hormone melatonin. This hormone is important in regulating the breeding season in species such as sheep that breed during specific times of the year. The objective of the Joint HVAC EMF Transmission Environmental Study was to determine whether exposure of sheep to EMF produced by a 500-kV transmission line could affect melatonin or reproductive cycles in this species. The study also investigated possible effects of a high voltage line on body and wool growth, on a stress hormone, and on behavior.

To provide meaningful results, EMF levels were quantified in both pens where control and exposed animals were kept. The integration of data on these fields was, therefore, a key part of the study.

To assist with coordination of the biological and electrical studies, BPA contracted with Dr. Dan Bracken, an authority on electrical exposure methodology.

Project Results

The study provided no evidence that female lambs exposed to EMF surrounding a normally operating 500-kV a-c transmission line were affected in any of the characteristics measured, compared to a control group raised under identical conditions. Under normal farming conditions, livestock are seldom confined directly under a transmission line, nor are they subject to the constant exposure received by the experimental animals. This study, therefore, was conducted under worst-case exposure conditions.

One of the basic tenets of scientific research is that a conclusion, to be valid, must be repeatable. With this in mind, BPA, with help from its sponsors, is undertaking a new study. A larger sample size is being used in experiment 2 in an attempt to test the results of experiment 1 presented in this report.

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ACKNOWLEDGEMENTS

The contributors to this report would like to thank the many other individuals and organizations that were important for the implementation and conduct of this research project. We particularly appreciate the support of those organizations that assisted the Bonneville Power Administration in funding this study. These were American Electric Power Service Corp., Columbus, OH; Houston Lighting and Power Co., Houston, TX; Hydro-Quebec, Montreal, Quebec; Salt River Project, Phoenix, AZ; and Western Area Power Administration, Golden, CO. Their sponsorship of this study demonstrates their commitment to actively help provide scientific answers to the questions raised about the possible effects of electric and magnetic fields.

Considerable effort was devoted to ensuring that this study was planned and conducted to meet high standards of scientific credibility. Two groups were important in this process and were key to the overall success of the study. First, we want to thank the members of the project's science advisory committee. The following members provided valuable independent review and advice: Dr. J. Richard Alldredge, Washington State University; Dr. Douglas L. Foster, The University of Michigan; and Dr. Russel J. Reiter, The University of Texas. The science advisors were sponsored by the Electric Power Research Institute (EPRI). Dr. Charles N. Rafferty was the EPRI contact, and we appreciate his effort on behalf of our study.

We also want to thank Dr. Foster and his colleagues in the Reproductive Sciences Program, for their help and advice during the early design phase of this study. Our study was modeled after their research in Michigan on factors that influence puberty in female lambs.

We thank Dr. Imre Guyk, U.S. Department of Energy, for providing the assistance of a second group to provide quality control review and advice during the study. We would like to acknowledge the help provided by members of this group, including Dr. William G. Wisecup, Dr. Richard Phillips and Mr. Fred Dietrich.

Several students from Oregon State University (OSU) assisted with collection of 48-hour blood samples and with other activities. These included Anna Cortell-Brown, Patrice Prater, LeeAnn Begley, Tresa Jones, Vicki Reichlein, Margaret Kaaekauhiwi, Teri Leichner, Lauren Henry, Steve Bateson, RaeAnne Chamberlin, Darla Jacob, Sonya Calvert, Dale Evers, and Candace Bailey. Two graduate students, Sue Leers Sucheta and OV Slayden, assisted in developing the melatonin assay.

Several people from BPA helped with the study in a variety of ways. A number of people were involved in the design of the research facilities, including Ed Olavarria, Shantini Ratnathicam, Dick Albrecht, Gary Tyler, Vic Shaw, David Fahrer and John Grover. Jim Lipscomb handled contractual arrangements with study sponsors; Ron Thorkildson provided information on sunrise/sunset times and night length; Lance Ward, operator at Ostrander Substation, provided assistance throughout the study; Gary Ihle helped with all 48-hour blood samples; and Monty Tuominen assisted in the completion of the research facilities required

for the study. Pam Odam's Field Services Section supported the special electrical studies, and Instrumentations and Standards Section provided the microclimate instrumentation and instrument calibration services.

A special thanks goes to Pam McPherron and Kerry Toda for typing the report and for handling the project correspondence and meeting arrangements. We also appreciate the assistance of Leroy Sanchez in preparing the project report for printing.

We also want to acknowledge the fine support given to this project by BPA management, especially by Chuck Clark, Assistant Administrator for Engineering, and Gary Parks, Division Director for Facilities Engineering. BPA supervisory personnel, Judy Woodward and Tim Murray were also influential in helping to initiate this study.

The facilities required for this study were successfully constructed on schedule, thanks to the efforts of Paul Berry and his construction crew and to the efforts of Jim Allan, who was hired by OSU to oversee the construction phase of the study.

We thank the Portland General Electric Company (PGE) for providing the necessary equipment and labor required for providing electric power to the research facilities. This work was coordinated through the efforts of PGE employee Stan Gray.

We greatly appreciate the assistance and cooperation of Dr. Steve Yellon, School of Medicine, Loma Linda University, in conducting a melatonin RIA of blood samples from this study for use in comparing to the results from the radioimmunoassay (RIA) conducted at OSU.

Special thanks also to Dr. Ruth Wood, The University of Michigan, for her help in setting up the melatonin RIA at OSU, and for demonstrating blood collection techniques to study personnel.

Additional personnel at Portland State University (PSU) provided reviews and advice during the design and implementation phases of the study. These included Dr. Randy Zelick, Dr. Pavel Smejtek and Dr. David Dunnette. They, along with Dr. Fred Stormshak (OSU) and Dr. Richard Forbes (PSU) also served as the Ph.D. Dissertation Committee for Jack Lee (BPA). This project report is the basis for a dissertation prepared in partial fulfillment of requirements for a degree in Environmental Sciences and Resources (Lee, 1992). Dr. Joan Whittier, now with the University of Queensland in St. Lucia, Queensland, Australia, was influential in assisting in development of the initial concept for the study while at PSU.

EXECUTIVE SUMMARY

This document describes the rationale, procedures, and results of a carefully controlled study conducted to establish whether chronic exposure of female (ewe) Suffolk lambs to the environment of a 500-kV 60-Hz transmission line would affect various characteristics of growth, endocrine function, and reproductive development. This experiment used identical housing and management schemes for control and line-exposed ewes, thus minimizing these factors as contributors to between-group experimental error. Further, throughout the 10-month duration of this study, changes in electric and magnetic fields, audible noise, and weather conditions were monitored continuously by a computerized system. Such measurements provided the opportunity to identify any relationship between environmental factors and biological responses.

Because of reports in the literature that electric and magnetic fields alter concentrations of melatonin in laboratory animals, the primary objective of this study was to ascertain whether a similar effect occurs in lambs exposed to a 500-kV a-c line in a natural setting. In addition, onset of puberty was monitored by measuring changes in serum concentrations of progesterone because changes in release of melatonin from the pineal gland are known to regulate annual reproductive cycles in sheep. Concomitantly, changes in body weight, wool growth, and behavior were also monitored. To determine whether the environment of a 500-kV line caused stress in the study animals, serum levels of cortisol were measured.

This study is characterized in the following important ways: 1) animals were exposed to EMF created by an operating a-c powerline outdoors rather than to fields artificially created in a laboratory; 2) multiple end points were monitored simultaneously in both control and exposed animals; and 3) the study employed a species of grazing animal that is exposed to powerline EMF on farms and which is significantly different evolutionarily from laboratory rodents.

Based on the results of this study, the following conclusions are advanced:

Exposure of ewe lambs from 8 to 52 weeks of age to the electrical environment of a 500-kV 60-Hz transmission line;

(1) did not interfere with body growth, wool growth or behavioral characteristics,

(2) did not significantly alter the nighttime duration, phase, or magnitude of serum melatonin,

(3) did not constitute a form of stress as evidenced by changes in serum concentrations of cortisol, and

(4) did not interfere with the attainment of puberty at the anticipated time.

The reported effect of electric and magnetic fields on melatonin in laboratory animals was not observed in this environmental study. This may be due to biological differences between laboratory rodents and sheep in their response to factors that affect melatonin. Alternatively, the different findings may involve the field exposures used in laboratory studies which, in general, do not duplicate actual field exposures produced outdoors by high voltage transmission lines.

Results of this study will be useful to persons interested in the possible effects of exposure of livestock to high voltage powerlines.

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LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

FIGURE PAGE

FIGURE PAGE

FIGURE PAGE

INTRODUCTION

This study investigated the possible effects of a high voltage transmission line on domestic sheep (Ovis aries L.), a species that can often be found near such lines. The study was prompted by growing interest in whether electric and magnetic fields produced by powerlines and other electrical sources may be harmful to animal or human health. Concerns were first raised in the early 1970s that electric fields from increasingly higher voltage electrical transmission lines might result in adverse biological effects. Much of the early concern was prompted by reports from the Soviet Union. Workers in newly-built 400-kV and 500-kV substations experienced a variety of ailments which were attributed to strong 50-Hz electric fields in the Soviet substations (Asanova and Rakov, 1966).

The Soviet reports, along with results from some studies of laboratory animals (e.g., Marino et al., 1976), led to initiation of several extensive electric field research programs in the U.S. and elsewhere. To date, these programs have found no evidence that power frequency (50- and 60-Hz) electric fields cause pathological effects in people or animals (see review by Lee et al., 1989). However, a number of effects have been confirmed by one or more laboratories. It is not known whether such effects could in some way adversely affect humans or animals exposed to electric fields under natural conditions.

More recently, interest in biological effects of magnetic fields has increased, and some effects have been reported from weak extremely-

low-frequency (ELF) magnetic fields of the strength produced by powerlines (Ahlbom et al., 1987). A major focus of attention is whether ELF magnetic or electric fields act as promoters of human cancers (Brown and Chattopadhyay, 1988).

To answer the questions that have been raised about the potential for ELF fields to cause deleterious effects on humans or animals, there is a need to move from broad screening studies to specific hypothesis-testing experiments (Carstensen, 1987). This environmental science study was designed to test an hypothesis which was developed based on published results of research on laboratory animals. Laboratory research has demonstrated the ability of weak electric and magnetic fields to decrease significantly the normally high nocturnal level of the pineal hormone melatonin. This hormone is associated with regulation of biological rhythms. Such an effect on melatonin, which may mimic effects of light, could have important implications for reproductive endocrinology, mental health, and carcinogenesis (Wilson et al., 1989).

Research is continuing on the effects of electric and magnetic fields on melatonin production under laboratory conditions. However, research is also needed to determine whether this effect occurs in animals exposed to actual power-frequency fields present in the environment. Thus, the purpose of this research project was to determine whether exposure of animals to 60-Hz fields under a natural setting would result in effects on the endocrine system.

The current study measured serum concentrations of melatonin and progesterone in female Suffolk lambs to assess whether 60-Hz electric

and magnetic fields alter the regulation of breeding cycles. The adrenal hormone cortisol was also measured to assess possible responses to stress. Sheep have been widely used in agricultural research and in research to model various aspects of human physiology (Hecker, 1983). Radioimmunoassays have been developed for several hormones of sheep. Also, many studies have been conducted to examine factors that affect melatonin, reproduction, and stress in this species (see review below).

Treatment animals in this study were kept outdoors and exposed to the 60-Hz fields produced by a commercial 500-kV transmission line resulting in realistic environmental exposures. The study provided important new information for evaluating the possible effects of an ubiquitous environmental agent on the mammalian endocrine system. While these study results have direct application for livestock, the study also adds to the data base for assessing the potential impact of electric and magnetic fields on human health.

LITERATURE REVIEW

Pineal Melatonin

The Pineal. The pineal, found in most vertebrates, is now considered to be a neuroendocrine transducer. In humans, the pineal is less than 1-cm long, and it is attached to the posterior wall of the third ventricle of the brain (Reiter, 1989). Although the pineal produces a variety of indoleamines and peptides, only the indoleamine melatonin (N-acetyl-5-methoxytryptamine) has been found, thus far, to have a specific function (Tamarkin et al., 1985). This hormone, discovered in 1958 (Lerner et al., 1958), exhibits a strong circadian rhythm with large changes in circulating concentrations corresponding to light-dark cycles. Melatonin functions as a time-keeping hormone in the regulation of reproduction in species which utilize photoperiod to regulate the breeding season.

Although melatonin synthesis occurs primarily in the pineal gland, in some species it is also found in other tissues including the retina and the Harderian gland (Pang et al., 1977). Melatonin is not restricted to vertebrates as it has been found in insects, planarians, and unicellular algae (Balzar and Hardeland, 1991). In vertebrates, melatonin is synthesized through a series of steps that include the pineal indoleamine serotonin as an intermediary. Serotonin N-acetyltransferase (SNAT) is the rate-limiting enzyme in melatonin synthesis, and the enzyme hydroxyindole-O-methyltransferase (HIOMT) is involved in the final step of the process. HIOMT is also involved in

the synthesis from serotonin of another pineal hormone, 5-methoxytryptophol (5-MTP).

With normal cycles of lighting, pineal and serum concentrations of melatonin peak during darkness while serotonin peaks during the light phase (Reiter, 1979). Exposure to even brief periods of light of sufficient intensity during the dark phase can abolish, at least temporarily, the normal nocturnal rise in melatonin (Reiter, 1985). In continuous darkness, the melatonin rhythm free-runs with a period of around 24 hours (Tamarkin et al., 1985).

Photoperiodic information reaches the pineal from the retina through the hypothalamic suprachiasmatic and paraventricular nuclei, the spinal cord, and the superior cervical ganglia (Tamarkin et al., 1985). Norepinephrine is then released in a rhythmic fashion, which corresponds to the light-dark cycle, from sympathetic nerves that terminate adjacent to pineal cells. SNAT is activated, and melatonin is synthesized and diffuses from the pineal into surrounding tissue. Moore and Klein (1974) found that destruction of all visual pathways in laboratory rats caused the serotonin rhythm to be free-running and no longer controlled by photoperiod. Their work also suggested that the suprachiasmatic nuclei functioned as a central rhythm generator. Reppert et al. (1988) found melatonin receptors in the suprachiasmatic nuclei of humans, now the assumed site of a biological clock that generates the melatonin rhythm.

A summary of key steps in the synthesis of melatonin is shown in diagramtic form in Appendix A.

Function of Melatonin

Mammals. The daily melatonin rhythm seems to apply to all mammals (including humans) studied to date (Tamarkin et al., 1985). This daily rhythm may be associated with a variety of mammalian physiological processes. Most research has investigated the role of melatonin in synchronizing reproductive cycles of species that are seasonal breeders (see section below). In rodents, melatonin may also have a role in coordinating seasonal changes in body weight, coat color, and torpor (Tamarkin et al., 1985).

Melatonin treatments inhibited the development of summer pelage in least weasels (Mustela rixosa) (Rust and Meyer, 1969). Duby and Travis (1972) used artificial short-day lighting cycles to accelerate development of winter pelage in mink (Mustela vison).

The laboratory rat (Rattus norvegicus) is generally considered a non-seasonal breeder. However, various responses to changes in photoperiod or melatonin have been reported for this species. Wallen et al. (1987) found that the photoperiodicity in rats can be "unmasked" by various experimental manipulations. For instance, they reported that pinealectomy blocked the inhibitory effects of short-days (limited light exposure) in testosterone-treated male rats. Rivest et al. (1985) injected melatonin into female Wistar rats from Swiss colonies and found a 10-day delay in vaginal opening. The effect was not replicated in another study in which rats from North American colonies were used (Badawi and Wilkinson, 1988).

Rats and other nocturnal mammals appear to be much more sensitive to light intensity than humans. Fluorescent light below 5 lux intensity

resulted in a 50% reduction in SNAT activity in rats (Minneman et al., 1974). As little as 1-minute of exposure to fluorescent light during the night caused SNAT levels in the rat to drop significantly (Vanecek and Illnerova, 1982). Red light had to be about 10-times stronger to have the same effect as white light.

Lewy et al. (1980) found that light of the intensity typically found in the home (500 lux) did not reduce nighttime serum melatonin levels in humans. Exposures to bright light (1500-2500 lux) at night caused melatonin levels to decrease within 10-20 minutes. Levels increased immediately after dark conditions were restored.

In humans, it has been suggested that changes in melatonin levels are associated with puberty. Waldhauser et al. (1984) found that nocturnal plasma melatonin levels are considerably higher in children than in adolescents and adults. Other studies, however, conflict with this finding (Klein, 1984; Tamarkin et al., 1985). A causative role of melatonin in regulating puberty in humans, therefore, has not been established (Tamarkin et al., 1985; Wurtman, 1986).

Other studies suggest that melatonin may also have a role in controlling sleep (Wurtman and Lieberman, 1985). Melatonin administered to young adults resulted in subjects feeling sleepy and experiencing diminished motor activity (Lieberman et al., 1984).

Melatonin also appears to be involved in the human "seasonal affective disorder syndrome" (SADS) (Rosenthal et al., 1984). Lewy et al. (1987) reported some success in using exposure to bright light (2000 lux) as a treatment for this syndrome. The treatment seemed to

correct an abnormal phase delay in the onset of nighttime production of melatonin in some patients.

Birds. The avian pineal appears to play a strong role as a pacemaker for circadian rhythms (Gaston and Menaker, 1968; Zimmerman and Menaker, 1975). Working with house sparrows (Passer domesticus), these researchers found that pinealectomy abolished free-running activity in constant darkness. Also, by transplanting pineal tissue into the anterior eye chamber, rhythmicity can be restored in pinealectomized sparrows (Zimmerman and Menaker, 1979). These results are compatible with the hypothesis that the avian pineal may contain a circadian pacemaker. As in studies with mammals, melatonin is also involved in circadian responses observed in birds. Turek et al. (1976) found that in intact sparrows treated with melatonin, the free-running period was shortened or activity was continuous. These effects mimicked the effects of constant dim light or constant bright light, respectively.

The pineal is also involved in avian reproduction, although, as in mammals, it is apparently not essential for gonadal development. The pineal, however, may have a transitory effect on avian reproductive development (Siopes and Halawani, 1989). Scanes et al. (1980) found that pinealectomy affected diurnal levels of luteinizing hormone (LH) and prolactin (PRL) in 8-week old cockerels (Gallus domesticus). However, LH levels were not found to be affected in another study of pinealectomized chickens (Johnson and Van Tienhoven, 1984) or in pinealectomized adult turkeys (Meleagris gallapavo) (Siopes and Halawani, 1989). In the latter study, however, PRL levels in hens were

reduced by the treatment. In an earlier study, Siopes and Underwood (1987) reported that pinealectomy delayed sexual maturity in turkey hens.

Mammalian Seasonal Breeders. Most animals breed only during specific times of the year. Various "ultimate factors" (e.g., food quality and quantity) act to determine the optimal breeding time (Whittier and Crews, 1986). This results in young being born during the season when conditions for survival are best (i.e., spring and summer). There is, therefore, a direct correlation between length of gestation and when breeding occurs (Karsch et al., 1984). Thus, horses (Equus caballus), with a gestation of around 11 months, and certain rodents, with a gestation of 1 month or less, normally breed during spring. Sheep and deer (Odocoileus sp.), with a gestation of 5-6 months, breed in the fall so that their young are born the following spring.

Seasonal breeders must use some means of keeping track of time so that breeding occurs at the correct season each year. "Proximate factors" are the cues that trigger and terminate the reproductive process (Whittier and Crews, 1986). The most important of such factors for mammals is photoperiod. Horses, ferrets (Mustela putorius), and some rodents are referred to as long-day (spring) breeders while sheep, deer, and goats (Capra hirca) are short-day (fall) breeders. In both groups, the gonads respond to photoperiods that either stimulate or inhibit development, giving rise to the annual reproductive cycle. The pineal and its hormone melatonin are thought to mediate the regulation of seasonal breeding.

The relationship between the pineal and photoperiod was first studied in long-day breeders. Pinealectomy, followed by a change from

long- to short-day photoperiods, prevented gonadal regression in Syrian hamsters (Mesocricetus auratus) (Hoffman and Reiter, 1965). Treatment with melatonin can cause the gonads to regress in the hamster, simulating the effect of short days (Goldman et al., 1979).

In Syrian hamsters, pinealectomy causes the gonads to remain permanently active since the melatonin signal is abolished. In contrast, pinealectomy in the Turkish hamster (Mesocricetus brandti) leads to gonadal regression (Carter et al., 1982).

Superior cervical ganglionectomy has also been shown to affect the reproductive cycle in the mare (Sharp et al., 1979). The date of first ovulation was delayed in the second breeding season after treatment, and there were delays in patterns of hair coat changes compared with controls.

Sheep And Other Short-Day Breeders. Results of research with long-day breeders led to interest in the role of the pineal in short-day breeders. Pinealectomy and ganglionectomy have been shown to affect reproductive hormones in deer (Odocoileus virginianus borealis) (Schulte et al., 1981), goats (Buttle, 1977), sheep (Bittman et al., 1983b), and kangaroos (Marcopus eugenii) (Renfree et al., 1981).

Studies of the kangaroo point out how sensitive seasonal breeders may be to changing photoperiod. In one population studied, reactivation of the corpus luteum occurred over a period of 7-13 days during which exposure to hours of daylight decreased by only 6 minutes (Tyndale-Biscoe and Renfree, 1987). Moon phase does not appear to affect the timing of this reactivation.

Most extensive research with short-day breeders has involved sheep. This work has produced a significant amount of information about photoperiodic activation of the hypothalamo-hypophyseal axis. The remainder of this section will review the studies of sheep that form the basis for this study.

Studies by Rollag and Niswender (1976) of ewes exposed to constant light (approximately 500 lux) showed that the normal nocturnal rise in melatonin was abolished. During continuous dark, however, the circadian rhythm of melatonin persisted.

Thibault et al. (1966) reported that continuous exposure to light did not induce permanent estrus in ewes or prevent the breeding season. Ducker et al. (1973) also found no evidence that exposure to permanent light (129-215 lux) affected seasonality of estrous cycles in ewes. An early study reported that pinealectomy did not affect estrus or the estrous cycle in ewes (Roche et al., 1970). However, in the previous three studies, estrus was detected by the use of vasectomized rams. Presence of rams may provide additional cues to aid ewes in timing the estrous cycle.

Bittman et al. (1983a) studied the effects of pinealectomy in ewes which were isolated from rams. Six of seven treatment ewes had sporadic ovulations during the anestrous season when they were maintained under natural photoperiods. By September, all the pinealectomized ewes began to cycle regularly, consistent with the pineal-intact animals. Pinealectomy, like constant light, also eliminated the nocturnal rise in melatonin.

Bittman et al. (1983b) also found that, in pinealectomized ewes, a change from long to short days did not reverse the capacity of estradiol to inhibit LH secretion during anestrous. By treating the ewes with melatonin, the reproductive response to photoperiod was restored. Bittman and Karsch (1984) extended the work to show that melatonin also mediates the inhibitory effect of exposure to extended daylight.

Kennaway et al. (1985) found that pinealectomy of female lambs at 10 weeks of age delayed the onset of puberty by 13 weeks. These investigators found no evidence that either exposure of the lambs to constant light up until 10 weeks of age, or pinealectomy of the mother before conception, affected timing of puberty. A ram was used in this study to detect estrus.

Other studies show that exposure to a certain sequence of long- and short-day photoperiods is required to initiate and sustain normal ovulatory cycles in female lambs (Yellon and Foster, 1985). Lambs kept outdoors initiated their first ovulation cycle (as measured by serum progesterone) at around 31 weeks of age. In comparison, lambs kept indoors and exposed to a constant 15L:9D photoperiod (15 hours light, 9 hours dark) did not initiate cycles until around 63 weeks of age. Those exposed to a constant short-day (9L:15D) photoperiod began cycling at around 54 weeks of age.

Yellon and Foster (1986) extended their earlier study to assess whether melatonin mediates the effects of photoperiod on sexual maturation in domestic ewe lambs. In lambs kept outdoors, surgical denervation of the pineal gland eliminated the normal nocturnal rise in melatonin and delayed the initiation of the reproductive cycle. Another

group of lambs was housed indoors and exposed to artificial short day photoperiods (9L:15D). Portable devices attached to these animals infused melatonin into a jugular vein in a manner designed to mimic levels present during normal photoperiods. In this group, the initiation of the reproductive cycle in three of four animals occurred close to that of the outdoor control ewes. Yellon and Foster (1986) suggested that the pineal melatonin rhythm is a necessary part of the photoperiodic process that times puberty in female sheep.

The following hypothesis for the neuroendocrine control and timing of puberty in the ewe lamb was proposed by Foster et al. (1985). In the lamb, the frequency of the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator is low. Consequently, the frequency of luteinizing hormone (LH) pulses from the anterior pituitary gland is low. When the ewe reaches a sufficient physiological size, and when a specific photoperiod sequence (long days followed by short days) is completed, the frequency of GnRH and LH pulses begins to increase sharply. This leads to development of an ovarian follicle, increased estradiol secretion, and eventually to ovulation. In the prepubertal lamb, the system is hypersensitive to an inhibitory feedback effect of estradiol. This hypersensitivity diminishes as the lamb ages. There is also a slight rise in circulating follicle-stimulating hormone (FSH) during puberty, but the significance of this change is not clear.

More recent studies have found that female lambs and mature ewes differ in response to photoperiods required for initiation of the breeding season (Ebling and Foster, 1988). Unlike their mothers, lambs required a decrease in hours of daylight for onset of the breeding

season. The authors suggested that mature ewes have previous experience with photoperiods, and their endogenous reproductive changes have been entrained.

There remain many unanswered questions about how melatonin actually influences reproductive events, such as the identification of target tissue for melatonin and the neuroanatomical basis for photorefractoriness. It is also not clear what aspect (amplitude, phase, or duration) of the nightly rise in melatonin codes for day length. Recent studies by Wayne et al. (1988) provided evidence to support the hypothesis that duration of the rise is the important feature.

This literature summary provides strong evidence that treatments which alter the nocturnal serum concentrations of melatonin in domestic sheep also affect the timing of reproductive events in both lambs and adults. Lambs appear to be particularly sensitive during the period leading to puberty.

Cortisol and Stress Responses

Response of animals to various environmental stressors can be divided into two general kinds of physiological reactions (Dantzer and Mormede, 1983). First are the short-term emergency reactions characterized by production of catecholamines (e.g., epinephrine) from the adrenal medulla. Second is the long-term reaction originally referred to as the "general adaptation syndrome" by Selye (1936). Hormones involved in this reaction include adrenocorticotropin (ACTH) from the pituitary which causes the adrenal cortex to secrete

corticosteroids (e.g., cortisol and corticosterone). Sheep, monkeys, and humans primarily secrete cortisol; whereas birds, rats, and mice secrete mainly corticosterone (Ganong, 1981).

Corticosteroids aid in the long-term synthesis of glucose from non-carbohydrates during periods of stress (Campbell, 1990). In many studies of livestock and other species, "stress" is defined as a condition characterized by higher-than-normal levels of plasma corticosteroid concentrations (Dantzer and Mormede, 1983).

Levels of serum cortisol have been used to assess effects of various stressors on sheep and other livestock (Dantzer and Mormede, 1983). For example, Shutt et al. (1987) reported significant increases in serum cortisol in lambs following various surgical procedures. Other studies of sheep found increases in cortisol due to fly bites and handling restraint (Shutt et al., 1988) and after yarding and vehicular transport (Fell et al., 1985).

In general, cortisol levels exhibit circadian rhythms. In humans, cortisol levels are highest during early morning before waking (Eckert et al., 1988). Studies of sheep produce inconsistent results. Bassett (1974), for example, found no evidence for a circadian cortisol rhythm in sheep. When ewes were kept indoors in crates, fed ad libitum, and blood samples collected hourly over 24 hours, a circadian cortisol rhythm was found with lowest levels during darkness (McNatty et al., 1972, 1973). Holley et al. (1975) measured cortisol at 4-hour intervals in confined ewes and found a circadian cortisol rhythm with peaks influenced by feeding time. Using very intensive blood sampling (10-minute intervals), Fulkerson and Tang (1979) found both ultradian

and circadian cortisol rhythms in sheep. In their study, however, peak levels occurred around midnight.

Several studies have also examined the effects of stress on melatonin production (see a review by Reiter, 1989). Various stressors have been reported either to have no effect or to increase or decrease melatonin levels in a number of species.

Electric and Magnetic Field Effects

Induced Currents. Alternating current (a-c) electric and magnetic fields induce currents in conducting objects, including people and animals. Kaune and Phillips (1980) provided estimates of induced current for grounded subjects in a 60-Hz 10-kV/m electric field (approximately the maximum field produced by transmission lines). Axial current density through a cross section of the head of a sheep-sized animal is approximately 18-40 nA/cm^2. The induced electric field can be calculated by dividing current density by an average tissue conductivity of 0.2 S/m. The above current densities are, therefore, associated with induced electric fields of around 0.9-2 mV/m. Amstutz et al. (1980) measured 40 uA of total current induced in a sheep standing under a transmission line in an 8-9 kV/m electric field.

Current induced by the electric field flows primarily in a direction parallel to the external applied field. However, there are deviations from this directionality. For example, where the legs connect to the body, the current from the whole body collects and flows to and through the legs. The current induced by an a-c magnetic field, however, flows in circular loops perpendicular to the applied field.

Electric fields impinge on the outer body and do not penetrate tissue to any significant degree. The magnitude and distribution of the induced current is greatly influenced by the size, shape, and orientation of the body. The induced electric field in the body is several orders of magnitude smaller than the external field.

Animals can be shielded from electric fields to varying degrees by the presence of nearby conducting objects, including other animals. Some researchers have estimated the amount of this shielding for animals in laboratory studies (Free et al., 1981). The "unperturbed" electric field strength is the value measured or calculated before the animal or cage is placed into the field. "Effective field strength" is the value that attempts to compensate for caging and multiple animal effects which reduce the amount of body current induced by the field. Unless stated otherwise, electric field strengths reported in most studies are for unperturbed fields.

As defined by Faraday's law of induction, a-c magnetic fields also induce electric fields inside organisms (Kaune, 1986). The maximum magnetic field from a transmission line (0.3 G) would induce an electric field in a sheep's head of around 0.3-0.5 mV/m. This would be added vectorially to the field induced by the external electric field for a sheep beneath a transmission line.

Organisms are essentially transparent to magnetic fields. Because the magnetic field penetrates the body, it is also possible that there could be direct effects of the field on magnetic material, in addition to any effects associated with induced currents.

A-C Fields and Melatonin. Although no hazardous effects of 60-Hz electric or magnetic fields have been confirmed, several effects have been reported which resulted in functional changes in laboratory animals. In one study, conducted at Battelle-Northwest in Richland, WA, adult male laboratory rats were exposed to 60-Hz electric fields (65 kV/m unperturbed, 39 kV/m effective). After 3 weeks of exposure, nocturnal pineal melatonin and SNAT concentrations were significantly depressed in exposed animals. Sacrifice times were 0200 hours (dark-phase) and 1400 hours (light-phase). The levels returned to normal less than 3 days after field exposure was terminated (Wilson et al., 1986).

Earlier work at Battelle had shown that even a relatively weak electric field (1.8 kV/m unperturbed, 1.1 kV/m effective) caused a reduction in rat nocturnal pineal melatonin, similar to that observed at the higher field strength (Wilson et al., 1983). On the other hand, those studies found that nocturnal pineal levels of 5-methoxytryptophol (5-MTP) increased in the field-exposed animals. It was suggested that this occurred because more of the serotonin was available for 5-MTP production, implying that field exposure had interfered with SNAT activity. Therefore, the authors also suggested that the enzyme hydroxyindole-O-methyltransferase (HIOMT) was not directly affected. Later research at Battelle using immature rats indicated that the threshold for the melatonin effect with 60-Hz unperturbed electric fields is between 200 V/m and approximately 2 kV/m (Anderson et al., 1988).

Nighttime pineal melatonin levels were also reduced in young rats exposed to 60-Hz electric fields in utero and for the first 23 days after birth (Reiter et al., 1988). Melatonin levels at 0200 hours were significantly reduced by fields of 10, 65, and 130 kV/m, with no indication of a dose response.

It has been hypothesized that effects on melatonin may in turn affect production of PRL and estrogen, thus possibly increasing the risk of mammary cancer (Stevens, 1987). Experiments were conducted to examine development of mammary tumors in rats (Leung et al., 1988a). The animals were administered a drug that induced mammary tumors and then exposed to a 40-kV/m 60-Hz electric field (effective). Results of two experiments revealed no differences between the exposed and control groups in the number of animals with tumors. However, when results of the two experiments were combined, the number of tumors in the animals with tumors was significantly higher in the exposed groups. This suggested that the electric field may promote the development of tumors. An earlier study by French researchers found no increase in mammary tumors in rats exposed to a 50-kV/m electric field (LeBars, 1983).

In another Battelle study, rats were exposed to unperturbed electric fields of up to 160 kV/m (Rommereim et al., 1988). Compared to control animals, the field-exposed group showed no significant increase in the incidence of litters with malformations. A curious rust-colored deposit was found on the ears of the field-exposed animals. The deposit was identified as a porphyrin secreted by the Harderian gland, which is also a source of melatonin (Leung et al., 1988b). It is not known

whether there is any relation between this finding and the effect found on nocturnal pineal melatonin.

In the most recent research at Battelle, melatonin in male and female laboratory rats was measured at 3, 5, and 7 hours into the dark period (Sasser et al., 1991). The rats were exposed to a 60-Hz electric field (39 kV/m effective) for 30 days. In this test, exposure to the field did not result in any significant reductions in nocturnal melatonin. The only significant difference was an increase in melatonin in the female rats at one time period (0400 hours) compared to sham controls.

The studies by Wilson et al. (1986) on pineal melatonin in rats exposed to 60-Hz electric fields prompted a study of neurotransmitters by Vasquez et al. (1988). As in the earlier work, rats were exposed to a 60-Hz electric field of 39 kV/m (effective). Animals were sacrificed at 4-hour intervals throughout the day, when samples were taken. In the field-exposed animals, dihydroxyphenylacetic acid (a primary dopamine metabolite) was significantly increased in the striatum during the dark phase. In the hypothalamus, exposure shifted the daily rhythms of three biogenic amines (norepinephrine, dopamine, and 5-hydroxyindoleacetic acid). The authors suggested that the latter effects were especially significant in relation to the melatonin effect. The hypothalamus contains the suprachiasmatic nuclei and a pathway for noradrenergic inputs to the pineal gland.

Three new preliminary reports suggest that power-frequency magnetic fields can affect melatonin levels under laboratory conditions. In one study, male laboratory rats were exposed for 6 weeks to rotating 50-Hz

magnetic fields of 10, 50, 500, and 2500 mG (spatial vector rms) (Kato et al., 1991). Pineal and serum melatonin levels were reduced in the field exposed animals, including serum melatonin in those exposed to the lowest level (10 mG).

Serum melatonin during the dark period was reduced in adult Djungarian hamsters (Phodopus sungorus sungorus) by a single 15-minute exposure to a 1-G 60-Hz magnetic field (Yellon, 1991). Exposure was applied 2 hours before lights off because this is reportedly the most effective time for melatonin to affect gonadal function.

In the third report, two baboons (Papio cynacephalus) were exposed to a 1-G 60-Hz magnetic field in combination with a 30-kV/m electric field (Rogers et al., 1991). The exposure had a rapid onset component. Exposures of 10 or 20 days resulted in a 15% suppression of nocturnal melatonin. In a second test of baboons, field exposure was applied without any rapid onset characteristics, and no effect on melatonin levels was found.

A well-known biological effect of strong a-c magnetic fields in humans is the induction of visual magnetophosphenes (Tenforde, 1985). However, this phenomenon, a flickering illumination within the visual field, occurs at strong field intensities (100 G or above). Evidence indicates that the retina is the site of action leading to the effect (Tenforde, 1985).

Possibly related to the effects reported on pineal hormones is another body of research which suggests that electric and magnetic fields can alter animal activity patterns and biological rhythms. Duffy and Ehret (1982) reported that 60-Hz electric fields of around 100 kV/m

caused phase shifts in circadian rhythms and, in some cases, caused torpor in male wild-type mice (Peromyscus leucopus). A 17-G 60-Hz magnetic field significantly increased locomotion activity in mice (Smith and Justesen, 1976). Laboratory rats exposed simultaneously to a 0.5-G 60-Hz magnetic field and to a 0.26-G d-c magnetic field responded differently in a behavioral test compared to a sham group (Thomas et al., 1986).

D-C Fields and Melatonin. Other studies suggest that artificial changes in the strength or direction of the earth's direct current (d-c) magnetic field can have remarkable effects on melatonin levels in mammals and birds. Welker et al. (1983) reported that nocturnal melatonin and SNAT concentrations were quickly depressed in laboratory rats when the horizontal component of the earth's d-c magnetic field was inverted. They reported similar effects when the d-c magnetic field intensity was increased to twice the strength of the natural field.

The magnetic field effect reported by Welker et al. (1983) was confirmed by other researchers (Olcese et al., 1985; Olcese and Reuss, 1986). These studies used exposure facilities to rotate the earth's d-c magnetic field by 50° which increases field strength up to 1 G (more than double the ambient field). After 30-minute exposure to the altered field, nocturnal melatonin was significantly reduced in two strains of rats but not in golden hamsters.

Reuss and Olcese (1986) reported that the effects on melatonin observed in rodents exposed to weak d-c magnetic fields depended on the presence of weak red light. These authors theorized that,

> If one could assume the photoreceptors in the retina to be the magnetoreceptors, then the combined presence of both red light and a weak MF [magnetic field] might generate sufficient activation of the photoreceptors to give rise to impulse activity in the visual system pathways, thereby leading to an inhibition of pineal function (Reuss and Olcese, 1986).

A study by Stehle et al. (1988) also provided evidence to support a role of the retina in magneto-perception. Pigmented and albino Mongolian gerbils (Meriones unguiculatus) of both sexes were exposed for 30 minutes to d-c magnetic fields of 0.3 G. The ambient earth field before exposure was 0.26 G. Pineal SNAT and melatonin were not affected by field exposure in either sex of the pigmented animals. In contrast, both these parameters were decreased in both sexes of the albino animals as a result of field exposure. Earlier studies reported that pigmented animals lack retinal melatonin, and albino rats have larger, more active pineals than pigmented rats (studies cited in Stehle et al., 1988).

Reuss and Semm (1987) studied pineal melatonin synthesis in pigeons (Columbia livia) exposed to d-c magnetic fields. The horizontal component of the 0.3-G natural field was changed to 0.4 G during exposures. Fifty-minute field exposures resulted in a 60% reduction in nocturnal pineal SNAT concentrations. Activity of HIOMT was not affected.

In vitro studies have also found evidence that the pineal responds to changes in d-c magnetic fields (Semm, 1983). In the guinea pig (Cavia porcellus), about 20% of cells in the pineal responded to artificial changes in the earth's d-c field. Cell activity returned to normal levels when the field strength was

returned to ambient. About 30% of pineal cells from pigeons showed a response to alterations in d-c magnetic fields.

A combination of a-c (33.7 Hz) and d-c magnetic fields tuned to ion-cyclotron-resonance conditions (for Ca^{2+}) and applied for 2.5 hours to isolated rat pineals significantly reduced melatonin levels in those glands (Lerchl et al., 1990).

New research indicates that the rapid change in switching d-c magnetic fields on and off, rather than presence of the field, is the factor responsible for reduction in nocturnal melatonin in exposed rodents (Lerchl et al., 1991). Nocturnal melatonin levels were reduced only in animals where the field power supply was rapidly switched automatically. Rapid switching causes rapid induced currents (eddy currents) in animals, which may affect neural input to the pineal gland (Lerchl et al., 1991).

Livestock Studies. It now appears that, under some conditions, electric and magnetic fields can significantly alter melatonin levels. It is important to further determine the possible biological significance of this phenomenon on animals under non-laboratory exposure conditions. A critical review of literature on ELF field effects for the utility organization West Associates (WAETF 1986) concluded:

> These data emphasize the need for long-term evaluation and more extensive consideration of the subtle disturbances, possibly with significant health effects that may develop from changes in biological rhythms. Until more is known, it cannot be predicted whether changes in biological rhythms will be expressed under practical exposure conditions, and if they will be benign or pathological.

It should be noted that past experimental studies of livestock exposed to electric and magnetic fields did not use sheep or other species generally considered to be seasonal breeders. These include studies of swine (Sus scrofa) in a laboratory setting (Sikov et al., 1987), swine near a 345-kV a-c line (Mahmoud and Zimmerman, 1984), cattle (Bos tarus) near a 400-kV a-c line (Algers and Hultgren, 1987), and cattle near a ±500-kV d-c line (Raleigh, 1988).

With the possible exception of the study of swine by Sikov et al. (1987), studies have not found evidence for any large effects of transmission line fields on livestock reproduction. Sikov et al. (1987) found evidence of increased incidence of fetal malformations in female swine chronically exposed indoors to a 30-kV/m 60-Hz electric field. However, in a second breeding of the second generation females, there were no differences in malformation rates between field-exposed and control swine.

Many transmission lines cross areas where sheep are raised. This includes the Willamette Valley in Western Oregon, where there is a large sheep industry. Although anecdotal reports of transmission lines affecting sheep and other livestock are known, there are apparently no documented reports that such lines are linked to reproductive problems. However, such effects, if present, may not be distinguishable by livestock producers from effects caused by numerous other factors.

A-C Fields and Stress. Results of studies of stress-related hormones in laboratory rats exposed to 60-Hz electric or magnetic fields are conflicting. Exposure of rats to a 25-kV/m electric field resulted in increased serum corticosterone levels in the first 5 minutes (Graves

et al., 1979). However, no increases were found over a 6-week exposure period. Seto et al. (1983) also reported increased levels of corticosterone levels in rats exposed to 80-kV/m electric fields. Mean corticosterone in rats exposed to a 64-kV/m electric field for 120 days was significantly less than in the control rats in one experiment, but there was no difference in a replicate experiment (Free et al., 1981). Free et al. (1981) suggested that differences in sampling times in their study may partly explain the inconsistency.

Wilson and Anderson (1990) suggested that effects of electric fields on pineal melatonin in laboratory rats are manifestations of stress. They cited studies by Leung et al. (1988b) in which secretion of a porphyrin from the Harderian gland was found in rats exposed to 39-kV/m electric fields. This, along with changes found in serum PRL levels, were reportedly similar to effects associated with the restraining of animals.

Based on research summarized above, it is possible that exposure to the electrical environment of a high-voltage transmission line could produce stress in growing lambs. The research also suggests the possibility of stress arising from handling and management activities and a possible interaction between stress and melatonin levels. For these reasons, serum cortisol levels were measured in this study to assess possible stress responses.

