8-1980

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BY

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A THESIS

SUBMITTED TO THE GRADUATE FACULTY

OF THE UNIVERSITY OF RICHMOND

IN CANDIDACY

FOR THE DEGREE OF

MASTER OF SCIENCE

IN BIOLOGY

AUGUST 1980

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B.S. UNIVERSITY OF RICHMOND 1974

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ABSTRACT

The reproductive effects of long (24L:0D), natural (14L:10D), and short (1L:23D) photoperiods were studied in the male mouse, Mus musculus, ICR strain. One third of the mice in each photoperiodic regimen were sacrificed after 45 days. Half of the remaining mice in each regimen received daily melatonin (5-methoxy-N-acetyltryptamine) injections for 45 additional days. Body weights, testicular and accessory sex organ weights were measured and microscopic examinations of the testes were made. Plasma testosterone levels were determined using radioimmunoassay. Other organ (pituitary, kidney, adrenal, thymus, thyroid) weights were also obtained.

Short photoperiods had a negative effect on the percent increase in body weight of mice that received melatonin, whereas long photoperiods had a positive effect. No significant differences in testis weights (expressed as percents of total body weight) were produced by the experimental treatments. The testes appeared histologically normal in all groups of mice. Plasma testosterone levels of mice sacrificed after 45 days were highest in the 1L:23D group and lowest in the 14L:10D group. The levels in mice that received melatonin and mice that received vehicle injections were highest in the 14L:10D groups and lowest in the 24L:0D groups.

The levels were higher in mice that received melatonin than in mice that received vehicle injections in each photoperiod.

There were a number of changes in accessory sex organ weights (expressed as percents of total body weight). Long photoperiods had positive effects on two of the accessory sex organs: ventral prostate gland weights of mice that received melatonin; and epididymis weights of mice that received vehicle injections. However, long photoperiods had inhibitory effects on four of the accessory sex organs: dorsal prostate gland weights of mice that were sacrificed after 45 days and mice that received melatonin; ventral prostate gland weights of mice that were sacrificed after 45 days; and penis weights of mice that received melatonin. No significant differences in sex accessory organ weights between animals that received melatonin and animals in the same photoperiod that received vehicle injections occurred.

There were a number of changes in other organ weights (expressed as percents of total body weight). Long photoperiods had inhibitory effects on the following parameters: kidney weights of mice that were sacrificed after 45 days and mice that received melatonin; and pituitary and thyroid weights of mice that received melatonin. Melatonin administration had a stimulatory

effect on thymus weight of mice that received melatonin as compared to the thymus weight of mice that received vehicle injections in the 1L:23D group.

In conclusion it was found that long photoperiods had a negative effect on testosterone levels and consequently accessory sex organ weights in four instances. The fact that melatonin alone produced no effects on accessory sex organ weights implies that the combination of long photoperiods and melatonin was necessary. The inhibitory effect of long photoperiods is probably mediated by the pineal gland with melatonin enhancing the synthesis or secretion of a pineal anti-interstitial cell stimulating hormone substance. No changes in testicular anatomy indicate that no anti-follicle stimulating hormone factor was involved. The inhibitory effect of long photoperiods on male mice found in the present study is an enigma as previous research by others indicates that long photoperiods are stimulatory to the reproductive system of female rodents.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to the following people who have helped me complete this thesis, especially Dr. Francis B. Leftwich, chairman of my committee, for his invaluable aid and suggestions. I am grateful to my committee members: Dr. David W. Towle, for the use of his laboratory and his criticism of this work; and Dr. Willie M. Reams, Jr., for his histological instruction and equipment and his criticism of the manuscript. Thanks are given also to Dr. R. Dean Decker for the use of his apparatus, Dr. Wilton R. Tenney for his help in photographing testis sections, and Dr. Jacob V. Bowen, Jr. (Mathematics Department, University of Richmond) for his aid in statistical analysis of data. Also I would like to thank Mr. James W. Newlin, Mr. Mark W. Hagood, and Mr. John E. Constantine (fellow graduate students) for their assistance with this study.

Finally, I would like to express my gratitude to my parents and husband for their support and encouragement during the course of this study.

INTRODUCTION

It is well documented that light is an important environmental factor in regulating reproduction in vertebrates (Itoh et al., 1962; van Tienhoven, 1968). Relatively few studies regarding the effect of photoperiod on reproduction in amphibians and male mammals (Hoffman and Reiter, 1965b; van Tienhoven, 1968) have been performed compared to the abundant studies on birds (Farner, 1964; van Tienhoven, 1968), reptiles (Hawley and Aleksiuk, 1976), and fish (van Tienhoven, 1968).

Most mammalian studies employed female rats (Itoh et al., 1962; Hoffman, 1973). Female rats placed in continuous light exhibited an increase in the incidence of estrus and hypertrophy of the uteri and ovaries (Wurtman et al., 1963a; Reiter and Klein, 1971; Bajpai et al., 1975; Singh et al., 1975). Exposure to short daily photoperiods, continuous darkness, or blindness resulted in a decrease in the incidence of estrus, frequency of ovulation, uterine weights and ovarian weights in rats and hamsters (Wurtman et al., 1963a; Hester, 1966; Reiter and Hester, 1966; Reiter et al., 1966; Reiter, 1967; Reiter, 1969a, 1969b; Osman et al., 1972; Reiter and Johnson, 1974; Singh et al., 1975; Tamarkin et al., 1977).

The role of the pineal gland in the reproduction of mammals has been studied primarily in rats and

hamsters (van Tienhoven, 1968). The pineal is believed to be a neuroendocrine transducer (Houssay et al., 1966b), converting neural input, via sympathetic neurons, to hormonal output (Minneman and Wurtman, 1975). It has been proposed that the pineal exerts an inhibitory effect on the reproductive system (Wurtman et al., 1968; Reiter and Fraschini, 1969; Fraschini and Martini, 1970). Pinealectomized rats and mice experienced acceleration of vaginal opening and an increased incidence of estrus (Chu et al., 1964).

The inhibitory effect of the pineal could be mediated by one of its products, melatonin, as melatonin synthesis is inversely controlled by environmental illumination (Minneman and Wurtman, 1975). Constant light enhanced activity of the female reproductive system in rats (Wurtman et al., 1963a; Reiter and Klein, 1971; Bajpai et al., 1975; Singh et al., 1975) while lowering activity of the pineal as demonstrated by decreased activity of the melatonin forming enzymes, hydroxyindole-O-methyltransferase (Wurtman et al., 1963b) and serotonin N-acetyltransferase (Rudeen and Reiter, 1977). Decreases in a number of other parameters also occurred following exposure to constant light: pineal weight (Wurtman et al., 1963b; Fiske, 1975), pinealocyte size (Kachi et al., 1971), number of pinealocyte

nucleoli (Wurtman et al., 1963b), and oxygen consumption of the pineal (Kitay, 1967).

Studies on the effects of melatonin in females are more numerous than in males and provide consistent results (Motta et al., 1967). Suppression of a number of parameters occurred following melatonin administration in rats: vaginal opening, frequency of estrus, uterine weights, ovarian weights, and frequency of ovulation (Wurtman et al., 1963a; Chu et al., 1964; McIsaac et al., 1964; Wurtman and Axelrod, 1965a, 1965b; Motta et al., 1967; Fraschini et al., 1971; Bajpai et al., 1975; Singh et al., 1975). Melatonin delayed estrus in the ferret (Herbert, 1971), decreased the incidence of estrus in mice (Chu et al., 1964), and inhibited compensatory ovarian hypertrophy following unilateral ovariectomy in mice (Vaughan and Benson, 1970; Vaughan et al., 1970; Vaughan et al., 1971; Reiter et al., 1972) and rats (Sorrentino, 1968; Vaughan and Benson, 1970). These studies suggest one of the most important functions of the pineal could be to regulate seasonal reproductive rhythms so that survival of the young is maximized (Reiter, 1973).

Studies on the effects of photoperiod in male mammals, particularly mice, are sparse (Hoffman, 1973) and available studies provide inconsistent results. Certain studies have found that rats exposed to short

daily photoperiods, constant darkness, or blindness experienced atrophy of the accessory sex organs (Browman, 1940; Reiter, 1968; Relkin, 1972) and/or testes (Itoh et al., 1962; Hester, 1966). However, there was no effect on the reproductive system of male Hooded strain rats exposed to short daily photoperiods (Kinson and Robinson, 1970) or rats exposed to continuous light (Hoffman, 1973). Hamsters exposed to short daily photoperiods, constant darkness, or blindness experienced atrophy of the testes (Hoffman and Reiter, 1965a, 1965b; Clabough and Seibel, 1968; Turek, 1977) and accessory sex organs (Reiter and Hester, 1966; Reiter, 1968; Berndtson and Desjardins, 1974; Reiter et al., 1974; Turek et al., 1975).

Pinealectomy of male mammals has been reported as having stimulatory, inhibitory, or no influence on the reproductive system (Reiter et al., 1975b). Pinealectomy in the rat and hamster resulted in increased testicular and accessory sex organ weights (Hoffman and Reiter, 1965a, 1965b; Clabough and Seibel, 1968; Reiter, 1968; Reiter et al., 1974; Tamarkin et al., 1977). Pinealectomy prevented the inhibitory effect of light restriction on the testes of rats (Sorrentino et al., 1971), seminal vesicles and ventral prostate glands of rats (Kinson and Robinson, 1970), and testes of hamsters (Reiter and Hester, 1966; Reiter, 1969a). However,

in hamsters exposed to stimulatory photoperiods. Weasels and grasshopper mice treated with melatonin exhibited testicular regression (Rust and Meyer, 1969; Turek et al., 1976). Debeljuk (1969) found that melatonin induced a decrease in testis and seminal vesicle weights in rats. Other studies of rats found that melatonin administration had no effect on the testes (Turek et al., 1976) but induced a reduction in accessory sex organ weights (Motta et al., 1967; Kinson and Robinson, 1970; Alonso et al., 1978). Melatonin has been shown to be progonadal as it prevented gonadal regression in male hamsters exposed to non-stimulatory situations (Reiter et al., 1974; Hoffman, 1975; Reiter et al., 1975a). Also melatonin induced precocious spermatogenesis and an increase in seminal vesicle weights in rats (Reiter et al., 1975b). Finally, other researchers found that melatonin had no effect on the male reproductive organs in rats (Ebels and Prop, 1965; Reiter et al., 1975b). No pattern to the occurrence of these effects is evident (Reiter et al., 1975b).

It was apparent that the previous studies produced inconsistent results and the purpose of the present study was an attempt to clarify the situation. Although a few studies regarding the effects of pinealectomy or melatonin administration in male mice have been performed, no previous research examined the combined

effects of different photoperiods and melatonin administration on the reproductive system of male mice.

Various organs were weighed in the present study to obtain an indication of the activity of these organs. Ventral prostate glands and coagulating glands were weighed because they are the most sensitive indicators of increased testosterone secretion in the rat and mouse (Vaughan and Reiter, 1971). Seminal vesicles, dorsal prostate glands, vas deferens, epididymis, preputial glands, and penis are also accessory organs and primary sites of testosterone action (Velardo, 1958). Previous studies have implicated the involvement of melatonin in the function of the pituitary, adrenal, thymus, and thyroid (Minneman and Wurtman, 1975) although the relationships were controversial (Collu and Fraschini, 1972). Therefore, the effects of the present experimental procedures on these organs were studied also. The kidney was included because adrenal corticoid hormones influence the kidney and studies regarding the adrenal were confusing (Reiter et al., 1975b).

MATERIALS AND METHODS

Seventy-two four-week-old male mice, Mus musculus, ICR strain, were obtained on October 18, 1979, from Flow Laboratories in Dublin, Virginia. The mice, weighing 13.3 - 19.1 gm upon arrival, were randomly divided into three equal groups. It was necessary to house three mice per cage due to limited space in the environmental chambers. The cages, measuring 28.5 X 17.5 X 13.0 cm, were plastic with wire tops.

One group was placed in a Biotronette Mark III Environmental Chamber in a 14L:10D photoperiod with lights on from midnight to 2:00 P.M. A second group was placed in a Biotronette Mark III Environmental Chamber in a 1L:23D photoperiod with lights on from 1:00 P.M. to 2:00 P.M. A third group was placed in the same room on a table 75.5 cm from the floor in a 24L:0D photoperiod with lights on continuously. Four 40 w fluorescent bulbs were used in the two environmental chambers. Five 40 w fluorescent bulbs mounted on a board were used above the table in the 24L:0D regimen in order to obtain the same illumination as in the two environmental chambers. Fluorescent bulbs provide wavelengths (3800 - 7500 Å) that are almost equivalent to the visible light spectrum (Reiter and Fraschini, 1969). Lights were placed 73 cm from the bottom of the cages. The illumination was 55.0 - 65.0 ft-c at the

bottom of the cages in all three environments and was adequate illumination as light is inhibitory to the pineal from 50 - 150 ft-c and probably at much lower intensities as well (Reiter and Fraschini, 1969). The temperature ranged between 20.0° and 29.5°C but at any particular time there was only a 2.0°C difference between any of the three environments. All mice were given Purina lab chow and water ad libitum, and were weighed every Monday, Wednesday, and Friday at approximately 1:00 P.M.

One mouse from each cage was sacrificed after 45 days in the previously described conditions. The mice were anesthetized with ether prior to sacrifice. Heart stabs were performed, using a heparinized 23 ga needle and 2.5 cc syringe, in order to obtain blood for testosterone determination. Plasma was obtained by centrifuging the blood for 10 min at 3000 RPM. The plasma was frozen until radioimmunoassay for testosterone determination was performed. It was necessary to combine plasma of animals that experienced identical regimens due to the small volume of blood obtained.

Testes, seminal vesicles, coagulating glands, dorsal prostate glands, ventral prostate glands, vas deferens, epididymis, preputial glands, penis, kidneys, adrenal glands, pituitary gland, thyroid gland, and thymus were removed and weighed. Testes were placed in

Bouin's fixative to be preserved for histological examination.

The 48 remaining mice were kept under the previously described conditions for an additional 45 days. Half of the mice in each group received injections containing 10 μg melatonin (5-methoxy-N-acetyltryptamine, Sigma Chemical Co.) in vehicle solution. The vehicle was 3% ethanol in Locke's physiological solution for mammals (Hoar and Hickman, 1967). Ethanol was necessary to dissolve melatonin (Quay, 1974). The remaining mice received vehicle injections. Mice received daily 0.05 ml subcutaneous injections of the appropriate solution between 1:00 P.M. and 2:00 P.M. Injections were given at the time indicated because it has been found that melatonin injections have no effect on the reproductive system of hamsters unless they are given late in the light period (Reiter et al., 1976; Tamarkin et al., 1977). Heart stabs were performed, mice were sacrificed, indicated organs were weighed, and testes were placed in Bouin's fixative after 45 days in the above regimens.

Testis preparation for histological examination

Testes were placed in Bouin's fixative for at least 2 days. The tissue was then dehydrated through a series of alcohols, from 35% to absolute, and placed in xylene. The tissue was then infiltrated with and

mounted in Paraplast tissue embedding medium and sectioned at 8 mu using an American Optical "820" Spencer microtome. Egg albumin was used as adhesive to attach sections to microscope slides. Slides were placed on a heating tray (50° - 55°C) overnight, rehydrated, and then stained for 23 min in Delafield's hematoxylin. Acid (HCl) water was used as a destaining medium. Lithium carbonate solution was used to stop destaining and blue the stain. The sections were dehydrated, mounted permanently in Canadian balsam, and examined microscopically.

Radioimmunoassay of plasma testosterone levels

Plasma testosterone levels were determined according to the method of New England Nuclear (1977). Radioimmunoassay in plasma extracts is a highly sensitive and specific method for measurement of plasma testosterone (Dufau et al., 1972). The Beckman LS-100C Liquid Scintillation System and Direct Data Readout Module were employed in the present study to determine tritium content of samples. A quenching curve was prepared using the combined External Standard—Channels Ratio Method (Beckman Instruments, 1967). This was necessary as all samples are quenched to some extent in the liquid scintillation process (Long, 1976).

The recovery tracer, testosterone (^{3}H), was dissolved in an appropriate amount of assay buffer to

obtain 1000 cpm/0.1 ml at approximately 30% efficiency. The assay tracer, testosterone (^{3}H), was dissolved in an appropriate amount of assay buffer to obtain 4000 cpm/0.1 ml at approximately 30% efficiency. The lyophilized antiserum, prepared in rabbits against testosterone-3-oxime-bovine serum albumin, was reconstituted and the titre determined.

The frozen mouse plasma was allowed to thaw at room temperature and recovery tracer was added as an internal standard. Methylene chloride was employed to extract testosterone from the plasma. The samples were vortexed and centrifuged for 5 min at 1500 x g. The extract was serially washed with 0.1 N NaOH, 0.1 N acetic acid, and distilled water and then dried with a gentle stream of air at 37°C. Multiple washings of the test tube wall with absolute ethanol and redrying as described above concentrated testosterone in the bottom of the tube. The residue was dissolved in absolute ethanol. Recovery determinations were made at that time. Duplicate 0.1, 0.2, and 0.4 ml aliquots were taken from each sample and dried down for assay. The residue was dissolved in assay buffer. Assay tracer and antiserum were added. The samples were vortexed and allowed to incubate for at least 2 hr at 4°C. Dextran-coated charcoal suspension was added to separate antiserum-bound and unbound testosterone after the

incubation period. The tubes were vortexed, placed in an ice bath for 5 min, and centrifuged for 20 min at 2000 x g in a refrigerated (4°C) centrifuge. The supernatant, containing antiserum-bound testosterone, was decanted into counting vials and counted in the liquid scintillation counter for 10 min under conditions for counting tritium. The radioimmunoassay was also performed on standard samples containing known amounts of testosterone. Calculations to determine the µg% of testosterone in the original plasma were made according to the method of New England Nuclear (1977).

Statistical analysis

Body weights were compared as percent increases in body weight because there were differences in initial body weights. Organ weights were expressed as percentages of total body weight. A one way analysis of variance (ANOVA) was performed on organ weights to determine statistical significance of the following comparisons: photoperiod differences after 45 days, photoperiod differences after 90 days in mice that received melatonin, and photoperiod differences after 90 days in mice that received vehicle injections. Duncan's multiple-range test was employed to order means and group means into similar groups after differences among means were determined (Walpole and Myers, 1972). A two way ANOVA was performed on organ weights to determine

statistical significance of photoperiod and treatment differences. Students' t tests were employed to specify the exact location of differences among means following melatonin treatment after differences were determined by ANOVA. All tests employed $\alpha=0.05$. Statistical analyses could not be performed on plasma testosterone levels because of the necessity of pooling plasma samples.

RESULTS

Mean body weights are given in Figure 1. The percent increase in body weight of mice that received melatonin was significantly larger in the 24L:0D group ($\bar{x}$=173.85) than in the 1L:23D group ($\bar{x}$=120.46) but did not differ significantly from the 14L:10D group ($\bar{x}$= 153.17) (Figure 2). There were no other significant differences in percent increases in body weight.

Testicular weights expressed as percentages of total body weight were used for all statistical analyses (Figure 3). There were no significant differences in testicular weights. Histological examination of the testes indicated that all groups of mice were reproductively competent at the end of the study (Figure 4). The testes appeared normal with well defined seminiferous tubules and interstitial cells. Spermatogenesis was evident in all testes and the majority of seminiferous tubules contained mature spermatozoa. Plasma testosterone levels of mice sacrificed after 45 days were 0.54 µg% in the 1L:23D group, 0.40 µg% in the 24L:0D group, and 0.28 µg% in the 14L:10D group (Figure 5). The levels in mice that received melatonin and mice that received vehicle injections were highest in the 14L:10D groups, 0.70 and 0.50 µg% respectively, and lowest in the 24L:0D groups, 0.27 and 0.05 µg% respectively. The µg percents were 0.42 and 0.40,

respectively, in the 1L:23D groups at the conclusion of the study. The levels were higher in mice that received melatonin than in mice that received vehicle injections in each photoperiod.

Accessory sex organ weights expressed as percentages of total body weight were used for all statistical analyses (Figures 6 through 13). There were a number of changes in accessory sex organ weights (expressed as percents of total body weight). Dorsal prostate gland weights of mice that were sacrificed after 45 days were significantly larger in the 1L:23D ($\bar{x}$=0.050) and 14L:10D ($\bar{x}$=0.045) groups than in the 24L:0D group ($\bar{x}$=0.024) (Figure 6). Ventral prostate gland weights of mice that were sacrificed after 45 days were significantly larger in the 14L:10D ($\bar{x}$=0.067) and 1L:23D ($\bar{x}$=0.054) groups than in the 24L:0D group ($\bar{x}$=0.039) (Figure 7). There were no other significant differences in accessory sex organ weights as influenced by photoperiod after 45 days.

Ventral prostate gland weights of mice that received melatonin were significantly smaller in the 1L:23D ($\bar{x}$=0.037) and 14L:10D ($\bar{x}$=0.043) groups than in the 24L:0D group ($\bar{x}$=0.058) (Figure 7). The dorsal prostate gland weights of mice that received melatonin were significantly larger in the 14L:10D group ($\bar{x}$=0.066) than in the 24L:0D group ($\bar{x}$=0.040) but did not

differ significantly from the 1L:23D group ($\bar{x}$=0.050) (Figure 6). Penis weights of mice that received melatonin were significantly larger in the 1L:23D ($\bar{x}$= 0.062) and 14L:10D ($\bar{x}$=0.058) groups than in the 24L:0D group ($\bar{x}$=0.049) (Figure 8). Epididymis weights of mice that received vehicle injections were significantly smaller in the 1L:23D ($\bar{x}$=0.232) and 14L:10D ($\bar{x}$=0.239) groups than in the 24L:0D group ($\bar{x}$=0.286) (Figure 9). There were no other significant differences in accessory sex organ weights as influenced by photoperiod after 90 days. There were no significant differences in accessory sex organ weights following melatonin administration.