METHODS AND MATERIALS

Study Area

This study was conducted near the BPA Ostrander Substation, 40-km southeast of Portland, Oregon (45° 24' north latitude). The site and facilities (Figures 1 and 2) are within 1 km of the Clackamas River at an elevation of 100 m. Average annual rainfall in the area is approximately 137 cm. Average low and high temperature for January and July are approximately 1° and 8°C and 11° and 27°C, respectively. Land use is primarily hay raising and grazing. The nearest grazing sheep were approximately 0.8 km from the site.

The line pen was located at the edge of a corridor containing three 500-kV transmission lines and two 230-kV transmission lines. The pen was located beneath the outer conductors of the outermost 500-kV line (named the Ostrander-Troutdale line). The location was chosen so that the pen was within the area of maximum field strength. To achieve field strengths typical of the maximums found beneath BPA 500-kV lines, the conductors in the span over the pen were lowered approximately 3 m. The resulting minimum conductor to ground clearance was around 12.5 m.

Study Animals

Twenty female Suffolk lambs were obtained from a commercial sheep producer in Western Oregon (Table 1). On arrival at the site on 2 April 1990, the animals were assigned randomly to two equal groups of 10. These comprised the control and line (treatment) groups. Mean ages

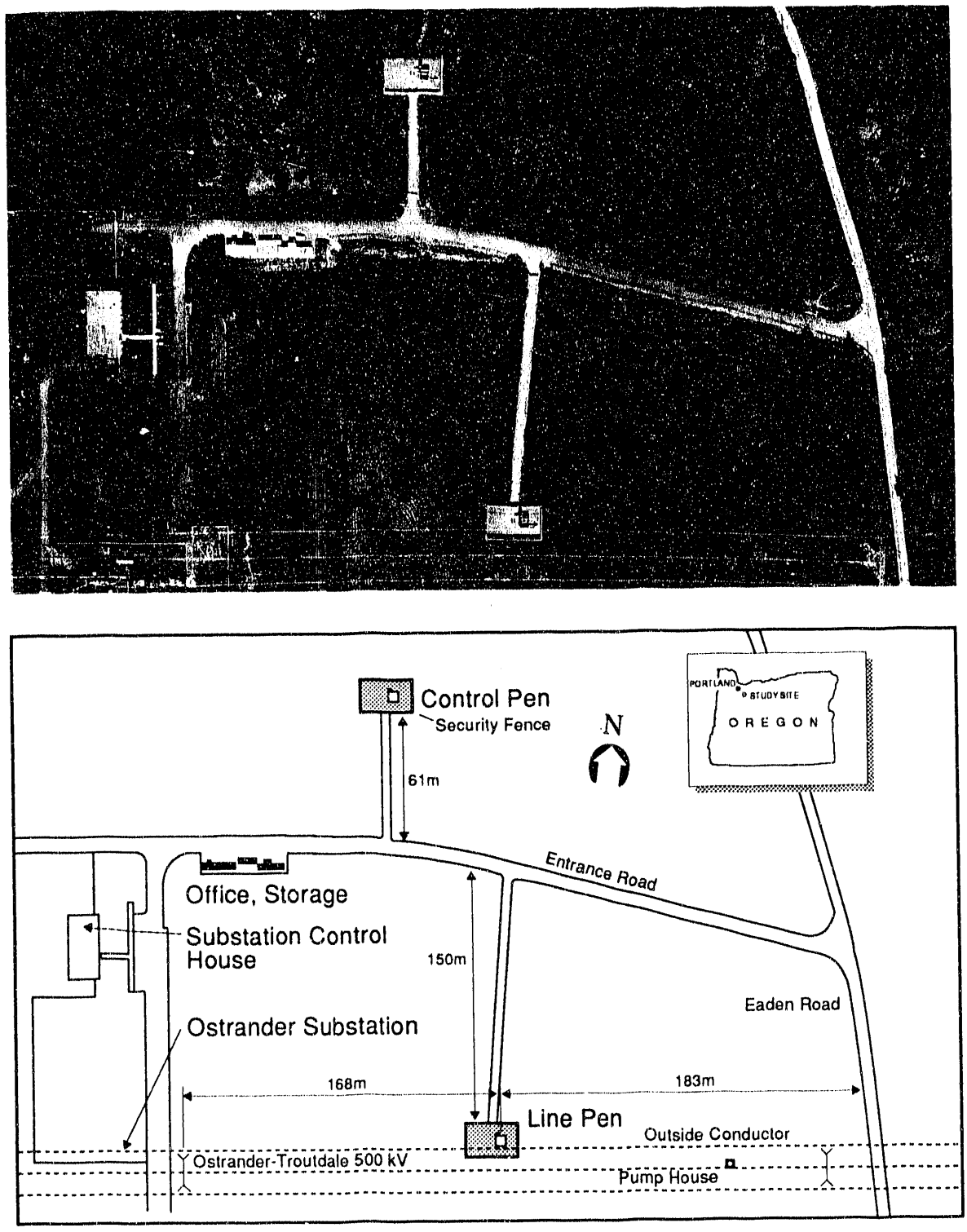


Figure 1. Study site for the Joint HVAC Transmission EMF Environmental Study. Aerial photograph of the site (upper) and sketch of facilities (lower).

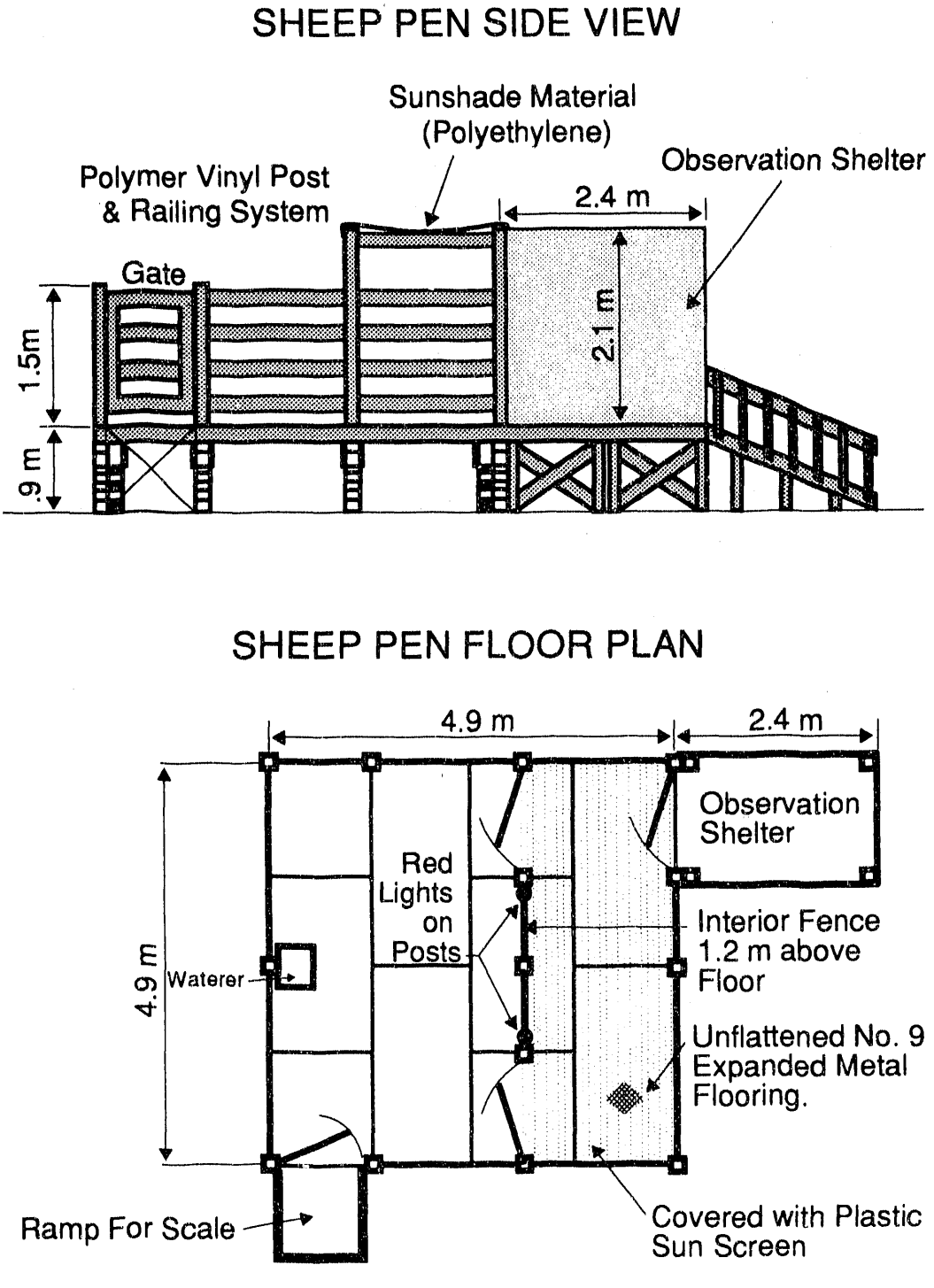


Figure 2. Design of the slatted-floor sheep pens.

TABLE I

CHARACTERISTICS OF FEMALE SUFFOLK LAMBS
USED IN THIS STUDY

Study No.[a]	Tag No.	Sire	Dam	Birth Date (Feb. 90)	Sibs[b]	Weight (kg) (2 April 90)
1	51	8134	5045	1	Tw c	35.3
2	117	8033	3195	5	Tw d	25.8
3	147	2166	5050	10	Tr	21.3
4	67	Gb2	6253	1	Tw	31.3
5	90	F3	6243	3	Tr	25.8
6	126	8033	6176	6	Tr	23.6
7	154	8134	4138	10	Tw	23.1
8	144	8033	4124	7	Tw	30.8
9	108	2166	7114	4	Tw	29.4
10	84	7262	6210	3	Tw	27.6
11	86	8134	7074	3	Tw	29.0
12	52	8134	5045	1	Tw c	28.1
13	171	8134	7107	10	Tw	26.7
14	63	8033	6264	1	Tw	25.8
15	116	8033	3195	5	Tw d	24.0
16	37	8033	5167	4	Tw	29.0
17	129	8033	6238	6	Tr	25.4
18	81	8134	7145	3	Tw	25.8
19	115	7262	515	5	Tw	31.3
20	102	GB2	7075	4	Tr	30.8

a Animals 1-10 were the control group, 11-20 the line group.
b Tw=twin, Tr=triplet.
c Twins in this study.
d Twins in this study.

(days ± SEM) for control and line groups were 56 ± 1.03 days and 56.8 ± 0.83 days, respectively. The age range was identical for the two groups (51-60 days).

The initial weights (kg ± SEM) for the control and line groups on 2 April were 27.4 ± 1.37 kg and 27.6 ± 0.77 kg, respectively. The range was 21.3 - 35.3 kg and 24.0 - 31.3 kg for the control and line groups, respectively.

Upon arrival at the site on 2 April, all lambs were initially placed in the control pen for collection of pre-exposure data. The treatment group was moved to the line pen on the afternoon of 6 April.

Facilities

Pens. The line and control lambs were kept in identical-sized pens (Figure 2). The control group was approximately 229 m from the 500-kV transmission line (Figure 1). The pens were made of elevated metal slatted-floors with plastic (non-conducting) sides (Figures 3 and 4). Slatted-floor pens are used by some sheep producers. For this study, this type of pen was used because it: 1) helps prevent health problems due to mud and parasites, 2) allows greater control over certain variables, e.g., food, 3) allows for greater site security, and 4) facilitates animal management and data collection activities.

The metal floor was grounded to prevent sheep from receiving shocks due to induced voltages from the electric field environment in the line pen. Plastic material was used above the pen floor so that the electric field would not be significantly shielded. A shelter was attached to the pen for use by personnel conducting behavioral observations.

Figure 3. Slatted-floor sheep pens in the control area (upper) and beneath the transmission line (lower).

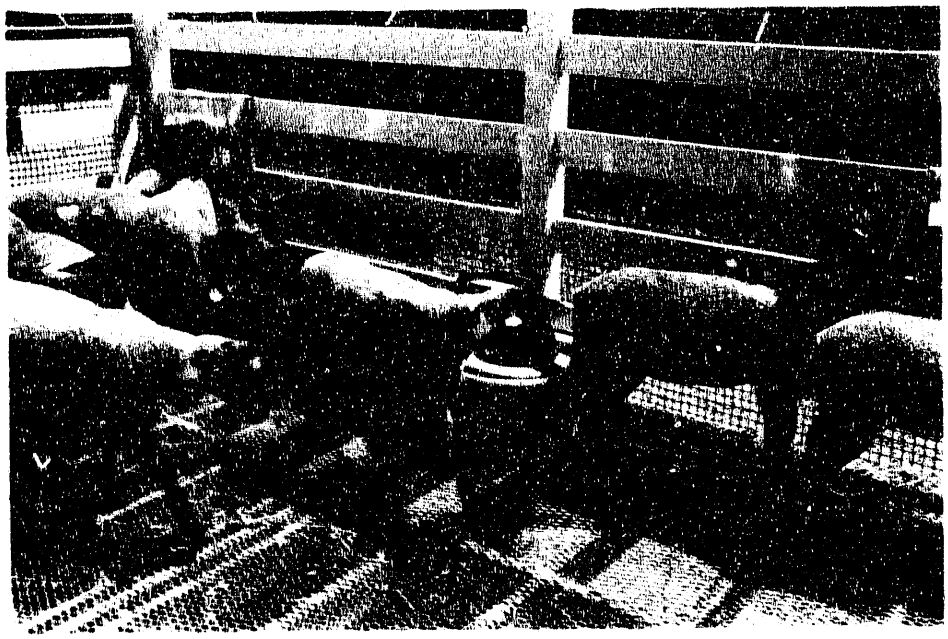


Figure 4. Lambs were maintained in pens with slatted metal floors and plastic sides.

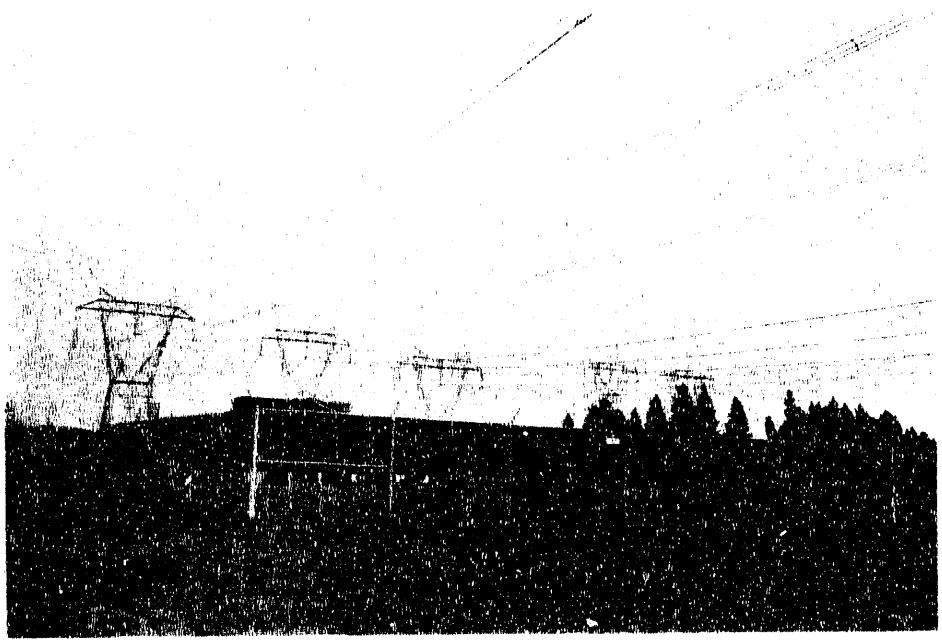


Figure 5. Black plastic was placed on the metal security fence surrounding the pens to block lights from vehicles and buildings.

Each pen had a floor area of approximately 24 m^2. This is about 2-1/2 times more than recommended floor space for sheep on slatted-floor pens (Johnson et al., 1981). Part of the pen was covered with plastic netting to act as a sunscreen. During prolonged rain, plastic tarps were also placed over a portion of the pen. A metal security fence 2.3 m high was constructed around each pen. It was kept at least 4.6 m away from the pen so that it would not reduce (shield) the electric field in the pen. A 1.3 m wide strip of black plastic was placed on the security fence to prevent exposure of animals to light from vehicles passing or entering the research site (Figure 5). As an extra precaution, drivers entering the site at night were told to use only parking lights.

Two 15-watt red lights (Sylvania) were installed in each pen to facilitate nighttime data collection. Red lights have commonly been used in previous studies of sheep with no reported effects on nighttime melatonin levels. The lights were turned on around sunset and off around sunrise by automatic time switches.

Plastic feeders for hay and grain were placed on the plastic rails at several locations throughout the pen, and a plastic freeze-proof tank was used to supply water (Figure 6). Water for both pens was obtained from a well which also supplied water to the office facilities and to the BPA substation.

Electric power was supplied to the pens by overhead wood poles from a nearby distribution line, with the final connection to the pens by underground cable.

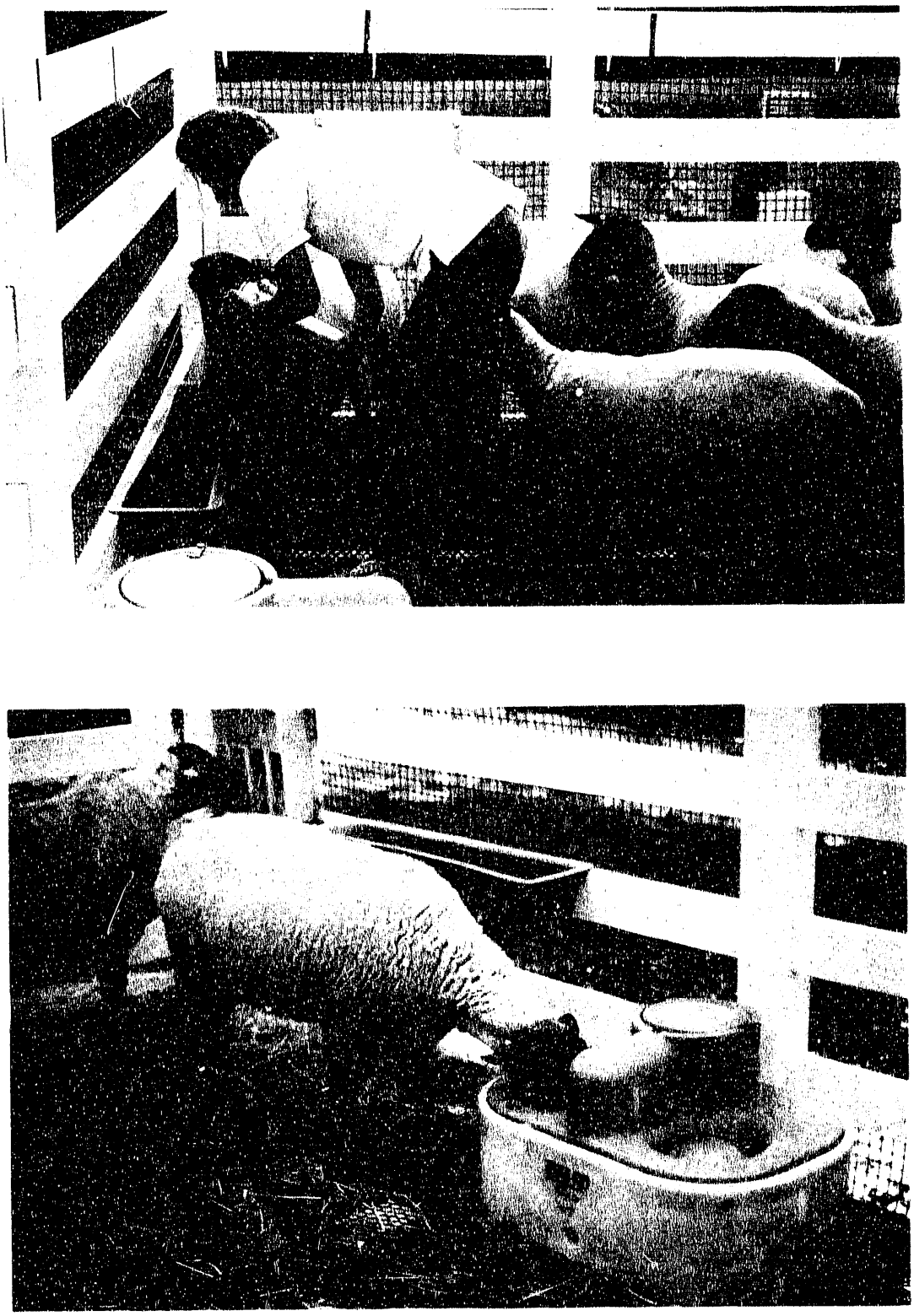


Figure 6. Plastic feed holders and a plastic freeze-proof water tank were located in each pen.

Office, Storage. Figure 1 shows the locations of the office and storage facilities. In addition, a mobile home served as the residence for the site manager. The site manager or other project personnel were essentially on site at all times for site security reasons. The BPA substation was also staffed during weekdays.

Blood Samples

Blood samples for use in assays to determine serum melatonin and cortisol levels were collected during eight 48-hour periods (Table II). The first 48-hour period occurred immediately prior to the start of exposure and the last period occurred 5 days after the end of exposure.

During each 48-hour period, blood samples were taken at 30-minute intervals during the first 2 hours after sunset and during the last 2 hours before sunrise. For the remainder of the night, samples were taken hourly. During the day, samples were taken at approximately 2-3 hour intervals. Sunrise and sunset times were obtained from data published by the United States Naval Observatory and corrected for location of the site.

The 48-hour sampling began at 1200 hours on a Thursday and ended at 1200 hours on the following Saturday. Four two-person teams worked 12-hour on-off shifts (Figure 7). Two teams, therefore, collected blood samples in the control and line pens simultaneously. Each team rotated between the control and line groups for the two 12-hour shifts. Synchronized clocks were located in each pen to ensure that samples were collected from both groups at the scheduled times.

TABLE II

TIMING OF 48-HOUR BLOOD SAMPLES

Sample No.	Dates	Time After Start of Exposure (wks)	Mean Age of Lambs (wks)
1	4-6 April 1990	Pre-exposure	8
2	19-21 April 1990	2	10
3	3-5 May 1990	4	12
4	14-16 June 1990	10	18
5	13-15 Sept. 1990	23	31
6	1-3 Nov. 1990	30[a]	38
7	31 Jan. - 2 Feb. 1991	43	51
8	7-9 Feb. 1991	1 wk Post-exp.	52

a This sample was taken at the end of a 2-week period when the 500-kV line closest to the line pen was out of service.

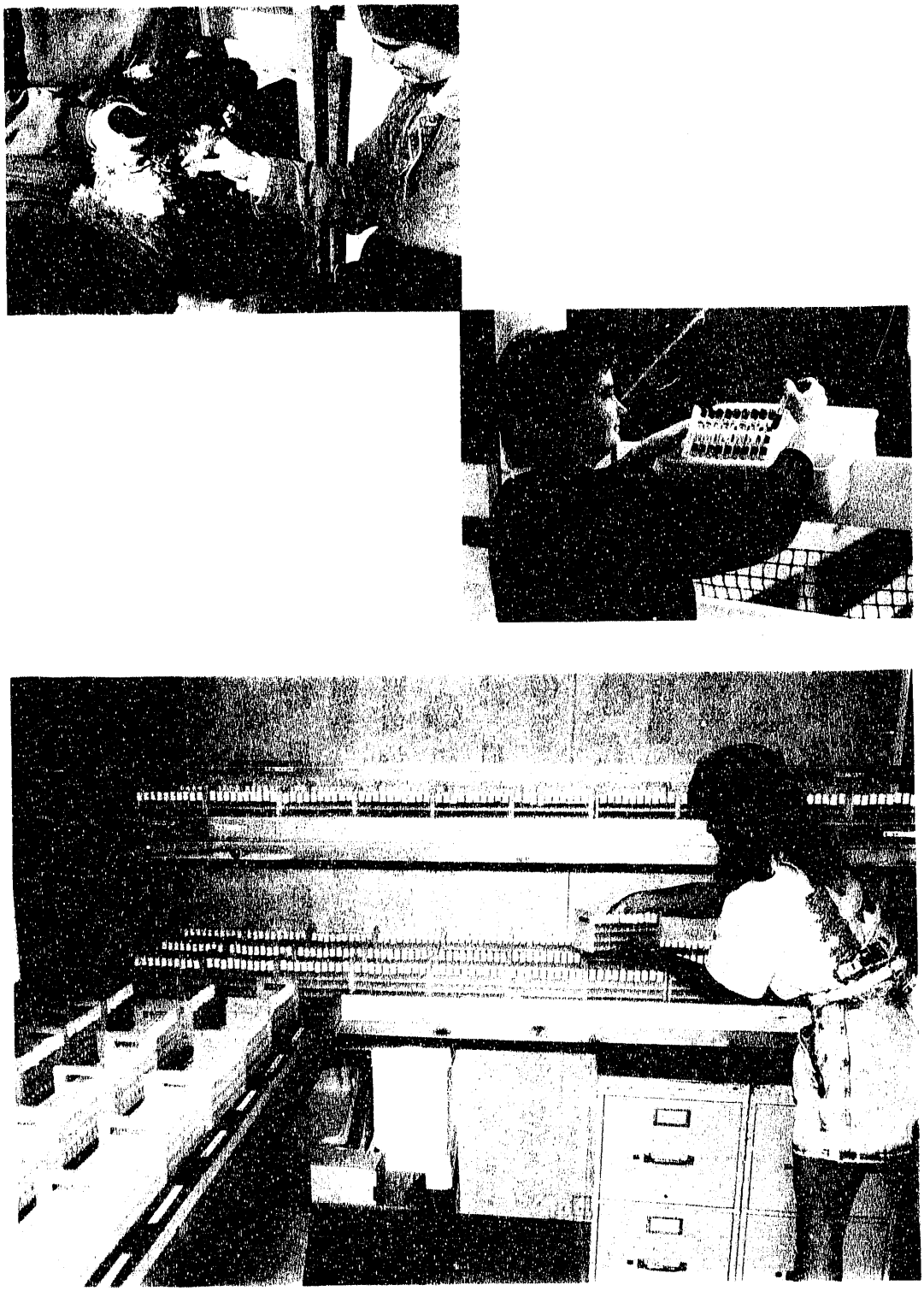


Figure 7. During 48-hour samples, two two-person teams collected blood samples simultaneously from catheterized lambs in control and line pens (upper). All tubes were pre-labeled before sampling began (lower).

Prior to taking the first blood samples from study animals, project personnel practiced the blood collection technique on non-study sheep. The practice sessions occurred at Oregon State University (OSU) in Corvallis and at the study site. The latter involved 10 lambs that were purchased specifically for use in the training sessions.

On the day preceding each 48-hour sample, catheters were placed in a jugular vein of each animal (Figure 7). The catheters were taped securely to the neck and were filled with an antibiotic citrate buffer solution to prevent blood coagulation. Catheters were removed immediately after each 48-hour sample was completed.

During the 48-hour sampling, personnel first withdrew and discarded the antibiotic citrate buffer and blood solution from the catheter. A 10-cc syringe was then used to withdraw 5-6 cc of blood. The blood was discharged into pre-labeled tubes and capped with rubber stoppers. For each blood sample, the animals were always taken in numerical sequence, i.e., control 1-10, line 11-20. After the 10 animals were sampled, syringes were washed by flushing them at least three times in clean water. A clean syringe was used for each animal.

If problems developed and blood could not be obtained from the catheter, blood was taken by jugular venipuncture using a 10-cc Vacutainer. After withdrawing the needle, the technician would apply finger pressure to the site to prevent hematoma.

Blood samples were taken to the site office for processing. Samples were allowed to clot for 2-4 hours at room temperature and then centrifuged at 1620 x g to separate serum. Serum for each animal from each sample was divided into two aliquots. One aliquot was used for the

melatonin assay and the other for the cortisol assay. The aliquots were contained in 3-dram plastic vials labeled with date, sample number, and lamb number. All sera samples were stored at -20°C until assayed for hormones.

Blood samples used for determining progesterone levels were collected during the morning twice a week (Monday and Thursday) beginning on 25 June 1990. Samples were collected by jugular venipuncture using 10-cc Vacutainers. Samples were processed as described above, except that only one aliquot was prepared.

Prior to removal from the site after conclusion of the study, all lambs were kept in temporary pens in the control area for collection of post-exposure blood samples. As shown in Figure 8, the animals were kept in their respective groups but were separated by only a wire-mesh fence. Blood samples were taken from each animal before and after they were transported to Corvallis, Oregon. The animals were all transported in a stock trailer in one group. Cortisol was assayed in these samples to obtain reference data on the effect of a known stressor.

Hormone Assays

Hormone concentrations were measured by radioimmunoassay (RIA). A number of RIAs have been developed and used in studies of reproductive endocrinology in sheep. Basically, this method requires an antibody specific for a particular hormone and a radioactive-labeled antigen (hormone). Each antibody molecule has a limited number of antigen binding sites. As the amount of unlabeled antigen in a sample increases, the number of binding sites for the labeled antigen

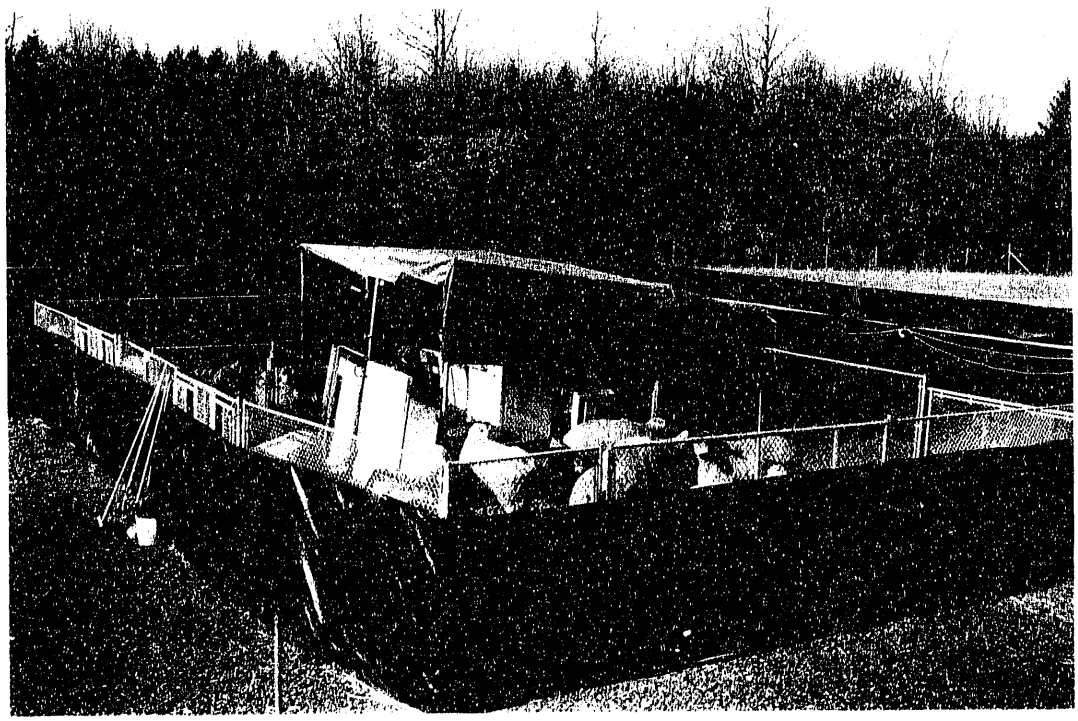


Figure 8. All control and line sheep were placed in temporary pens in the control area for collection of post-exposure data.

decreases. The amount of radioactivity in the supernatant from the assay tube is determined. Curves are prepared relating the radioactivity levels to known concentrations of the unlabeled hormone of interest. Lower radioactivity levels indicate higher hormone concentrations in the unknown sample.

Melatonin RIA. After each 48-hour sampling period, serum samples were transported from the study site to Oregon State University where they were randomized (except that night and day samples were kept separate) and then coded as follows: The vials were first placed randomly into racks that held 42 samples each. A code number was assigned to each and a list made of these numbers and the corresponding sample numbers and lamb numbers. Sera were then assayed using these code numbers to identify them. Personnel conducting the RIA, therefore, did not know whether samples were from the control or line group.

Serum melatonin concentrations were determined by an RIA as described by English et al. (1986) and modified by Malpaux et al. (1987) and by Foster et al. (1988). The procedure was further modified slightly during this study in regard to equipment, reagents, and amount of serum used. It was validated for purposes of this study by demonstrating parallel displacement using different amounts (n=5) of the same ewe serum. Recovery of mass was determined by adding various amounts of melatonin (n=6) to ewe serum containing known melatonin concentrations; recovery ($\pm$ SD) averaged 96.3% $\pm$ 4.0%. The antibody used (Guildhay G/S/704-6483) has been shown to be specific for melatonin in sheep plasma by its manufacturer, Stockgrand, Ltd., Guildford Surrey, U.K.

In addition to demonstrating parallel displacement and recovery of mass, on one occasion the same 250 samples were assayed in two laboratories (Oregon State University and the laboratory of Dr. Steven M. Yellon, Division of Perinatal Biology, School of Medicine, Loma Linda University, Loma Linda, CA). Although values from Loma Linda University were lower, because that laboratory did not extract plasma with organic solvent, patterns of secretion were similar.

One ml of chloroform (Photrex, J.T. Baker) was added to duplicate 350 µl aliquots of night serum, and 2 ml of chloroform were added to duplicate 700 µl aliquots of day serum, using a Hamilton Digital Diluter. The samples were vortexed 30 seconds; 80% of the extracted phase was withdrawn, and the diluter tubing was washed with 1 ml of chloroform (and placed into the tubes with the extracted phase). Extracted samples were then dried for 25 minutes using air and a water bath (38°C).

The dried residue containing melatonin was resuspended in 500 µl of 1.8% tricine (Sigma T-0377) buffered saline (TBS) (with 0.1 % 300-bloom gelatin, Sigma G-2500), pH 5.5. Samples were vortexed 15 seconds and 200 µl of antisera, diluted 1:6000 with stripped serum (24.96%) and TBS (74.88%), were added to each sample. The samples were vortexed briefly and incubated for 20 minutes at room temperature. One hundred microliters of ^{3}H-melatonin (NEN Research Products, NET-801), diluted to 6000 cpm with TBS, were added to each sample. The samples were vortexed briefly and incubated 18-22 hours at 4°C.

A standard curve was constructed for each assay using 12 dilutions of melatonin (Sigma M-5250) in TBS ranging from 0 to 500 pg/tube.

Antisera and ^{3}H-melatonin were added to these in the same amounts and at the same time as to the lamb sera samples.

Pools of ewe serum were collected every few months from animals at Oregon State University during the day and during the night. Night and day sera were mixed 1:2 for the low quality control (QC) pool, which averaged ($\pm$ SD) 41.1 $\pm$ 6.2 pg/ml; night serum was used for the high QC pool and averaged 122.4 $\pm$ 13.3 pg/ml. Approximately 18% of assays were done with a high pool averaging 66.8 $\pm$ 5.4 pg/ml. When assaying daytime unknowns, 700 µl aliquots of QC sera were also assayed; when assaying nighttime unknowns, 350 µl aliquots of QC sera were used. Twelve aliquots of each pool were included in each assay, six of each following the standard samples at the beginning of the assay and six of each following the unknowns at the end of the assay.

A separate recovery step was conducted with each assay. This included two total count tubes with 400 µl of TBS and 100 µl of ^{3}H-melatonin (6000 cpm), two blank tubes containing 800 µl of TBS, four low QC tubes and four high QC tubes (350 µl if night serum; 700 µl if day serum). One hundred microliters of ^{3}H-melatonin were added to each QC tube, and these were incubated at room temperature for a minimum of 1 hour; then, the hormone was extracted in the same manner as the unknowns, as described above. Three hundred microliters of TBS were then added to all but the blank tubes so that all recovery tubes contained a total volume of 800 µl, and the recoveries were incubated 18-22 hours at 4°C. After the incubation, 500 µl of TBS were added to each sample. Samples were then poured into vials containing 6 ml of

scintillation cocktail (Ecolume, ICN Biomedicals, Inc.), shaken briefly, and counted in a Beckman beta-scintillation counter for 2 minutes each.

Extraction efficiency was determined by correcting for the 20% of the extracted phase that was left behind (80% of extractant had been removed and dried down) and dividing mean counts per minute (cpm) for the QC samples by mean cpm for the total count samples. Average extraction efficiency was 84% for day serum and 86% for night serum. This extraction efficiency was then taken into account when concentrations of the unknown samples were determined.

Following the 18-22 hour incubation, free and bound fractions were separated using dextran-coated charcoal. The charcoal (Sigma C-5385, Activated, Neutralized), 2% charcoal and 0.02% dextran (Dextran T70, Pharmacia) in TBS, was stirred on ice for a minimum of 30 minutes prior to proceeding. A timer was set for 20 minutes; 500 µl of the charcoal were added to each sample, and all samples were vortexed within 5 minutes. The samples were incubated at 4°C for the remainder of the 20 minutes and then centrifuged at 2540 x g for 15 minutes.

The supernatant, containing the bound fraction, was then poured into 20 ml glass scintillation vials containing 6 ml of scintillation cocktail, shaken briefly, and counted 2 minutes each in a Beckman beta-scintillation counter.

Standard curves, backfit, percentage nonspecific binding, total binding, midpoints of standard curves, and melatonin concentrations (pg/ml) were determined by the RIA AID computer program (Robert Maciel Associates, Arlington, Massachusetts). Intraassay coefficients of variation (CV) were manually calculated, as this software calculates a

CV for every set of two samples, rather than for the total of 12 of each QC pool. Mean intraassay CV was 8.9% with a mean interassay CV of 12.4% for 222 assays. The limit of detection, determined by Student's t-test for paired samples, was 0.5 pg/tube (500 µl) or 1.0 pg/ml ($p < 0.01$).

Cortisol RIA. Cortisol and progesterone RIAs were performed by the Hormone Assay Core Laboratory at the Oregon Regional Primate Research Center.

Cortisol was analyzed by extracting duplicate 20 µl serum aliquots diluted with 180 µl of 0.1% gelatin phosphate buffered saline (0.1% GPBS - pH 7.0). The diluted samples were added to 13 x 100 mm glass culture tubes and extracted with 6.0 ml of diethyl ether (Burdick and Jackson: either freshly opened or redistilled) by shaking vigorously for 5 minutes. Extraction losses were monitored by determining the recovery of ^{3}H-cortisol (10-12,000 cpm) added to diluted serum samples and extracted in parallel with unknown samples. The organic phase was decanted into 13 x 100 mm glass culture tubes after freezing the aqueous phase in a dry ice/ethanol bath. The organic phase was evaporated under an air stream and the steroid concentrated to a tip of each assay tube by three sequential washes with 0.5 ml of ether, evaporating the ether after each wash. A Micromedic Digiflex automatic pipetter was used to add 100 µl each of cortisol antisera (R2P8, ICN Biomedicals, Costa Mesa, CA) and ^{3}H-cortisol (8-10,000 cpm; NET-396, NEN Research Products) to all tubes including a set of duplicate cortisol standards (12 doses ranging from 10 to 1000 pg). The cortisol standard was prepared in redistilled ethanol (1 pg/µl) and

appropriate aliquots dried before antiserum/tracer addition. Tubes were vortexed vigorously and incubated overnight at 4°C. The bound and free steroid in each sample were separated by centrifugation for 10 minutes after addition of 100 µl 0.5% GPBS, 1.0 ml dextran-coated charcoal, vortexing and incubation for 15 minutes at 4°C. The supernatants were decanted into individual 7 ml plastic scintillation vials; 3.5 ml of scintillation fluid (ScintiVerse BD, Fisher Scientific) was added, and each vial was counted for 5 minutes in a Packard scintillation counter. The radioactivity in each tube was concurrently recorded on a floppy disk and on a printer. The stored assay data were transferred to either a micro or mini computer and concentrations calculated for unknown and quality control samples from the standard curve after logit-log transformation of the data. All samples were corrected for method blanks (200 µl of 0.1% GPBS; extracted and assayed in parallel with sheep samples), extraction recovery, aliquot volume and the data reported as ng/ml of the original sample. Varying aliquots of a sheep serum pool gave parallel displacement curves to the authentic cortisol standard. The average ($\pm$ SEM) recovery and method blank for the 43 cortisol assays were 90.21 $\pm$ 0.69% and 1.94 $\pm$ 0.33 pg. Intra- and interassay coefficients of variation for these assays were 5.83% and 13.66%, respectively.

Progesterone RIA Progesterone was analyzed by extracting duplicate 100 µl aliquots of sheep serum diluted with 150 µl of 0.1% GPBS and treating the extract as described above for cortisol. The progesterone antisera (R12) was obtained from Dr. A. Surve (Sandoz Pharmaceuticals,

Summit, NJ) and the ^{3}H-progesterone from NEN Research Products (NET-381). The progesterone standard was assayed in duplicate (11 doses ranging from 5 to 750 pg) and assay calculations for standards, unknowns and quality control pools were done using the same logit-log transformation program. Varying aliquots of a luteal phase sheep serum pool gave parallel displacement curves to the authentic progesterone standard. The average ($\pm$ SEM) recovery and method blanks for the eight progesterone assays were 93.35 $\pm$ 2.46% and 5.71 $\pm$ 1.15 pg, respectively. Intra- and interassay coefficients of variation for the assays were 4.73% and 9.63%, respectively.

Weights

All animals were weighed once weekly throughout the study. A portable steel scale (Mosdal Feed Carts, Model 3000A) was moved into the pen on weigh day (Figure 9). Scale weights were given by an electronic digital readout, and values were recorded on data sheets. Scale readings were verified at the beginning and end of each weigh session using a constant weight source (either a person or weight-lifting weights). When not in use, the scales were moved away from the pens so they would not produce any shielding of the electric field.