There were a number of changes in other organ weights (expressed as percentages of total body weight). Organ (pituitary, kidneys, adrenals, thymus, thyroid) weights expressed as percentages of total body weight were used for all statistical analyses (Figures 14 through 18). The kidney weights of mice that were sacrificed after 45 days were significantly larger in the 1L:23D group ($\bar{x}$=1.575) than in the 24L:0D group ($\bar{x}$= 1.273) but did not differ significantly from the 14L:10D group ($\bar{x}$=1.462) (Figure 14). There were no other significant differences in non-reproductive organ weights that were examined after 45 days.

Mean pituitary weights of mice that received melatonin were all significantly different from one another (Figure 15). The mean pituitary weight was 0.003 in the 24L:0D group, 0.005 in the 1L:23D group, and 0.006 in the 14L:10D group. Kidney weights of mice that received melatonin were significantly larger in the 14L:10D ($\bar{x}$=1.526) and 1L:23D ($\bar{x}$=1.459) groups than in the 24L:0D group ($\bar{x}$=1.336) (Figure 14).

Thyroid weights of mice that received melatonin were significantly larger in the 1L:23D ($\bar{x}$=0.007) and 14L:10D ($\bar{x}$=0.006) groups than in the 24L:0D group ($\bar{x}$= 0.003) (Figure 16). There were no significant differences in adrenal (Figure 17) or thymus (Figure 18) weights as influenced by photoperiod. The thymus weights were significantly larger in mice that received melatonin ($\bar{x}$=0.150) than in mice that received vehicle injections ($\bar{x}$=0.103) in the 1L:23D group (Figure 18). There were no other significant differences in non-reproductive organ weights that were examined following melatonin administration.

DISCUSSION

Short photoperiods had an inhibitory effect and long photoperiods had a stimulatory effect on body weights of mice that received melatonin in the present study. The inhibitory results are in agreement with other studies on male and female rats and male hamsters (Browman, 1940; Hoffman and Reiter, 1965b; Hester, 1966; Reiter and Fraschini, 1969; Osman et al., 1972). Darkness increases the activity and metabolic rate of nocturnal animals (Hoffman and Reiter, 1965b) and consequently decreases body growth. Light deprivation could also inhibit body growth through a decrease in the synthesis or secretion of growth hormone (GH) at the pituitary or growth hormone-releasing factor (GH-RH) at the hypothalamus (Reiter and Fraschini, 1969; Reiter et al., 1975b).

Reduced pituitary and plasma GH levels in male rats exposed to constant darkness (Relkin, 1972) were reversed by pinealectomy (Malm et al., 1959; Hester, 1966; Reiter and Fraschini, 1969) while melatonin inhibited GH secretion (Minneman and Wurtman, 1976). However, there were no differences in body growth of mice that received melatonin and mice in the same photoperiod that received vehicle injections in the present study. It is possible that another pineal substance could be the inhibitory factor involved as the pineal

produces peptides and other methoxyindoles (Wurtman and Moskowitz, 1977).

The present results indicate that only the combination of long photoperiods and melatonin treatment was inhibitory to the pituitary gland. Previous research found that short photoperiods, melatonin treatment, or pinealectomy alone had no effect on pituitary weights of male hamsters (Reiter et al., 1974), rats (Holmes, 1956; Motta et al., 1967; Kinson and Robinson, 1970), or mice (Houssay et al., 1966a, 1966b). However, others found that pinealectomy had a stimulatory effect on pituitary weight in male and female rats (Hester, 1966) and mitotic activity (Bindoni and Raffaele, 1968) in male rats. Melatonin could be the inhibitory factor that was removed by pinealectomy as Adams and associates (1965) reported decreased functional activity of the pituitary associated with melatonin treatment. Certain authors have found decreased pituitary weights in male hamsters (Reiter and Hester, 1966) and male and female rats (Browman, 1940; Hester, 1966; Kinson and Robinson, 1970) exposed to short photoperiods or blindness, instances that stimulate melatonin synthesis. The present study is unique in observing an inhibitory effect on the pituitary when a combination of long photoperiods and melatonin administration were employed.

Long photoperiods alone or with melatonin treatment had an inhibitory effect on kidney weights in the present study. However, Vaughan (1971) found no effect on kidney weights in male mice that were pinealectomized. The present author has no explanation for the above results.

Different photoperiods, melatonin treatment, or combinations thereof had no effect on adrenal weights in the present study. Data from previous research are inconsistent. Houssay and others (1966b) found that pinealectomy had no effect on adrenal weights in male mice. However, exposure to continuous light led to adrenal regression in mature female rats (Reiter and Klein, 1971) and there are reports that pinealectomy inhibited adrenal growth in rats (Reiter et al., 1975b). Melatonin administration had positive effects on adrenal weights in female rats and male mice (Houssay et al., 1966b; Reiter and Fraschini, 1969) and corticosterone production in rats (Gromova et al., 1967).

Conversely, others found short photoperiods or blinding had an inhibitory effect on adrenal weights in male and female rats (Hester, 1966), male and female hamsters (Reiter and Hester, 1966; Reiter et al., 1966), and female mice (Farmer, 1974) while pinealectomy induced adrenal hypertrophy in female rats (Wurtman et al., 1959; Fraschini et al., 1968; Dickson and Hasty, 1972)

and male mice (Vaughan, 1971; Vaughan and Reiter, 1971; Vaughan et al., 1972). Melatonin suppressed adrenal hypertrophy in male and female mice (Fraschini et al., 1968; Vaughan, 1971; Vaughan et al., 1972). It is possible that the pineal inhibits corticotrophin (ACTH) secretion through melatonin (Collu and Fraschini, 1972; Reiter et al., 1975b; Shaw, 1977) in view of the latter data. It is also possible that conditions of stress, such as crowding, influence adrenal weights and therefore produce confusing results in the aforementioned studies.

Melatonin administration had a stimulatory effect on thymus weight in the 1L:23D group of mice in the present study and is consistent with the finding that pinealectomy of male mice accelerated involution of the thymus (Vaughan, 1971; Vaughan and Reiter, 1971). Increased androgen secretion from the testes or adrenals, a consequence of pinealectomy, enhances thymic involution (Vaughan, 1971; Vaughan and Reiter, 1971).

Information regarding the pineal - thyroid relationship is sparse and inconsistent (Reiter and Fraschini, 1969; Reiter et al., 1975b). The combination of long photoperiods and melatonin administration in the present work had a negative effect on thyroid weight. Likewise, constant light depressed thyroid weight and I^{131} uptake in male and female mice (Puntriano and Meites, 1951) and thyroid hypertrophy

followed melatonin treatment in prepuberal rats (Collu and Fraschini, 1972). Melatonin injections alone had no effect on thyroid weight of male mice in the present or other studies (Houssay et al., 1966a, 1966b). Pinealectomy of rats did not affect thyroid function in one study (Mess, 1968). However, other research found that darkness decreased thyroid activity in female hamsters (Reiter et al., 1966) and melatonin decreased such activity in male and female rats (Braschieri et al., 1963; Ishibashi et al., 1966; Houssay and Pazo, 1968; Panda and Turner, 1968). Pinealectomy of male and female rats induced thyroid activity and growth (Ishibashi et al., 1966; Houssay and Pazo, 1968; Pazo et al., 1968). The latter results suggest that a stimulated pineal suppresses thyroid activity via melatonin.

Testicular anatomy was unaffected by photoperiod in the present study. Long photoperiods appeared to have an inhibitory effect on plasma testosterone levels in mice that received melatonin or vehicle injections while short photoperiods appeared to have a stimulatory effect on testosterone levels in mice that were sacrificed after 45 days. The anatomical results are in agreement with certain studies on rats by Kinson and Robinson (1970) and Hoffman (1973). However, other studies have reported testicular atrophy in rats (Itoh

et al., 1962; Hester, 1966) and hamsters (Hoffman and Reiter, 1965a, 1965b; Clabough and Seibel, 1968; Turek, 1977) in response to short photoperiods.

Melatonin treatment alone had no effect on testicular anatomy in the present study. This finding agrees with some studies on rats (Ebels and Prop, 1965; Motta et al., 1967; Kinson and Robinson, 1970; Reiter et al., 1975b; Turek et al., 1976; Alonso et al., 1978). However, melatonin induced testicular regression in rats (Debeljuk, 1969), hamsters (Turek et al., 1976; Turek, 1977), weasels (Rust and Meyer, 1969), and grasshopper mice (Turek et al., 1976) in other works. Conversely, others found that melatonin prevented testicular regression in hamsters (Reiter et al., 1974; Hoffman, 1975; Reiter et al., 1975a) and stimulated spermatogenesis in rats (Reiter et al., 1975b). Similarly, melatonin administration alone appeared to have a stimulatory effect on mouse plasma testosterone levels in each photoperiod in the present study. Melatonin may exert progonadal effects by inhibiting the synthesis or release of the true antigonadotrophic substance, by rendering the sites of action less sensitive to the antigonadotrophic substance, or by directly stimulating the gonads (Reiter et al., 1974). Inconsistent results from various studies could be due to the following experimental factors that varied widely:

dosage of melatonin, time of administration, mode of administration, age of animals, species of animals, duration of the study, and photoperiod employed.

The two instances (ventral prostate and dorsal prostate glands) of inhibitory effects of long photoperiods and two instances (dorsal prostate gland and penis) of inhibitory effects of the combination of long photoperiods and melatonin treatment were probably due to decreased plasma testosterone levels that were found after exposure to long photoperiods in the present study. A decrease in the weight of accessory organs is indicative of a decrease in the production or secretion of testicular androgen (Motta et al., 1967; Fraschini, 1970) as androgen is necessary to maintain the structural integrity and functional activity of accessory sex structures (van Tienhoven, 1968).

The inhibitory effects of long photoperiods, alone or in combination with melatonin, found in the present study are contrary to the inhibitory effects of short photoperiods or melatonin administration, or stimulatory effect of long photoperiods on the reproductive system of female mice (Vaughan and Benson, 1970; Vaughan et al., 1970; Chu et al., 1964; Vaughan et al., 1971; Reiter et al., 1972), rats (Wurtman et al., 1963a; Chu et al., 1964; McIsaac et al., 1964; Wurtman and Axelrod, 1965a, 1965b; Hester, 1966; Motta et al.,

1967; Sorrentino, 1968; Reiter, 1969b; Vaughan and Benson, 1970; Fraschini et al., 1971; Reiter and Klein, 1971; Osman et al., 1972; Bajpai et al., 1975; Singh et al., 1975), hamsters (Reiter and Hester, 1966; Reiter et al., 1966; Reiter, 1967; Reiter, 1969a; Reiter and Johnson, 1974; Tamarkin et al., 1977), and ferrets (Herbert, 1971). The fact that melatonin alone produced no effects on accessory sex organs in the present study implies that the combination of long photoperiods and melatonin was necessary. Other researchers also found that melatonin alone had no effect on male accessory sex organs in rats (Ebels and Prop, 1965; Reiter et al., 1975b) and hamsters (Turek et al., 1975; Reiter et al., 1976). However, other studies found that melatonin had an inhibitory effect on male accessory sex organs in rats (Motta et al., 1967; Debeljuk, 1969; Kinson and Robinson, 1970; Alonso et al., 1978) and hamsters (Turek et al., 1975; Reiter et al., 1976; Sackman et al., 1977). Conversely, melatonin had a stimulatory effect on male accessory sex organs in hamsters (Reiter et al., 1974; Reiter et al., 1975a) and rats (Reiter et al., 1975b). Widely varying experimental conditions are one explanation for the conflicting results.