Wool Growth

At the beginning of the study, wool was clipped to skin level on two 10x10 cm areas on the side and britch of each lamb. Growth (staple length) was measured in these patches every 2 weeks. The measurement was made by placing a small metal ruler flush against the skin

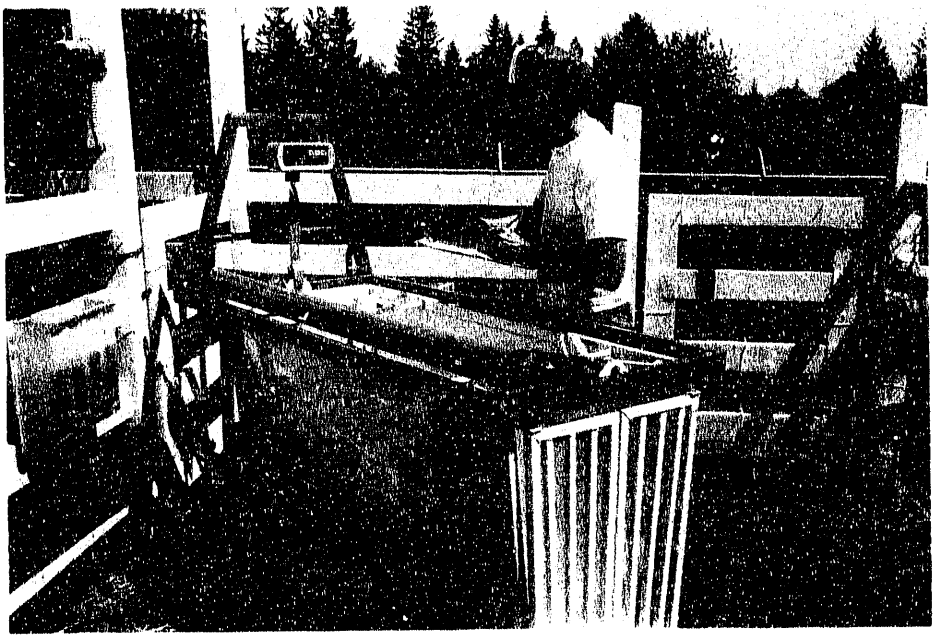


Figure 9. A portable platform scale was used to weigh all lambs weekly.

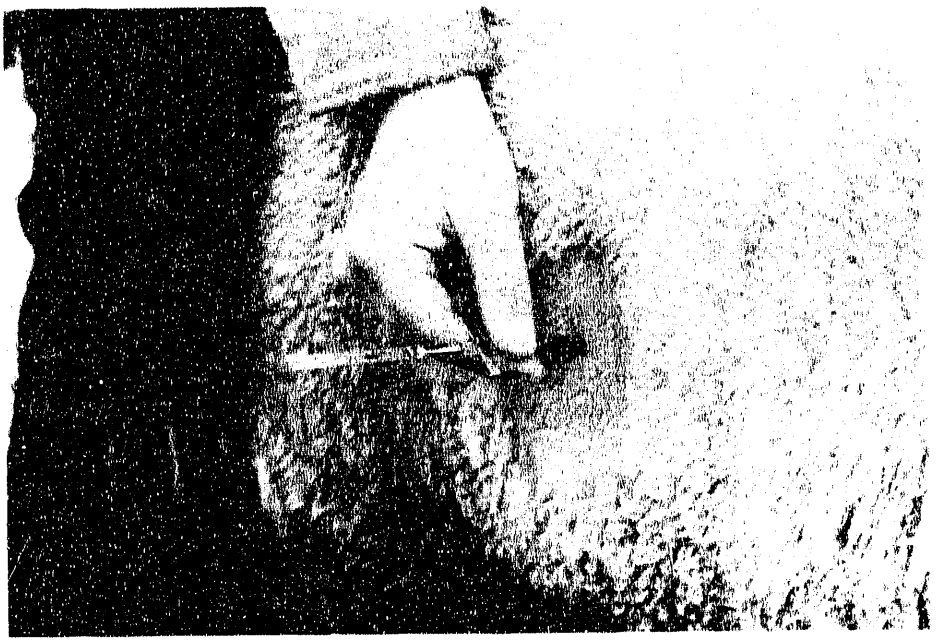


Figure 10. Wool growth was measured in two areas located on the side and britch of each animal.

(Figure 10). Five measurements were made within each 10x10 cm patch, and the final value used for analysis was the mean of these five measurements. All measurements were made by the same person (site manager). A test was conducted on 5 June 1990 to determine the repeatability of the measurements.

All sheep were sheared on 19 or 20 June 1990 by the site manager, and the wool was weighed on 25 June. Samples of wool from each animal were sent to Yocom-McColl Testing Laboratories, Inc., Denver, CO, for analysis of fiber diameter.

Behavior

Once each month from May through January, 24-hour observations were made of general behavioral activities of lambs in each pen. The observations began at 1200 hours and were made from an enclosure attached to the pen (Figure 3). Two people, one each for the control and line pens, made the observations simultaneously. A total of four people participated in each 24-hour observation session. Each person worked two 6-hour shifts alternating between the control and line groups.

For purposes of behavioral observations, the pens were divided into four sections (Figure 11). Every 15 minutes, the observers counted and recorded on data sheets, the number of sheep within each section involved in one of five basic behaviors. (Individual sheep were not identified when the behavior observations were recorded.) These behaviors consisted of standing, walking, resting, feeding, and

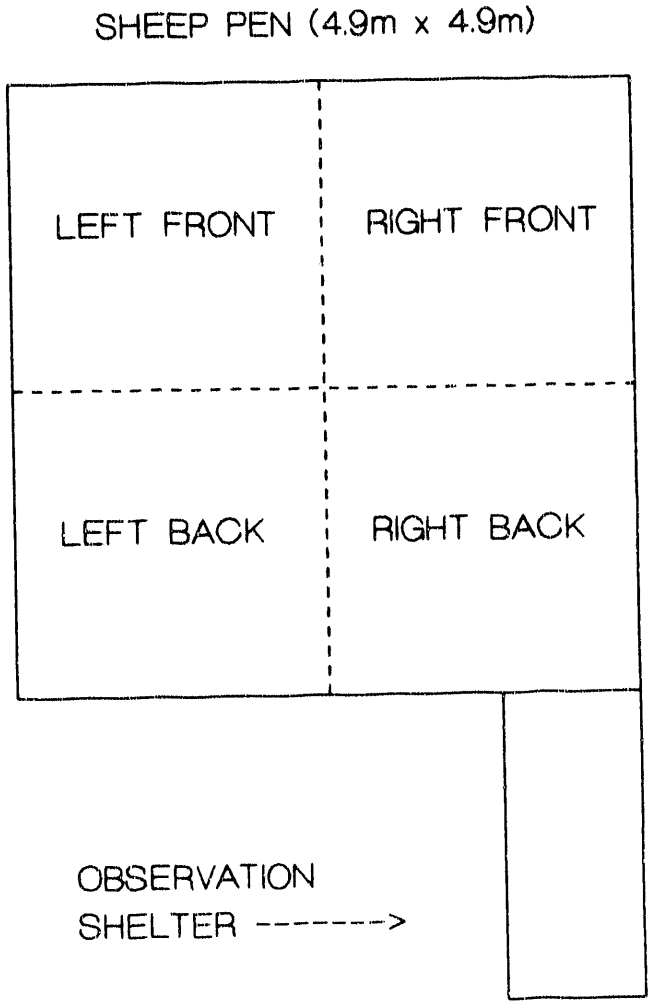


Figure 11. During 24-hour behavioral observations, animal locations were recorded by dividing the pen into four sections.

drinking. Observations of sheep behavior were possible at night because of the two 15-watt red lights within each pen.

Animal Management

Feeding. The sheep were fed twice daily, once in early morning and once in late afternoon. Rations consisted of hay and grain with a mineral supplement. The amount of food provided was varied as needed to produce average weight gains considered typical for Suffolk lambs under good farm management. Both line and control groups received the same amount of rations at each feeding; however, near the end of the study, the control group rations were reduced to compensate for the loss of one animal on 16 January 1991.

Grain rations consisted of varying mixtures of creep feed, alfalfa pellets, and soybean meal. Depending on stage of growth, the amount of grain fed daily per pen ranged from 5 to 23 kg. Approximately 4-6.5 kg of grass hay per pen was also fed to sheep daily. Water and a sheep mineral salt with selenium were provided ad libitum.

Health Program. All animals were examined at the start of the study by the project veterinarian. All animals appeared in good health and no unusual problems or conditions were noted. Blood and fecal samples were also taken from each animal and analyzed to detect any abnormalities, including indication of internal parasites.

Project personnel monitored the health and condition of the animals daily. If problems were noted, the project veterinarian was consulted about the need for treatment.

Each sheep was sheared once during the study on 19 or 20 June 1990.

Electrical and Environmental Monitoring

The purpose of this study was to determine whether the electrical environment of a 500-kV transmission line affects various biological end points in sheep. Therefore, an extensive program was developed which included long- and short-term measurements of various components of the electrical environment. The locations of the long-term monitoring instruments are shown in Figure 3. Instrument layout is further depicted in Figures 12 and 13.

The magnetic field is directly related to current on the transmission line so it varies as line loading changes. Therefore, the magnetic field near both the line and control pens was measured continuously with a monitoring system designed by Electric Research and Management, Inc. (ERM). This is a computer-controlled system which automatically measures and stores data for later analysis.

Measurements of the magnetic field were taken every 10 minutes along three orthogonal axes over a frequency range of 60-780 Hz. In addition to field strength, the direction, phase and waveshape of the field were also recorded.

Audible noise (AN) due to corona on line conductors is also highly variable, with the highest noise levels occurring during inclement weather. A-weighted audible noise was monitored with B&K 4921 all-weather microphones located near both the line and control pens. Measurements were made every 10 minutes. This measurement system has

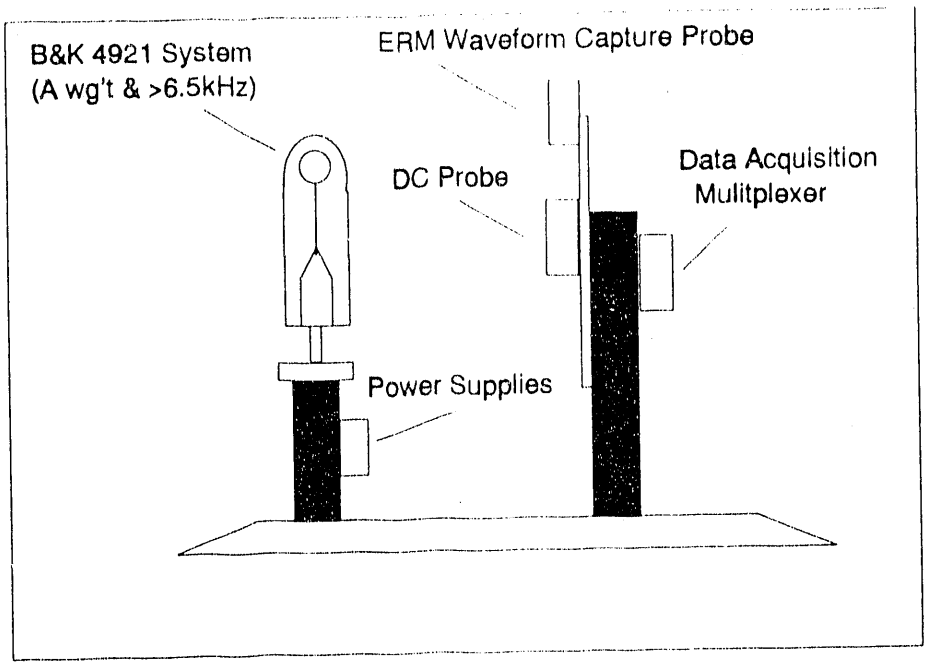


Figure 12. Instruments located in control and line pens used for monitoring audible noise (dBA and >6.5 kHz) and the magnetic field.

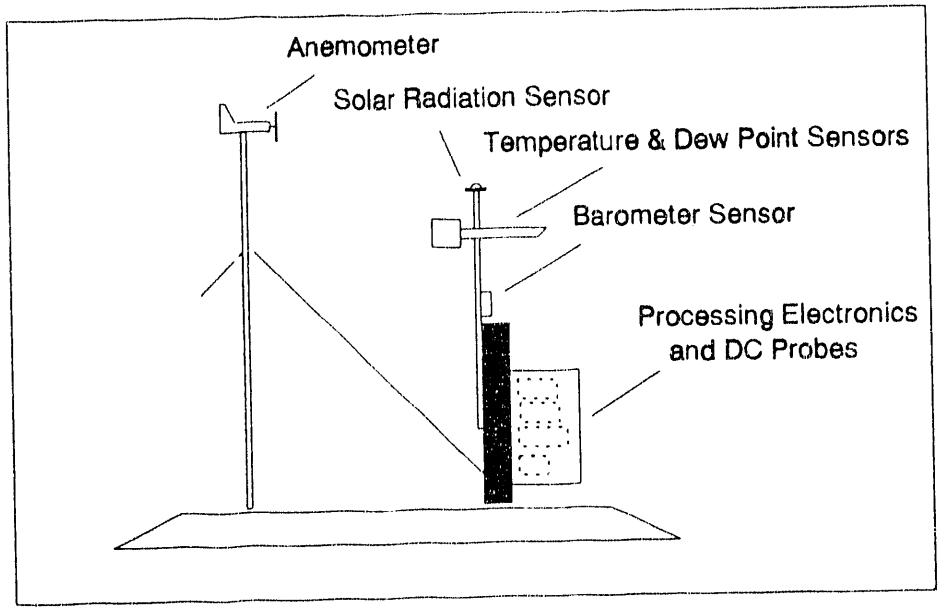


Figure 13. Instruments located near the control pen used for monitoring microclimate.

been successfully used by BPA in several other environmental research projects.

The A-weighted system models how the human ear perceives sound. The most sensitive hearing range in sheep is shifted toward higher frequencies compared to the human (Ames, 1978). Therefore, long-term measurements were also made of noise at 6.5 kHz and above.

Several microclimatic factors were also sampled every 10 minutes. These were ambient temperature, dew-point temperature, wind speed and direction, solar radiation, and barometric pressure.

Data recorded by all permanent electrical and microclimate monitors were transmitted by cable to the data acquisition computer at the site office. Data were stored at the site until transfer to the BPA laboratories in Vancouver, Washington for analysis.

The electric field, due to voltage on conductors, is relatively more stable than the magnetic field, since line voltage is fairly constant. As with the magnetic field, however, electric field strength near ground level is influenced by conductor height. Conductor height changes with changes in air and conductor temperature. Electric field strength was measured periodically in both pens with a hand-held meter (EMF Model 110). For the line pen, electric field strength was periodically mapped at specific locations throughout the pen. To minimize electric field

shielding, all pen material above the metal floor consisted of non-conducting plastic material.

Measurements of nighttime light levels in the pens were made with a hand-held instrument (International Light, Model 710 A). This instrument had a photoropic (eye response) type sensor (photodetector SE015), a diffuser (W #2091), and a filter (Y #3079). Light intensity measured in the pens was primarily produced by the two 15-watt red lamps in each pen. Black plastic was placed on the metal security fence surrounding the pen to block entry of white light from vehicles and buildings. The illumination intensity and spectral response of the red lamps were also measured in a laboratory test by Dr. David McIntyre, Department of Physics, Oregon State University.

The d-c geomagnetic field was measured at a height of 1 m at four locations at the study site: in the center of the line pen; 25.9 m east of the line pen; in the center of the control pen; and 16.5 m west of the control pen. Measurements of three orthogonal components of the d-c field were made with a Rawson Lush Model 906 Rotating Coil Gaussmeter. The sensor coil in the this meter rotates at 27 Hz to avoid interference from 60-Hz magnetic fields. Measurements of the two field components perpendicular to the axis of the spinning coil are made by switching the phase of the voltage output from the coil. The third component is measured by physically rotating the axis of the probe through 90°.

Prior to the measurements, BPA surveyors established survey lines through the center of the two pens and parallel to the outer conductor of the Ostrander-Troutdale 500-kV line. The magnetic field sensor was located on these lines for measurements and the survey targets marking the lines were used to align the axis of the probe. In this orientation, the probe measured the horizontal field component perpendicular to the line and the vertical field component. The other orthogonal horizontal component (parallel to the line) was obtained by rotating the probe through 90° in a specially constructed mount. The horizontal and vertical components at each orientation were read by switching the meter display. Each field component was measured with the probe in two orientations 180° apart and the results averaged. The orientation of the Ostrander Troutdale 500-kV line is 77°44' east of north as determined by the BPA surveyors.

Electric Field Exposure

Both magnetic and electric fields induce electrical current in conducting objects, including the bodies of animals. The amount of induced current is maximal when animals are in good contact with ground. This was the case in this study where the sheep were maintained on a grounded metal slatted floor.

The amount of current induced by the electric field is significantly affected by the size and orientation of the body and by the presence of nearby conducting objects, including other animals. To obtain an estimate of electric field exposure, data were collected on one occasion by placing a portable electric field monitor (EMDEX-C) on

one of the line group sheep. The monitor was contained in a small backpack provided by Dr. Douglas Foster, The University of Michigan. The EMDEX-C measured the current from a small parallel plate transducer which was glued to the wool on the top of the sheep's head. The current from the transducer is a measure of the electric field at the sheep's head.

Data Analysis

This section describes the data analysis and statistical tests used for the parameters investigated in this study. In general, methods used were those reported in similar studies. Parametric tests (analysis of variance, t tests) were used because of their increased power. For data which consisted of multiple observations on the same animals, repeated measures analysis of variance (ANOVA) was used.

Melatonin. Data on melatonin from blood samples collected over eight 48-hour periods were analyzed using three basic parameters of the nighttime elevation: 1) mean duration, 2) mean amplitude, and 3) mean phase. Prior to analysis, concentration data were transformed to natural logarithms to reduce heterogeneity of variance related to the melatonin concentration level in the blood samples. Because some raw data values were 0 or were less than 1, 1 was added to each value prior to transformation, a procedure which is also preferred on theoretical grounds (Zar, 1984).

The determination of the beginning and end of the night elevation was derived from the method of Earl et al. (1985) and Malpaux et al. (1987). The former defined onset and termination of night melatonin

secretion as points when plasma melatonin concentration was first greater than, and then less than 2 standard deviations of assay sensitivity, respectively. In the latter study, night melatonin elevation was defined as the period between the first and last serum concentration values that exceeded 3 standard deviations of the daytime concentrations.

For this study, the mean day melatonin value was determined for each animal. Day was assumed to extend from the first sample taken after sunrise to the last sample before sunset. The day mean and standard deviation were calculated for each animal and two standard deviations were then added to the day mean. This value, calculated for each animal, was used as an index to judge the onset and end of the nightly melatonin elevation.

The studies by Earl et al. (1985) and Malpaux et al. (1987) used adult ewes and the melatonin concentrations were generally very low and stable during the light periods. In this study of lambs, there were several high daytime values that occurred during the first four 48-hour samples. It was, therefore, necessary to adjust the method to account for these occasional high daytime levels. Values greater than 30 pg/ml were not included when the day mean index was calculated. In some cases, inclusion of these high values resulted in the above method indicating no night melatonin elevation when visual inspection showed an obvious elevation had occurred. The total number of these high values for the control and line groups was nearly identical (10 and 11, respectively). These values were also nearly evenly distributed among several animals in both groups. Therefore, this adjustment to the

calculation method was not expected to result in any bias to group comparisons for night melatonin parameters. The high levels were included for comparison of day melatonin levels between groups.

Melatonin data for each ewe were listed on a spreadsheet by sample time for each 48-hour sample period. Beginning at sunset, the first sample time value to equal or exceed the index value was designated as the onset of the night elevation. The last sample before the first sample after sunrise which equaled or exceeded the index value was designated as the end of the elevation. The duration of the night elevation for each animal was determined by calculating the time difference between these onset and ending sample times.

The mean amplitude of the night melatonin elevation, as determined above, was calculated for each animal. This was done by calculating the mean of all sample values between and including the onset and ending values.

Determination of the phase of the night melatonin elevation was based on the method of Claypool et al. (1989). First, for each sample night, the time midpoint between sunset and sunrise was determined. Then, for each animal, the midpoint of the nighttime elevation was determined. The difference between these two midpoints represented the phase. If the two values were equal, then the phase exactly coincided with natural dark period. A positive difference meant that the melatonin elevation was phase-advanced (midpoint preceded midpoint of the dark period). Likewise, a negative difference indicated a phase delay (midpoint followed midpoint of the dark period).

Group means for melatonin duration, amplitude, and phase measured on multiple dates were tested by analysis of variance (ANOVA) (completely randomized design with dates as repeated measures). Appendix B shows the ANOVA table for this design. Post-hoc tests were done on adjacent dates to compare pre-exposure, exposure, and post-exposure times. All tests were performed using a statistical software program (SYSTAT/SYGRAPH, Inc., Evanston, IL). In all tests, $p < 0.05$ was considered statistically significant.

The SYSTAT program provides Greenhouse-Geiser and Huynh-Feldt statistics which adjust the probability of the univariate test when compound symmetry fails. SYSTAT recommends that if the adjusted probabilities lead to different conclusions than the univariate probabilities, the multivariate statistics provided by SYSTAT should be used. The latter statistics do not require the compound symmetry assumption. The repeated-measures ANOVA cannot handle missing data, because error terms must be computed from subject-trial interactions. Therefore, missing values were estimated for the last two 48-hour sample periods for the control animal that died, using the method described by Zar (1984).

Cortisol. Cortisol concentration data were also log-transformed prior to statistical analysis. Cortisol secretion may follow a circadian rhythm, although not with the sharp on-off patterns observed for melatonin. Therefore, the basic interest, in addition to possible group differences, was whether there was a day-night difference in cortisol levels.

Cortisol measured during the 48-hour sample periods was divided into day and night components. Night was defined for each animal as all sample times from sunset to sunrise. Day consisted of the remaining sample times. Repeated-measures ANOVAs were used to test for group and for day and night differences in mean cortisol levels. No further analysis was done to examine circadian rhythmicity. Comparisons were also made between adjacent 48-hour samples corresponding to various exposure conditions.

Progesterone. Progesterone was measured in blood samples collected twice each week. Puberty was defined by the method described by Yellon and Foster (1985, 1986). The onset of the first and subsequent normal ovarian cycles was indicated by a rise in serum progesterone above 0.3 ng/ml in at least three consecutive samples, one of which exceeded 1 ng/ml. The date of the first sample in this series was considered the puberty date for an individual animal. Age at puberty was then determined for each animal, and group means were analyzed with an independent samples t-test. A one-tailed test was used because the study hypothesis predicted that exposure to the transmission line would increase the time required for lambs to reach puberty. A one-tailed t-test was also used to compare the number of estrous cycles between groups.

Growth Rate. All animals were weighed weekly throughout the study. Group mean weights at the start of the study, at puberty, and at the conclusion of the study were compared with repeated-measures ANOVA.

Wool Growth. Final wool length, fiber diameter, and clipped wool weights were all analyzed with two-tailed independent samples t-tests.

The two sets of wool length measurements taken on the same day to evaluate the measurement technique were analyzed with a repeated-measures ANOVA.

Behavior. The repeated observations of behavior of individual lambs are not independent events. Therefore, behavior was not analyzed by formal statistical tests. Rather, data from monthly behavior observations were totalled, plotted, and inspected for major differences between the control and line groups. The same approach was applied to data on the distribution of lambs within the pens.

Electrical and Environmental Data. Descriptive statistics such as means, medians, maximums, minimums, and standard deviations were developed for all the data that was continuously monitored using standard statistical analysis techniques. Cumulative probability distributions were determined using the bin approach which is commonly used in developing histograms. Specific exceedance levels were created using the ordering technique. In other words all the measurements for a particular parameter were sorted from the lowest to the highest value. Then the computer program searched through this sorted data base for the following exceedance levels: L_{99}, L_{95}, L_{90}, L_{75}, L_{50}, L_{25}, L_{10}, L_5, and L_1.

RESULTS

Animal Health and Condition

All animals were examined by a project veterinarian shortly after their arrival at the study site on 2 April 1990. This examination, along with results of blood sample analyses, revealed no health problems. The animals were again examined on 6 April following the first 48-hour blood sampling period, and no problems were found.

On 6 May, following the third 48-hour sample, control animal 3 developed a temperature, loss of appetite, and hardness around the neck. Combiotic (antimicrobial preparation) and propylene glycol were administered, and the animal recovered.

On 11 July, line animal 12 began limping on her right hind leg, and line animal 13 was also observed limping. Examination and blood sample analysis showed no abnormality in either animal. A selenium (BO-SE) and a vitamin E injection along with Combiotic were given to both animals. Animal 13 eventually recovered, but number 12 continued to limp for the remainder of the study.

Enterotoxemia vaccinations were given to all sheep on 21 May, and all received Ivermectin drench (for internal parasite control) on 16 July.

On 30 September, control animal 6 showed symptoms of depression and loss of appetite. She lost approximately 11 kg of weight through mid-October, when her weight began to increase. During this time, she was given Combiotic, propylene glycol, electrolytes, vitamin B-complex, and a rumen starter.

Loss of appetite was again observed in control animal 6 on 9 January 1991. Over a period of 5 days, the following treatments were administered: Probios, vitamin B-complex, propylene glycol, electrolytes, ampicillin, gentamicin, mineral oil, lactated ringers, dextrose, and calcium. On 16 January, she was found dead.

A necropsy was performed on the animal at the OSU Veterinary Diagnostic Laboratory. A copy of the laboratory report is included as Appendix C. The final diagnosis was vegetative valvular endocarditis and scarred myocardial infarcts, probably due to infection associated with the indwelling jugular catheters.

In late January and early February, control animal 4 appeared depressed, showed poor appetite, and limped on her right hind leg. Combiotic was administered, and she recovered.

Also in early February, several animals in both control and line groups showed nasal discharge. Only line animal 14 was treated with ampicillin, because in that case the discharge caused breathing difficulty.

Growth Rate

Weekly weight gain is shown in Figure 14. Mean growth rate for both control and line groups was nearly identical throughout the study. Mean weights (kg $\pm$ SEM) for the control and line groups on 2 April 1990 at the beginning of the study were 27.4 $\pm$ 1.4 kg and 27.6 $\pm$ 0.8 kg, respectively. Final weights on 15 February 1991 were 92.7 $\pm$ 2.8 kg and 91.0 $\pm$ 2.2 kg for the control and line groups, respectively. At puberty, mean weights were 74.8 $\pm$ 2.3 kg and 74.4 $\pm$ 1.8 kg for the two

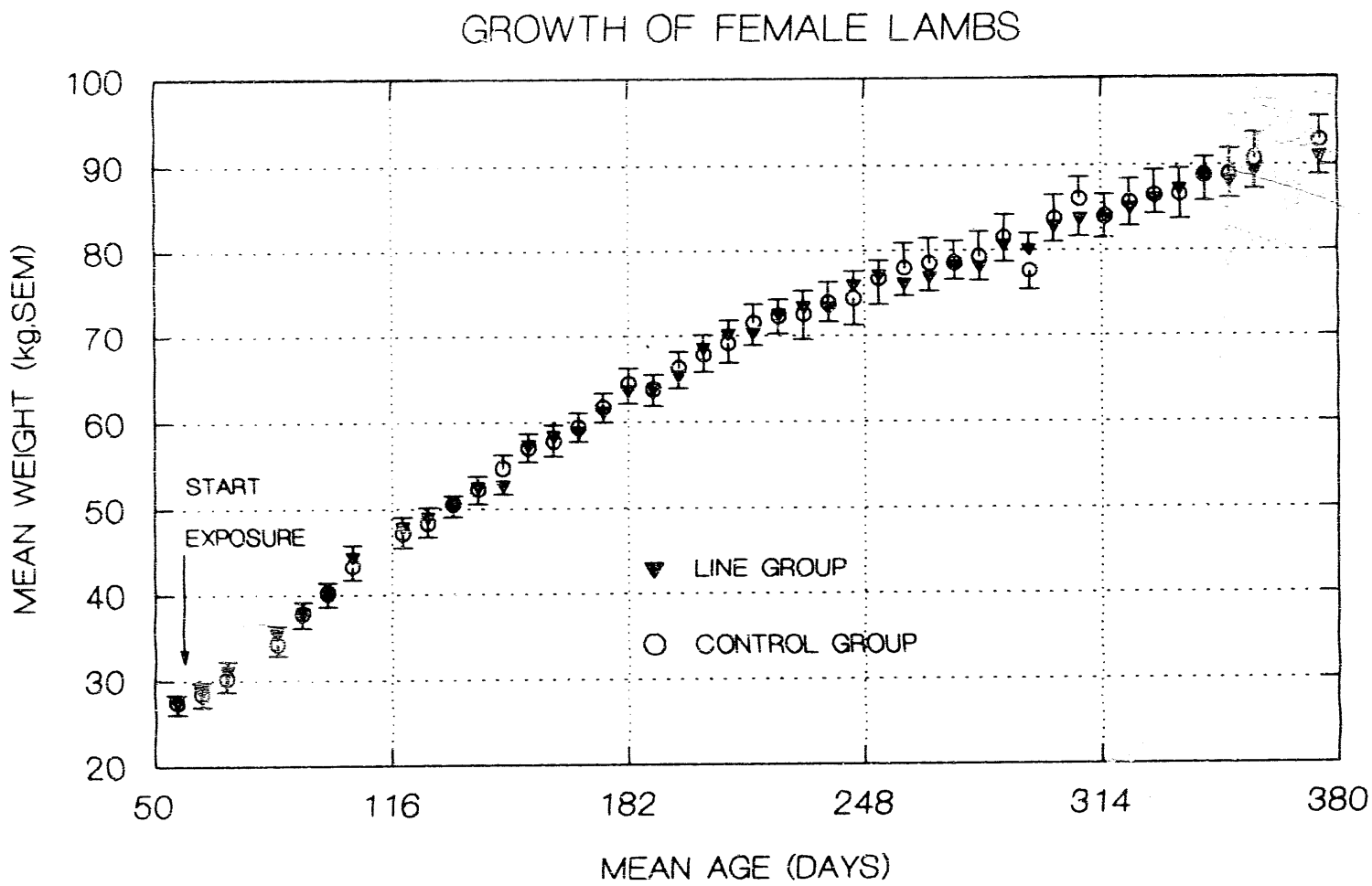


Figure 14. Mean weights of line and control lambs. Weights were taken weekly from 2 April 1990, to 15 February 1991.

groups, respectively. Analysis of variance indicated no group differences for beginning, ending, and puberty weights ($F = 0.08$, $df = 1,18$, $p = 0.78$).

Serum Melatonin

Blood Samples. Table II lists the dates when the eight 48-hour blood samples were taken. A total of 6618 blood samples were collected during these eight periods. Also listed are mean ages for each group and cumulative time of exposure corresponding to each sample. Overall, few problems were experienced with the jugular catheters. Some catheters had to be replaced during each sampling period due to various malfunctions. Typically, if no problems developed, a blood sample was taken in approximately 1 minute. Therefore, sampling all 10 sheep usually took 10-15 minutes.

Most of the problems were encountered during the first 48-hour sampling period. This was due to the relative inexperience of most of the personnel in collecting blood samples at night in dim red light. The lambs appeared to adapt quickly to the handling and procedures associated with the sampling.

The initial problems resulted in some blood samples not being collected. The numbers of missing data by group for all eight 48-hour samples are presented in Table III. The total number of missing samples represents only 0.7% of the total samples collected.

Melatonin Patterns. Mean serum melatonin concentration (pg/ml) for control and line groups for each of the eight 48-hour samples (Figure 15) showed the expected general melatonin pattern of low levels during

TABLE III

MISSING DATA IN 48-HOUR BLOOD SAMPLES

48-Hour Sample Dates	Number Samples[a]			
	Control		Line	
	Missing	Collected	Missing	Collected
4-6 April 1990	22	388	14	396
19-21 April 1990	0	390	0	390
3-5 May 1990	0	390	2	388
14-16 June 1990	0	370	3	367
13-15 September 1990	0	430	0	430
1-3 November 1990	0	450	1	449
31 January-2 February 1991	2	421	0	470
7-9 February 1991	3	420	1	469
Totals	27	3259	21	3359

a One sample is one blood sample for one lamb for one of the sample times over 48-hours.

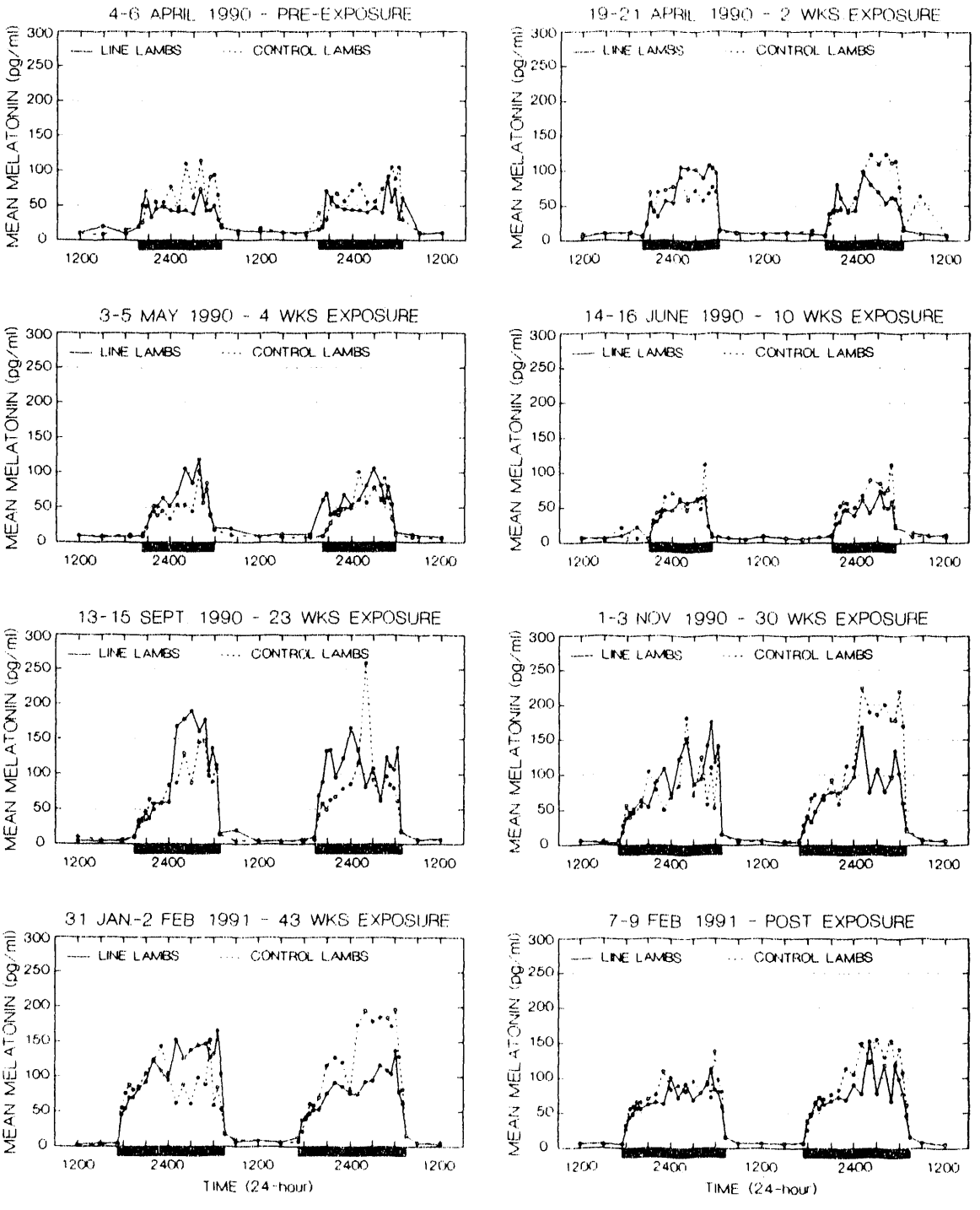


Figure 15. Mean serum melatonin concentrations of line and control lambs over eight 48-hour periods. For clarity, standard error bars are not shown. See Figure 16 for an example of typical variation.

the day and elevated levels at night. (In this and in all other figures depicting data from 48-hour samples, the black bars represent the sunset to sunrise period.) Combining all data, the mean melatonin concentration at night was 6.8 times higher than the mean day concentration (77.0 pg/ml vs 11.4 pg/ml, respectively).

Typically, melatonin levels increased sharply near sundown and decreased sharply near sunrise. During the night, considerable individual variation occurred at a given sample time as suggested by some large standard errors (Figure 16). The two highest melatonin levels measured during the study occurred on the night of 15 September 1990. At 0200 hours, serum melatonin in control animal 7 was 1377.5 pg/ml, and at 0617 hours the level in line animal 12 was 835.9 pg/ml. Melatonin patterns for individual lambs for all eight 48-hour samples are included in Appendix D.

Repeated-measures ANOVA of mean duration of the elevated melatonin levels for all 16 sample nights (Figure 17, upper) revealed no significant difference between groups ($F = 0.02$, $df = 1,18$, $p = 0.882$). However, there was a highly significant effect of season ($F = 228.9$, $df = 7,124$, $p < 0.001$). The melatonin elevation reflected the change in night length occurring from April through February. This relationship between night melatonin duration and length of the night (sunset to sunrise) is shown in Figure 17 (lower).

Analysis of group means for the amplitude of the night melatonin elevation for the 16 sample nights (Figure 18) revealed no significant difference between the two groups ($F = 0.26$, $df = 1,18$, $p = 0.619$). The mean amplitude did change through the study period, with higher

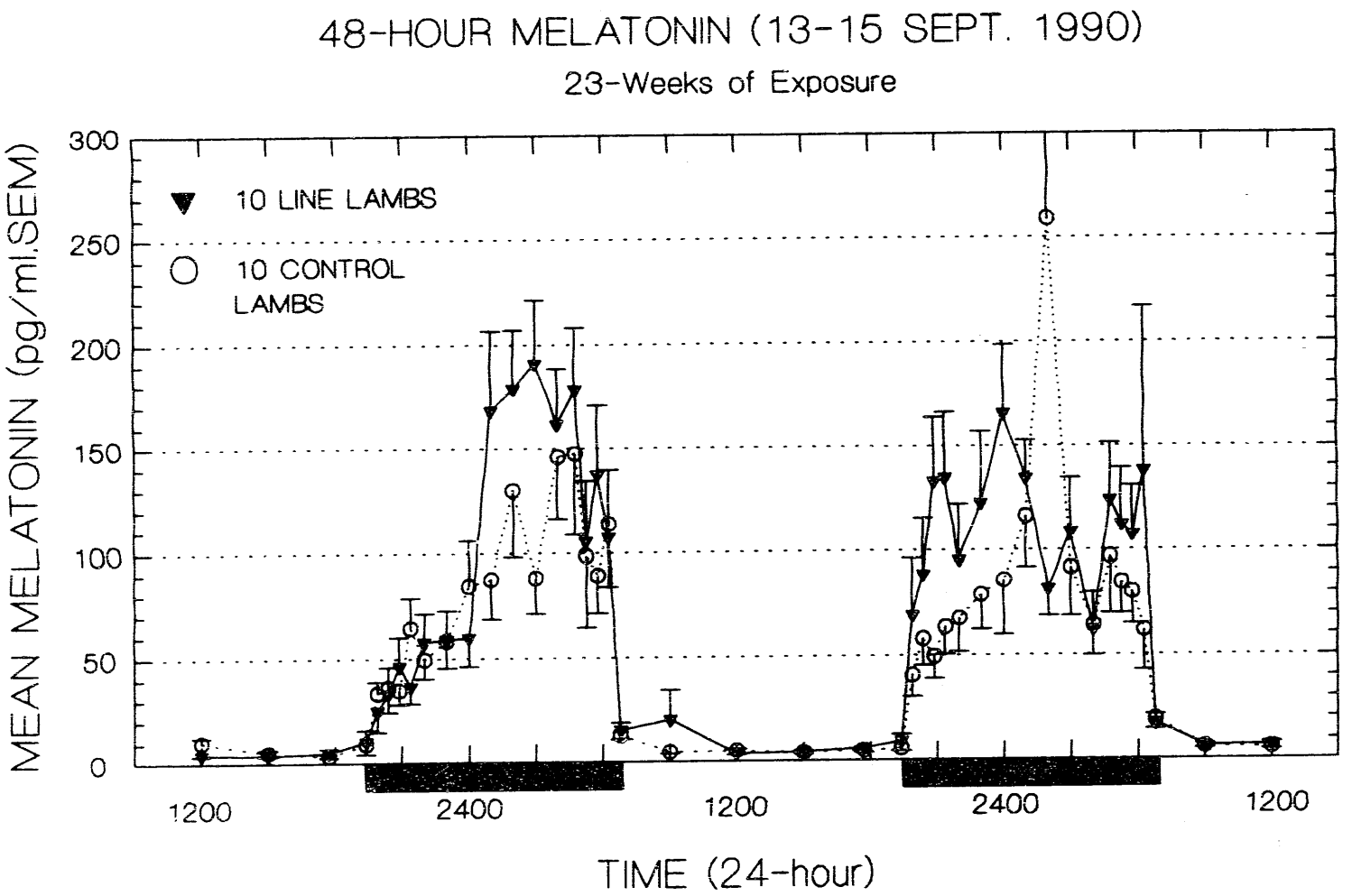
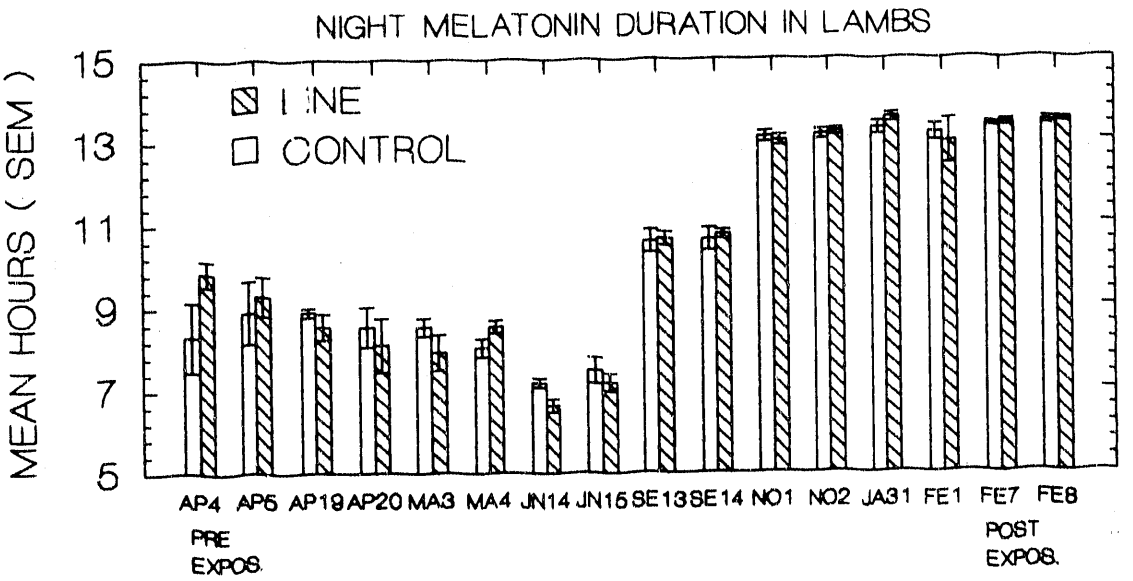


Figure 16. Example of typical variation in serum melatonin in blood samples collected over 48-hour periods.



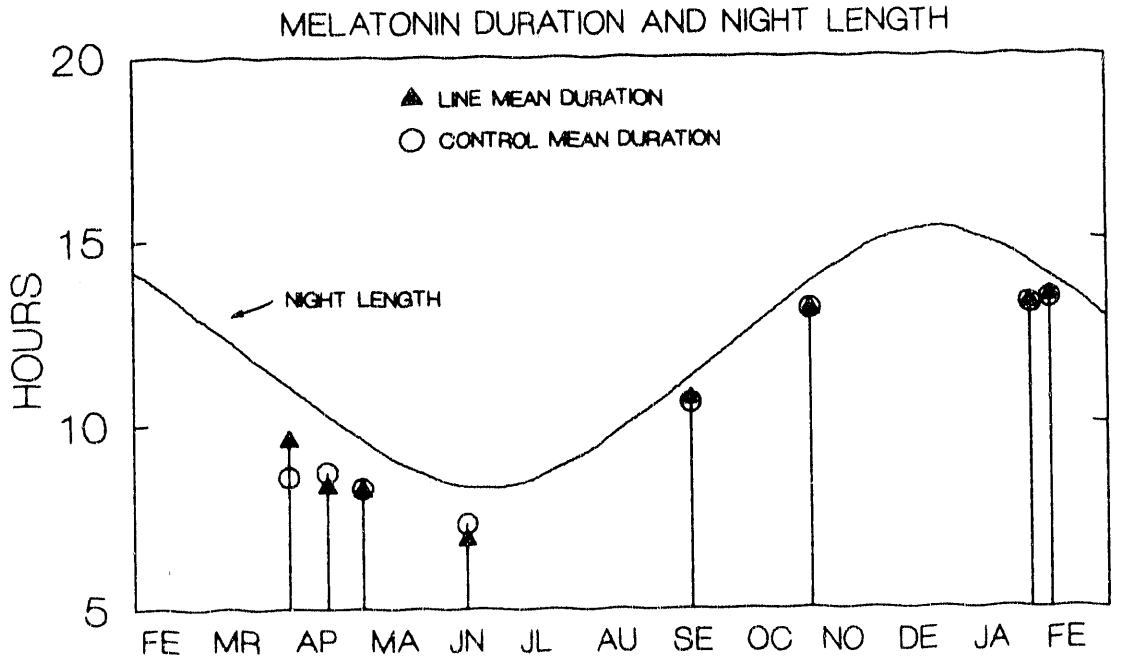


Figure 17. Duration of night melatonin elevation in line and control lambs. Duration for all 16 sample nights (upper). Duration (two night averages) compared to night length (sunset to sunrise) (lower).

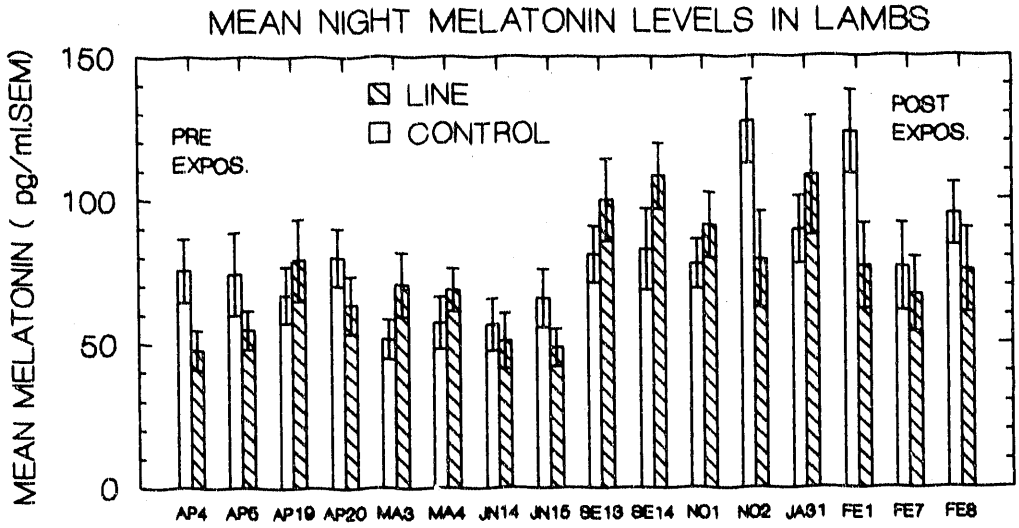


Figure 18. Mean amplitude of night serum melatonin concentrations in line and control groups for 16 nights.

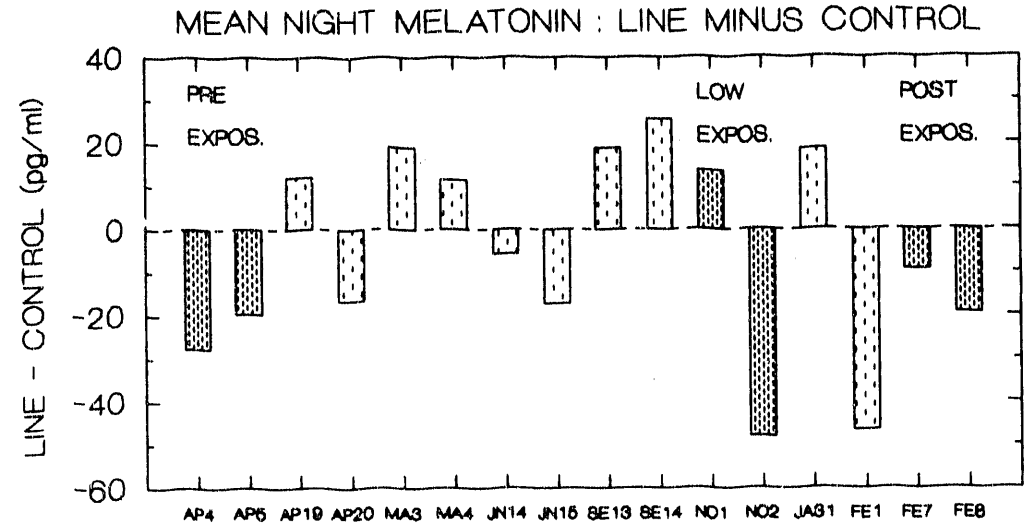


Figure 19. Comparison of mean night melatonin between line and control groups (line minus control). Bars below/above the 0 line indicate that the line value was lesser/greater than the control.

melatonin levels in the latter part of the period. This corresponded to longer night length and to increasing age of the lambs. This time effect was highly significant ($F = 5.85$, $df = 7,124$, $p < 0.001$).

Inspection of Figure 18 suggests numerous reversals in the relative mean amplitudes for the two groups on various sample dates. Figure 19 depicts the same data in a form that allows these reversals to be more easily observed. Figure 19 also shows the data in relation to the pre- and post-exposure periods, and a low exposure period from 22 October to 5 November when the 500-kV line crossing the pen was out of service. There is no clear suggestion of a consistent pattern distinguishing between exposure conditions.

The grand mean nocturnal melatonin for each lamb combining data from all 16 sample nights is shown in Figure 20. The distribution and range were similar in both groups. In each group of lambs, there was about a twofold difference in the mean melatonin between the lowest and highest individual values.

To further explore possible differences in night melatonin amplitude related to pre-, low-, and post-exposure, data were averaged for each of the two nights in the eight sample periods (Figure 21). Then post-hoc tests were used to compare adjacent samples by group. For the line group, there were three differences between adjacent samples which were significant (Figure 22). Of these, mean melatonin in the first exposure sample (19-20 April) was significantly higher than in the pre-exposure sample ($F = 7.01$, $df = 1,18$, $p = 0.016$). This is opposite of the effect predicted by the study hypothesis. For the control group, only one adjacent sample comparison was significant. This did not

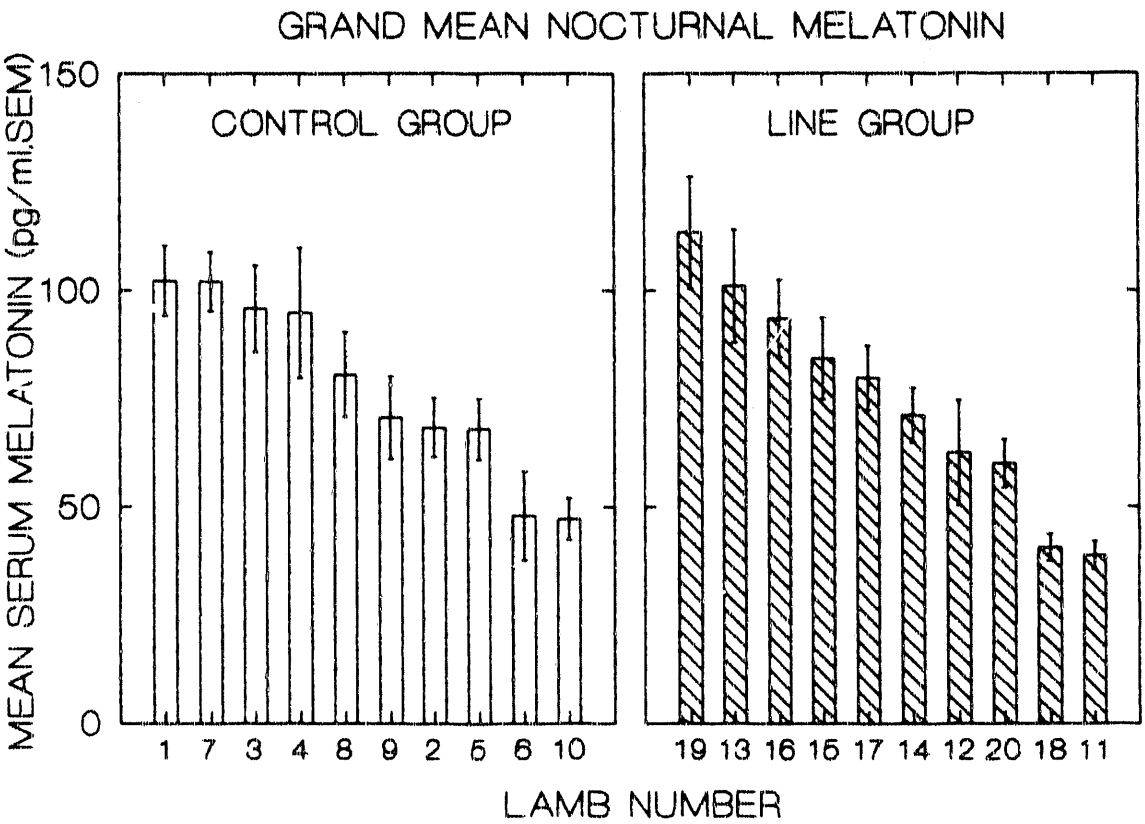


Figure 20. Grand mean nocturnal melatonin for each lamb for all 16 sample nights combined. Lambs 1 and 12 and lambs 2 and 15 were twins.

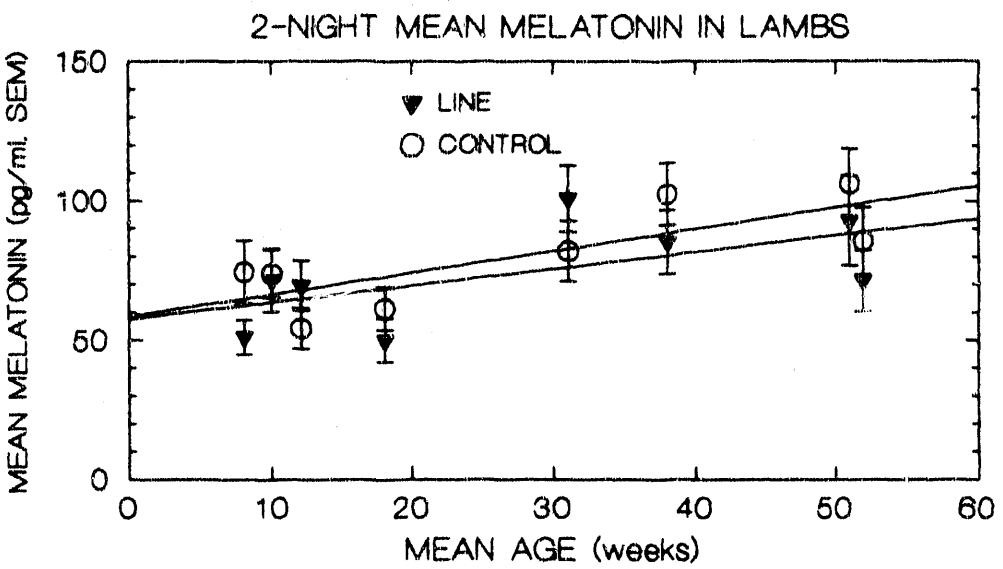


Figure 21. Night melatonin concentrations averaged for each of the two nights in the eight 48-hour samples. The upper/lower diagonal lines are linear regression lines for the control/line groups.

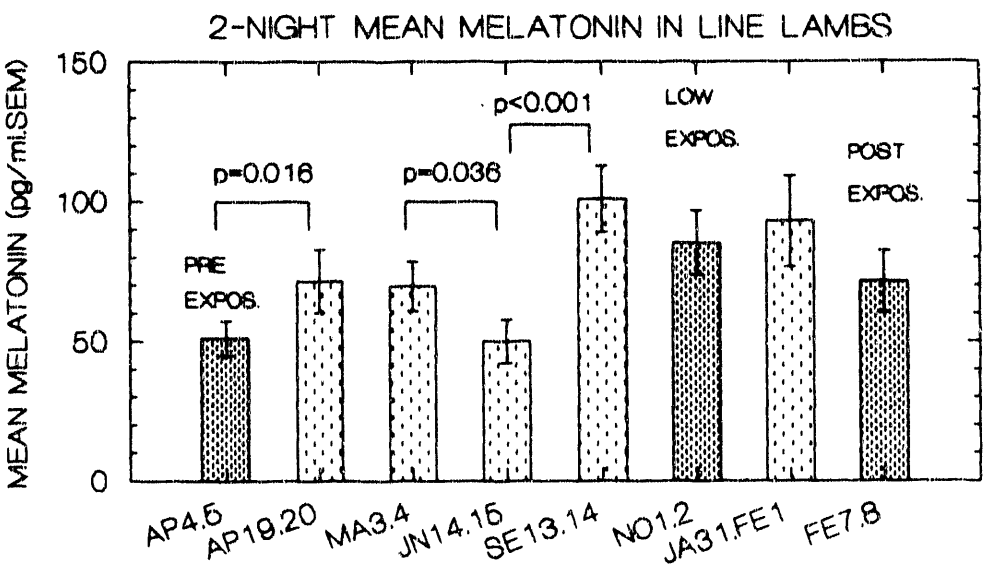


Figure 22. Comparison of adjacent two-night melatonin concentrations in line lambs in relation to exposure conditions. Differences between adjacent samples with connecting lines above the columns are statistically significant.

involve times corresponding to the above three line group exposure conditions (19-20 April vs. 3-4 May) ($F = 3.52$, $df = 1,18$, $p = 0.014$).

Regression lines in Figure 21 have a positive slope showing that mean melatonin amplitude was increasing over the duration of the study. This time period corresponds to a changing photoperiod and increasing age of the lambs. The correlation with age was statistically significant for the control group (r^2 0.61, $p = 0.023$) but not for the line group ($r^2 = 0.35$, $p = 0.120$).

Mean night melatonin for each of the line lambs over the eight 48-hour samples was also examined (Figure 23). Visual inspection of these data showed no obvious patterns that would indicate differing individual animal responses to exposure. The corresponding data for the control lambs are provided for comparison (Figure 24).

Data on the phase of the melatonin night elevations suggest that, except for the pre-exposure sample, means for the 16 nights tend to be phase-advanced (Figure 25). However, the midpoints of the night melatonin elevations preceded the midpoint of the natural dark period by only 1/2 hour or less, on average. As in the other melatonin parameters, there was no group difference for phase ($F = 0.36$, $df = 1,18$, $p = 0.556$). However, for this parameter, there was also no effect of sample time (nights) ($F = 0.239$, $df = 7,124$, $p = 0.975$).

Comparison of mean day melatonin levels showed that the control and line groups did not differ significantly from each other ($F = 0.304$, $df = 1,18$, $p = 0.588$).

In summary, no statistically significant differences between control and line groups were found for nighttime melatonin amplitude,

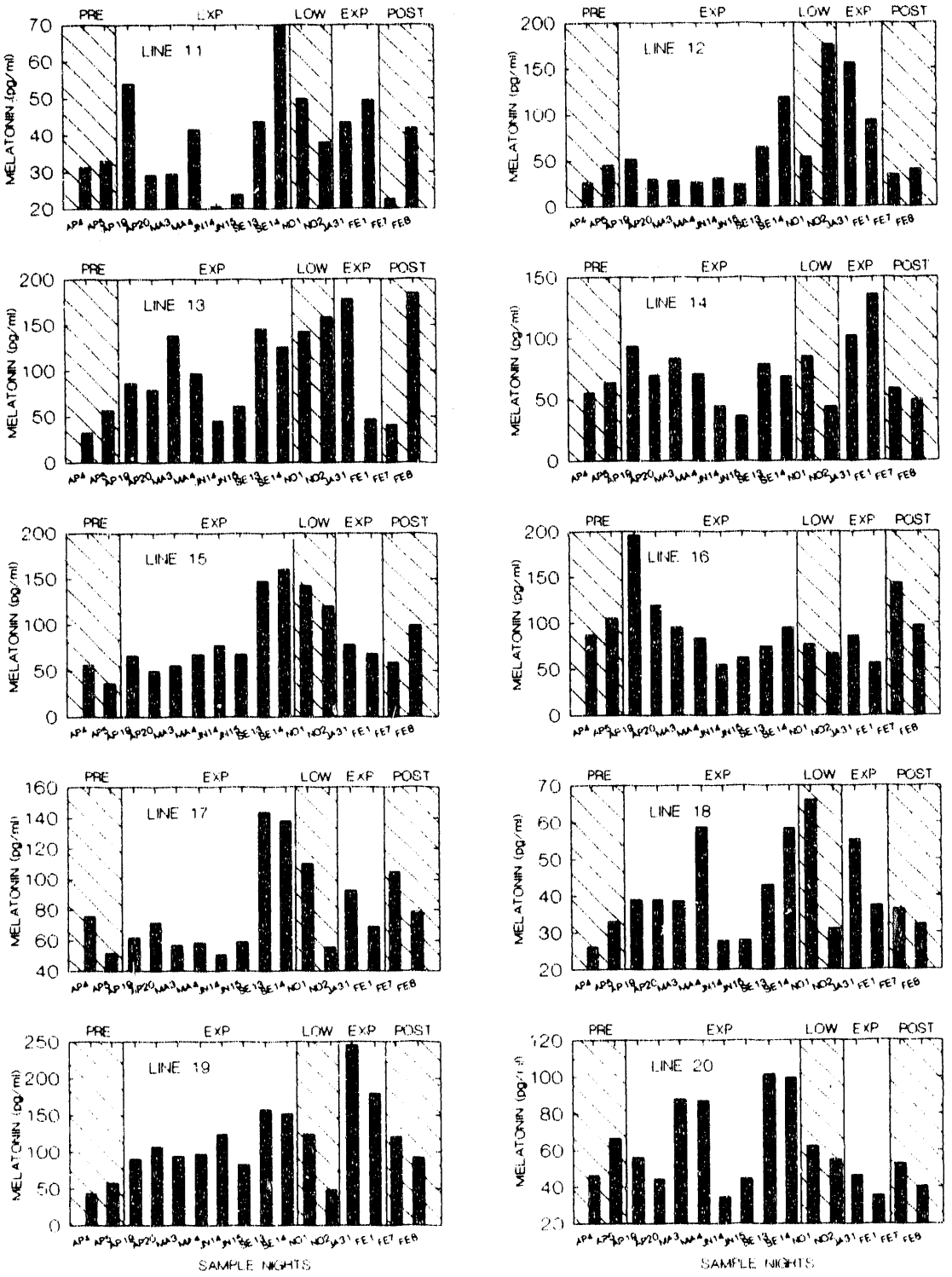


Figure 23. Mean night melatonin concentration in line lambs for the 16 sample nights in relation to exposure conditions. (Pre = pre-exposure, Exp. = exposure, low = low exposure, post = post-exposure).

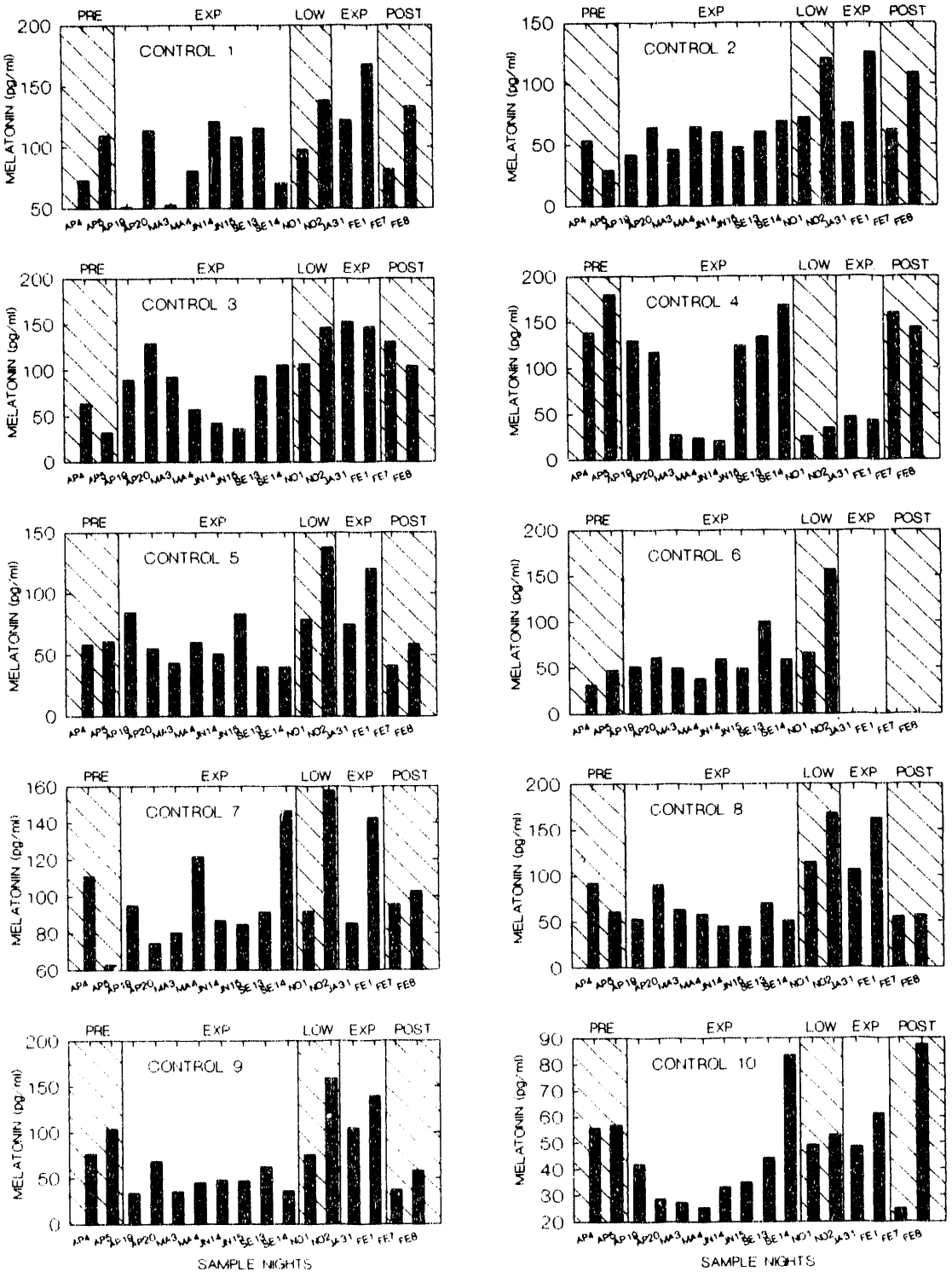


Figure 24. Mean night melatonin concentration in control lambs for the 16 sample nights in relation to exposure times for line lambs (see Figure 23).

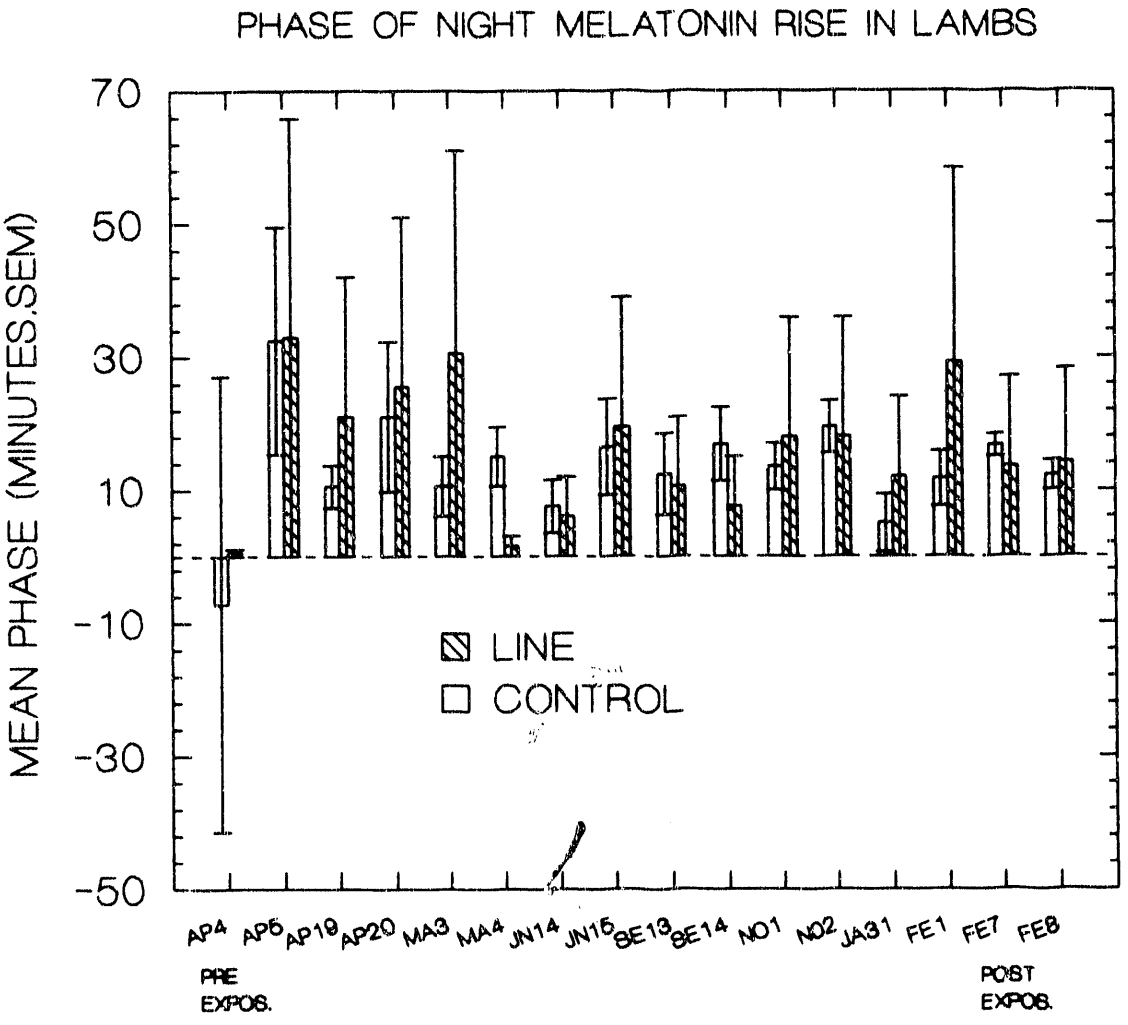


Figure 25. Phase of night melatonin elevation in relation to sunset-sunrise. Positive values represent a phase advance; i.e., midpoint of night elevation precedes the midpoint of the sunset-sunrise period.

duration, or phase. For the line group, mean nighttime melatonin amplitude was significantly higher in the first 48-hour sample after start of exposure compared to the pre-exposure level.

Progesterone and Estrous Cycles

Progesterone was measured from blood samples collected twice each week from June 1990 through February 1991. Progesterone levels for each control lamb throughout this period are shown in Figure 26. Animal no. 7 showed consistently elevated progesterone with no clear interval between cycles. Figure 27 depicts the progesterone levels for the line animals.

Age at puberty for all control and line lambs is shown in Figure 28. Mean age ($\pm$ SEM) at puberty for the control and line groups was 241.2 $\pm$ 2.1 days and 236.2 $\pm$ 3.3 days, respectively. These ages were not significantly different from each other ($T = 1.26$, $df = 18$, $p = 0.112$). Exposure to the line did not increase mean age at puberty. The mean number ($\pm$SEM) of estrous cycles detected for the control and line groups was 8.8 $\pm$ 0.5 cycles, and 8.9 $\pm$ 0.4 cycles, respectively. These values did not differ significantly from each other ($T = -0.19$, $df = 17$, $p = 0.425$).

Serum Cortisol

Cortisol was measured from the same blood samples taken over eight 48-hour periods as described above for melatonin. Figure 29 shows the mean serum cortisol levels for these periods for the control and line groups. Although the cortisol data are highly variable, inspection of

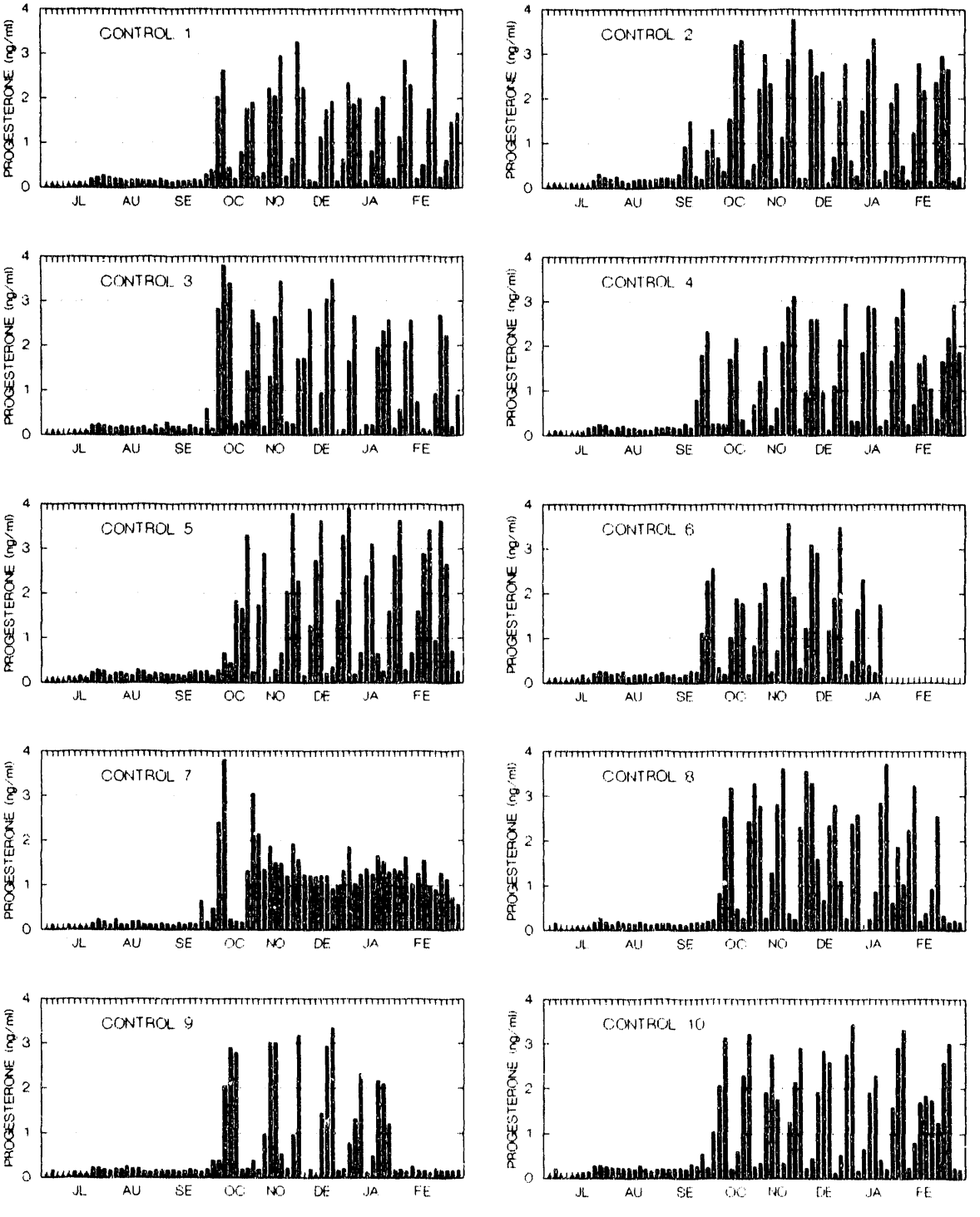


Figure 26. Serum progesterone in control lambs from 25 June 1990 to 7 March 1991. Blood samples were collected on Monday and Thursday.

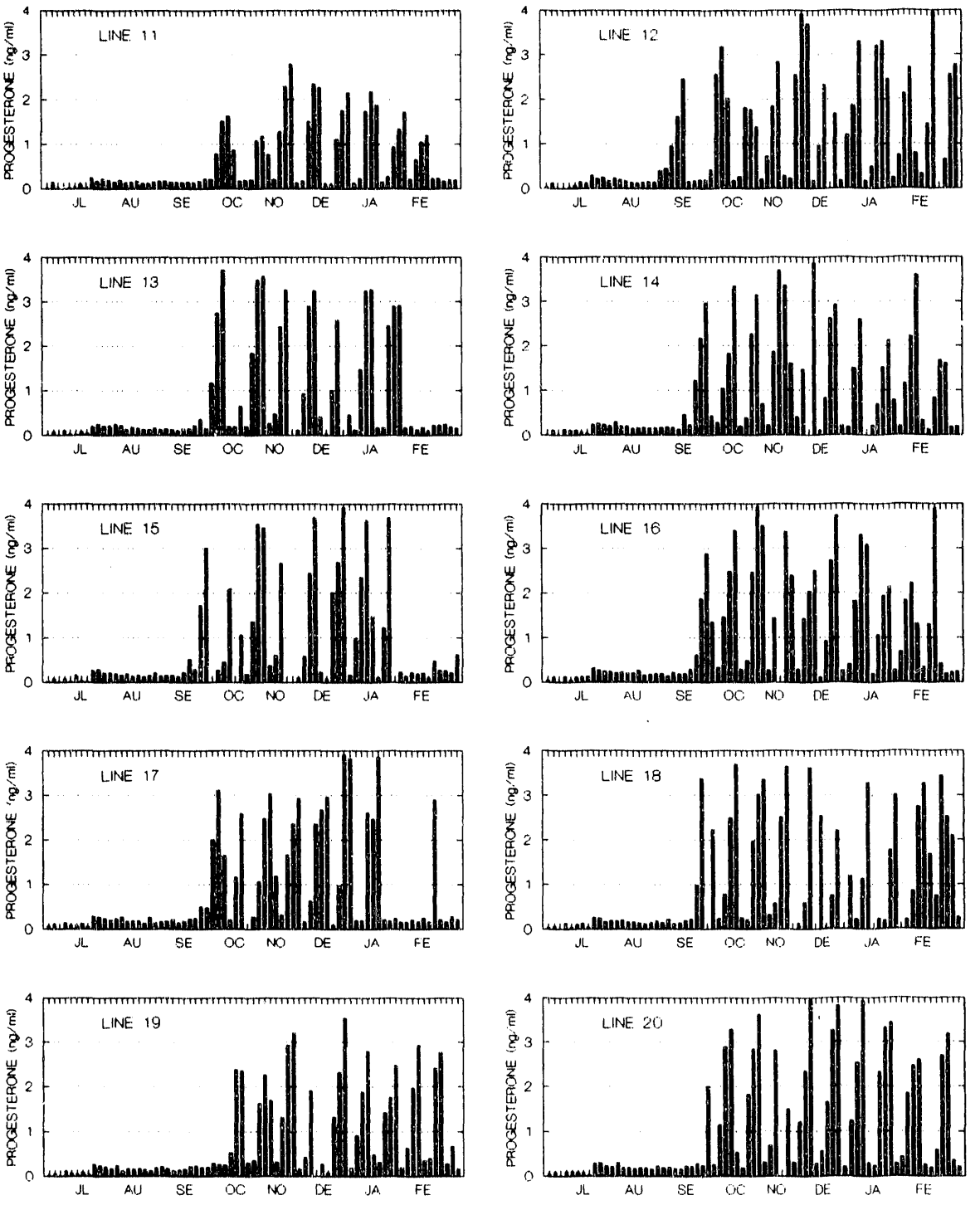


Figure 27. Serum progesterone in line lambs from 25 June 1990 to 7 March 1991. Blood samples were collected on Monday and Thursday.

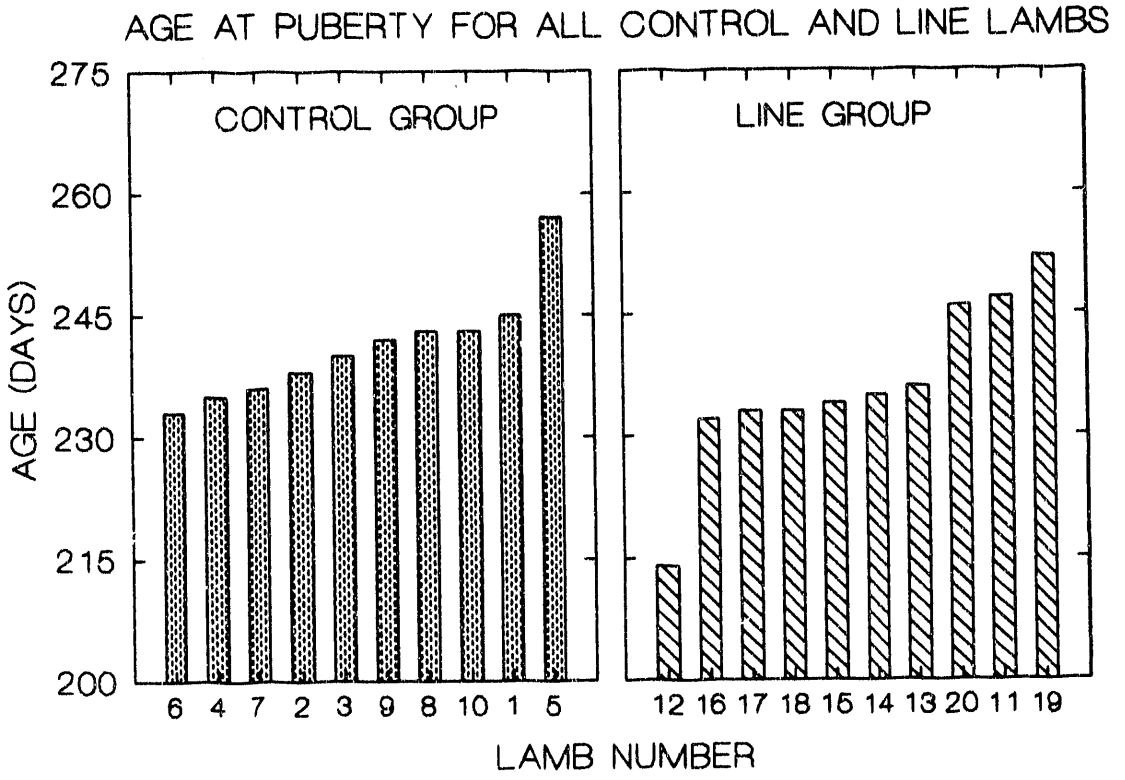


Figure 28. Age at puberty for all control and line lambs.

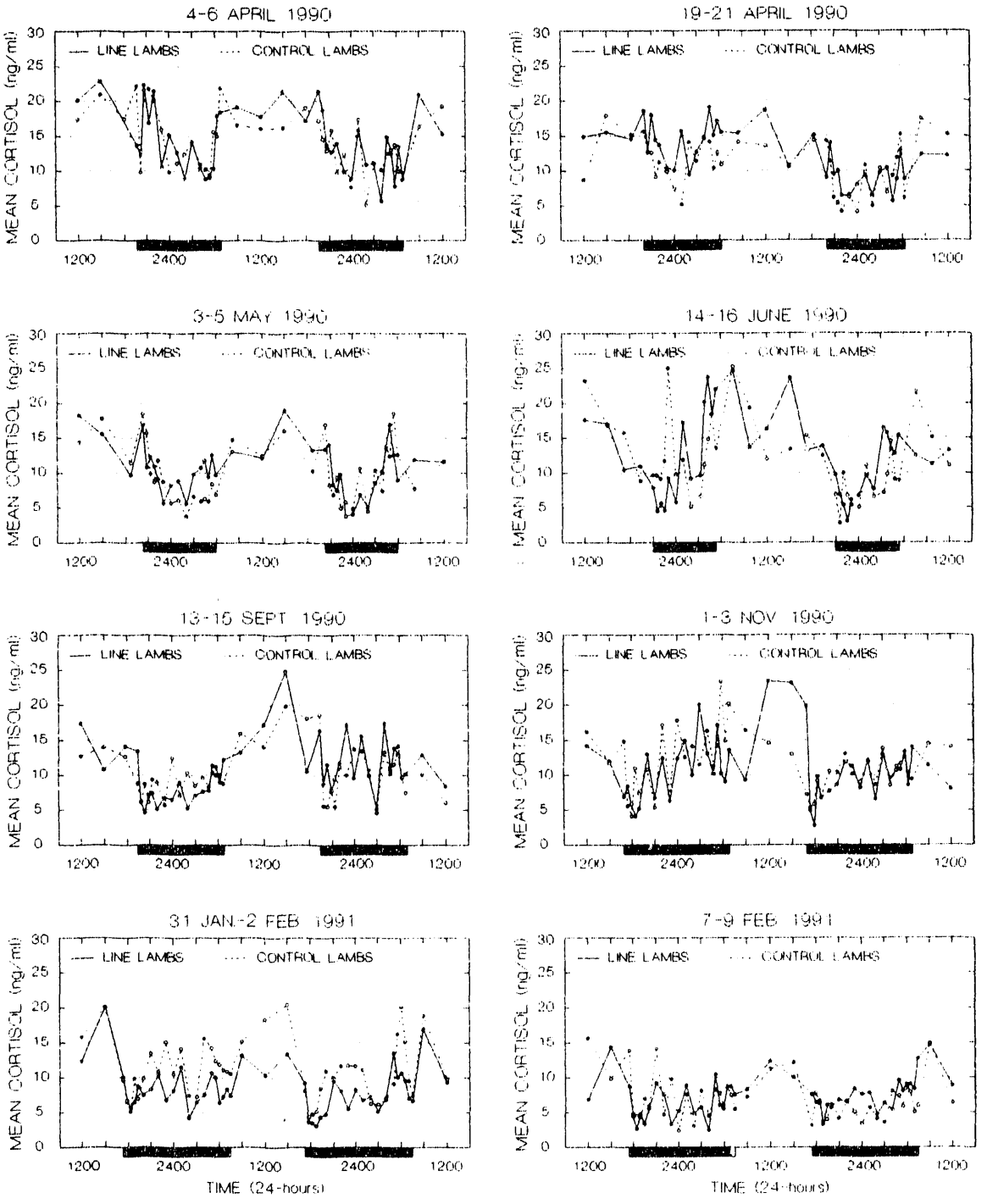
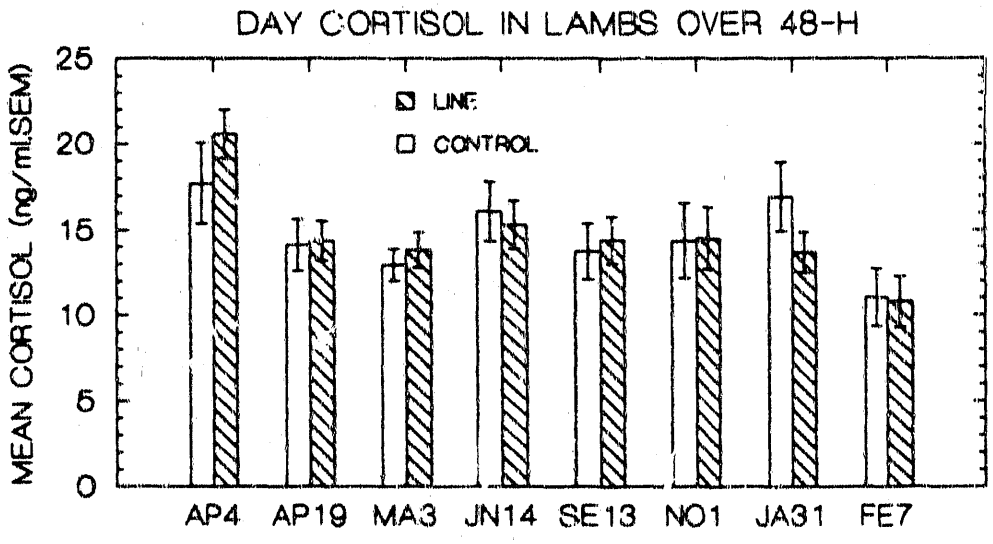


Figure 29. Serum cortisol patterns in line and control lambs over eight 48-hour periods. For clarity, standard error bars are not shown.