There are various explanations for the necessity of a combination of long photoperiods and melatonin

treatment in order to produce antigonadotrophic effects. One explanation is that long photoperiods stimulate the pineal to produce a substance that enhances the antigonadotrophic effect of melatonin. A second explanation is that long photoperiods stimulate the pineal to produce an antigonadotrophic substance that is activated by melatonin. A third explanation involves serotonin, a precursor of melatonin. Serotonin is synthesized in the pineal in a circadian rhythm that experiences maximum concentrations during the daytime, continues in darkness, but ceases in rats exposed to continuous light (Wurtman and Axelrod, 1965b; Axelrod, 1974; Strang, 1977). Serotonin is an essential substance in the biosynthesis of melatonin (Axelrod, 1974) and a decrease in pineal serotonin content therefore reduces melatonin production. Perhaps long photoperiods are necessary to examine the antigonadotrophic effect of melatonin because exogenous melatonin and short photoperiods produce such a high melatonin content that it is progonadal. Chronic administration of melatonin in male hamsters was progonadal (Reiter et al., 1976) and is in agreement with the above idea.

A fourth explanation involves granulated vesicles of the pinealocytes and possibly serotonin. The mouse pineal differs from that of the rat in that it contains large concentrations of granulated vesicles (Upson et

al., 1976; Strang, 1977). The vesicles probably represent the packaged or secretory form of the main antigonadotrophic product of the pineal (Upson et al., 1976; Benson and Krasovich, 1977). Similarities between serotonin and granulated vesicle rhythms provide evidence that the vesicles could store serotonin (Upson et al., 1976; Strang, 1977). There was a circadian rhythm in number of pinealocyte vesicles of male mice exposed to a 12L:12D photoperiod with maximum numbers found late in the light period (Benson and Krasovich, 1977). Also, a dramatic decrease in the number of vesicles in male mice exposed to continuous light occurred (Upson et al., 1976). Benson and Krasovich (1977) suggested an association between the synthesis or secretion of the vesicles and melatonin based on the following evidence: melatonin, levels of which are highest during darkness, enhanced the reduction of number of vesicles when given during darkness; and, melatonin enhanced the increase of number of vesicles when given during the light period of a diurnal cycle (Benson and Krasovich, 1977). Perhaps melatonin stimulates the release and synthesis of the vesicles and antigonadotrophic factor they contain (Upson et al., 1976; Benson and Krasovich, 1977). This concept is consistent with results of the present study on male mice and previous findings (Reiter et al., 1976; Tamarkin et al., 1977) that melatonin

produces antigonadotrophic effects in male hamsters only if given late in the light period. The implication is that the pineal gland itself is a primary site of melatonin action and that melatonin influences the release of pineal compounds that inhibit reproduction (Reiter et al., 1976; Benson and Krasovich, 1977).

Long photoperiods were stimulatory to the epididymis of mice that received vehicle injections and ventral prostate of mice that received melatonin in the present study. The two cases are certainly inconsistent with the above results and unexplained by the author. While the results are contradictory to the above, they are consistent with some works of other researchers on rats (Browman, 1940; Itoh et al., 1962; Hester, 1966; Reiter, 1968; Relkin, 1972) and hamsters (Reiter and Hester, 1966; Reiter, 1968; Berndtson and Desjardins, 1974; Reiter et al., 1974; Turek et al., 1975).

The pineal contains other substances, such as peptides and other methoxyindoles, that could be antigonadotrophic. For example, the peptide arginine vasotocin inhibited growth of accessory sex organs in male mice (Vaughan et al., 1974b; Vaughan and Blask, 1978) and hamsters (Vaughan et al., 1974a). Further research on the effects of different pineal principles on the reproductive system of male mammals is necessary to clarify the present state of knowledge.

Long photoperiods had no effect on testicular anatomy but appeared to have an inhibitory effect on plasma testosterone levels implying that the pineal gland could mediate the effect by secreting an anti-interstitial cell stimulating hormone (anti-ICSH) substance (Tamarkin et al., 1977). ICSH stimulates the interstitial cells of Leydig in the testis to produce and secrete androgen (Turner and Bagnara, 1976). Melatonin was probably not the anti-ICSH substance involved in the present study as it appeared to have a stimulatory effect on plasma testosterone levels.

It appears that long photoperiods had a negative effect on testosterone levels and consequently accessory sex organ weights in the present study. The inhibitory effect is probably mediated by the pineal gland with melatonin enhancing the synthesis or secretion of a pineal anti-ICSH substance. No changes in testicular anatomy indicate that no anti-follicle stimulating hormone (anti-FSH) factor was involved as changes in testicular weights and spermatogenesis are indicative of changes in FSH secretion (Motta et al., 1967; Fraschini, 1970).

The inhibitory effect of long photoperiods found in this study on male mice is an enigma as long photoperiods are stimulatory to the reproductive system of female rodents. The biological significance of the

inhibitory effect on males is unclear and apparently in contrast to the concept of optimum timing for successful mating and survival of offspring. Further research in the area should be enlightening.

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Figure 1. Weekly mean body weights (expressed with initial body weights as 100.0) for each experimental group of mice. (N=24 until one third of the mice in the photoperiodic regimen were sacrificed; N=8 thereafter).

*LEGEND.

Photoperiod.

1L:23D - Lights on from 1:00 P.M. to 2:00 P.M.

14L:10D - Lights on from midnight to 2:00 P.M.

24L:0D - Lights on continuously.

Experimental Group.

45 Day - Mice that were sacrificed after 45 days in the indicated photoperiodic regimen.

90 Day Vehicle - Mice that were sacrificed after 90 days in the indicated photoperiodic regimen and received daily 0.05 ml vehicle injections from day 46 through the end of the experimental period.

90 Day Melatonin - Mice that were sacrificed after 90 days in the indicated photoperiodic regimen and received daily 0.05 ml melatonin (10 µg) injections from day 46 through the end of the experimental period.

Symbol	Experimental Group and Photoperiod
▲	45 Day, 24L:0D
□	90 Day Vehicle, 24L:0D
■	90 Day Melatonin, 24L:0D
∇	45 Day, 14L:10D
○	90 Day Vehicle, 14L:10D
●	90 Day Melatonin, 14L:10D
△	45 Day, 1L:23D
▼	90 Day Vehicle, 1L:23D
x	90 Day Melatonin, 1L:23D

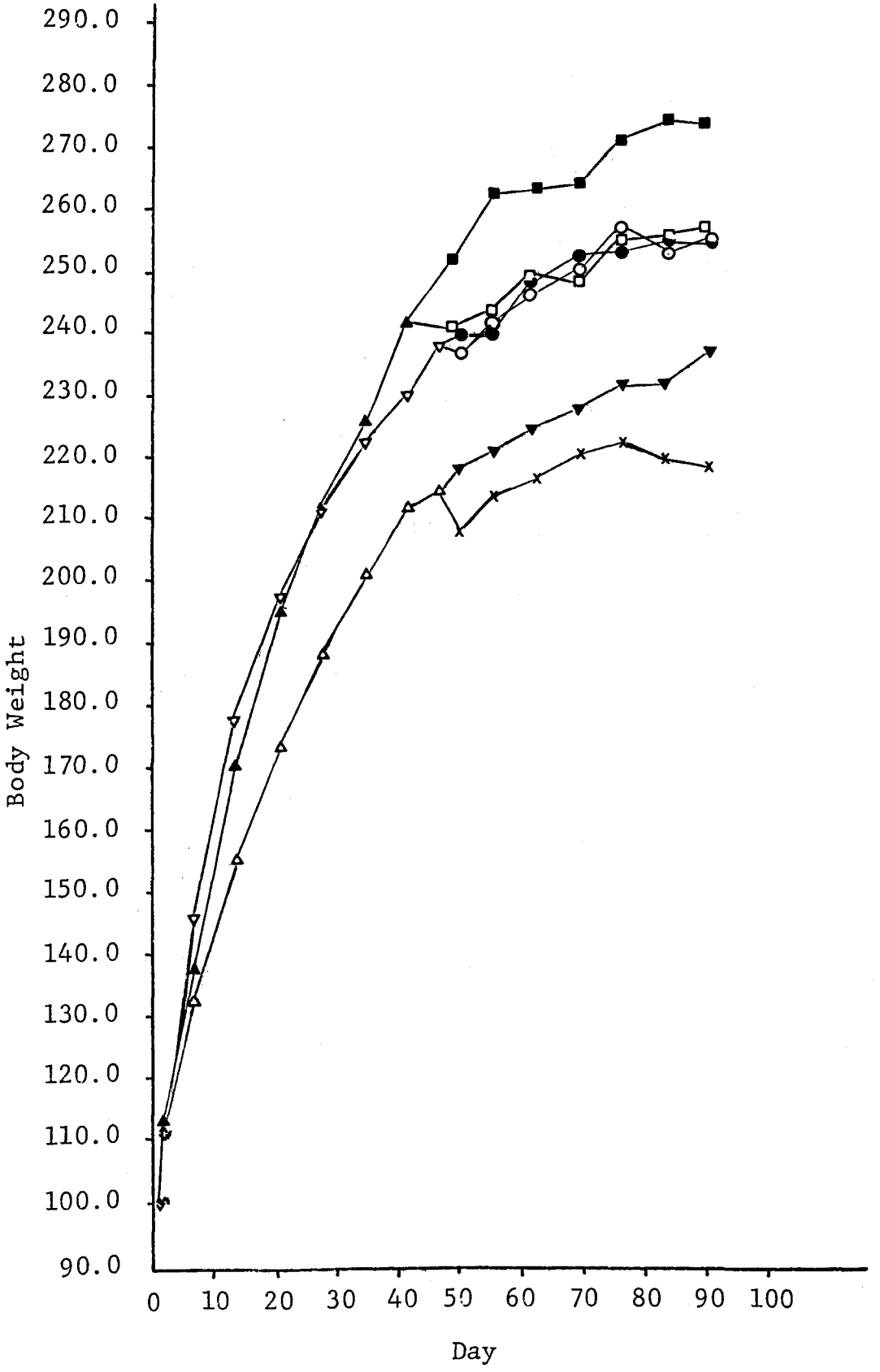

290.0
280.0
270.0
260.0
250.0
240.0
230.0
220.0
210.0
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
Body Weight
0
10
20
30
40
50
60
70
80
90
100
Day

Figure 2. Mean percent increase in body weight ± Standard Deviation (N=8).

Results of Duncan's multiple-range test of mean percent increases in body weight. Means underscored by the same line do not differ significantly at α=0.05.

*See Legend, Figure 1.