Figure 29 suggests a circadian rhythm, with highest levels during the day. This is further supported by the data (Figure 30) which shows mean day and night cortisol levels. Analysis of variance revealed no group difference ($F = 0.08$, $df = 1,18$, $p = 0.783$) but showed that day cortisol levels were significantly higher than night levels ($F = 109.6$, $df = 1,18$, $p < 0.001$).

Adjacent 48-hour samples were compared to see whether cortisol levels changed with changes in exposure conditions. Mean day cortisol levels for control and line groups (Figures 31 and 32) exhibited the same pattern. Cortisol levels in the first exposure sample (19-21 April) were significantly lower than in the pre-exposure sample (Control: $F = 5.68$, $df = 1,18$, $p = 0.028$; Line: $F = 17.65$, $df = 1,18$, $p = 0.001$). Also, cortisol levels in the final post-exposure sample were significantly lower than in the preceding sample (Control: $F = 12.49$, $df = 1,18$, $p = 0.002$; Line: $F = 4.54$, $df = 1,18$, $p = 0.047$).

This pattern was not consistent in the night cortisol data (not graphed). The control group again showed a significant drop in cortisol in the post-exposure sample ($F = 6.41$, $df = 1,18$, $p = 0.021$) but not in the sample following pre-exposure. For the line group, neither the pre- or post-exposure samples differed from adjacent ones. However, night cortisol was significantly lower in the first sample following the low-exposure sample ($F = 5.42$, $df = 1,18$, $p = 0.032$). These results suggest no effects of exposure since similar patterns were observed in both line and control groups.



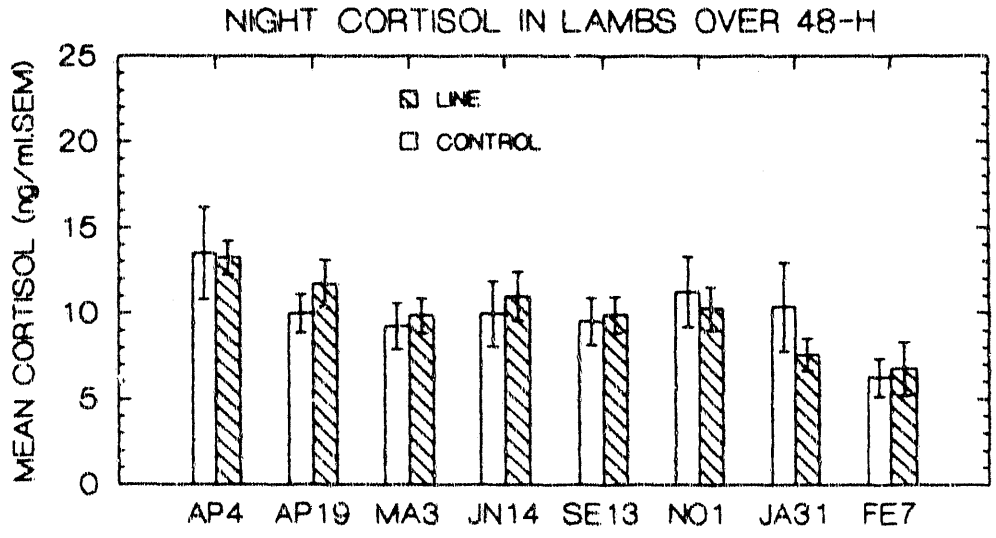


Figure 30. Mean day (upper) and night (lower) cortisol in line and control lambs over eight 48-hour periods.

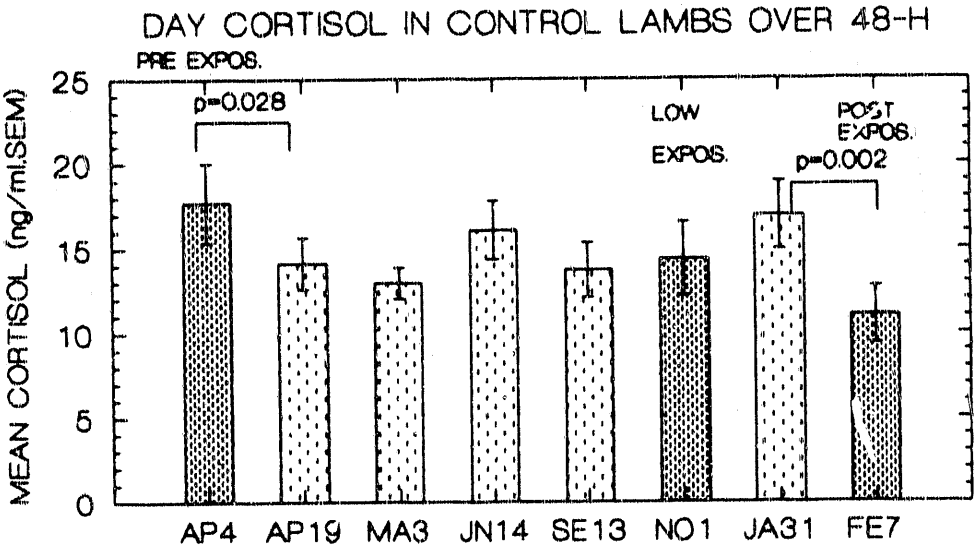


Figure 31. Comparison of mean day cortisol concentration in control lambs between adjacent sample times corresponding to different line group exposure times. Differences between adjacent samples with connecting lines above columns are statistically significant.

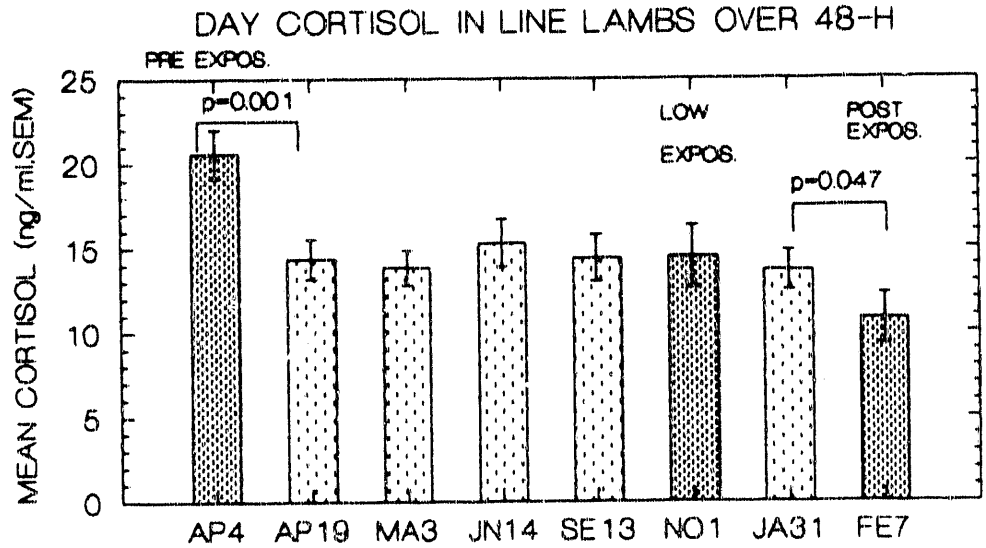


Figure 32. Comparison of mean day cortisol concentration in line lambs between adjacent sample times corresponding to different exposure conditions. Pre-exposure, low exposure, and post exposure samples are indicated by dark shading. Differences between adjacent samples with connecting lines above columns are statistically significant.

As a positive control, cortisol was measured in blood samples collected on 15 February 1991, before and after transport of sheep from the site at the conclusion of the study. "Before" samples were collected between 1110 and 1130 hours; "after" samples were collected between 1545 and 1620 hours. The sheep were hauled in a stock trailer a distance of approximately 160 km over hard-surface roads.

Since cortisol levels normally vary throughout the day, the transport samples were compared to samples taken on 8 February at 1200 and 1500 hours (Figure 33). Mean cortisol levels in both groups of sheep more than doubled after transport. Analysis of variance confirmed a highly significant difference between cortisol levels before and after transport ($F = 22.44$, $df = 2,17$, $p < 0.001$). There was no difference between the levels taken on 8 February during similar times of day ($F = 0.44$, $df = 2,17$, $p = 0.648$). There was no difference in cortisol levels between the control and line groups ($F = 0.26$, $df = 1,17$, $p = 0.617$).

Wool Growth

Measurements of wool growth taken every 2 weeks on side and britch patches (Figures 34 and 35) were very similar for each group throughout the period. Final wool length was measured on 17 January 1991. Mean side wool length (mm $\pm$ SEM) for control and line groups was 79.9 $\pm$ 4.7 mm, and 82.7 $\pm$ 5.4 mm, respectively. These values do not differ significantly ($T = -0.39$, $df = 17$, $p = 0.703$). The corresponding britch wool growth for the two groups was 83.4 $\pm$ 3.8 mm, and 88.0 $\pm$ 5.6 mm, respectively. These values were also not significantly different ($T = -0.66$,

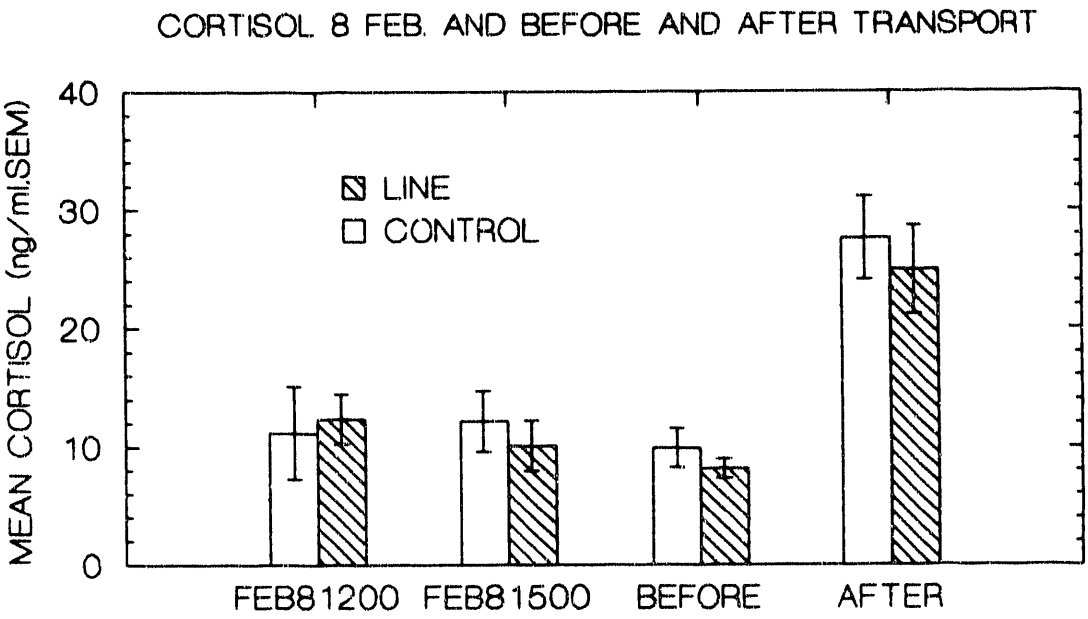


Figure 33. Serum cortisol in line and control lambs on 15 February 1991 before (1110 hours) and after (1545 hours) transport 160 km to Corvallis, OR. Shown for comparison are cortisol levels on 8 February at 1200 and 1500 hours.

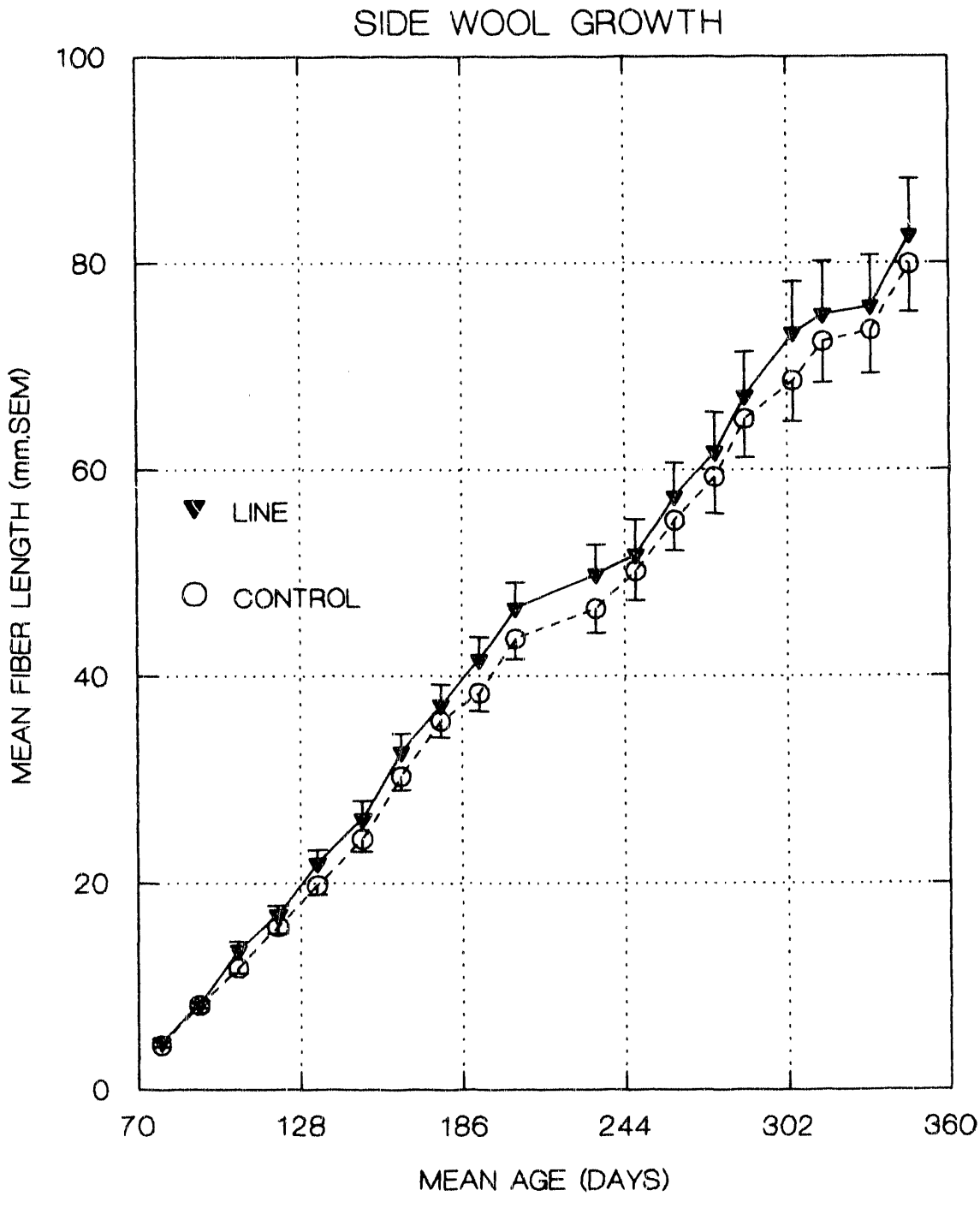


Figure 34. Mean side wool growth for line and control lambs measured every 2 weeks from 5 June 1990 to 17 January 1991.

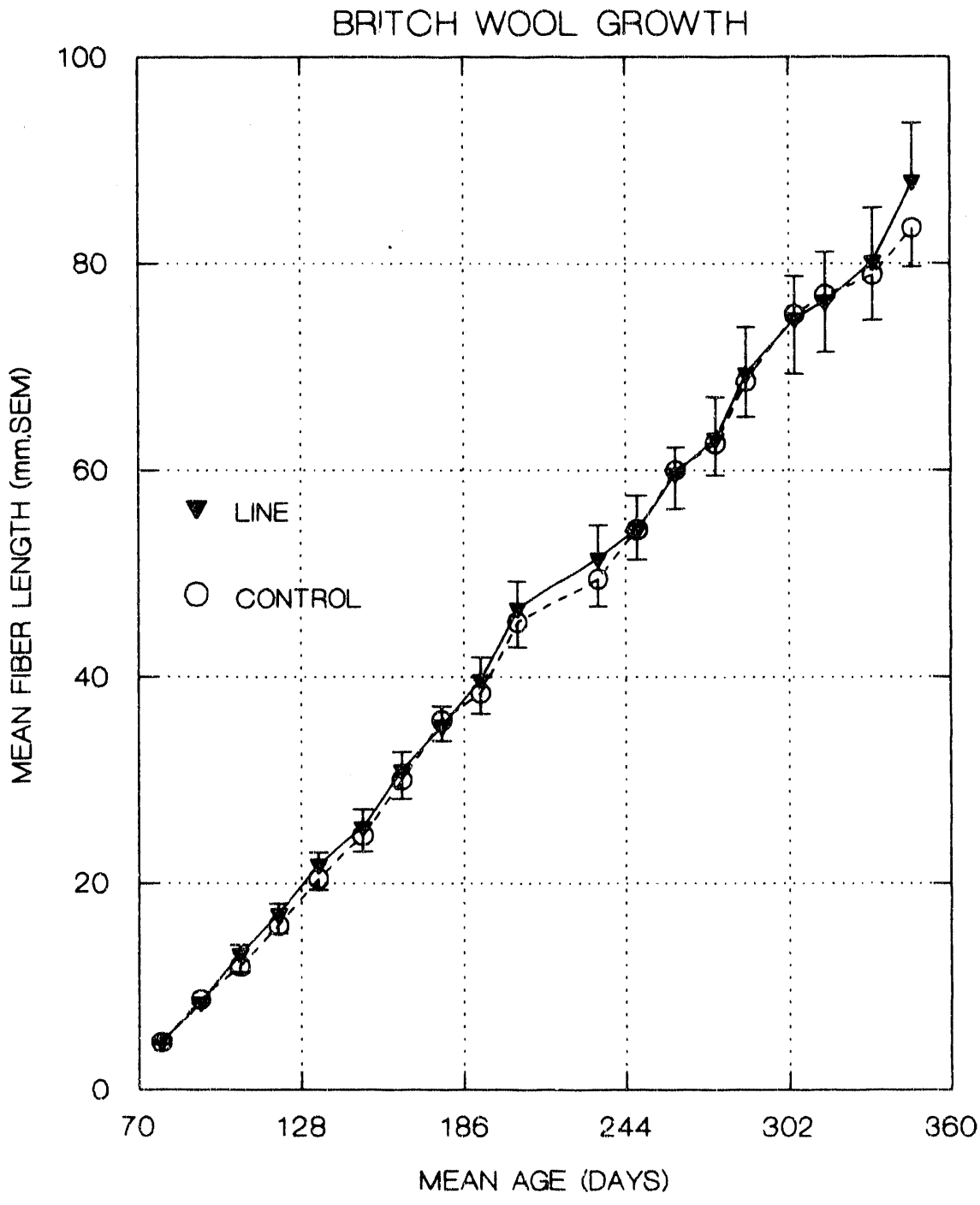


Figure 35. Mean britch wool growth for line and control lambs measured every 2 weeks from 5 June 1990 to 17 January 1991.

df = 17, p = 0.519). The test of the wool growth measurement technique conducted on 5 June 1990 revealed no differences in the mean length obtained in the two consecutive measures (F = 0.222, df = 1,18, p = 0.643).

After the sheep were sheared the wool was weighed on 25 June 1990. Mean wool weight (kg ± SEM) for the control and line groups was 0.89 ± 0.04 kg, and 0.99 ± 0.04 kg, respectively. These weights were not significantly different (T = -1.76, df = 18, p = 0.096.

There was also no significant difference between groups in the diameter of wool fibers from samples of wool collected on 19 and 20 June 1990. Mean fiber diameters (μm ± SEM) for the line and control groups were 41.3 ± 1.4 μm and 42.6 ± 0.7 μm, respectively (T = -0.8, df = 17, p = 0.435).

Behavior

Results of the nine monthly 24-hour behavioral observations (Figure 36) show that the greatest percentage of observations was of resting animals. The two groups showed nearly identical behavior when the mean values for each activity were inspected. There is no evidence suggesting any noticeable differences in general behavior between the groups.

Figure 36 also shows behavior separated for day and night (sunset-sunrise). As with activity type, there are no apparent day-night differences between groups. The animals were fed twice during the day and this is reflected in the higher percentage of daytime feeding observations. Daytime resting percentages are lower than for night in both groups as a result of the feeding schedule.

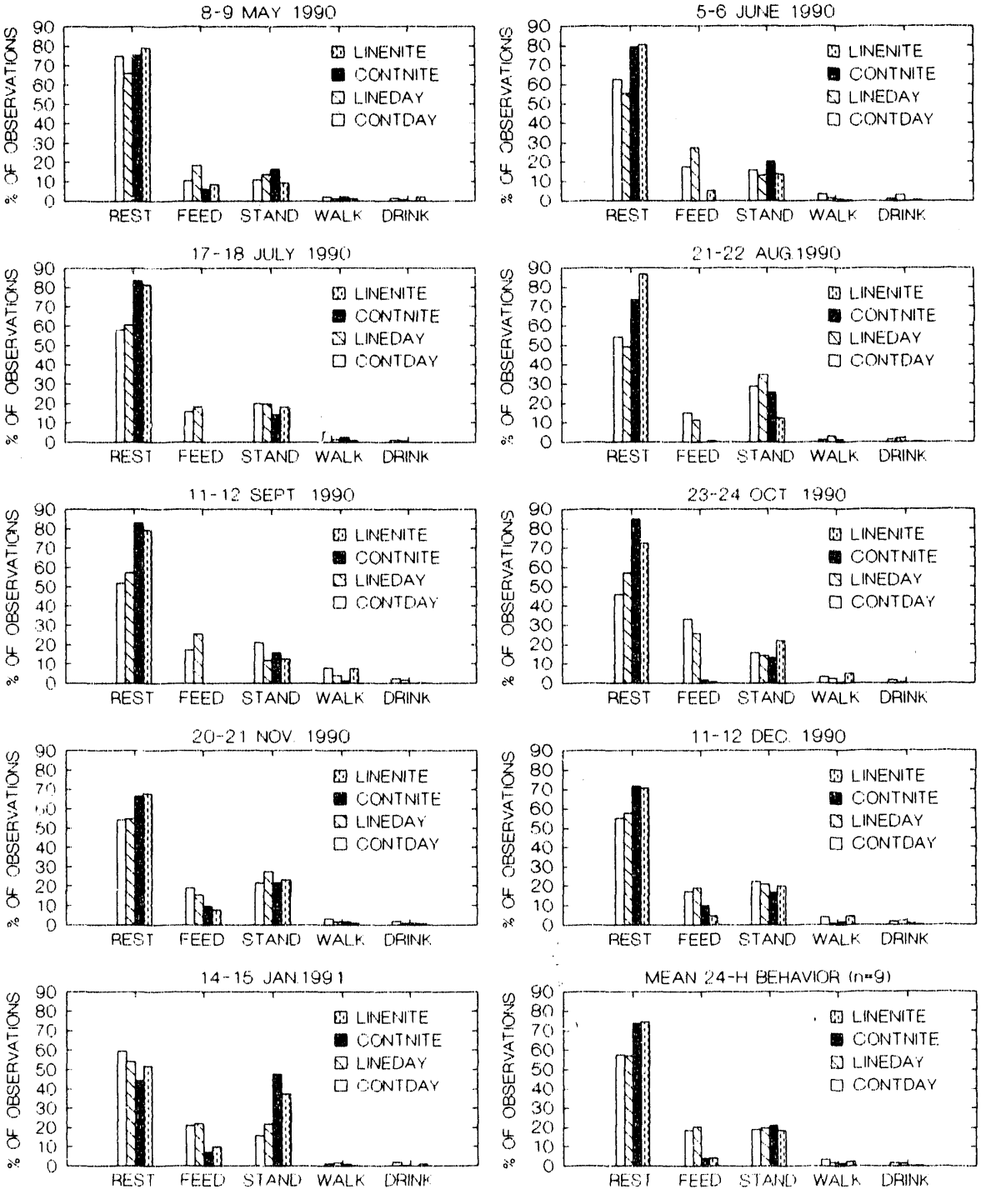


Figure 36. Comparison of general behavioral activities of line and control lambs. Data are from observations made at 15-minute intervals over 24 hours, once a month. The group means are shown in the lower right. "Nite" = sunset-sunrise.

In addition to type of behavior, location of animals within the pens was also recorded. Figure 37 shows where animals were observed in the four main areas of the pens. In both groups, animals tended to spend a slightly greater percentage of their time in the section of the pens near the observation shelter. This may, in part, be because of shade or wind protection offered by the presence of the shelter. Also, personnel typically entered this part of the pen during feeding times.

Electrical and Environmental Monitoring

Because of the capabilities of the ERM field measurement system, it is possible to conduct very extensive data analyses. To determine daily and weekly magnetic field variation, it is particularly interesting to examine weekly plots of the 60-Hz magnetic fields. An example of such plots for the line and control pens for two different weeks in June and July are shown in Figure 38. The maximum magnetic field levels during daytime are about two-times higher than during nighttime. This is a relatively small ratio as ratios as high as 10 have been seen on at least one BPA 230-kV line. The diurnal variations in the magnetic field are associated with the diurnal load patterns and changes in conductor height. The magnetic fields in the control pen also show a day and night variation pattern but at a much lower level (mean of 0.25 mG compared to a mean of 35-38 mG in the line pen).

Similar weekly plots of AN (audible noise) are shown for the same 2 weeks in Figure 39. AN increases with rain, and it is easy to identify the AN levels during rain on these plots. A period of steady rain

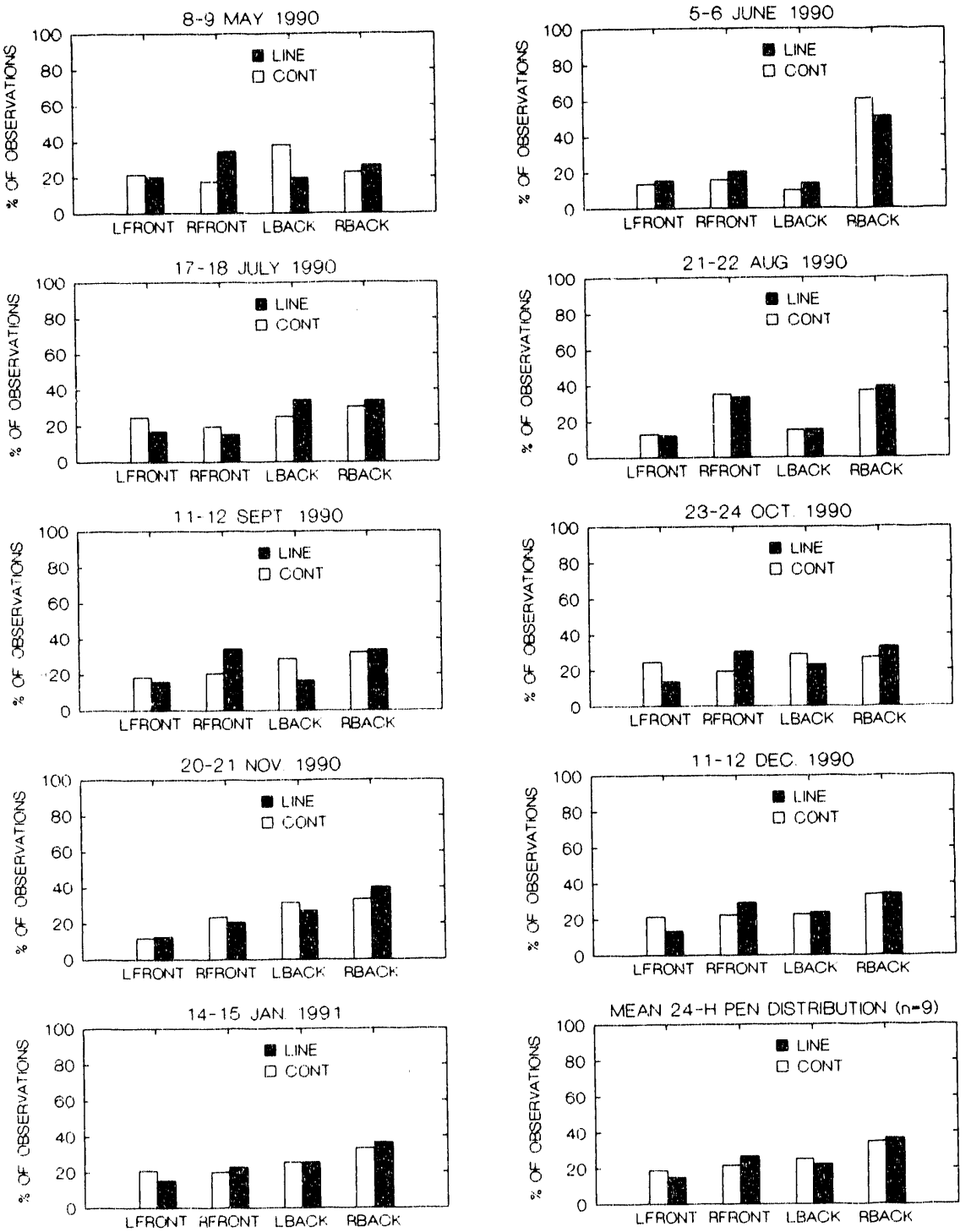


Figure 37. Comparison of animal distribution within the line and control pens. Data are from observations made at 15-minute intervals over 24 hours, once a month. The group means are shown in the lower right.

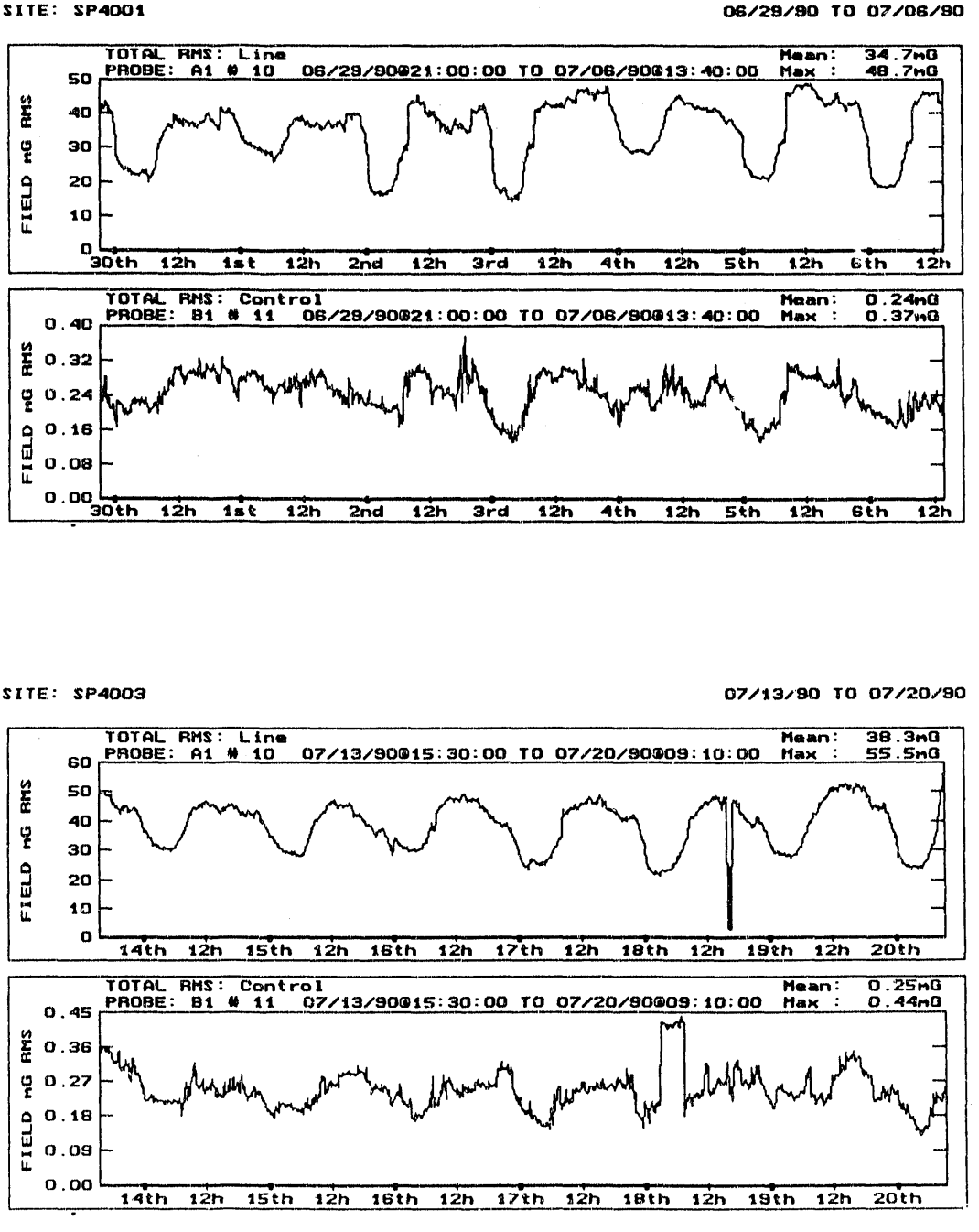


Figure 38. Magnetic field levels recorded in the line and control pens for two typical weeks.

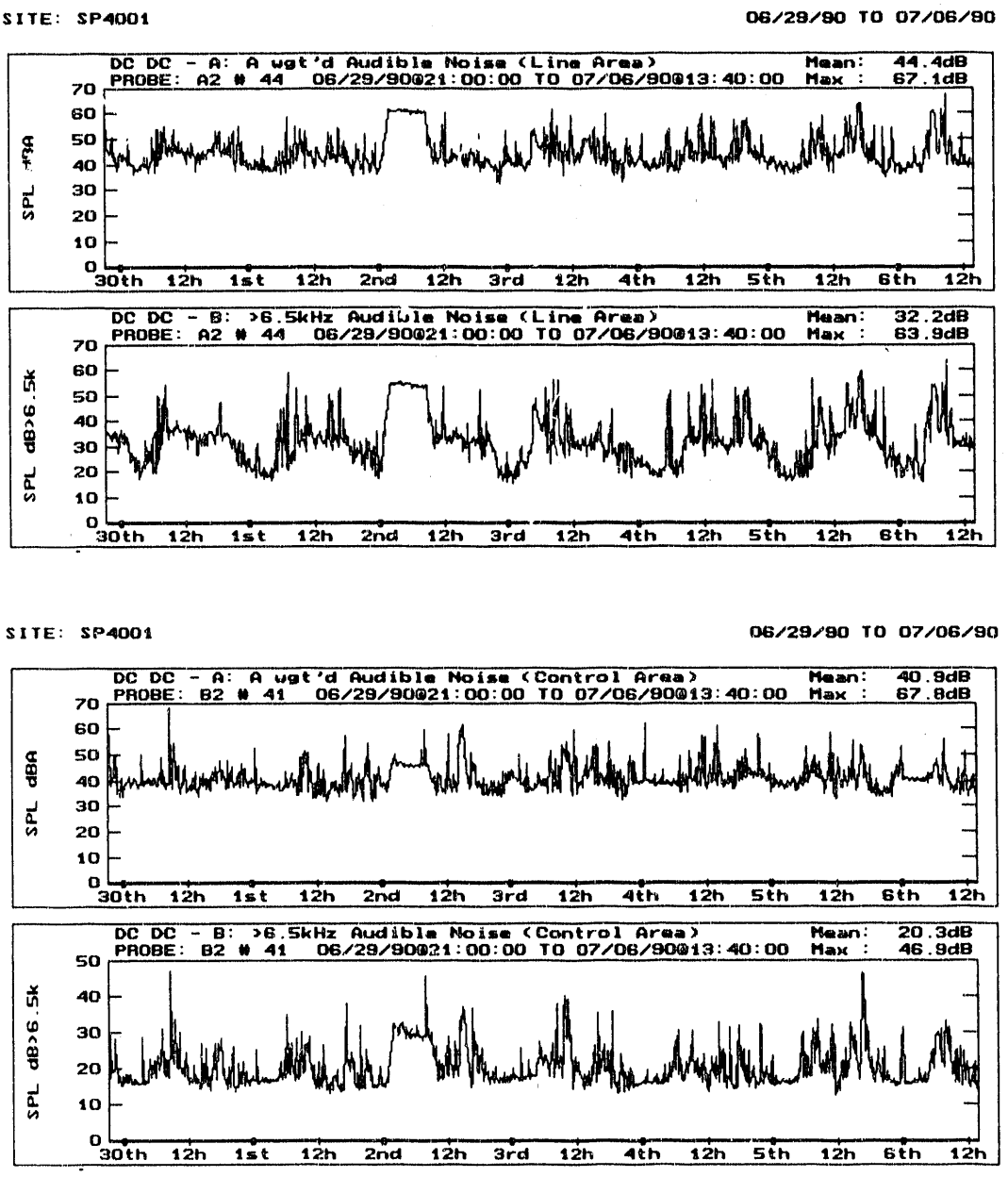


Figure 39. Audible noise (dBA and >6.5 kHz) recorded in line and control pens from 29 June to 6 July 1990.

occurred on 2 July 1990. Since the AN falls off slowly with distance, the AN during the rain period could also be detected in the control pen.

Figure 40 shows similar plots for each of the weather variables that were measured from 26 June to 6 July 1990.

As expected, harmonic fields were very low from this line. An example of these fields for the period from 29 June to 6 July 1990 for both the line and control pens is shown in Figure 41.

Figures 42-43 show the cumulative probability distribution of the 60-Hz magnetic fields, A-weighted and 6.5 kHz audible noise, and the weather parameters, respectively for the entire weather period. The distributions are plotted on normal probability paper which has been traditional for transmission line electrical environmental measurements. Specific exceedance levels can be obtained from these plots and are shown in Tables IV and V. Also, shown on Tables IV and V are the following descriptive statistics: mean, standard deviation, maximum and minimum levels, and the number samples.

Table IV shows that the median values of the magnetic fields in the line and control pen were 41.2 and 0.261 mG, respectively, whereas the mean values were 40.0 and 0.289 mG. The proximity of the mean and median values to each other suggests that the magnetic fields in both the line and control pens for the most part were normally distributed over the duration of the study period. Normal distributions on probability paper will plot as straight lines. Examination of Figure 42 (upper), however, shows that there are actually two normal probability distributions for the line pen. The one with the lower magnetic field values is during the time periods when the line was off.

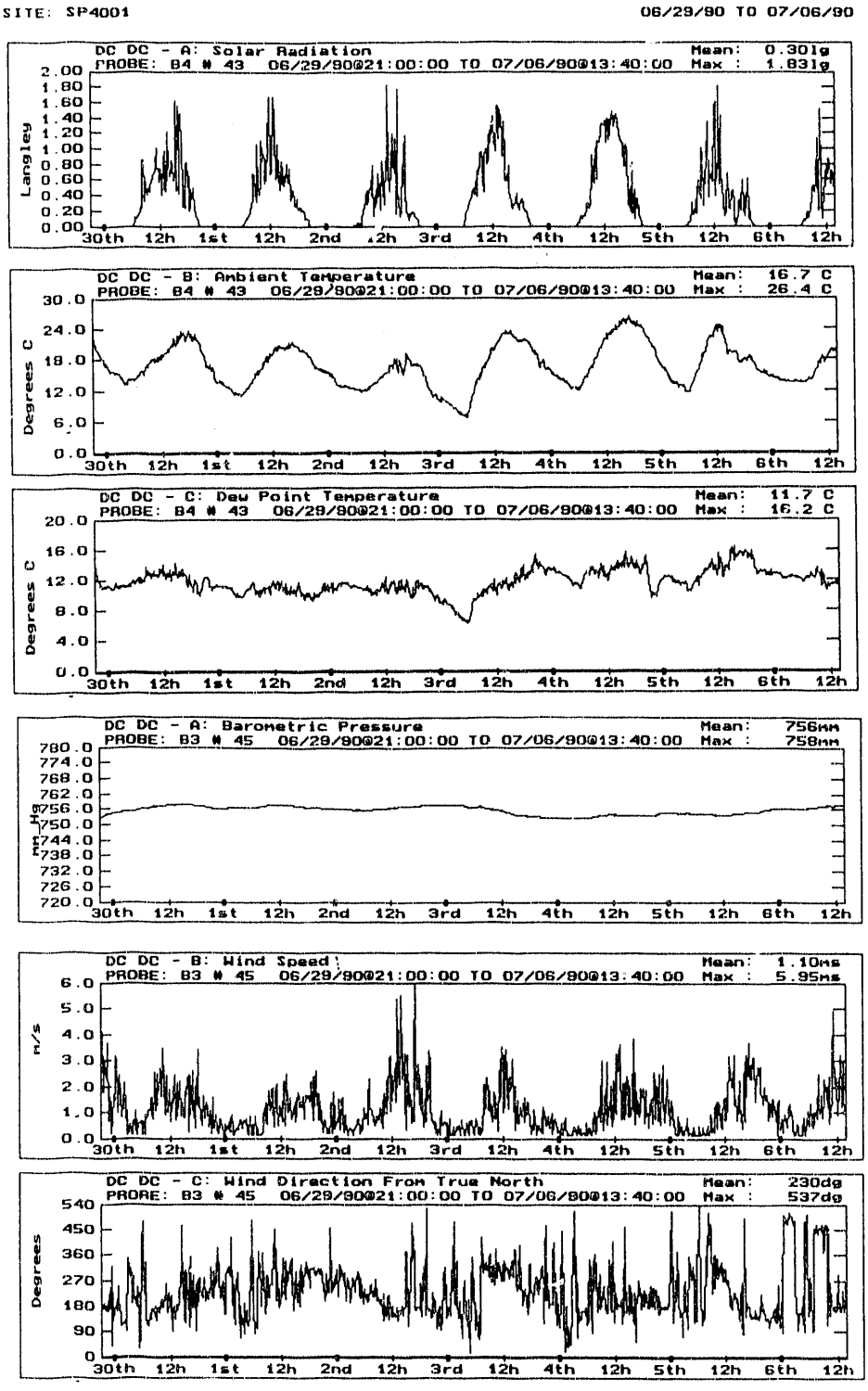


Figure 40. Microclimate variables recorded from 29 June to 6 July 1990.