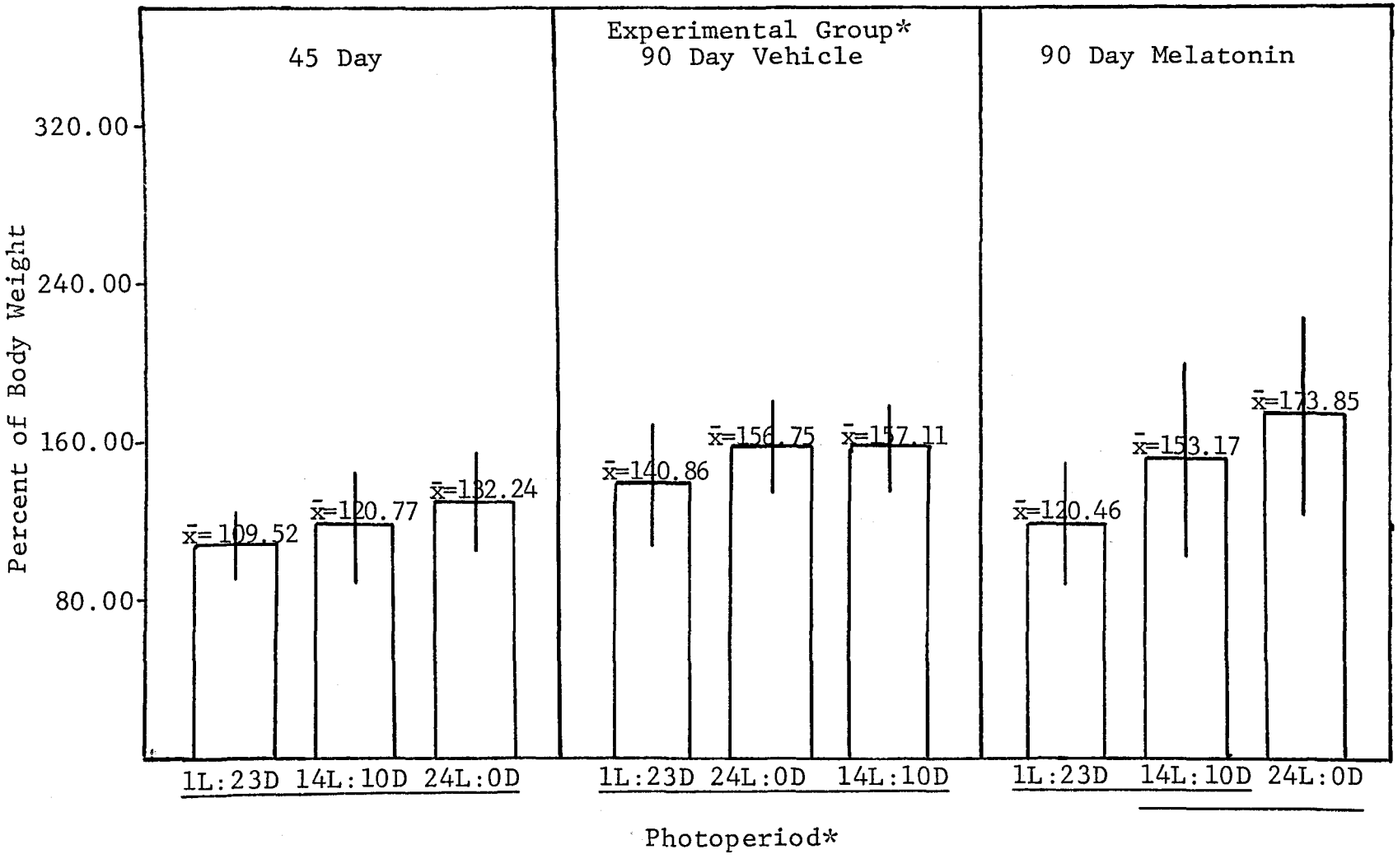

45 Day
Experimental Group*
90 Day Vehicle
90 Day Melatonin
320.00
240.00
160.00
80.00
Percent of Body Weight
x̄=109.52
x̄=120.77
x̄=132.24
x̄=140.86
x̄=156.75
x̄=157.11
x̄=120.46
x̄=153.17
x̄=173.85
1L:23D 14L:10D 24L:0D
1L:23D 24L:0D 14L:10D
1L:23D 14L:10D 24L:0D
Photoperiod*

Figure 3. Mean testis weights (expressed as percents of total body weight) ± Standard Deviation (N=8).

*See Legend, Figure 1.

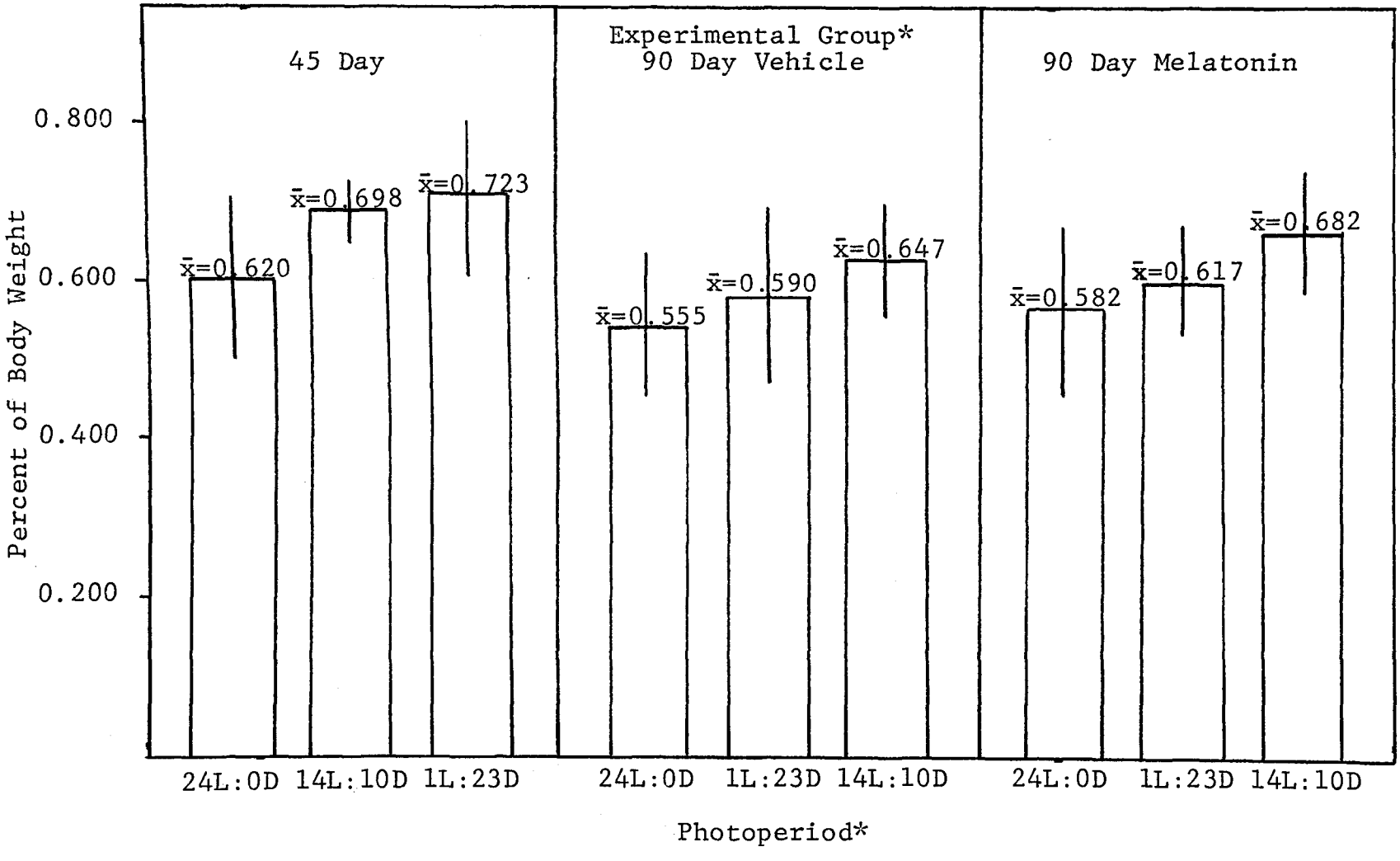

45 Day
Experimental Group*
90 Day Vehicle
90 Day Melatonin
0.800
0.600
0.400
0.200
Percent of Body Weight
x̄=0.620
x̄=0.698
x̄=0.723
x̄=0.555
x̄=0.590
x̄=0.647
x̄=0.582
x̄=0.617
x̄=0.682
24L:0D 14L:10D 1L:23D
24L:0D 1L:23D 14L:10D
24L:0D 1L:23D 14L:10D
Photoperiod*

Figure 4. Photomicrographs of seminiferous tubules in section showing stages of spermatogenesis. A representative photomicrograph is shown for each experimental group of mice. Magnification is 311X.

Photomicrograph	Experimental Group and Photoperiod*
1	45 Day, 24L:0D
2	45 Day, 14L:10D
3	45 Day, 1L:23D
4	90 Day Vehicle, 24L:0D
5	90 Day Vehicle, 14L:10D
6	90 Day Vehicle, 1L:23D
7	90 Day Melatonin, 24L:0D
8	90 Day Melatonin, 14L:10D
9	90 Day Melatonin, 1L:23D

*See Legend, Figure 1.

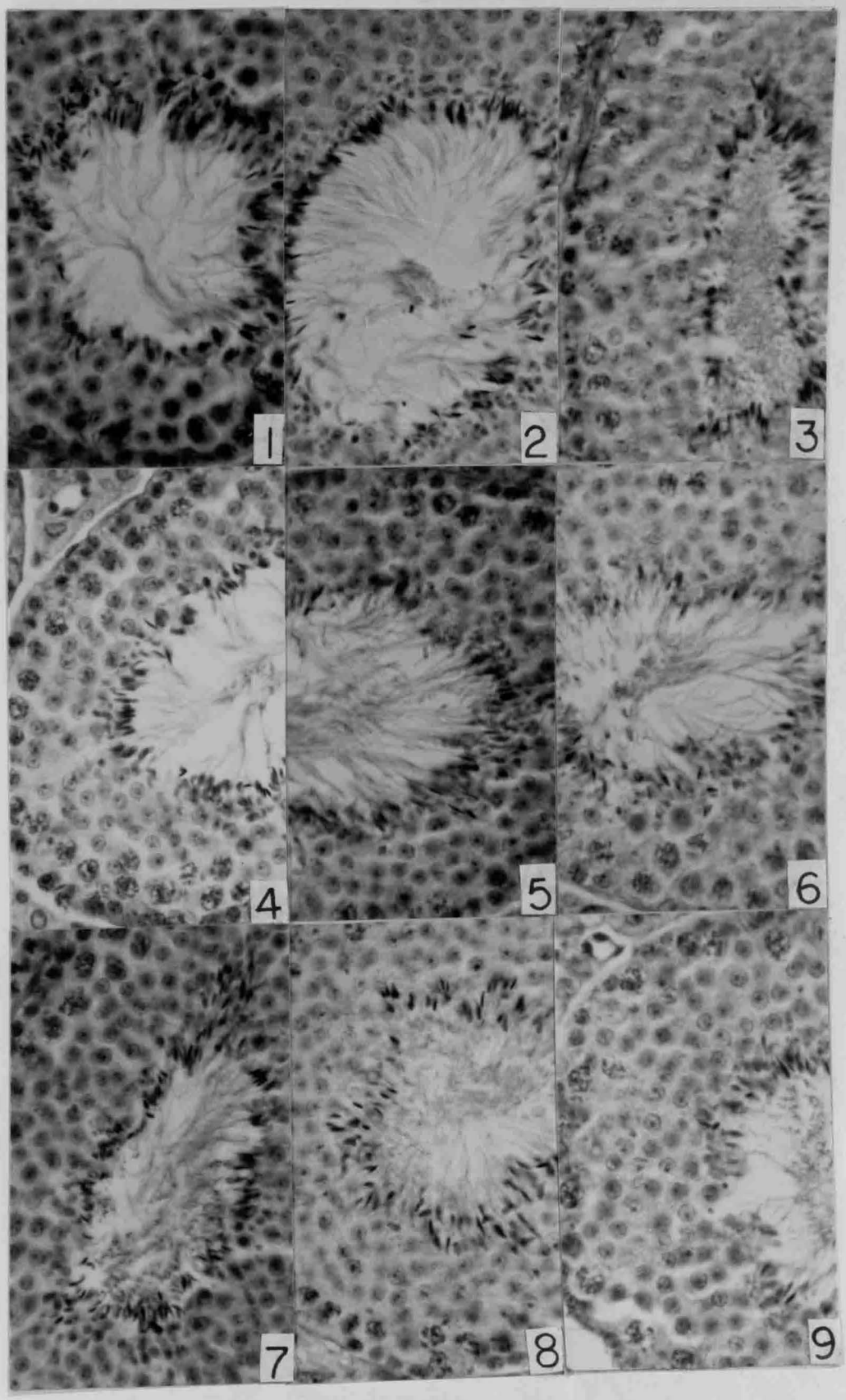

1
2
3
4
5
6
7
8
9

Figure 5. Plasma testosterone levels given as µg% of testosterone in plasma as determined by radioimmunoassay. Plasma from animals (N=8) that experienced identical regimens was combined prior to radioimmunoassay.

*See Legend, Figure 1.

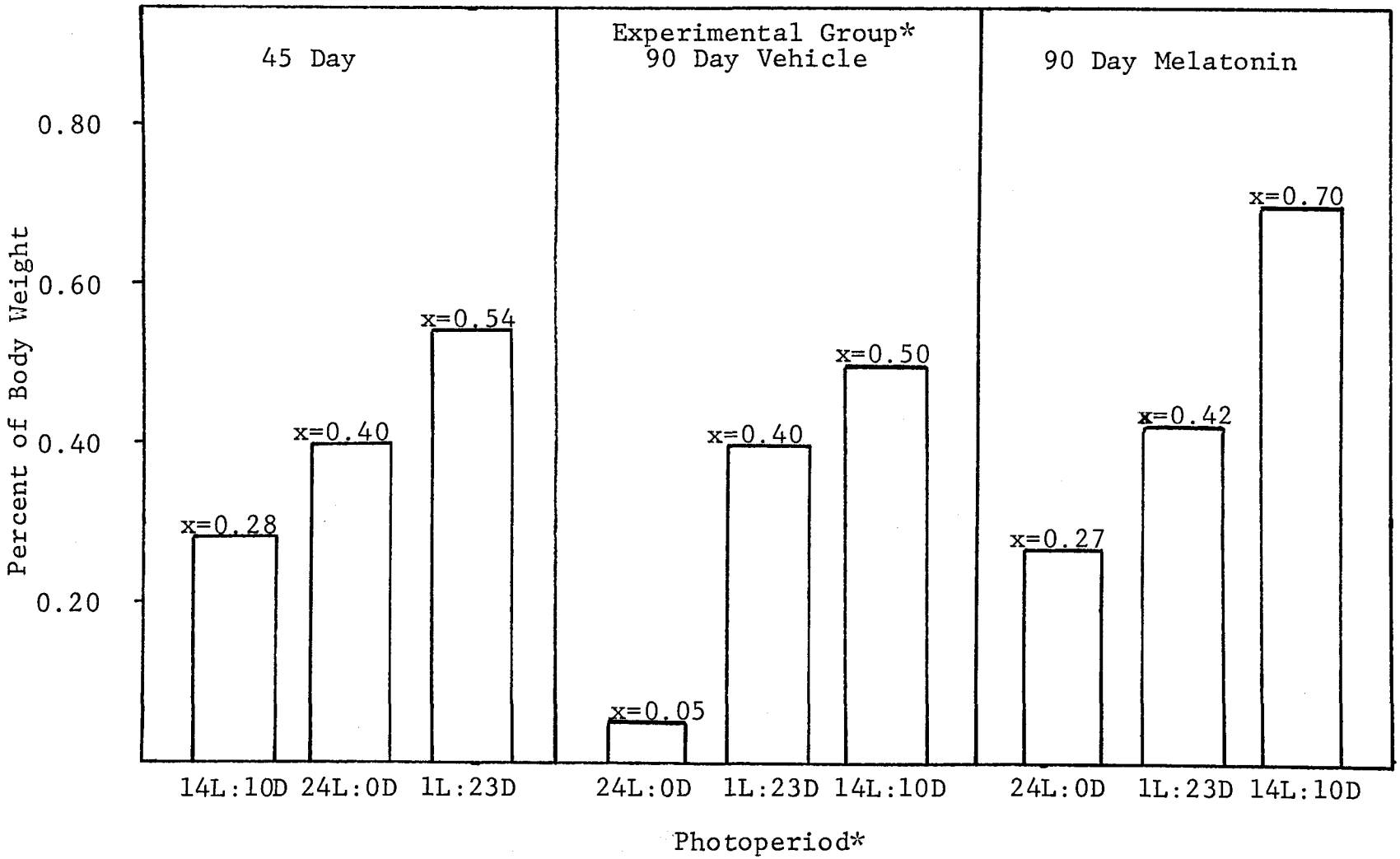

45 Day
Experimental Group*
90 Day Vehicle
90 Day Melatonin
Percent of Body Weight
0.80
0.60
0.40
0.20
x=0.28
x=0.40
x=0.54
x=0.05
x=0.40
x=0.50
x=0.27
x=0.42
x=0.70
14L:10D
24L:0D
1L:23D
24L:0D
1L:23D
14L:10D
24L:0D
1L:23D
14L:10D
Photoperiod*

Figure 6. Mean dorsal prostate gland weights (expressed as percents of total body weight) ± Standard Deviation (N=8).

Results of Duncan's multiple-range test of mean dorsal prostate gland weights. Means underscored by the same line do not differ significantly at α=0.05.

*See Legend, Figure 1.

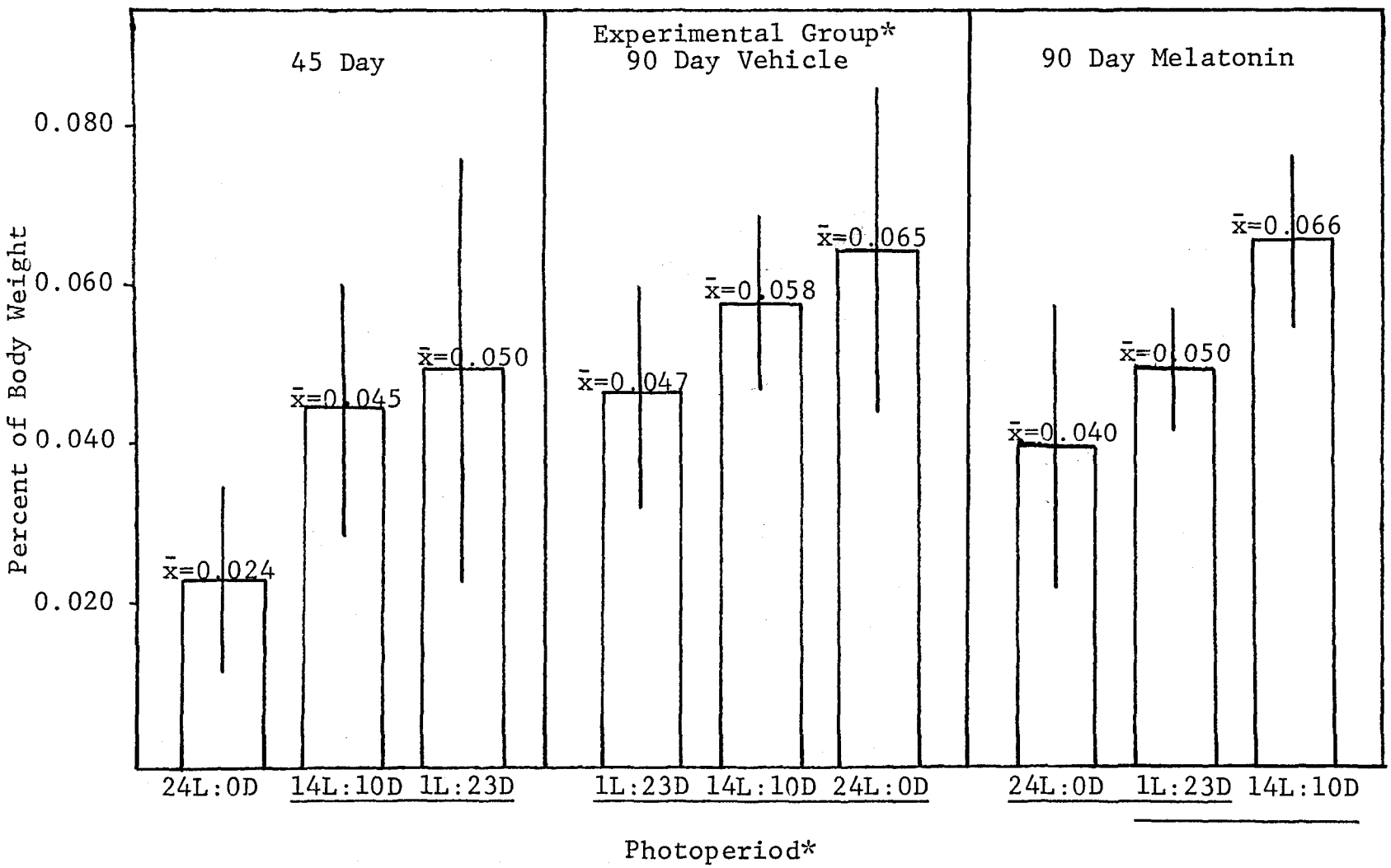

45 Day
Experimental Group*
90 Day Vehicle
90 Day Melatonin
Percent of Body Weight
0.080
0.060
0.040
0.020
x̄=0.024
x̄=0.045
x̄=0.050
x̄=0.047
x̄=0.058
x̄=0.065
x̄=0.040
x̄=0.050
x̄=0.066
24L:0D
14L:10D
1L:23D
1L:23D
14L:10D
24L:0D
24L:0D
1L:23D
14L:10D
Photoperiod*

Figure 7. Mean ventral prostate gland weights (expressed as percents of total body weight) ± Standard Deviation (N=8).

Results of Duncan's multiple-range test of mean ventral prostate gland weights. Means underscored by the same line do not differ significantly at α=0.05.

*See Legend, Figure 1.