SITE: SP4001 06/29/90 TO 07/06/90

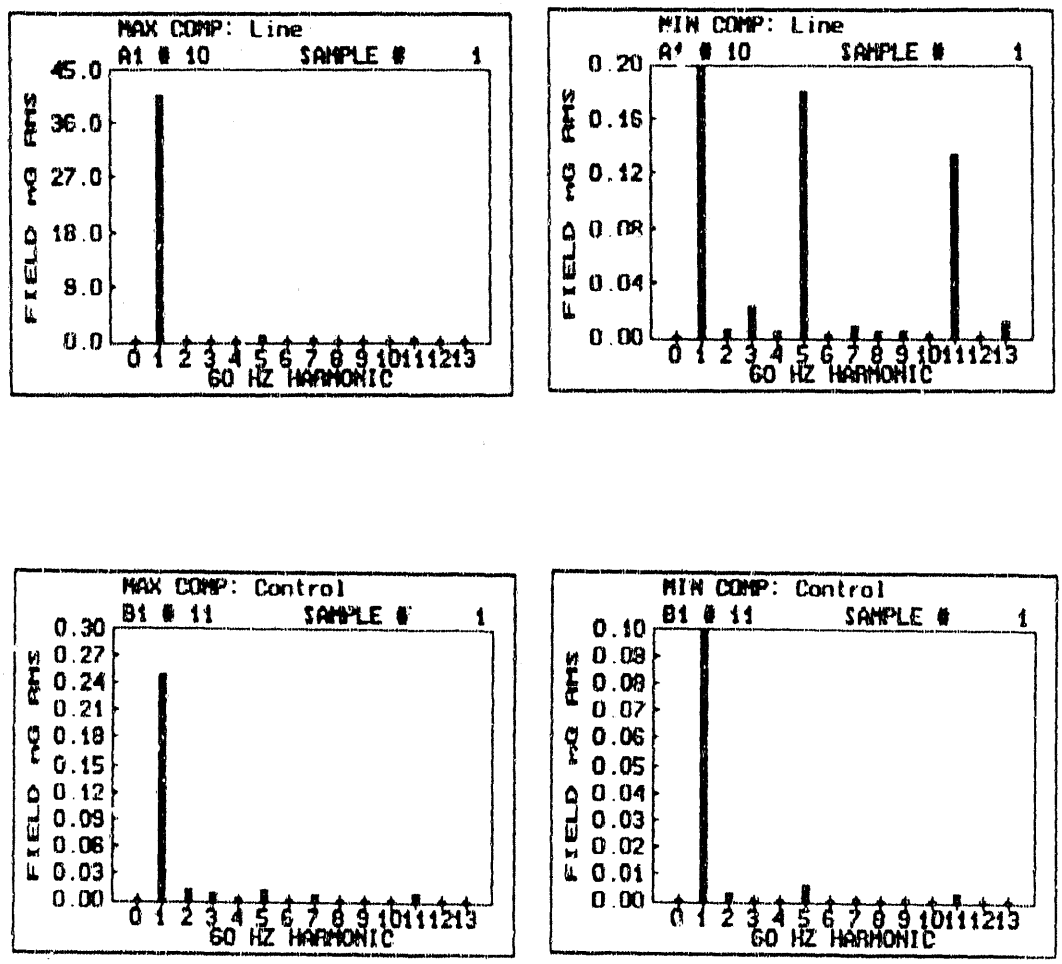


Figure 41. Examples of magnetic field harmonics recorded from 29 June to 6 July 1990.

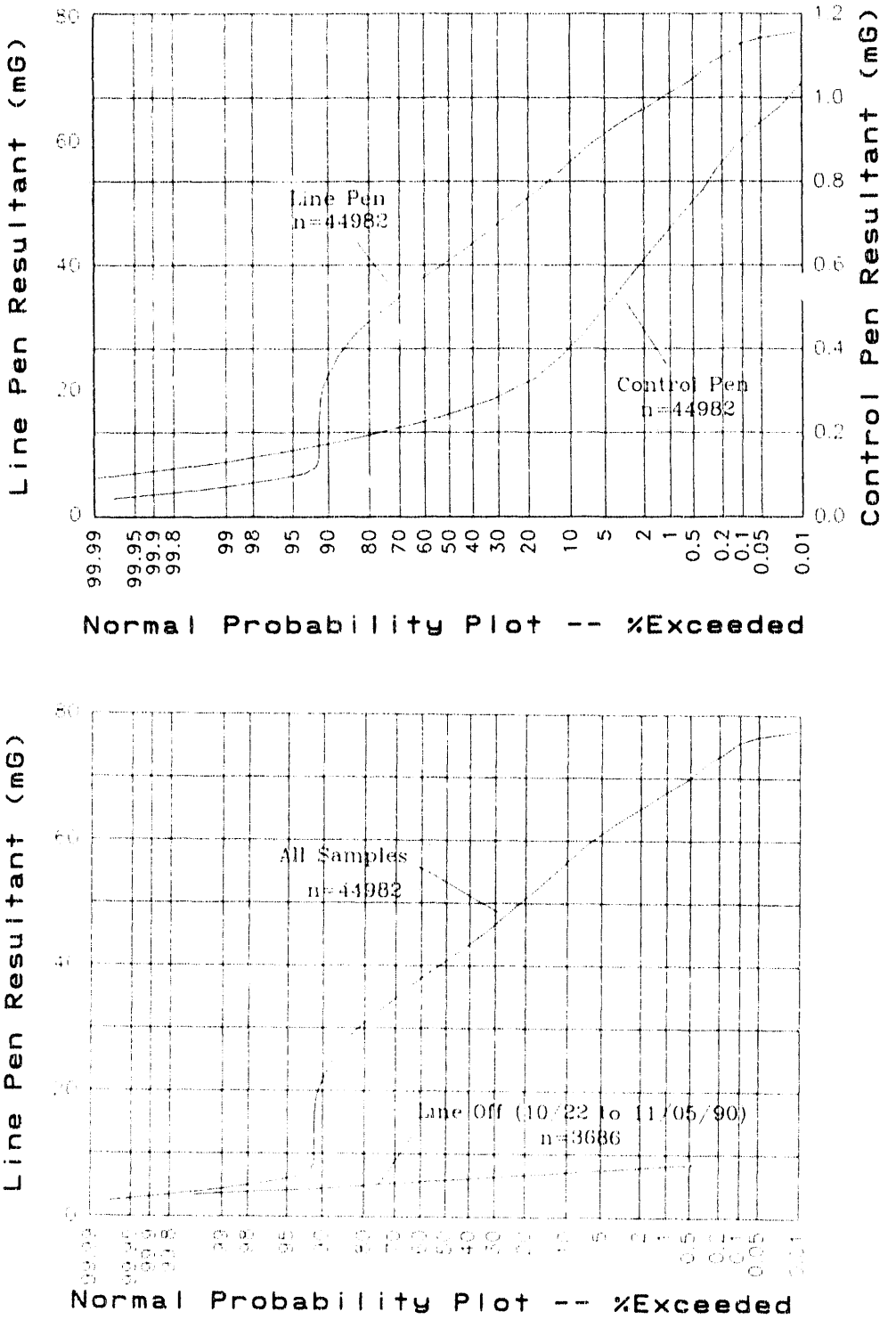


Figure 42. Cumulative probability distributions of the magnetic field measurements for the entire study period (upper) and for the low exposure period from 22 October to 5 November 1990 (lower).

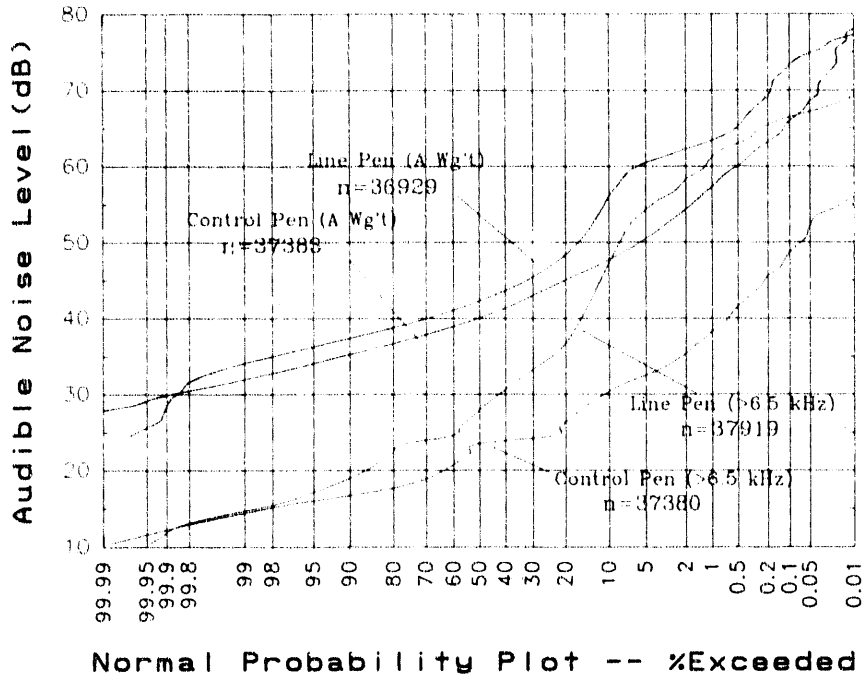


Figure 43. Cumulative probability distributions of the audible noise measurements for the entire study period.

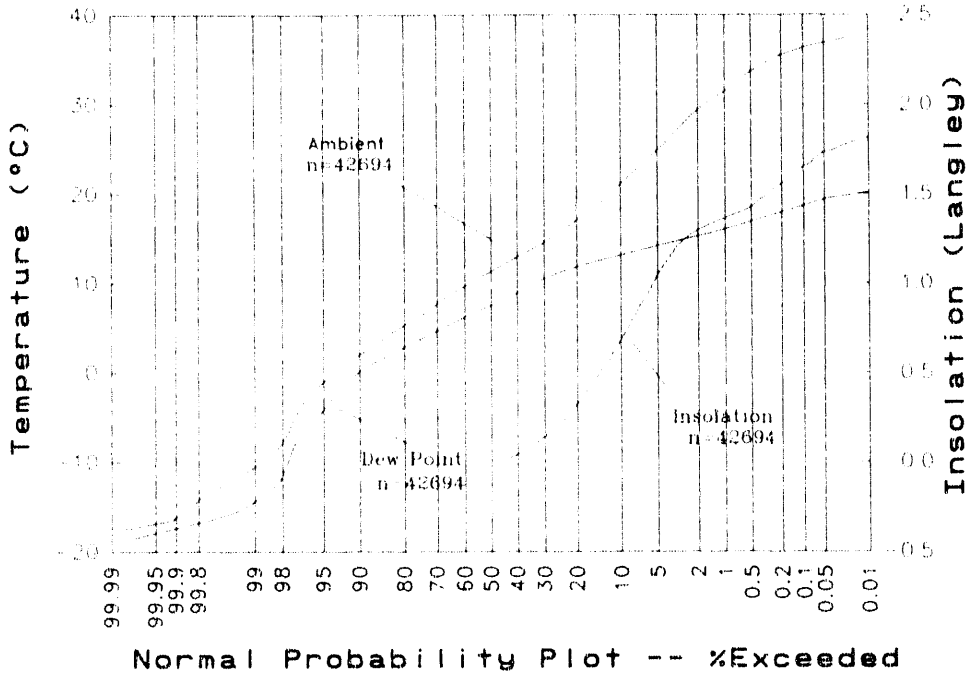


Figure 44. Cumulative probability distributions of the temperature and insolation measurements for the entire study period.

TABLE IV

EXCEEDANCE LEVELS AND DESCRIPTIVE STATISTICS
FOR MAGNETIC FIELD AND AUDIBLE NOISE MEASUREMENTS

Exceedance Percentage (% exceeded)	Magnetic Field		Audible Noise A-Wtd.		Audible Noise >6.5 kHz	
	Line (mG)	Control (mG)	Line (dB)	Control (dB)	Line (dB)	Control (dB)
			Exceedance Levels			
99	4.9	.150	34.6	32.5	15.2	14.9
95	6.6	.171	36.8	34.6	17.7	16.5
90	22.3	.190	37.9	35.7	19.5	17.2
75	33.4	.220	39.9	37.8	24.0	18.8
50	41.2	.261	42.8	40.5	28.4	24.0
25	49.1	.320	47.2	44.5	35.1	24.6
10	57.0	.422	56.6	48.2	47.7	30.9
5	61.6	.519	61.0	51.0	54.8	33.0
1	68.2	.708	63.9	57.8	61.6	38.7
			Descriptive Statistics			
Mean	40.0	.289	44.7	41.5	30.8	23.2
S.D.	14.3	.10	7.0	5.2	10.7	5.3
Max.	79.0	1.14	82.6	84.3	74.8	71.0
Min.	2.8	.083	24.0	24.8	10.1	10.1
Samples	44982	44982	36929	37383	37919	37380

TABLE V

EXCEEDANCE LEVELS AND DESCRIPTIVE STATISTICS
FOR THE WEATHER PARAMETERS MEASUREMENTS

Exceedance Percentage (% exceeded)	Temperature Ambient (°C)	Dew Pt. (°C)	Barometric Pressure (mm Hg)	Wind Speed (m/s)	Insolation (Langley)
		Exceedance Levels			
99	-10.1	-13.8	748.0	.097	0.0
95	- 0.65	- 3.59	751.8	.120	0.0
90	2.48	0.64	753.3	.140	0.0
75	6.98	4.31	755.5	.340	0.0
50	11.8	8.05	757.9	.712	0.13
25	16.2	11.8	760.7	1.42	0.22
10	21.6	13.6	764.0	2.45	0.69
5	25.4	14.8	765.3	3.27	1.05
1	32.0	16.6	768.0	5.07	1.37
		Descriptive Statistics			
Mean	11.8	7.32	758.1	1.07	1.191
S.D.	7.9	5.8	4.1	1.0	0.30
Max.	39.0	21.2	770.0	10.9	1.930
Min.	-17.3	-18.6	714.6	0.070	0.0
Samples	42694	42694	42691	42694	42694

There are also two normal distributions for the magnetic fields in the control pen. The lower distribution is probably the statistics for the noise floor of the measurement system. The upper distribution is the magnetic fields in the control pen that are higher than the noise floor.

Also shown on Figure 42 are the cumulative probability distributions for a 2-week period when the 500-kV line closest to the line pen was de-energized. During this period the other lines were still on. The median field during this period was 8 mG. In the analysis of melatonin data, the period when the line was out of service is referred to as "low-exposure." A 48-hour blood sample was taken on 1-3 November at the end of the low-exposure period. Electric field levels measured during this time are given below.

The audible noise data in Figure 43 and Table IV show that the differences between the mean AN values at the line and control pens are 3.2 and 7.6 dB in A-weighted and >6.5 kHz, respectively. If the measured AN was only from the line, the theoretical differences should have been at least 10 dB in A-weighted and at least 12 dB in >6.5 kHz. The reason the actual differences were much smaller than the theoretical differences is because the data base was contaminated with other noise; people working and talking, sheep bleating, airplane flyovers, etc.

Because of the high AN from the corona activity from these 500-kV lines, the microphone at the control pen was able to measure this noise; especially in foul weather. This can be seen in Figure 39 on 2 July from about midnight to 12 noon. This was during a period when there was fairly steady rain. The microphones in both the line and control pens

were able to measure this AN in both A-weighted and >6.5 kHz. The levels that occurred during this foul weather period were very typical for most of the rainy weather periods over the duration of the study. As can be seen, the A-weighted level was about 62 dBA whereas the >6.5 kHz level was about 53 dBA. In the control pen the A-weighted level during this same rainy weather period was about 46 dBA and the >6.5 kHz level was about 30 dBA. As can be seen from this data, the measured differences between the line and control pens during this rainy period when other noise sources were not prevalent are greater than the theoretical differences. This is because the theoretical difference is based upon an infinite line source which isn't the case at Ostrander. The span in which the measurements are being made terminates at the Ostrander Substation.

As can be seen in Figure 39, the fair weather AN data base is contaminated by man-made noise sources. A close examination of Figure 39 shows the trend of the fair weather AN. During the summer months, the daily variation of the fair weather AN appeared to follow the daily variation of the magnetic field. This relationship can't be firmly established because the magnetic field being measured comes primarily from the 500-kV line closest to the line pen whereas the AN comes primarily from the adjacent 500-kV line. The 500-kV line next to the line pen is much quieter than the adjacent 500-kV line. This is because each phase of the noiser line has a single 2.5-inch conductor whereas each phase of the quieter line has a three conductor bundle; each subconductor is 1.302-inches in diameter.

What appears to be relatively good correlation between fair weather AN and magnetic field was probably caused by the heating of the conductors due to the load current which, of course, is what produces the magnetic field. It is well known that corona increases as the relative air density decreases and relative air density is inversely proportional to temperature. This heating effect on the AN was more pronounced in the summer months because of the higher day-time ambient temperature. This is supported by the smaller variation of the fair weather AN in the winter months.

Figures 44 through 46 show the cumulative probability distribution's for the weather parameters that were collected during the study.

Figure 47 shows the mean of the electric and magnetic field levels that were measured in the line pen at 25 fixed locations when all transmission lines were energized. These measurements were conducted on a weekly basis over the duration of the study period. The overall averages for these electric and magnetic field measurements were 6 kV/m and 44 mG, respectively. The plastic tarp placed over a portion of the pen during prolonged rain reduced the electric field strength by around 10%. The tarp was in place about 25% of the time when electric field measurements were made. Therefore, the electric field variation over the pen was greater than the magnetic field variation.

Electric field strength in the control pen was typically < 10 V/m.

From 22 October to 5 November 1990, the Ostrander-Troutdale 500-kV line was de-energized for maintenance work. The line pen is located beneath this line. Electric field strength in the pen was mapped on

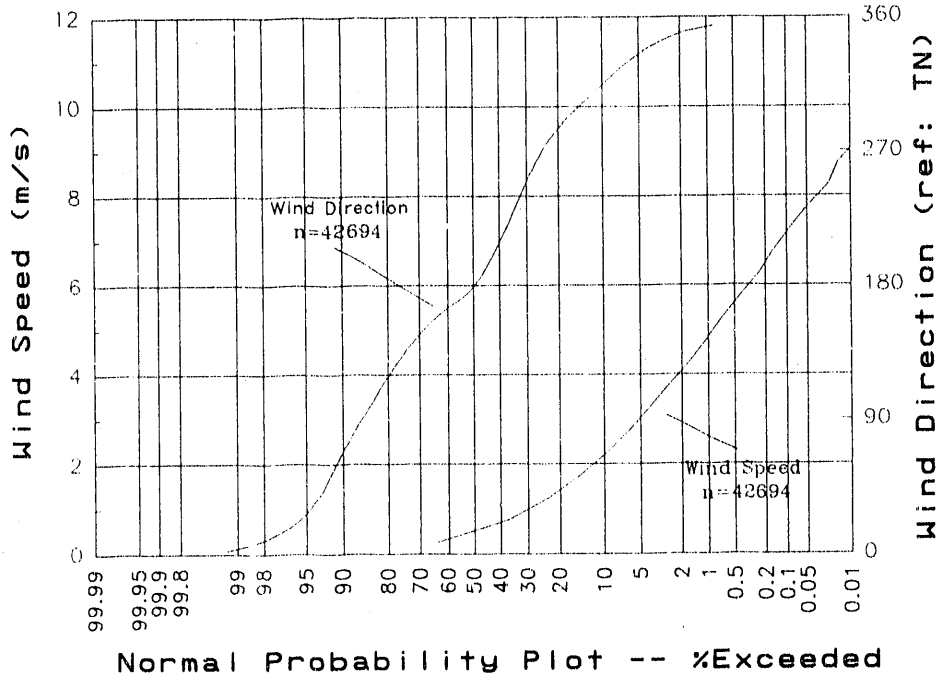


Figure 45. Cumulative probability distributions of the wind speed and direction measurements for the entire study period.

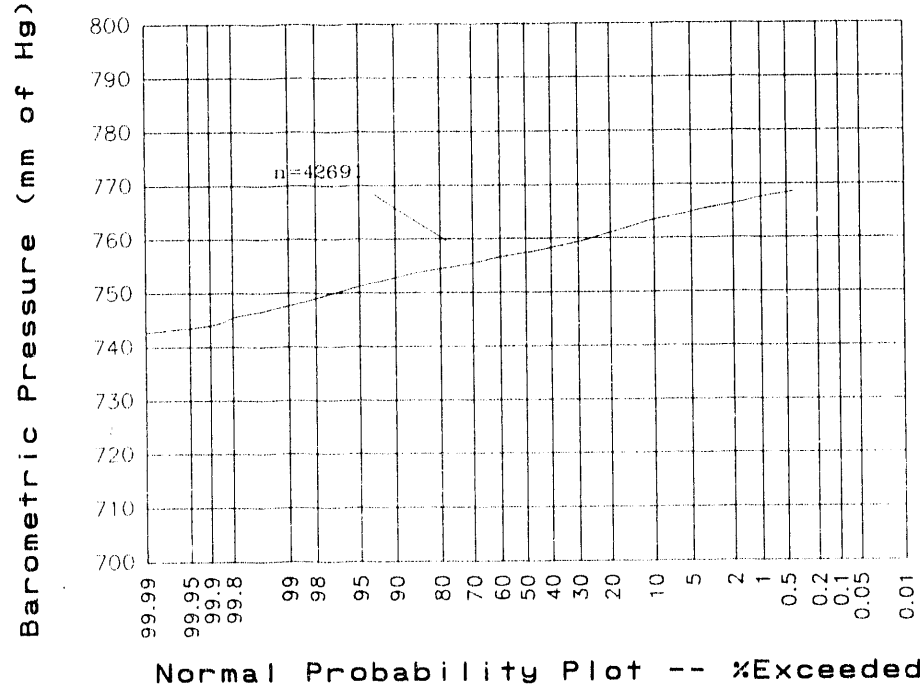


Figure 46. Cumulative probability distributions of the barometric pressure measurements for the entire study period.

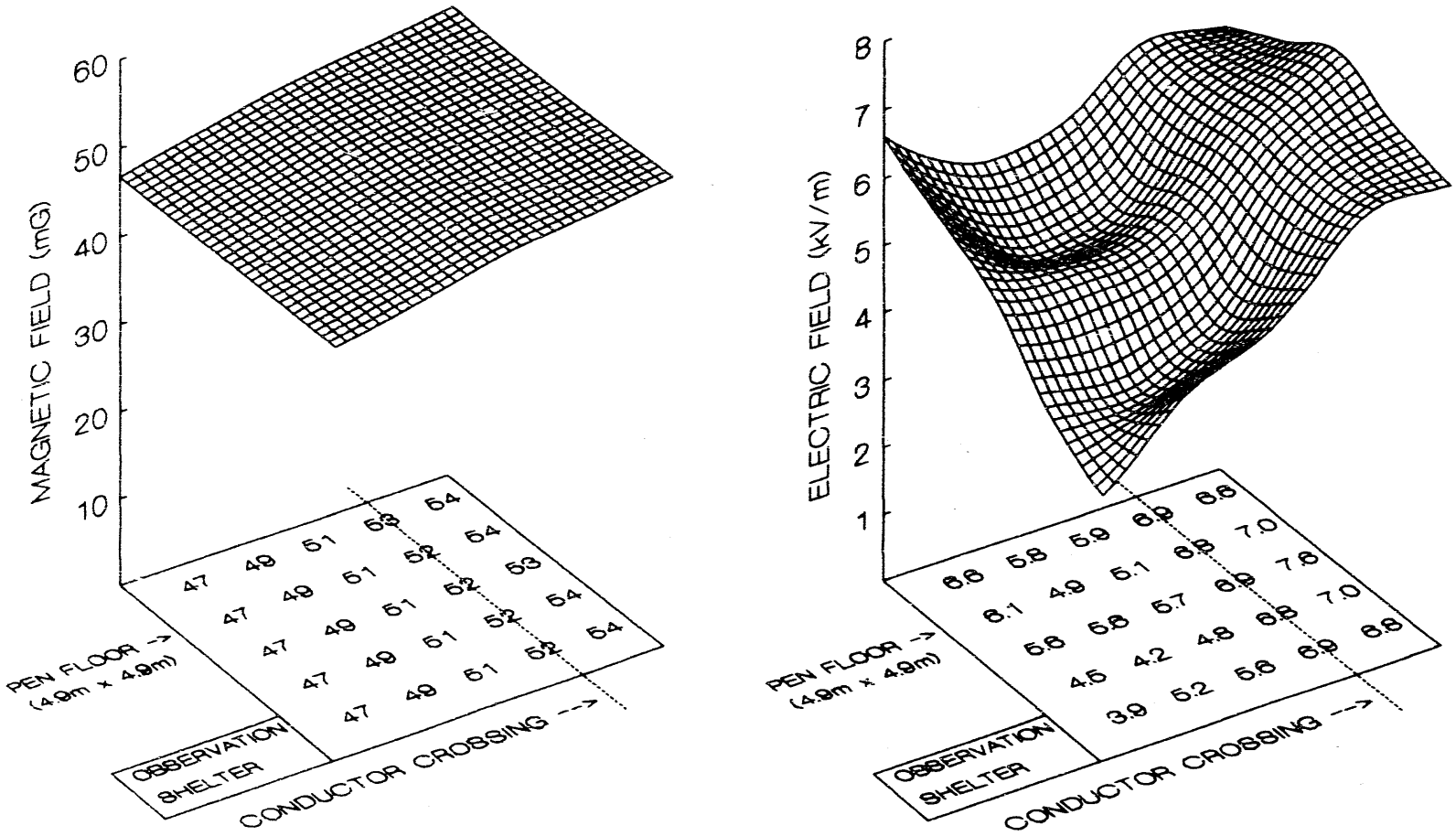


Figure 47. Magnetic (left) and electric (right) field measurements within the line pen. Measurements were made at 1 m above the floor at 25 locations. Average fields for these locations are shown in plan and 3-dimensional views.

three occasions during this low-exposure period. The overall mean electric field strength for the 25 measurement locations throughout the pen was 0.6 kV/m (range 0.3 - 0.9 kV/m).

A summary of outages for the five transmission lines in the corridor is shown in Table VI. Outages coinciding with 48-hour sampling periods and switching operations occurring during the sampling are listed in Table VII.

On 17 July 1990, photometric measurements were made in the line pen to determine the nighttime illumination levels of two 15-watt, red-coated incandescent light bulbs (Dickson, 1990). The measurements were made at a height to simulate eye level for a standing sheep at various locations within the pen. The results of these measurements are shown in Figure 48. Typical illumination levels in the pens were found to be about 13 lux, with a maximum of 22.5 lux. These levels are primarily due to the pen lights since the night-sky background illumination was only 0.6 lux.

The illumination levels from the red lights used in this study were greater than reported in other studies that measured nocturnal melatonin in sheep. However, in all other studies reviewed in this report, none described how red light illumination levels were measured. Yellon and Foster (1985) used a 15-watt red bulb that reportedly produced < 2 lux of illumination. Other studies simply reported that a "dim red light" was used (e.g., Kennaway et al., 1983). Earl et al. (1985) reported the use of a 40-watt red globe, but no illumination level was given.

Measurements of the relative intensity and spectral response of the red light bulbs were conducted by David McIntyre, Department of Physics,

TABLE VI

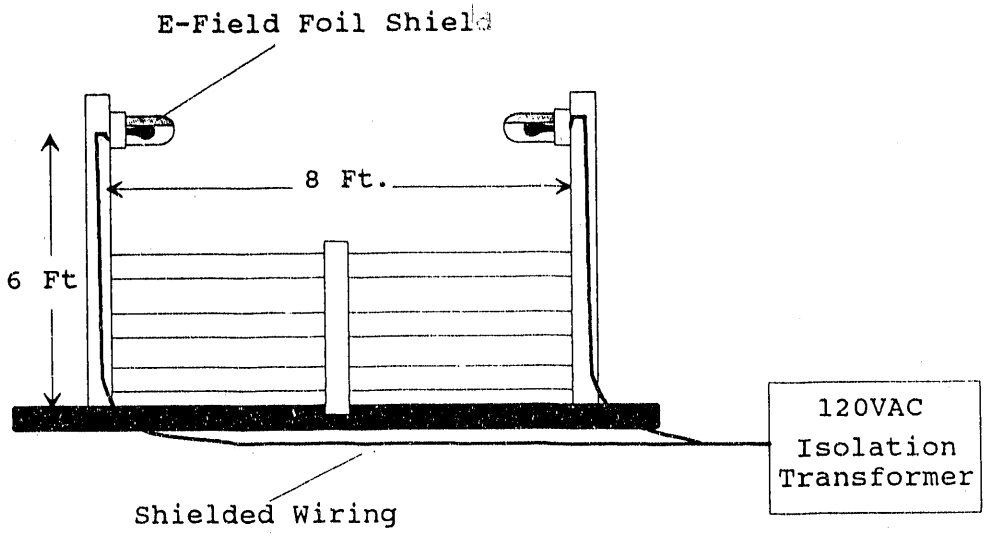
SUMMARY OF TRANSMISSION LINE OUTAGES
DURING THE STUDY PERIOD

Line Name	No. of Outages	Total Hours
Ostrander-Troutdale 500 kV (Crosses Line Pen)	9	375
Ostrander-Hanford 500 kV	19	58
Ostrander-Big Eddy 500 kV	5	8
Big Eddy-McLoughlin 230 kV	8	26
Big Eddy-Chemawa 230 kV	3	11

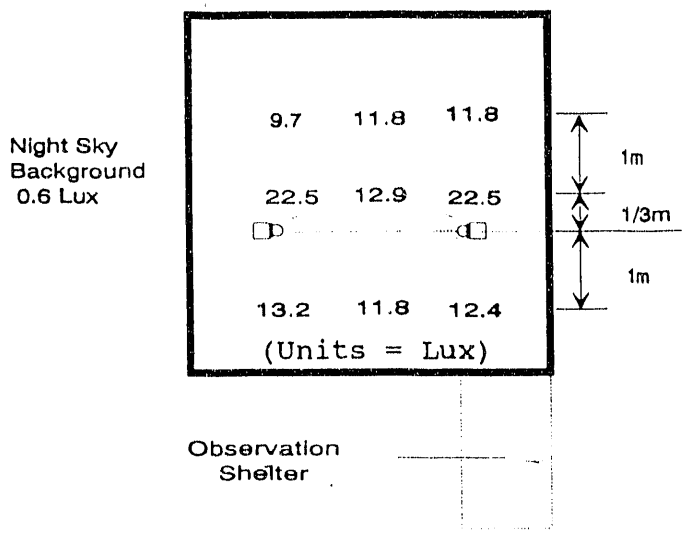
TABLE VII

LINE OUTAGES COINCIDING WITH 48-HOUR SAMPLES

Sample Dates	Line	Outage/Switching During Sample
1-3 Nov. 1990	Ostrander-Troutdale 500 kV	Outage 10 Oct. - 5 Nov. No Switching Operations
1-3 Nov. 1990	Ostrander-Hanford 500 kV	Outage 1 Nov., 0800-1630 h 1 Switching Operation
31 Jan. - 2 Feb. 1991	Ostrander - Hanford 500 kV	Outage 31 Jan., 1513-1515 h 2 Switching Operations



Sheep Pen Illumination Fixtures



Line Pen Illumination (07/17/90)

Figure 48. Sheep pen red light fixtures (upper) and nighttime illumination levels measured in the line pen on 17 July 1990 (lower).

Oregon State University. The results are shown in Figure 49. The red light bulbs did not transmit significant light below a wavelength of 600 nm.

Measurements of the earth d-c magnetic field were made at the site on 23 January 1992. The d-c field was not expected to vary over the duration of the project and the changes to the pens made between Phase 1 and when the fields were measured were not extensive. Therefore, the measurements made at that time were assumed to be representative of magnetic field levels during Phase 1 and the remainder of the study. The results of the d-c magnetic field measurements are given in Table VIII. The measurements are reported in terms of horizontal components perpendicular and parallel to the line, total horizontal component, vertical component and total field. The a-c fields from the line have essentially all vertical and perpendicular horizontal components. The calculated values for field inclination from horizontal and field declination from true north are also given in Table VIII for comparison with reported values.

The components and total field in the line and control pens were all comparable (within 1.6%). The total d-c field measured in the pens was 0.543 G in the control pen and 0.549 G in the line pen. The horizontal field components in the pens were consistently lower than the same components remote from the pens, as expected due to the influence of the steel flooring. However, the difference between the pen values and ambient fields is only slight. The vertical components in the pens were not consistently different from remote values. The measurements at the two locations remote from the pens are essentially identical and

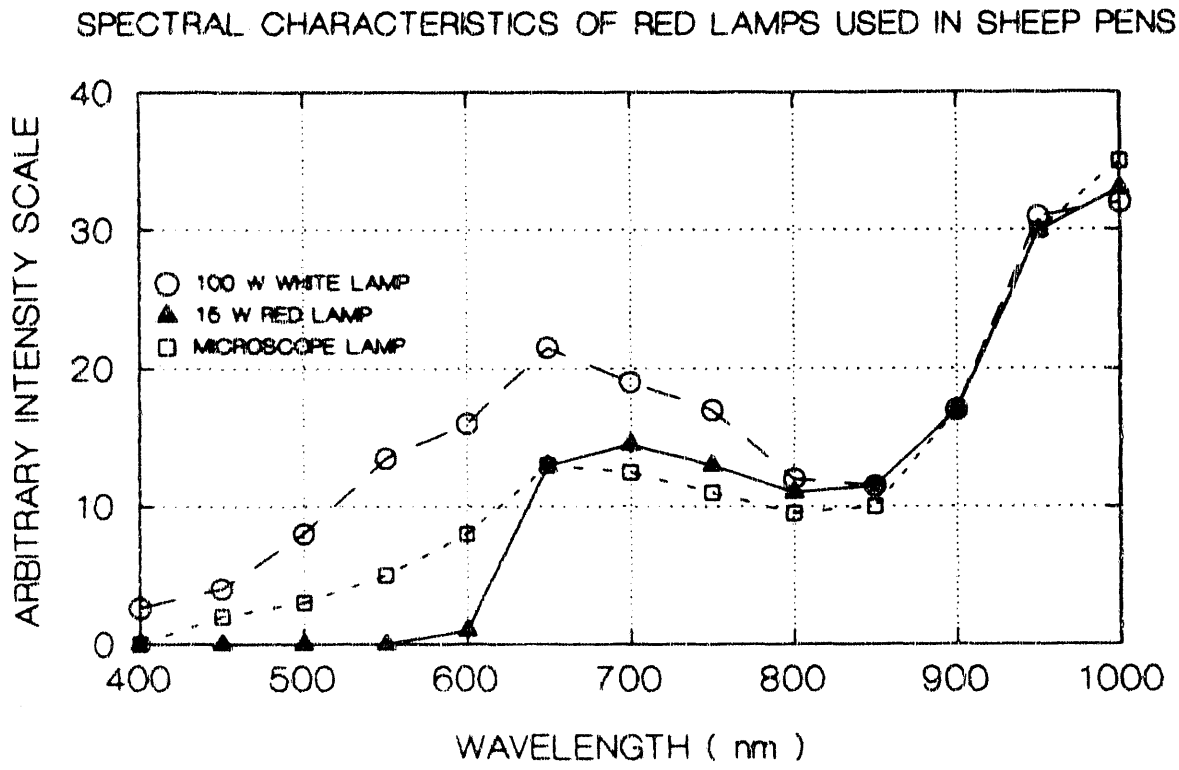


Figure 49. Relative intensity and spectral response of red light bulbs used in line and control pens.

TABLE VIII

RESULTS OF D-C MAGNETIC FIELD MEASUREMENTS IN GAUSS (G)
AT THE OSTRANDER STUDY SITE

	Magnetic Field, G					Orientation	
	Horizontal						
Location	Perpendicular to line	Parallel to line	Total horiz	Vertical Compon.	Total Field	Incli-nation	Decli-nation
Control pen	.154	.107	.188	.509	.543	69.8	22.5
Control Area 16.5 m west of pen	.172	.113	.205	.512	.551	68.1	21.0
Line pen	.153	.108	.187	.517	.549	70.1	22.9
Line area 25.9 m east of pen	.172	.112	.205	.512	.551	68.1	20.8

Reported declination: 19.0 east of north.
Boren, 1992.[a]

Reported inclination: 68.0 - 69.0 (1911 data).
Hodgman et al., 1956.[b]

Reported horizontal intensity: .21 G (1911 data).
Hodgman et al., 1956.[b]

a Boren, Gary, U.S. Geological Survey, Vancouver, WA. Private communication. 24 January 1992.

b Hodgman, C. D. ed. 1956. Handbook of Chemistry and Physics, 38th Edition. Chemical Rubber Publishing Co., Cleveland, OH 2423.

provide field magnitude and orientation values that are in agreement with reported values.

Electric Field Exposure

As discussed earlier, the electric field in the sheep pen with no animals or other conducting objects present is the unperturbed field. The field is perturbed by the presence of animals and can be enhanced (stronger) near the surface of the body. Also, the amount of current induced in the sheep by the electric field depends on body position and proximity of other animals. A short test was conducted to obtain some preliminary data on the electric field exposure received by an individual lamb.

Figure 50 shows data collected over a 15-minute period by an EMDEX-C meter attached to a small plate placed on line lamb number 15. The system was first calibrated by placing the plate at a fixed location in the pen where the unperturbed electric field was 7.5 kV/m. The current collected by the plate amounted to 0.018 μA/(kV/m). Next, the plate and meter were attached to number 15 and a measurement was taken while she stood alone with her head upright in a 7.5-kV/m field (unperturbed). Attached to the animal, the current collected by the plate increased to 0.197 μA/(kV/m). This value is the factor used for converting current to an equivalent electric field.

The current collected by the plate on the sheep, divided by the current collected by the plate alone represents the enhancement factor for the electric field. The enhancement factor was, therefore, 11 (0.197/0.018 = 11). This means that the enhanced electric field at the

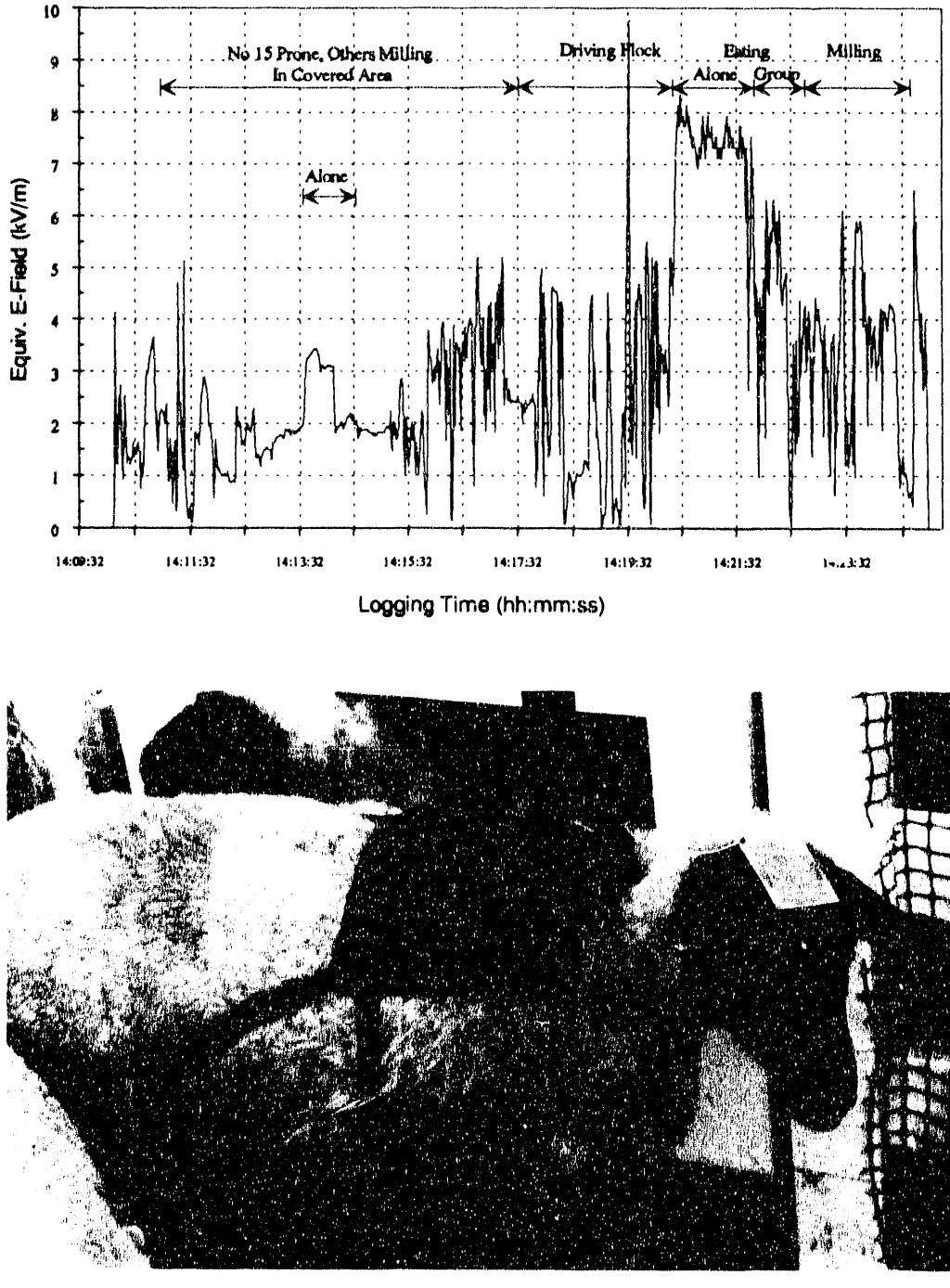


Figure 50. Results of a test to monitor electric field exposure received by an individual sheep (upper). A 100 x 50 mm sensor plate was glued to the top of the animal's head and connected to an EMDEX-C data logger placed in a pouch on the animal's back (lower).

top of a lamb's head, when she was standing alone in an unperturbed electric field of 7.5 kV/m, was approximately 82.5 kV/m.