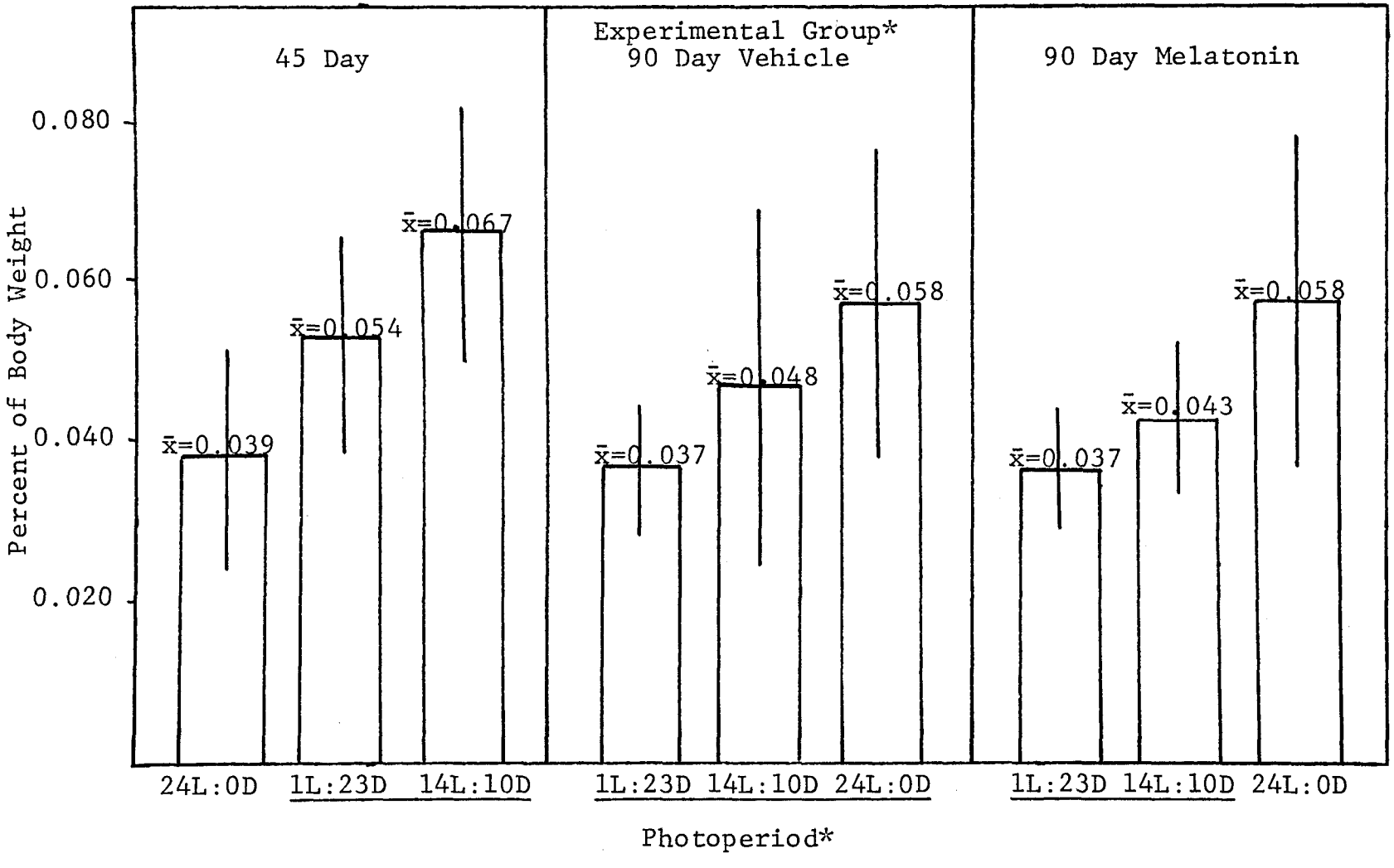

45 Day
Experimental Group*
90 Day Vehicle
90 Day Melatonin
Percent of Body Weight
0.080
0.060
0.040
0.020
x̄=0.039
x̄=0.054
x̄=0.067
x̄=0.037
x̄=0.048
x̄=0.058
x̄=0.037
x̄=0.043
x̄=0.058
24L:0D
1L:23D
14L:10D
1L:23D
14L:10D
24L:0D
1L:23D
14L:10D
24L:0D
Photoperiod*

Figure 8. Mean penis weights (expressed as percents of total body weight) $\pm$ Standard Deviation (N=8).

Results of Duncan's multiple-range test of mean penis weights. Means underscored by the same line do not differ significantly at α=0.05.

*See Legend, Figure 1.

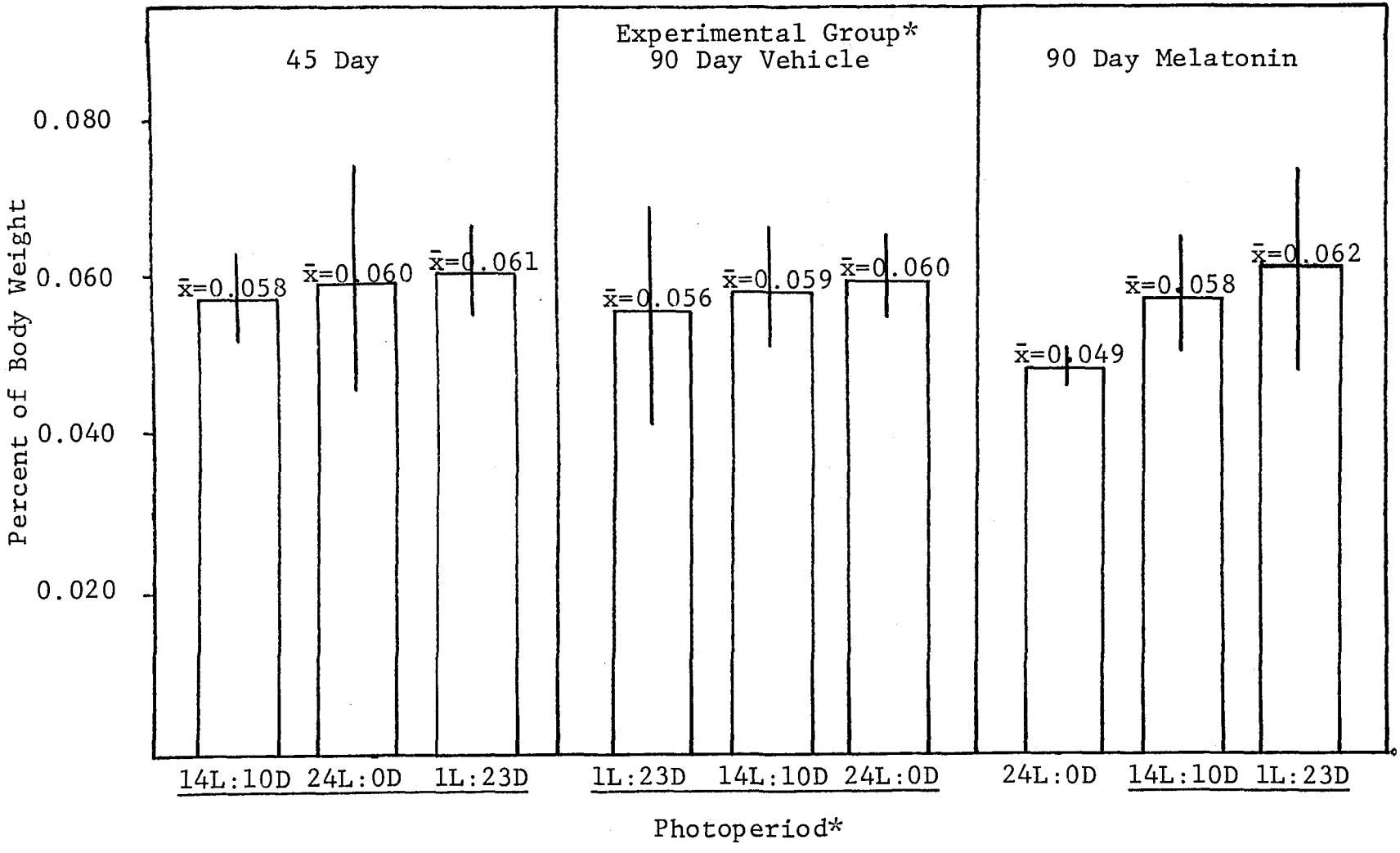

45 Day
Experimental Group*
90 Day Vehicle
90 Day Melatonin
0.080
0.060
0.040
0.020
Percent of Body Weight
x̄=0.058
x̄=0.060
x̄=0.061
x̄=0.056
x̄=0.059
x̄=0.060
x̄=0.049
x̄=0.058
x̄=0.062
14L:10D 24L:0D 1L:23D
1L:23D 14L:10D 24L:0D
24L:0D 14L:10D 1L:23D
Photoperiod*

Figure 9. Mean epididymis weights (expressed as percents of total body weight) ± Standard Deviation (N=8).

Results of Duncan's multiple-range test of mean epididymis weights. Means underscored by the same line do not differ significantly at α=0.05.

*See Legend, Figure 1.