During the 15-minute test, lamb number 15 was allowed to move about the pen with and without other lambs near her. During this period, the activity and proximity of the other lambs were noted. The average equivalent electric field strength during the test was 2.8 kV/m. As shown in Figure 50, the posture of number 15 and the presence of nearby lambs both affect the equivalent field exposure considerably. The highest exposure occurred while number 15 was standing up and eating alone. When she was lying down alone, the equivalent field was reduced by about half.

DISCUSSION

Electrical Monitoring

Results of the electrical monitoring program demonstrate that electric and magnetic field levels in the line pen during the study are typical of BPA 500-kV lines. The average electric field (6 kV/m) is a typical maximum for such lines. Measurements made of 359 spans of BPA 500-kV lines showed an average electric field strength of 3.7 kV/m (Bracken and Ray, 1980). Less than 5% of the measured spans had maximum fields greater than 6 kV/m.

The plastic pen components, including the sun screen and the tarps used during heavy rain, resulted in only a small reduction of the electric field strength. More significant shielding in one corner of the pen was caused by the presence of the attached observation shelter. Even so, the average electric field measured in the line pen near the shelter (3.9 kV/m) was nearly identical to the average level for 500-kV lines in the study by Bracken and Ray (1980).

The mean magnetic field strength measured near the line pen (40 mG) during the study is a representative level for BPA 500-kV lines. Spot measurements made in 1989 of magnetic fields for several BPA 500-kV lines showed that the average field ranged from 32 to 61 mG on the right-of-way (Perrin et al., 1991). Calculations in a recent analytical study indicated that the average magnetic field on the right-of-way for BPA 500-kV lines ranged from 26 to 65 mG (Stearns and Tuominen, 1992).

Sheep in this study were, therefore, exposed to field strengths that are typically encountered outdoors near 500-kV lines.

The field monitoring and measurement program for this study showed that electric and magnetic field levels in the line pen were two orders of magnitude greater than in the control pen. This demonstrates that there was a large difference between the exposures received by lambs in the line and control groups. The field levels measured in the control pen are on the low end of the ambient range of levels typically measured in homes and offices (Lee et al., 1989).

Both mean and maximum A-weighted audible noise within line and control pens were similar. Mean noise > 6.5 kHz was somewhat higher in the line pen although maximum noise levels in this frequency range were similar between line and control pens. Since no effects were documented in this study between line and control sheep, these noise levels apparently had no measurable biological influence.

Melatonin

The serum melatonin of all lambs in this study from 8 weeks of age on showed the typical pattern reported for lambs (Claypool et al., 1989) and for most other species studied (Reiter, 1986). Levels were very low and stable during the day with a very rapid increase shortly after sundown. Levels typically remained elevated throughout the night, although with high fluctuations, until declining rapidly around sunrise. Claypool et al. (1989) found that melatonin patterns under natural photoperiods were established in most lambs in their Michigan

study by 1 week of age. All lambs in their study showed the typical pattern from 6 weeks of age on.

Melatonin amplitude tended to be higher during winter than during spring and summer (Figure 21). The former corresponds to periods of longer nights and increased age of the lambs. Claypool et al. (1989) also found that night melatonin levels tended to increase with age of lambs from 6 to 27 weeks. Two studies of adult ewes in natural photoperiods revealed no consistent differences in mean night melatonin levels throughout the year (Rollag et al., 1978; Kennaway et al., 1983).

Partly because of high variation, many investigators question the biological significance of the shape or amplitude of melatonin elevation in regulating sheep reproductive cycles (Yellon and Foster, 1986). As shown in Figure 20, there was about a twofold difference in the overall mean melatonin range between high and low individuals in both control and line lamb groups. All lambs, however, reached puberty at about the same time. The duration of the elevation appears to be the critical factor. The duration of the melatonin elevation in lambs in this study closely reflected the length of the dark period (Figure 17). Thus, the melatonin signal appeared to provide information about the sequence of long days followed by short days required for initiation of the fall breeding season (Foster et al., 1985).

Except for the first night of the pre-exposure period, mean phase of the melatonin elevation tended to be advanced in both line and control groups (Figure 25). There was no difference in phase between groups, however. Younger lambs in Michigan had phase delays in melatonin at 1 and 6 weeks of age (Claypool et al., 1989). By 27 weeks

of age, however, the Michigan lambs showed either no phase change or a slight phase advance. Results of the present study are, therefore, generally consistent with that study (Claypool et al., 1991).

Studies of rodents suggest that melatonin phase may have some influence in regulating reproductive cycles. However, the prevailing view is that this is probably not the case for sheep (Wayne et al., 1988).

This study was conducted to test the hypothesis that chronic exposure of sheep to a 500-kV transmission line results in a large depression of nighttime melatonin, as reported in rodents exposed to electric or magnetic fields under laboratory conditions. Results provide no evidence to support such an effect in lambs under conditions of this study. Results of some early studies of rodents showed that the normal melatonin elevation in rats was essentially abolished by exposure to 60-Hz electric fields (Wilson et al., 1981). In theory, if such a large effect occurred in sheep and no nighttime melatonin elevation occurred, there could be a significant effect on the timing of the reproductive cycle in sheep.

This study also found no evidence for a significant phase delay in melatonin, as reported in rats exposed to 60-Hz electric fields (Reiter et al., 1988).

Statistical analyses revealed no differences between group means for night melatonin amplitude, duration, or phase. Patterns of melatonin of the line group were also examined in relation to exposure conditions. The only statistically significant difference between two-night average samples adjacent to pre-, low-, or post-exposure was

an increase in mean amplitude in the first exposure sample (Figure 22). Studies cited earlier, of rodents exposed to electric and magnetic fields, generally report decreases in melatonin amplitude.

In most studies, melatonin analyses typically involved testing group means. Wilson et al. (1990) studied a urinary melatonin metabolite in people who used various types of electric blankets. They found evidence that certain types of exposure from specially-constructed electric blankets affected melatonin in some sensitive individuals. A typical pattern in such individuals was an increase in melatonin following cessation of exposure. Inspection of Figure 23 indicates that only one line lamb (no. 16) showed a noticeable increase in melatonin in the post-exposure sample. One of the control lambs (no. 4) also showed an increase during this period (Figure 24). Although there was no indication of melatonin responses associated with samples adjacent to various exposure conditions, it is possible that responses may occur within time intervals shorter than the adjacent periods in this study.

There are a number of possible reasons why effects of electric and magnetic fields reported on melatonin in other species were not apparent in this study. One general consideration is interspecies differences in susceptibility of pineal melatonin patterns to alteration by environmental factors. One of the factors most studied is light. Although light during the dark period can significantly depress melatonin levels, there is great variation among species in the minimum light intensity required for suppression (Reiter, 1985).

In general, nocturnal species appear to be much more sensitive to effects of light on melatonin suppression than diurnal species. For

albino laboratory rats, 15 minutes of exposure to white light irradiance as weak as 0.0005 $\mu W/cm^2$ can significantly suppress pineal melatonin (Webb et al., 1985). In domestic rams, 0.04 $\mu W/cm^2$ of white light for 1 hour did not suppress melatonin, while a significant decrease was observed at an intensity of 0.3 $\mu W/cm^2$ (Arendt and Ravault, 1988).

In most cases, normal room lighting does not have a significant effect on night melatonin levels in humans (Lewy et al., 1987). Much higher levels (2500 lux) are required to depress levels in humans (Lewy et al., 1980). Reiter (1990) estimated that the 2500 lux level is approximately equivalent to 150 $\mu W/cm^2$.

Studies of rodents (e.g., Wilson et al., 1981), humans (Wilson et al., 1990), baboons (Rogers et al., 1991), and this study of sheep suggest interspecies differences in melatonin suppression from electric and magnetic fields. Of possible interest in this regard are differences between rodents and sheep in the functions of pineal alpha and beta adrenergic receptors (Morgan et al., 1989; Howell and Morgan, 1991). Cell surface electrical currents induced by 60-Hz fields may interact with surface receptors in different ways. Only further research will clarify whether interspecies effects of electric and magnetic fields on melatonin parallel those of light.

Another general difference between this environmental study and laboratory studies involves the type of electric and magnetic field exposures used. In this study, lambs were exposed to the actual fields produced by three-phase transmission lines. In contrast, laboratory studies used a variety of a-c and d-c electric and/or magnetic fields of various intensities, none of which exactly duplicated actual powerline

fields. There is some evidence that rapid field changes associated with switching fields on and off may be more significant in affecting melatonin than continuous field exposure (Lerchl et al., 1991). During this study, only a few line-switching operations occurred during 48-hour samples, and none of these were at night (Table VII).

In terms of field intensity, unperturbed electric field strength in this study was well above the 2 kV/m level reported to affect melatonin in laboratory rats (Wilson et al., 1983). The magnetic field exposure in the study was also comparable to levels of 50-Hz magnetic fields recently reported to affect melatonin in laboratory rats (Kato et al., 1991).

Mutual shielding of animals in this study reduced, to varying degrees, the effective electric field exposure received by the lambs. However, the same general effect occurs with laboratory animals in electric field studies. For example, young rats in the study by Reiter et al. (1988) presumably were significantly shielded at times while near their mother and littermates, yet their nighttime melatonin was still reduced by 60-Hz electric fields as low as 10 kV/m.

At this time, it is not possible to reconcile the negative results of this study with positive results of some laboratory animal studies. However, the effect of electric fields on melatonin in laboratory animals is not as robust as was initially believed. The researchers who originally found the effect of 60-Hz electric fields on melatonin in laboratory rats have recently reported that they were not able to reproduce the effect in their latest study (Sasser et al., 1991).

Results of this environmental study point out the limitations of extrapolating from data obtained from laboratory rodents exposed to some form of electric and/or magnetic field. Laboratory studies are useful in screening for possible kinds of field effects and for investigating mechanisms of interaction between organisms and applied fields. A major goal, however, is to determine whether people and animals are affected adversely by fields produced by electric power facilities and equipment. Studies in which the species of interest are exposed to the fields that actually occur in the environment are, therefore, essential for ultimately assessing the impacts, if any, of these fields.

Progesterone and Estrous Cycles

Since melatonin patterns of lambs in this study appeared to be normal, and their weight gains were good, puberty would be expected to occur in the fall. Progesterone levels, in fact, showed that all lambs in both groups reached puberty by September or October. We could find no published data on puberty in spring-born Suffolk lambs in Western Oregon. However, discussions with sheep producers in this area revealed that our findings are consistent with their experience.

In Michigan, spring-born female Suffolk lambs raised in natural photoperiods reach puberty at around 26-35 weeks of age (Foster and Ryan, 1981). In this study, in both control and line groups, puberty occurred at a mean age of around 34 weeks. After the first ovulation, most of the lambs cycled at the usual interval of approximately 16 days. Through 7 March, there was no difference in the mean number of cycles in the control and line groups.

After an initial normal estrous cycle, control lamb no. 7 exhibited elevated progesterone levels through the remainder of the study (Figure 26). This condition may have been caused by an abnormality in the production of prostaglandin from one of the uterine horns. Prostaglandin is thought to play a local role in causing regression of the corpus luteum on the ipsilateral ovary in domestic animals, and consequently the cessation of progesterone secretion (Hecker, 1983; Alila and Dowd, 1991; Gibori and Miller, 1982). Some of the prostaglandin released from the uterine horn which enters the uterine vein, diffuses into the closely apposed ovarian artery (Hecker, 1983). If one uterine horn is removed in the ewe, luteal function is prolonged in the ipsilateral ovary, but there is no effect on the contralateral ovary (Gibori and Miller, 1982).

In the case of lamb no. 7, it is possible that the first normal ovulation occurred from an ovary associated with a functioning uterine horn. The next ovulation, however, may have occurred from the other ovary where there was some abnormality in the associated uterine horn production of prostaglandin. Thus, luteal regression did not occur, and the continued production of progesterone prevented further estrous cycles from occurring. Because no necropsy was performed on lamb no. 7, the above explanation for the results shown in Figure 26 is only speculation.

Cortisol

Results of this study provide evidence for a circadian rhythm of serum cortisol levels in growing lambs. However, previous studies of

cortisol in sheep report either no evidence for diurnal rhythms (Bassett, 1974) or a rhythm with levels elevated at night (Fulkerson and Tang, 1979) or during the day (McNatty et al., 1973). The frequency of blood sampling no doubt had some role in the outcome of these earlier studies. Because of the highly variable nature of the cortisol secretion, high sampling rates are necessary to detect the rhythm. This probably explains the rhythms found in the study by Fulkerson and Tang (1979) in which samples were taken every 10 minutes. Those authors suggested that the elevated nighttime cortisol levels they found may reflect the intense rumination occurring at that time. Lambs in this study also were presumably ruminating at night, but lowest cortisol levels tended to occur at that time. Differences in facilities, animal management, feeding times, or data collection techniques between the various cortisol studies are probably responsible for the differences among their results.

The animals in this study may have been responding to the metal slatted floor of their pens. In both the line and control group, cortisol levels were significantly lower in post-exposure when all animals were taken off the metal floors and placed on sawdust. Although the metal flooring used in the study is frequently used by sheep producers, we are not aware of any previous research on cortisol levels in sheep maintained on such facilities.

The elevated cortisol in both groups during the pre-exposure period seems consistent with the presence of multiple known stress factors during this time. These include weaning, handling and restraint, introduction to new facilities, and transport (Dantzer and Mormede,

1983). It appears, however, that following this initial stress, mean stress levels (as suggested by serum cortisol) decreased and then remained relatively constant throughout the exposure part of the study.

The sharp increase in cortisol levels following transport in a stock trailer is consistent with results of other studies. For example, 30 minutes of transport caused a threefold increase in total serum cortisol in ewes (Fell et al., 1985).

Overall, this study provides no indication of stress responses associated with chronic exposure of lambs to a 500-kV transmission line. In this study, the animals were purposely placed on a grounded, metal floor to prevent or minimize the occurrence of shocks due to induced voltages. Different results might be expected if animals were frequently receiving spark discharge shocks in an ungrounded facility. Possible stress effects in some studies of laboratory animals, reviewed previously, may also have been related to shocks or to perception effects from the strong electric fields used in some studies.

Wool and Body Growth

Wool growth and body weight gain in both groups of animals were nearly identical throughout the study. Since no effects were noted on melatonin patterns, no effect would be expected on the seasonal growth of wool. Growth rate throughout the study was excellent, and all animals were well above minimum weights reported as necessary for lambs to achieve puberty. This study provides no evidence that exposure to electric and magnetic fields affects growth as reported in some studies

of laboratory rodents (Marino, 1990) and monkeys (Grissett and Lotz, 1985).

Behavior

The overall behavior of lambs throughout the study was very consistent, and there were no apparent differences between groups. More than half the observations during each 24-hour sample were of resting animals. Time spent feeding accounted for less than 20% of all observations. When on pasture, sheep may spend an average of 10 hours per day grazing, and 8-10 hours ruminating (Hecker, 1983).

In one laboratory study, sheep stood up 70% of the time over 24 hours (Ruckebusch, 1972). They were awake for about 16 hours per day.

Done (1975) reported that sheep confined in a laboratory animal house spent about 20% of the time lying down. The percentage of time animals spent lying down in the present study is significantly greater than in these two studies. This suggests that animals may have responded negatively to standing for long periods on the slatted metal floor.

There were no obvious differences in distribution between line and control lambs throughout their pens. There was a tendency for animals to spend a slightly greater amount of time in the "right back" pen section. This was most apparent during the June observation. The use of this section of the pen was probably influenced by the presence of personnel in the attached observation shelter. Study personnel also typically entered through a gate in this section to feed the animals.

CONCLUSIONS

During the course of the past 2 decades a large number of studies have been conducted to evaluate the possible effects of electric and magnetic fields on biological systems of various animal types under both in vitro and in vivo conditions. Except for a few studies, results have been equivocal for various reasons. In this project, every attempt was made to conduct a rigorous study in which the results could not be challenged because of inappropriate design or other mitigating factors. This study was specifically aimed at examining those endogenous factors that could, if altered by exposure, interfere with the normal development and attainment of puberty.

Based on the results of this study, the following conclusions are advanced:

Exposure of ewe lambs from 8 to 52 weeks of age to the electrical environment of a 500-kV 60-Hz transmission line:

(1) did not interfere with body growth, wool growth or behavioral characteristics,

(2) did not significantly alter the nighttime duration, phase, or magnitude of melatonin secretion,

(3) did not constitute a form of stress as evidenced by changes in serum concentrations of cortisol, and

(4) did not interfere with the attainment of puberty at the anticipated time.

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APPENDIX A

PATHWAY OF NEURAL CONNECTIONS BETWEEN THE EYES AND THE PINEAL GLAND AND A SUMMARY OF BASIC STEPS IN THE SYNTHESIS OF MELATONIN

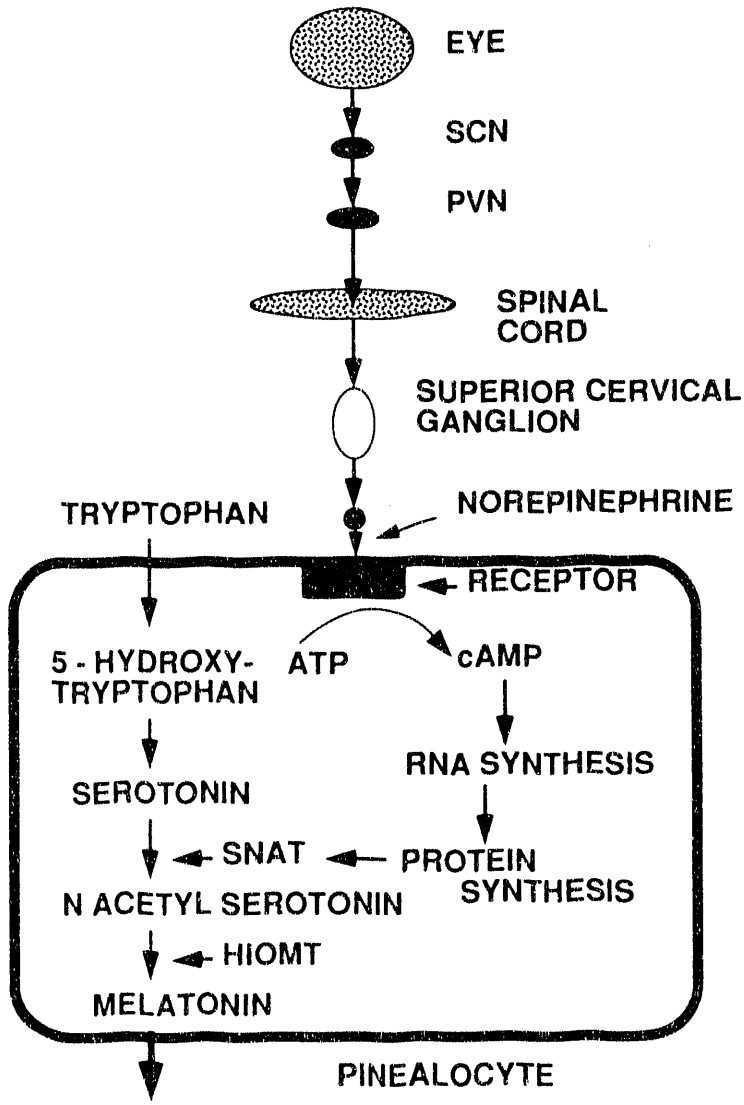


Figure 1. Neural connections from the eye to the pinealocytes in the pineal gland and the overall process for the synthesis of melatonin. Abbreviations: SCN = suprachiasmatic nuclei, PVN = paraventricular nuclei, ATP = adenosine triphosphate, cAMP = cyclic adenosine monophosphate, SNAT = serotonin N-acetyltransferase, HIOMT = hydroxyindole-O-methyltransferase. Sources: Reiter, 1989; Wilson et al., 1981.

APPENDIX B

ANALYSIS OF VARIANCE TABLE FOR THE MELATONIN STUDY DESIGN

TABLE I

ANALYSIS OF VARIANCE TABLE FOR THE MELATONIN STUDY DESIGN. THE DESIGN INVOLVES TWO GROUPS OF 10 LAMBS EACH, EIGHT 48-HOUR SAMPLES, AND 2 NIGHTS IN EACH SAMPLE

Source of Variation	Degrees of Freedom	F (0.05)
Group	1	4.41
Error	18	
Sample	7	2.09
Sample x Group	7	2.09
Error	124[a]	
Night	1	4.41
Night x Group	1	4.41
Error	18	
Sample x Night	7	2.09
Sample x Night x Group	7	2.09
Error	124[a]	

a Error df reduced from 126 because of the loss of one control lamb for the last two 48-hour samples.

APPENDIX C

NECROPSY REPORT ON CONTROL LAMB 6

VETERINARY DIAGNOSTIC LABORATORY
College of Veterinary Medicine
Oregon State University
P.O. Box 429
Corvallis, Oregon 97339-0429 (503) 737-3261

Accession Number: D91-07114

REPORT OF LABORATORY EXAMINATIONS

FRED STORMSHAK

Specimens submitted: 1 SHEEP
Animal ID: SUFFOLK EWE
Species: OVINE
Age: 1 YEAR
Sex: F

Mail to - Account:
OSU ANIMAL SCIENCE
OREGON STATE UNIVERSITY
CORVALLIS OR 97331

Date Specimen Received: 01/16/91
Preliminary Report On:
Final Report On: 01/30/91

Communications: |__| oral |XX| phone

Dr. Thompson 1-16-91 SPS

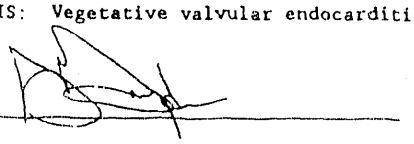
PATHOLOGIST REPORT:

NECROPSY: Presented for necropsy in good postmorten condition is a yearling Suffolk ewe with a somewhat distended abdomen and "bottle jaw." There is marked subcutaneous edema in the intermandibular area, ventral neck, brisket, and ventral abdomen. The thoracic and abdominal cavities are distended with several liters of yellow fluid which clots readily upon standing, indicating high protein content. The lungs are wet, heavy and edematous. The pericardial sac is distended with fluid containing clots of fibrin. The heart is round in shape due to marked dilatation of the left ventricle. Irregular scarred myocardial infarcts are in the left ventricle at its apex and in the free wall. These range from 1-2 cm diameter and have resulted in marked thinning of the heart wall. Vegetative valvular endocarditis affects all three cusps of the aortic valve. This also crowds the orifices into the coronary circulation and likely represents the pathogenesis for the old myocardial infarcts seen. The liver is swollen and passively congested.

MICROBIOLOGY: See attached report.

COMMENT: This is a rare lesion in sheep and only seen with any frequency at all in sheep that have had indwelling jugular catheters. The bacteria cultured are typical skin bacteria. Presumably bacteria gain entrance via the catheter and colonize the valve, causing the valvular endocarditis. In this case emboli from the valvular lesions probably passed into coronary circulation causing myocardial infarcts. This occurred several weeks to months ago, as these infarcts are now fully scarred.

FINAL DIAGNOSIS: Vegetative valvular endocarditis and scarred myocardial infarcts

SIGNED: 

Stanley P. Snyder, DVM, PhD

APPENDIX D

MELATONIN CONCENTRATION FOR INDIVIDUAL LAMBS FOR ALL 48-HOUR SAMPLE PERIODS

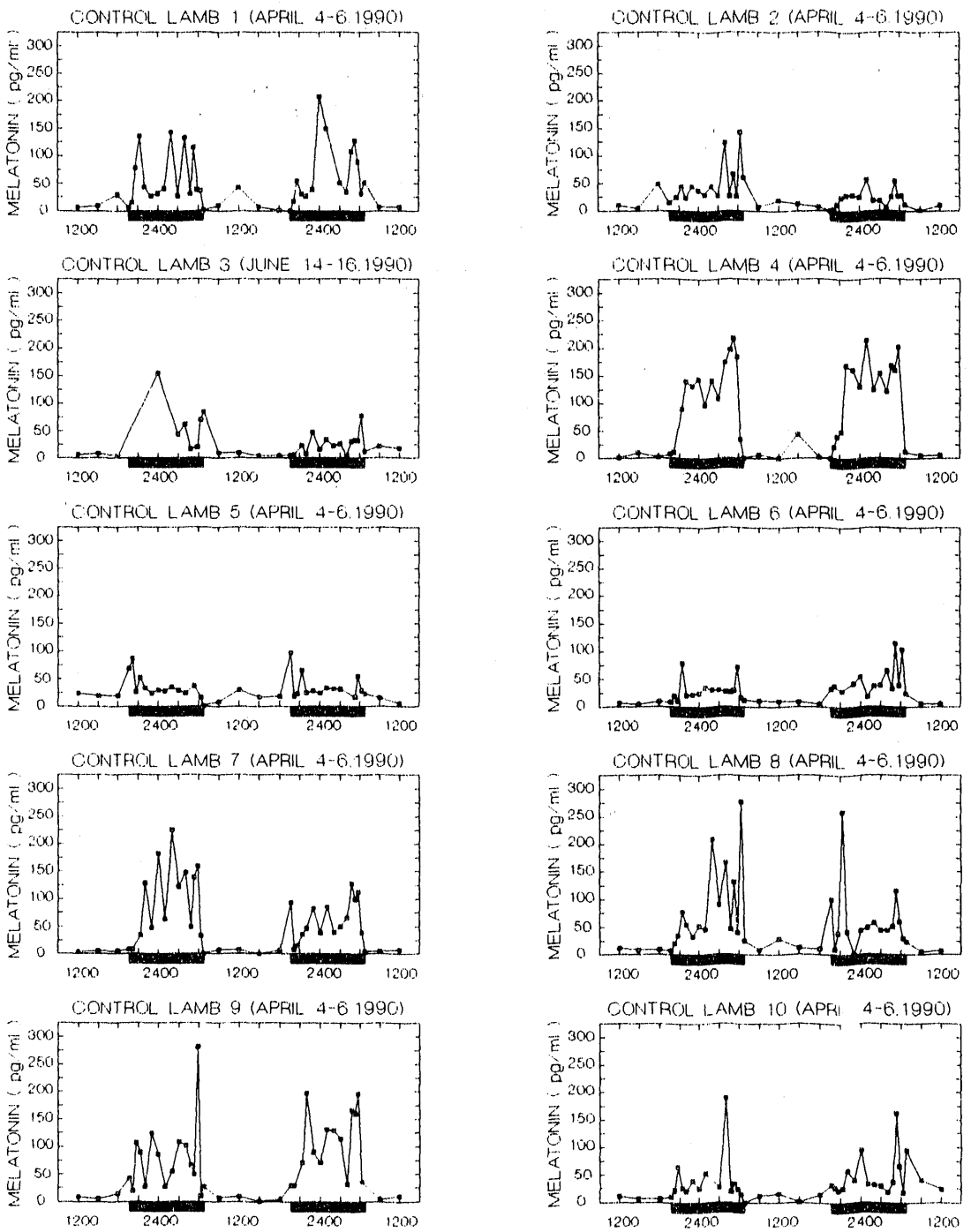


Figure 1. Melatonin concentration (pg/ml) in control lambs for 4-6 April 1990 (Pre-exposure).

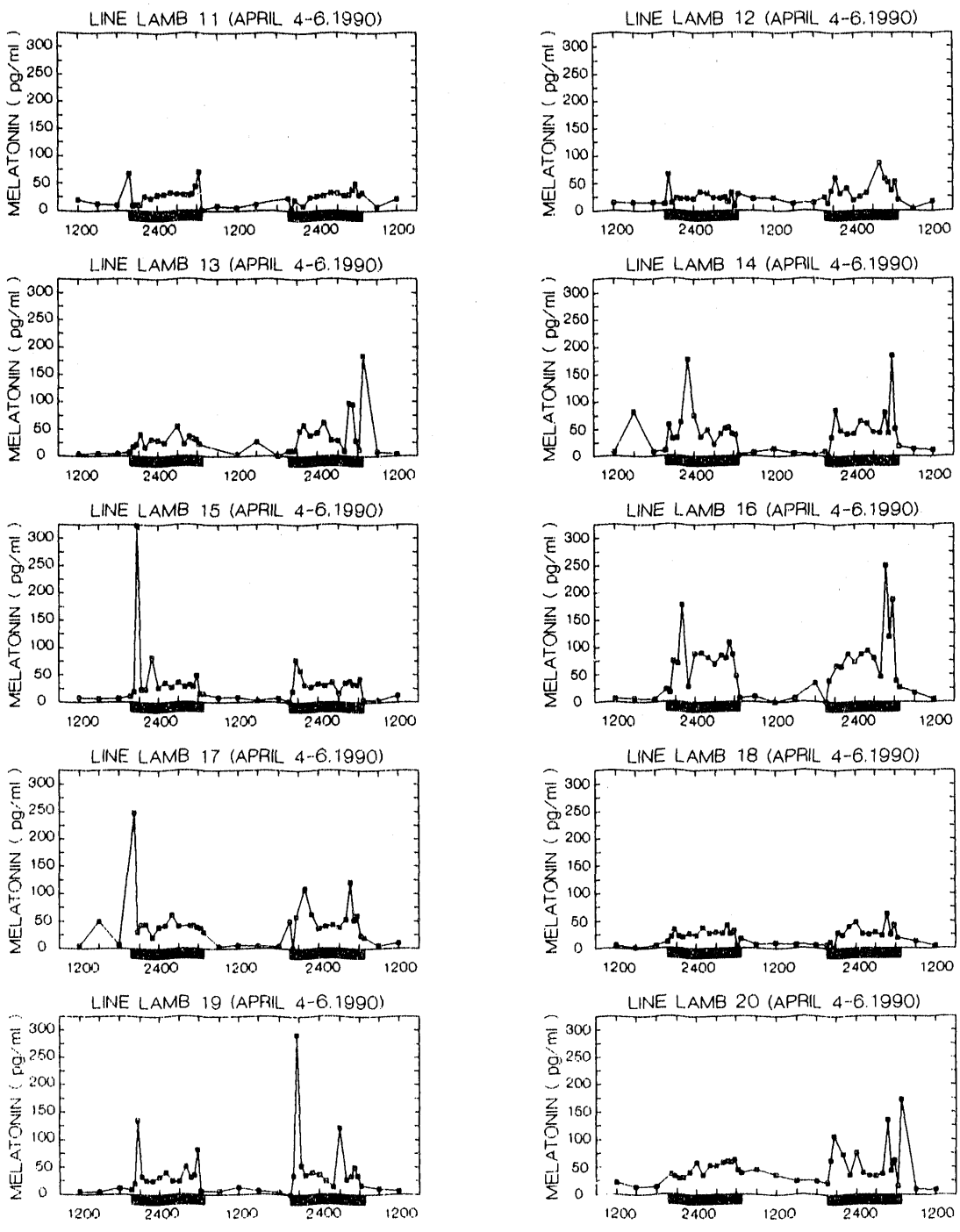


Figure 2. Melatonin concentration (pg/ml) in line lambs for 4-6 April 1990 (Pre-exposure).

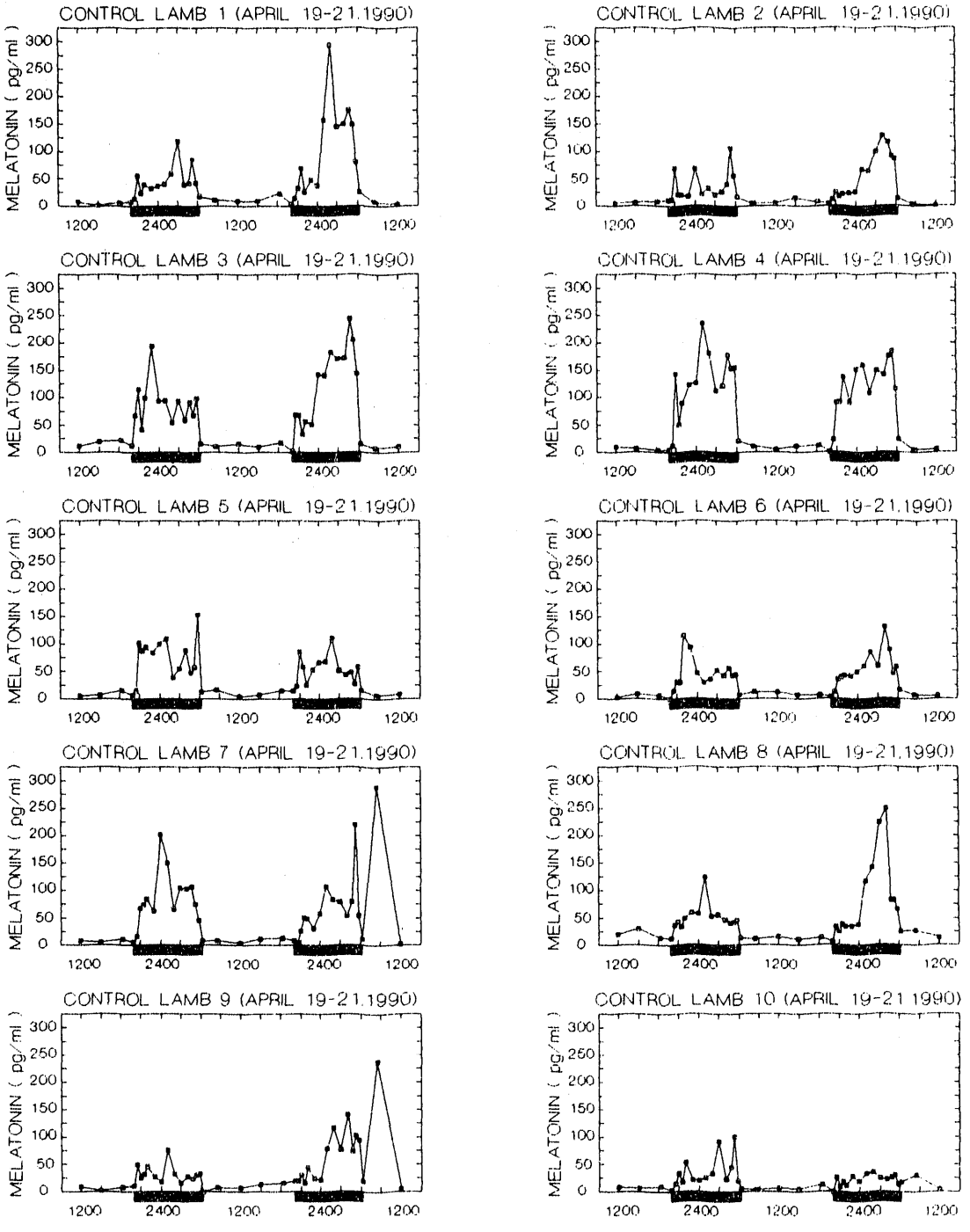


Figure 3. Melatonin concentration (pg/ml) in control lambs for 19-21 April 1990.

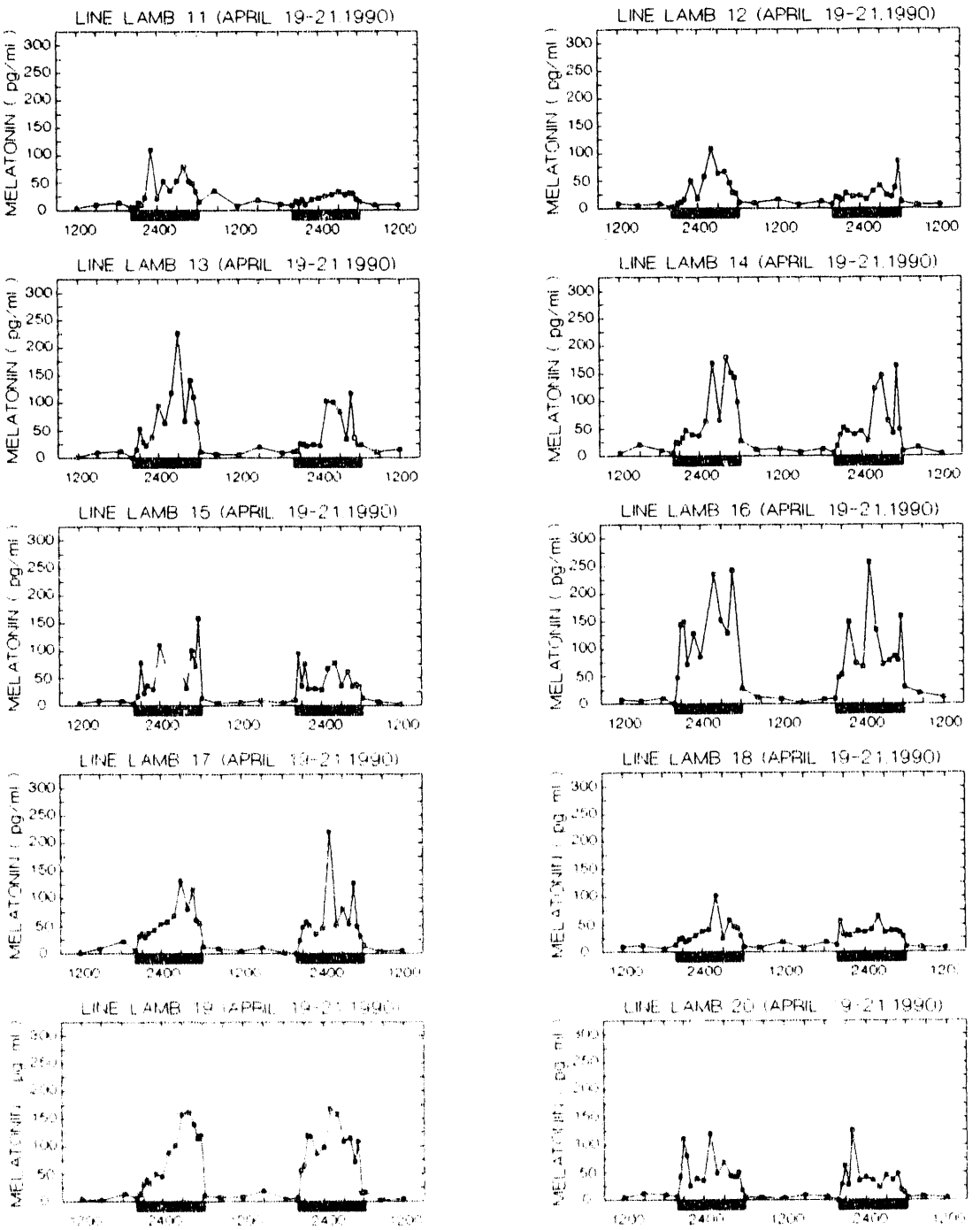


Figure 4. Melatonin concentration (pg/ml) in line lambs for 19-21 April 1990.

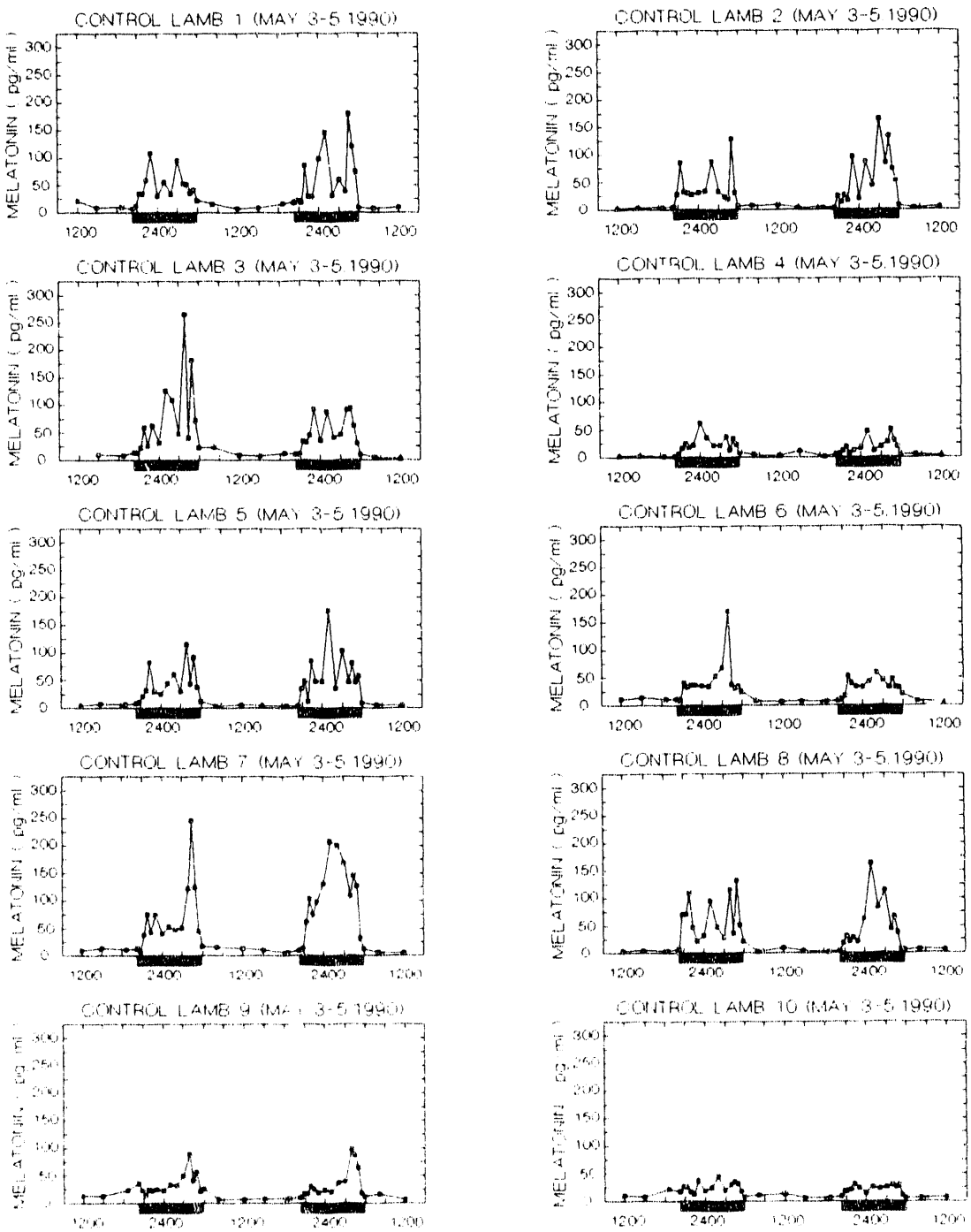


Figure 5. Melatonin concentration (pg/ml) in control lambs for 3-5 May 1990.

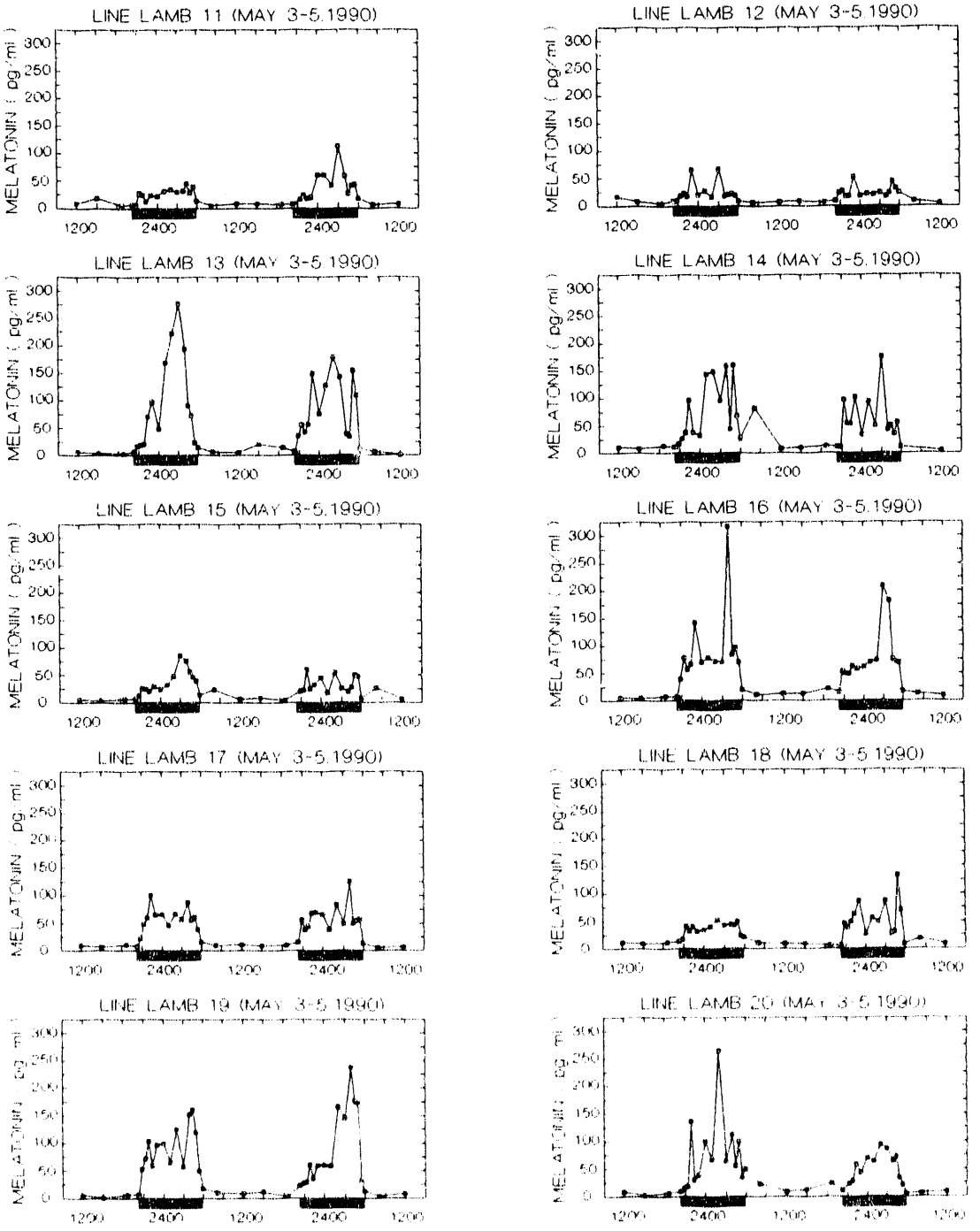


Figure 6. Melatonin concentration (pg/ml) in line lambs for 3-5 May 1990.

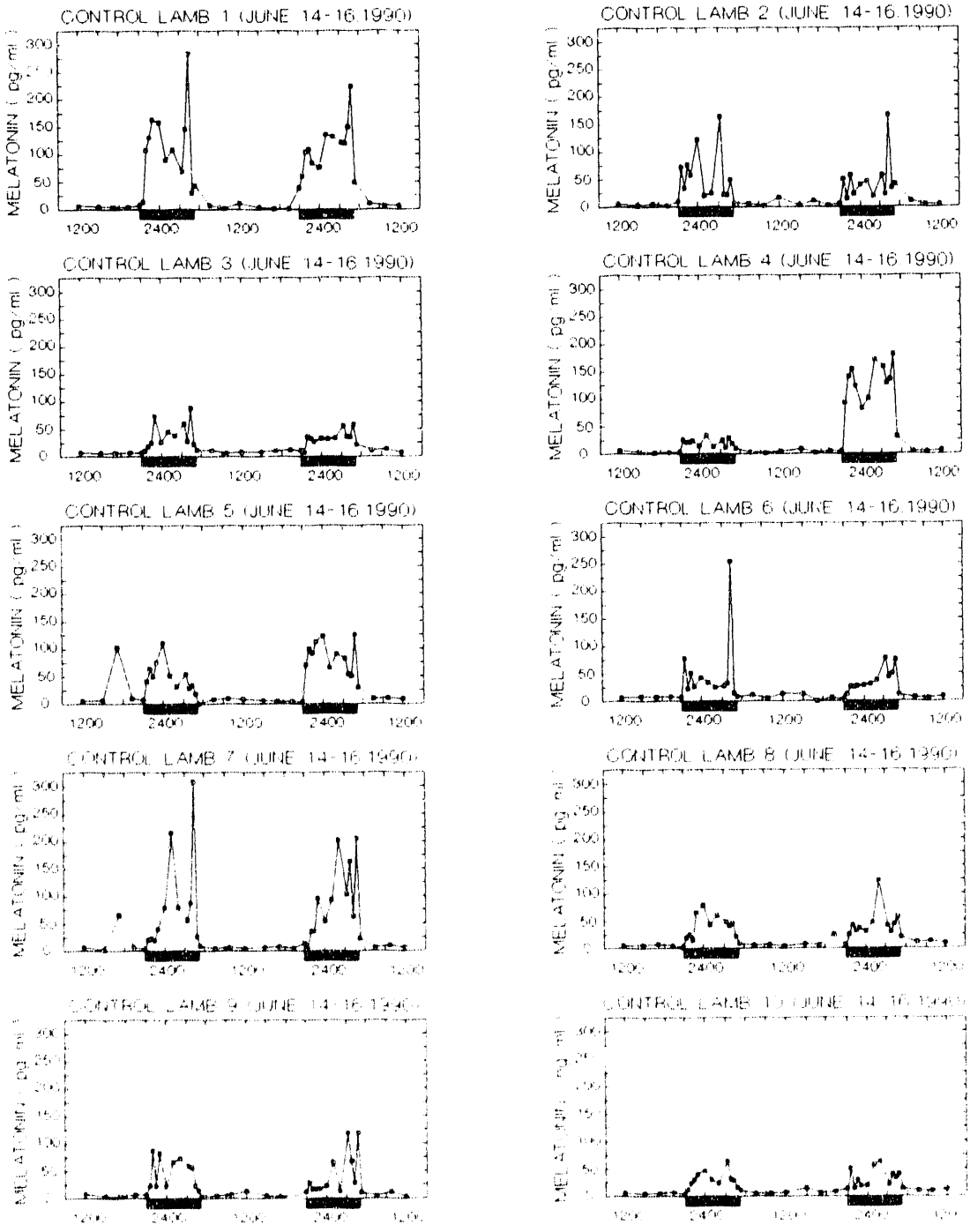


Figure 7. Melatonin concentration (pg/ml) in control lambs for 14-16 June 1990.

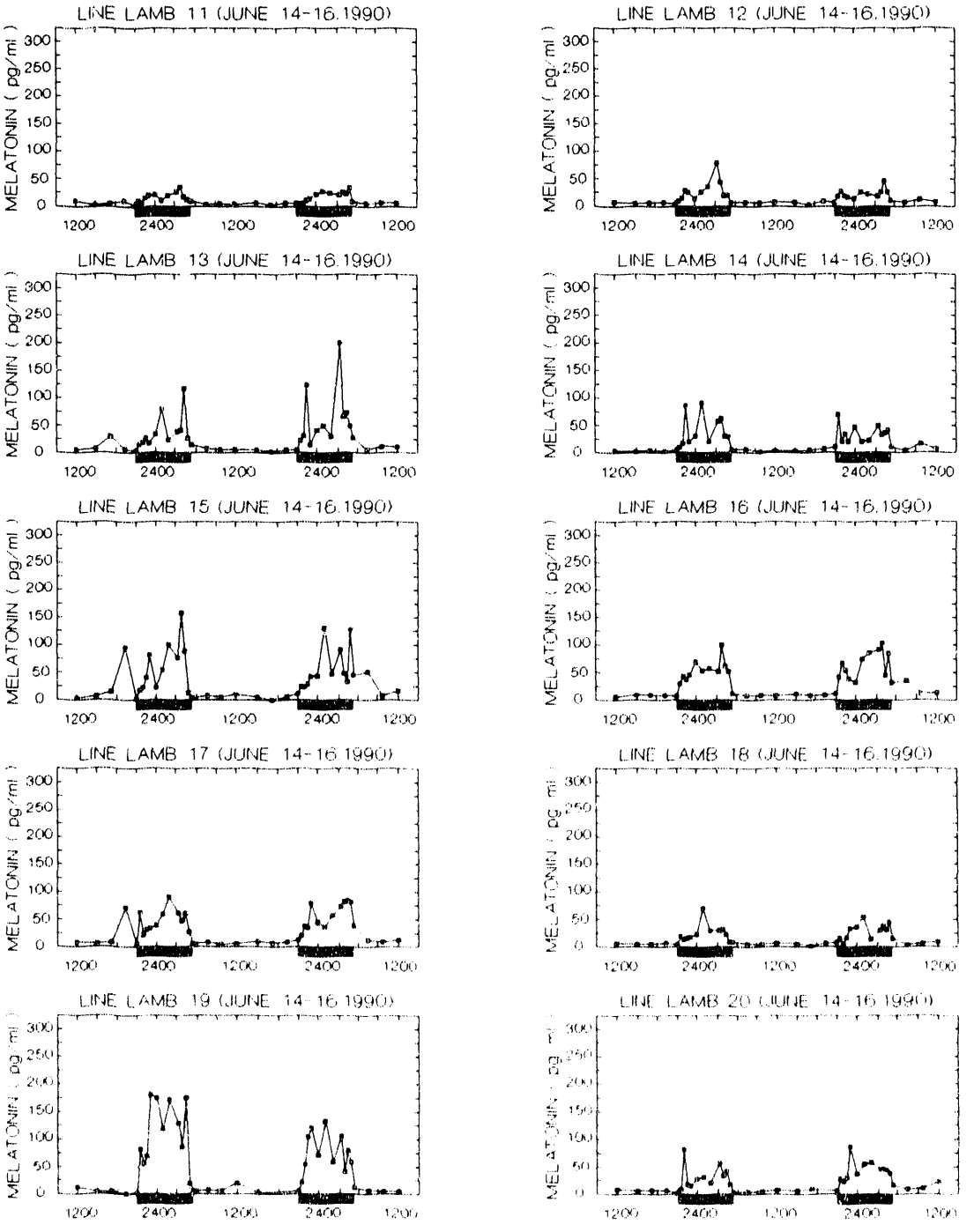


Figure 8. Melatonin concentration (pg/ml) in line lambs for 14-16 June 1990.

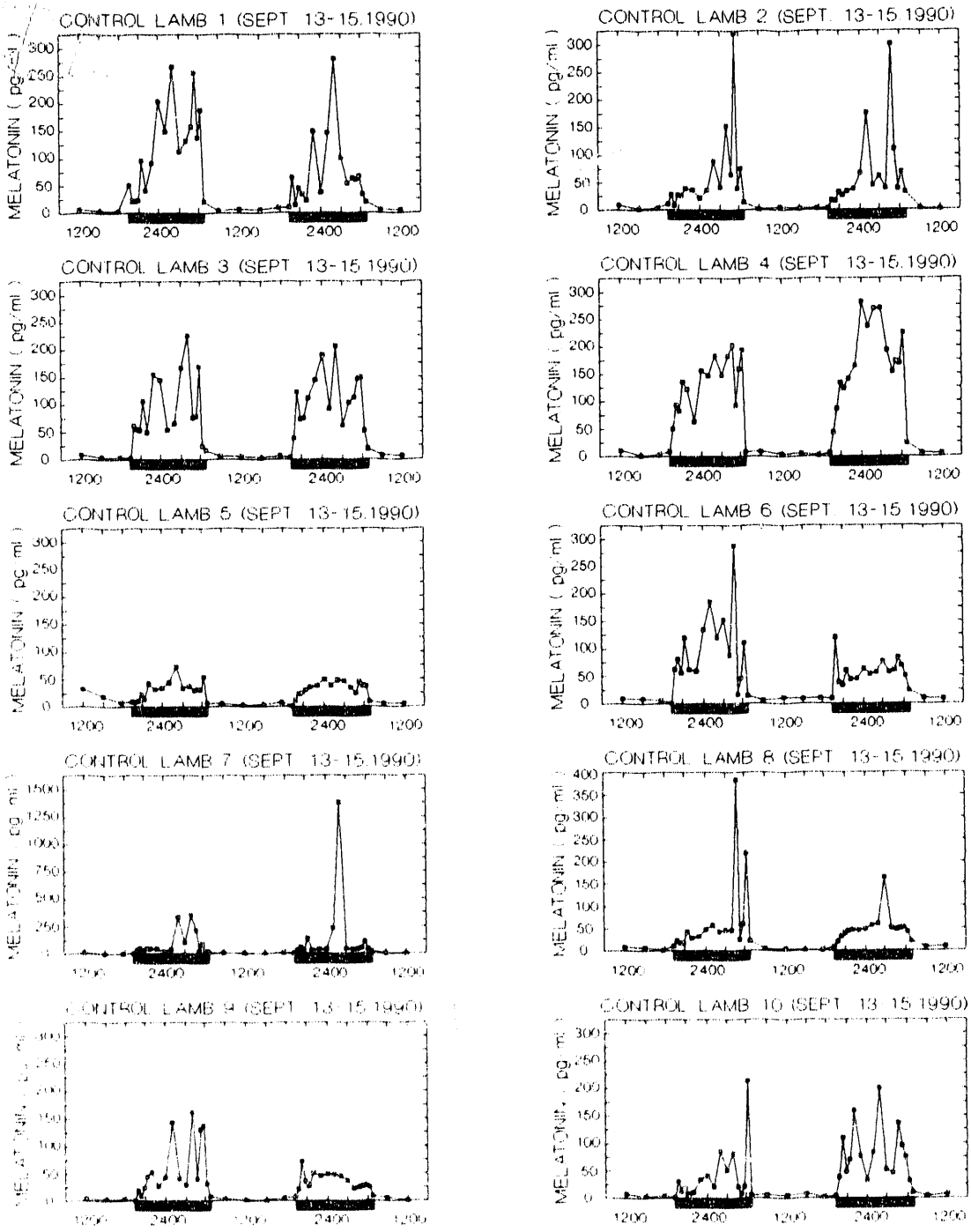


Figure 9. Melatonin concentration (pg/ml) in control lambs for 13-15 Sept. 1990.

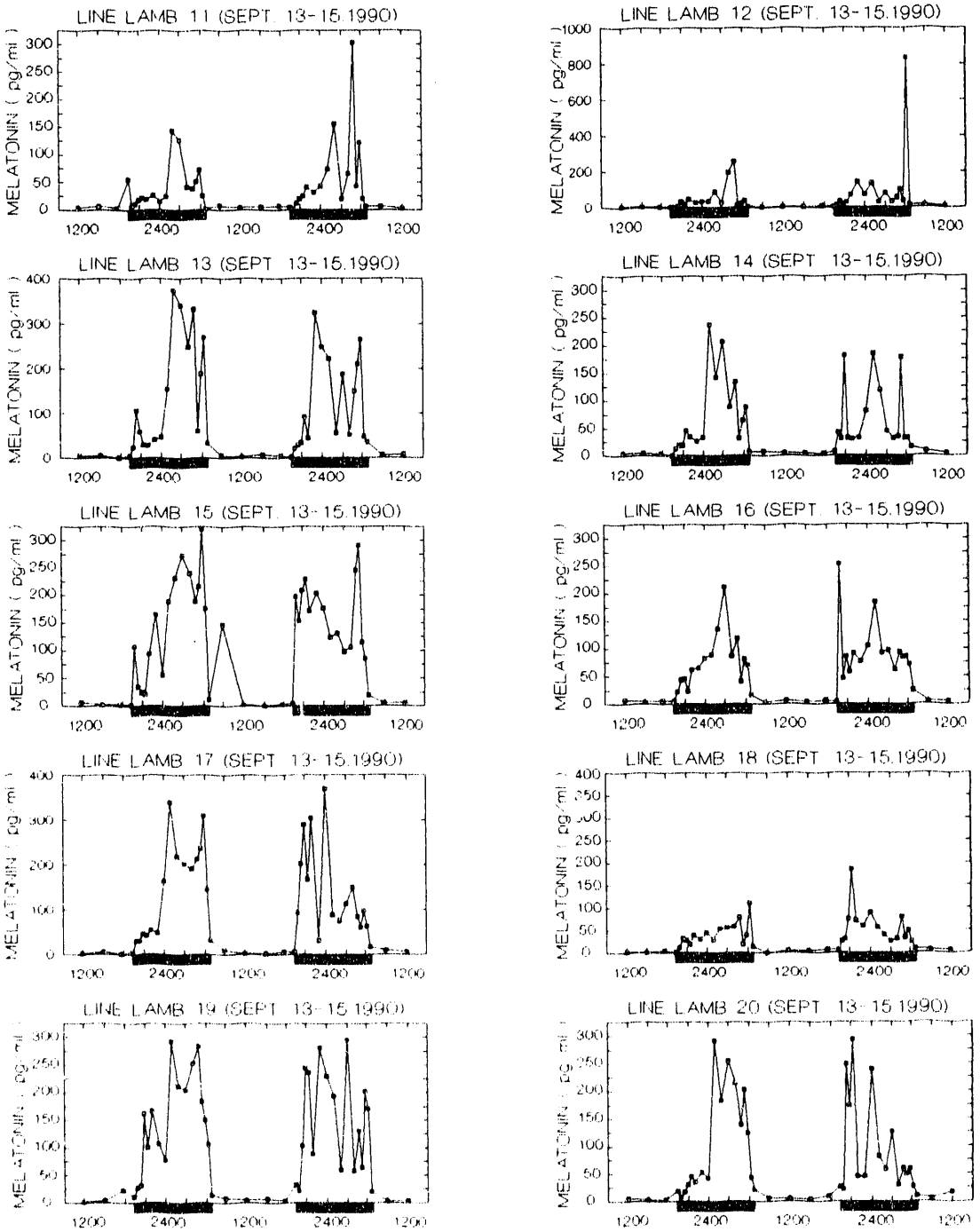


Figure 10. Melatonin concentration (pg/ml) in line lambs for 13-15 Sept. 1990.

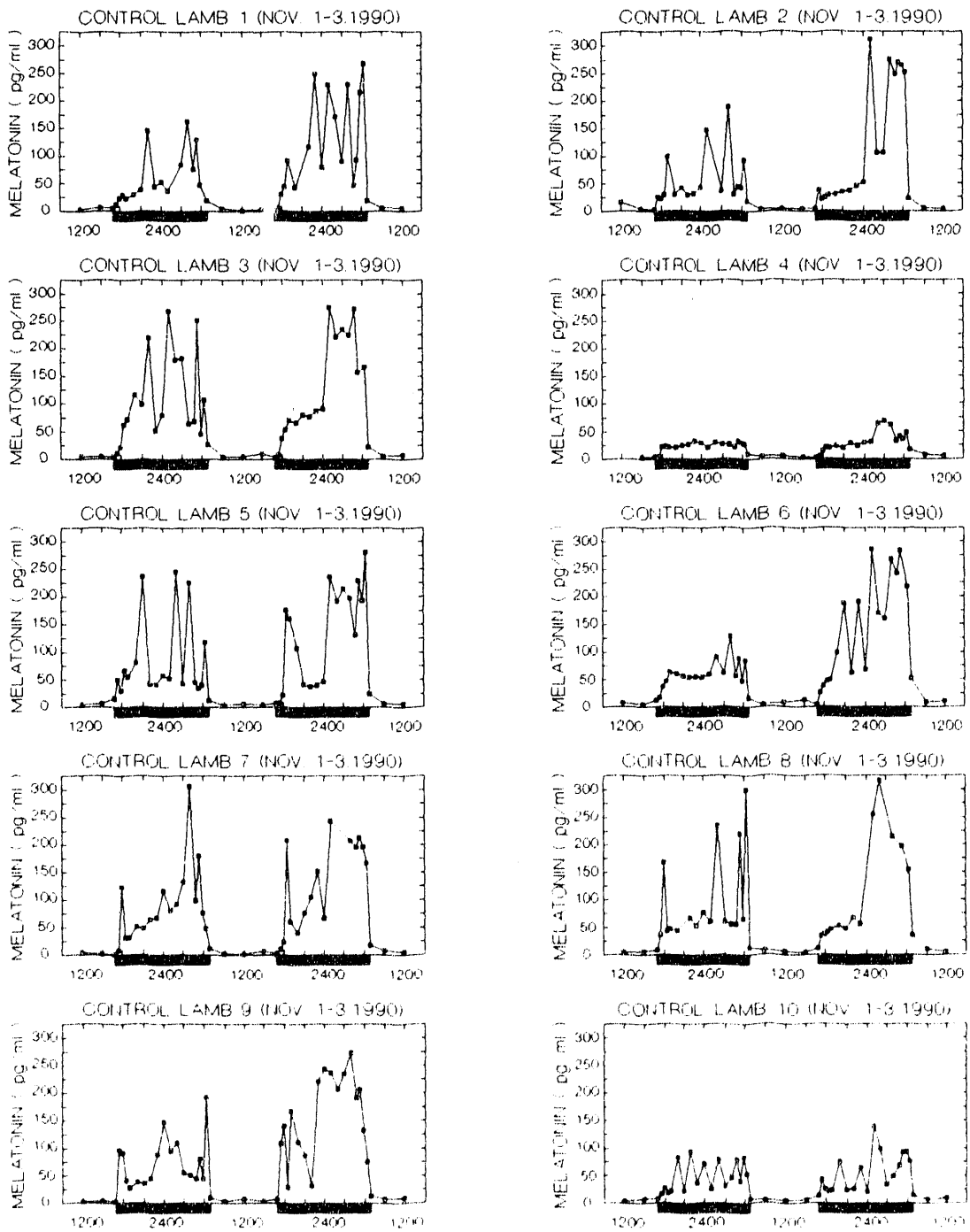


Figure 11. Melatonin concentration (pg/ml) in control lambs for 1-3 Nov. 1990.

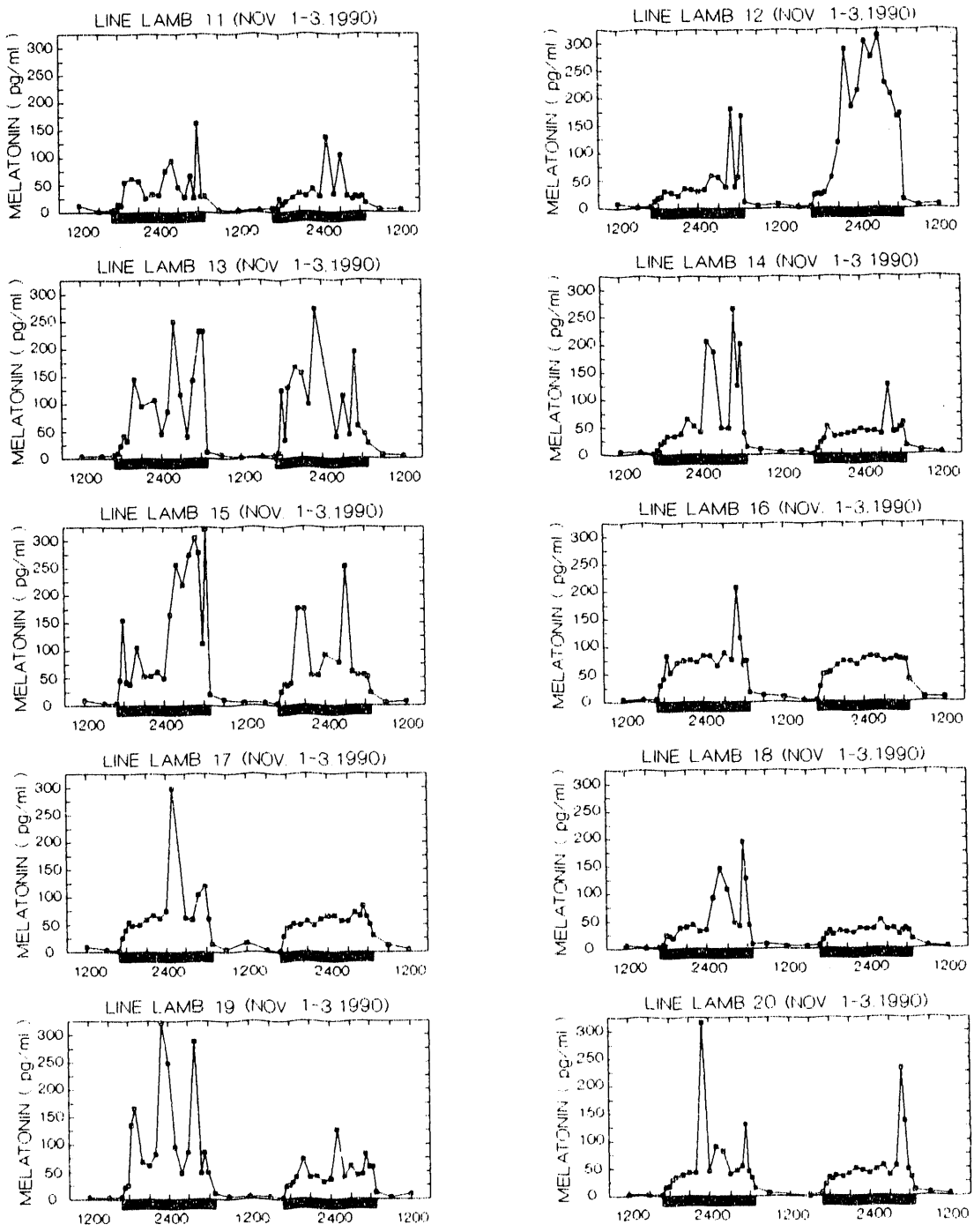


Figure 12. Melatonin concentration (pg/ml) in line lambs for 1-3 Nov. 1990.

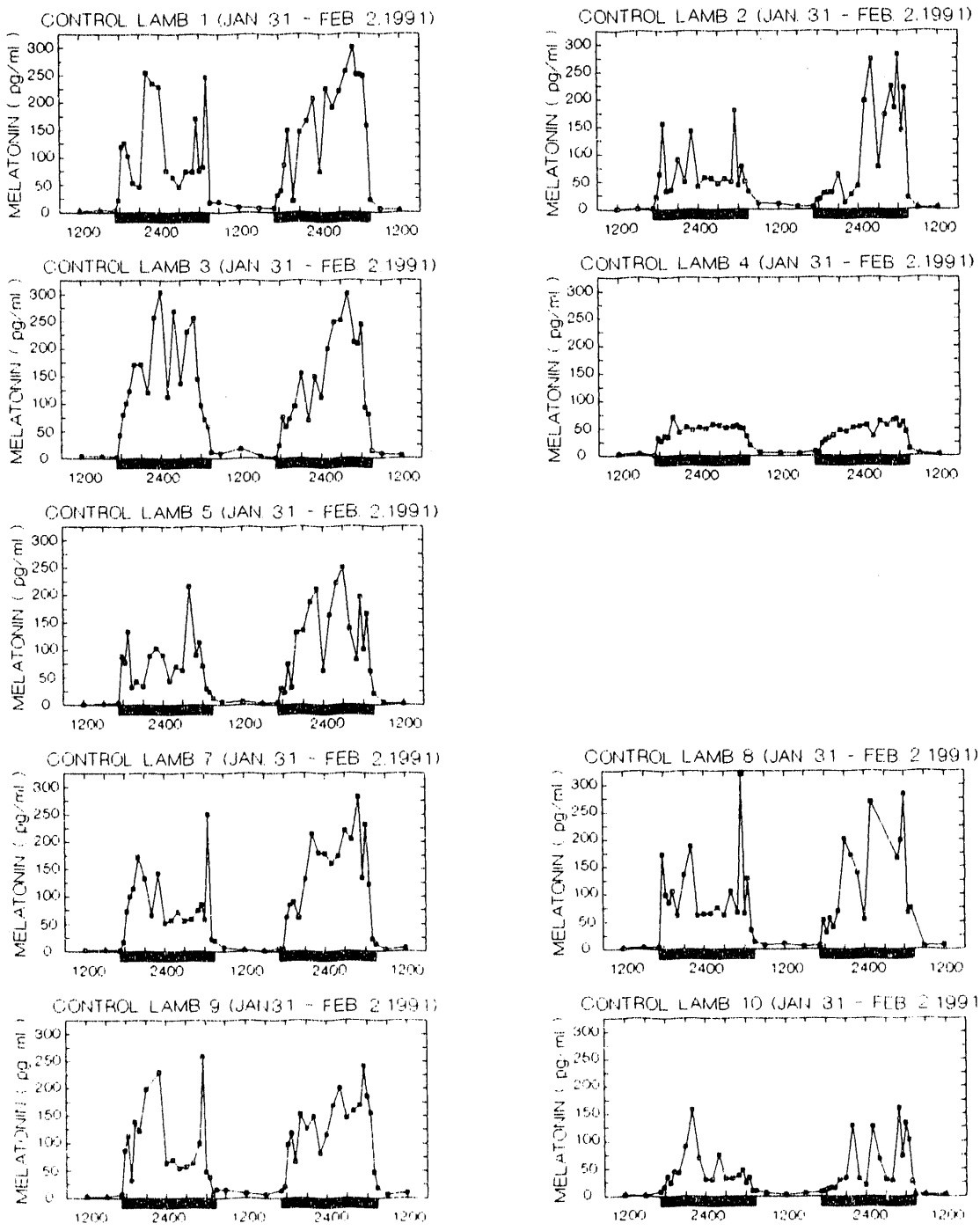


Figure 13. Melatonin concentration (pg/ml) in control lambs for 31 Jan. - 2 Feb. 1991.

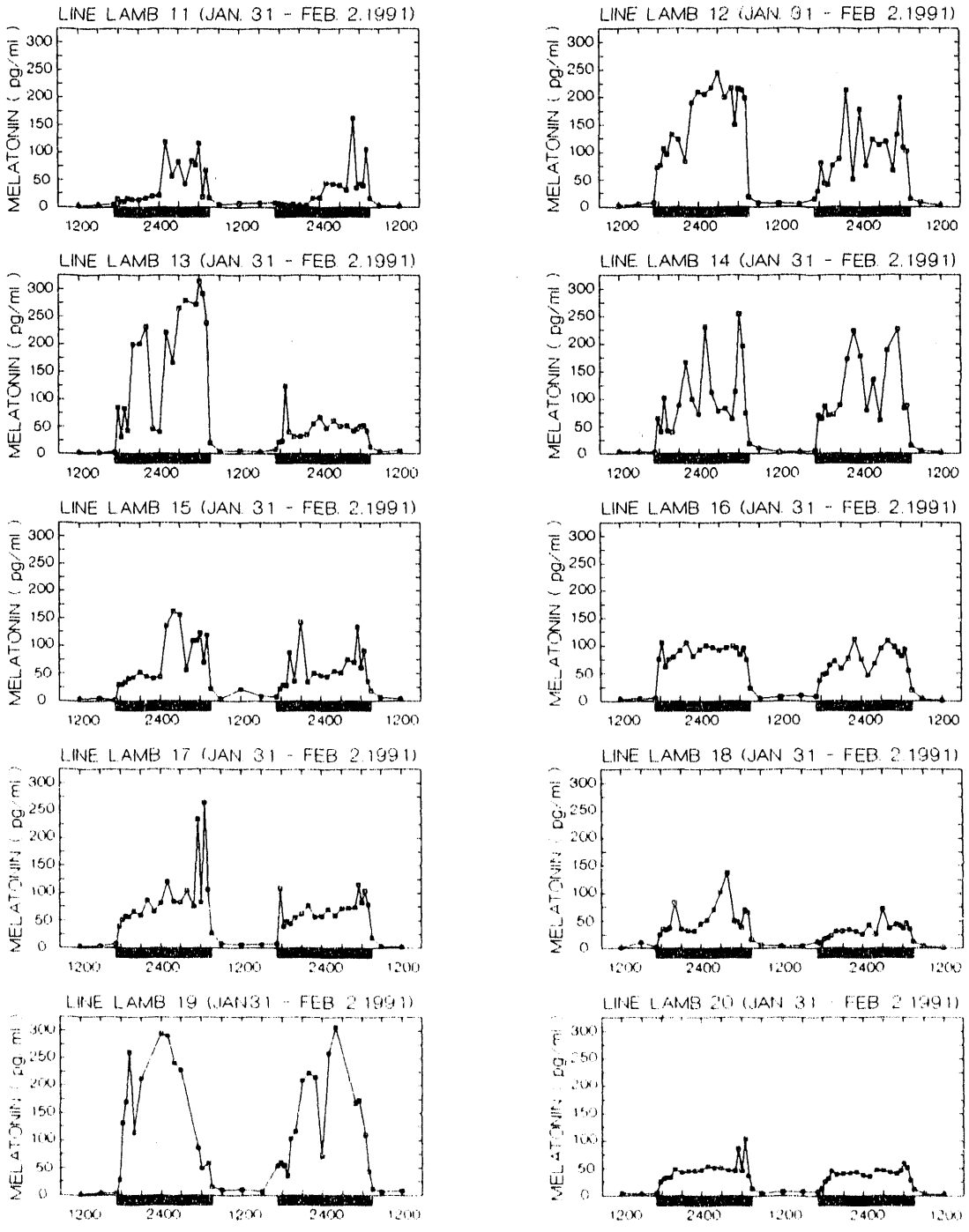


Figure 14. Melatonin concentration (pg/ml) in line lambs for 31 Jan. - 2 Feb. 1991.

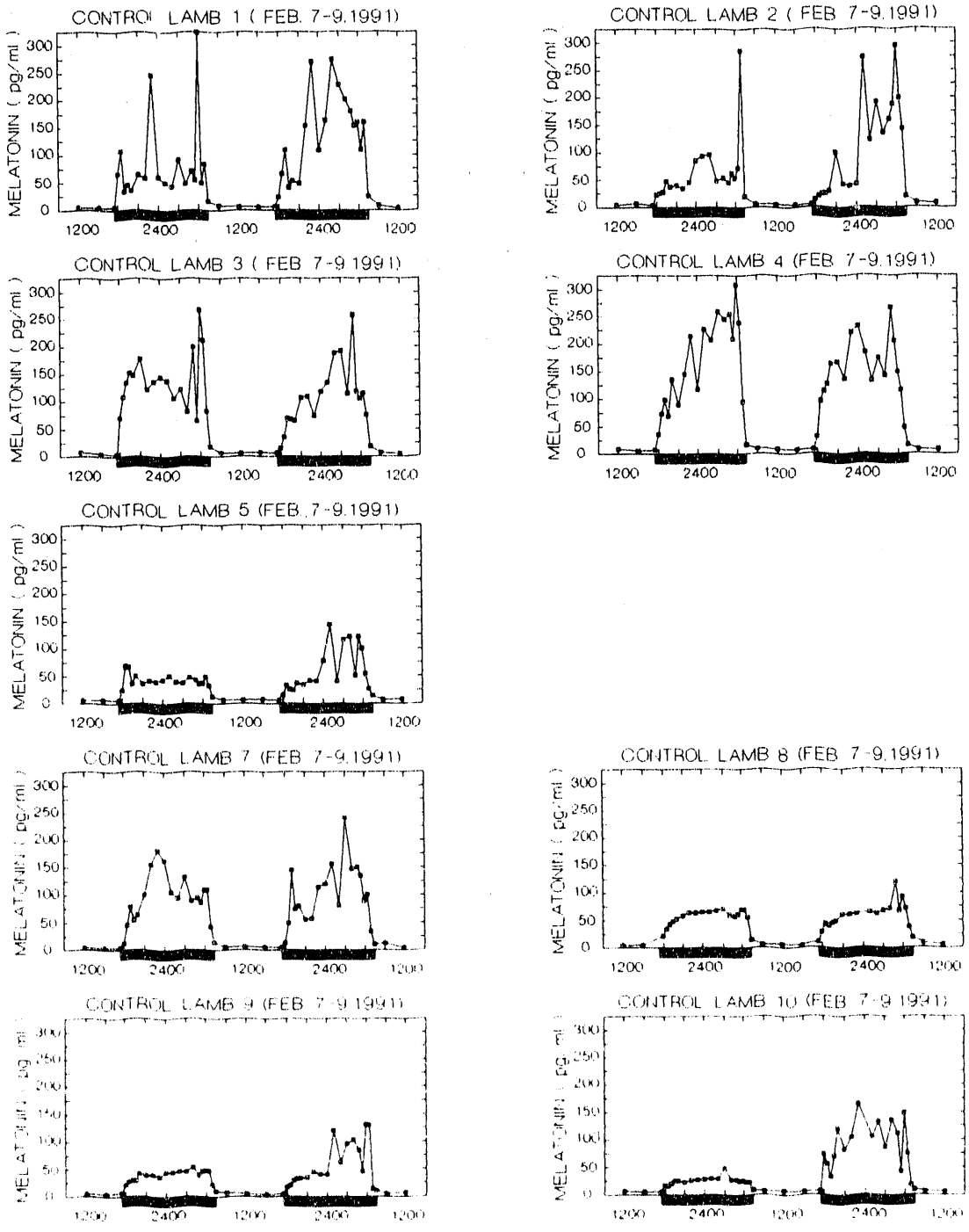


Figure 15. Melatonin concentration (pg/ml) in control lambs for 7-9 Feb. 1991.

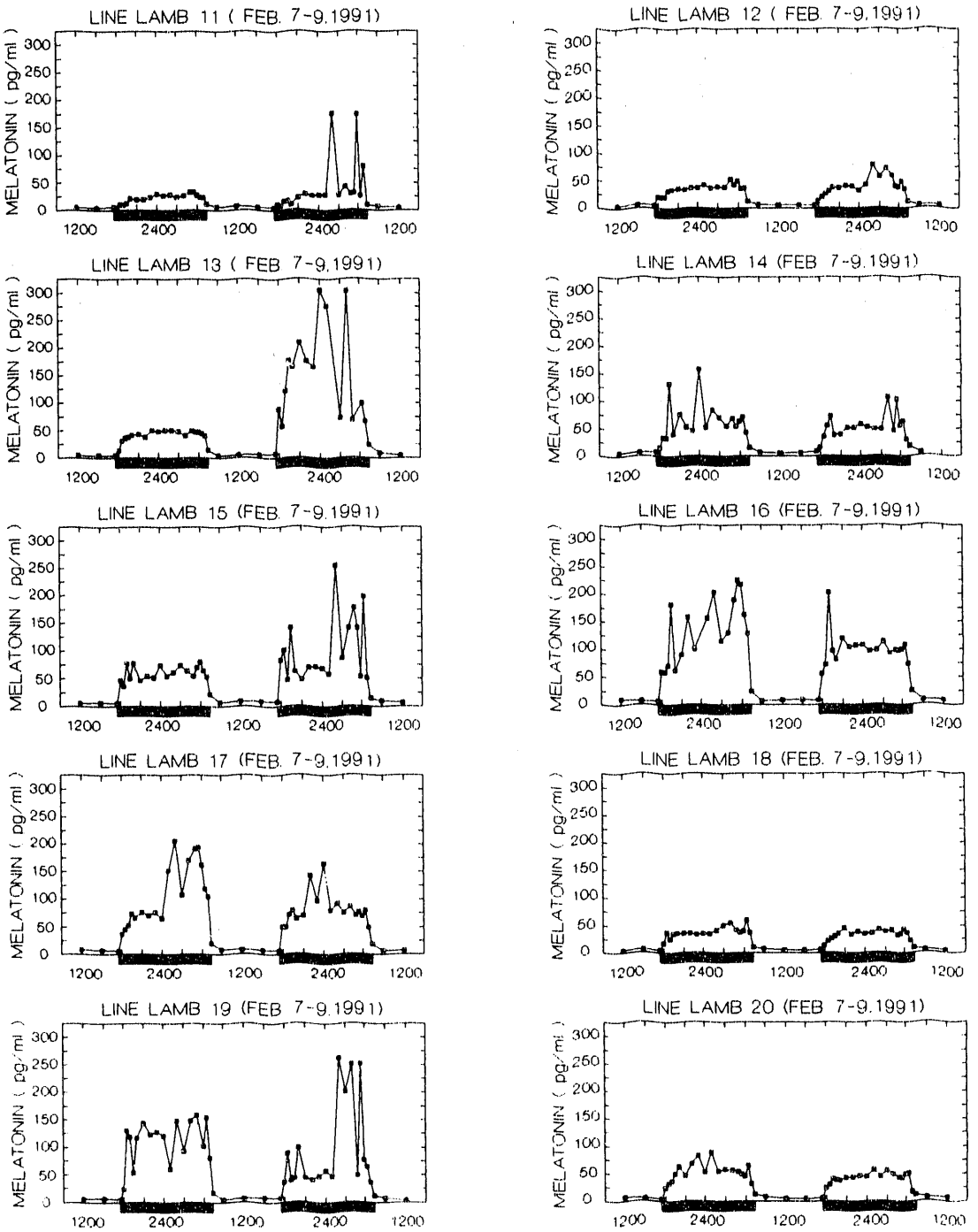


Figure 16. Melatonin concentration (pg/ml) in line lambs for 7-9 Feb. 1991.

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9/2/92