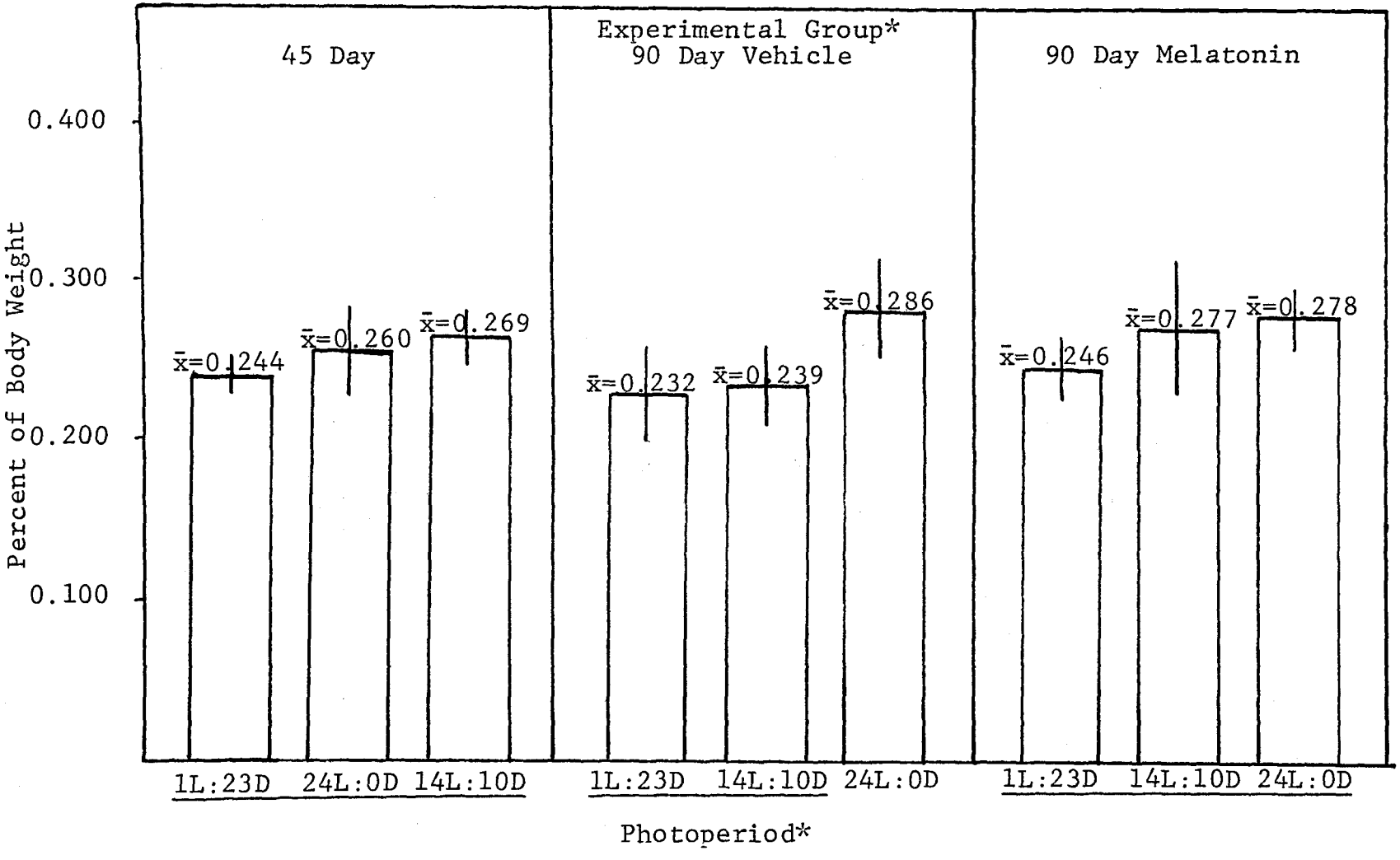

45 Day
Experimental Group*
90 Day Vehicle
90 Day Melatonin
0.400
0.300
0.200
0.100
Percent of Body Weight
x̄=0.244
x̄=0.260
x̄=0.269
x̄=0.232
x̄=0.239
x̄=0.286
x̄=0.246
x̄=0.277
x̄=0.278
1L:23D
24L:0D
14L:10D
1L:23D
14L:10D
24L:0D
1L:23D
14L:10D
24L:0D
Photoperiod*

Figure 10. Mean seminal vesicle weights (expressed as percents of total body weight) ± Standard Deviation (N=8).

*See Legend, Figure 1.

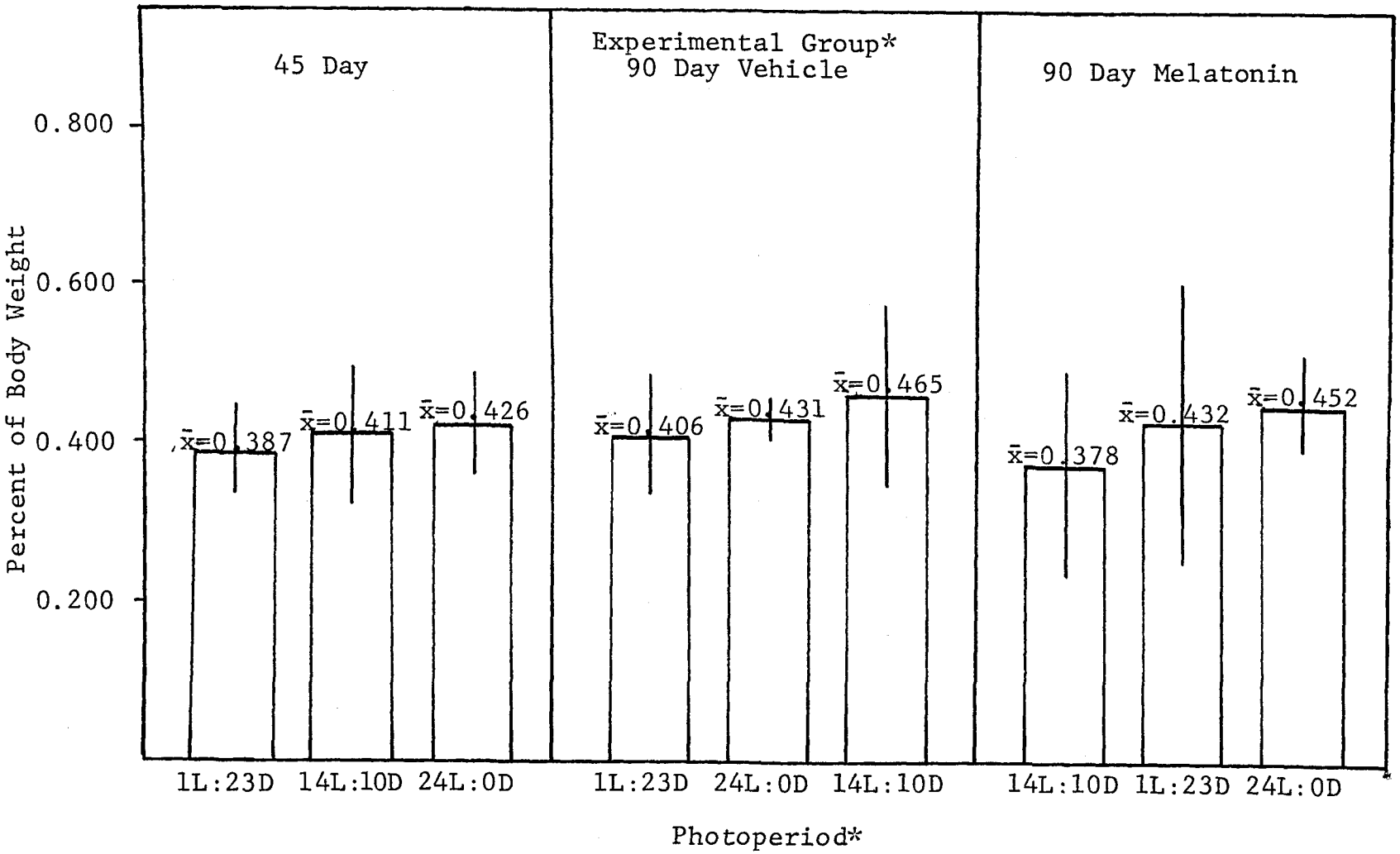

45 Day
Experimental Group*
90 Day Vehicle
90 Day Melatonin
0.800
0.600
0.400
0.200
Percent of Body Weight
x̄=0.387
x̄=0.411
x̄=0.426
x̄=0.406
x̄=0.431
x̄=0.465
x̄=0.378
x̄=0.432
x̄=0.452
1L:23D
14L:10D
24L:0D
1L:23D
24L:0D
14L:10D
14L:10D
1L:23D
24L:0D
Photoperiod*

Figure 11. Mean coagulating gland weights (expressed as percents of total body weight) ± Standard Deviation (N=8).

*See Legend, Figure 1.

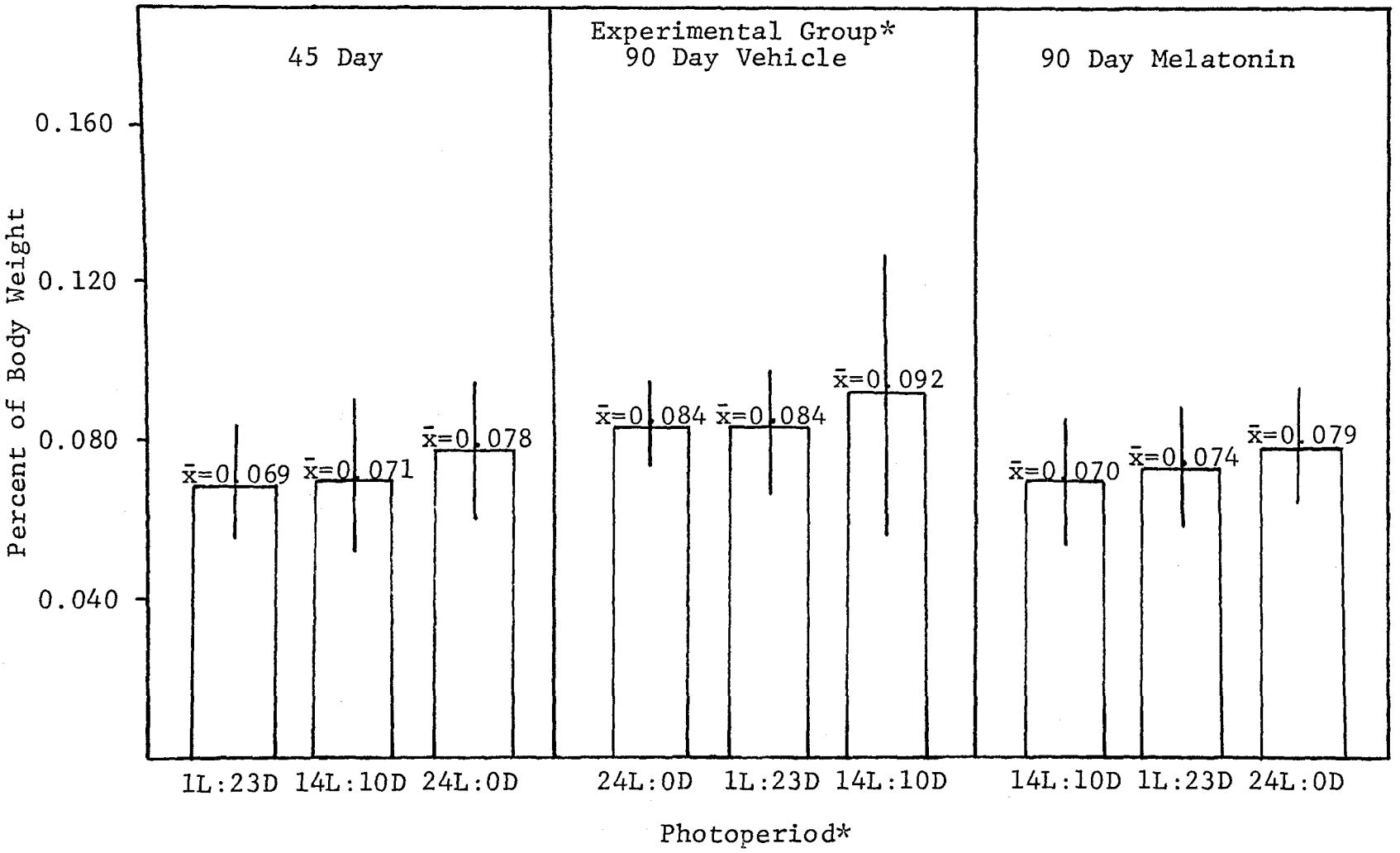

45 Day
Experimental Group*
90 Day Vehicle
90 Day Melatonin
0.160
0.120
0.080
0.040
Percent of Body Weight
x̄=0.069
x̄=0.071
x̄=0.078
x̄=0.084
x̄=0.084
x̄=0.092
x̄=0.070
x̄=0.074
x̄=0.079
1L:23D 14L:10D 24L:0D
24L:0D 1L:23D 14L:10D
14L:10D 1L:23D 24L:0D
Photoperiod*

Figure 12. Mean preputial gland weights (expressed as percents of total body weight) ± Standard Deviation (N=8).

*See Legend, Figure 1.

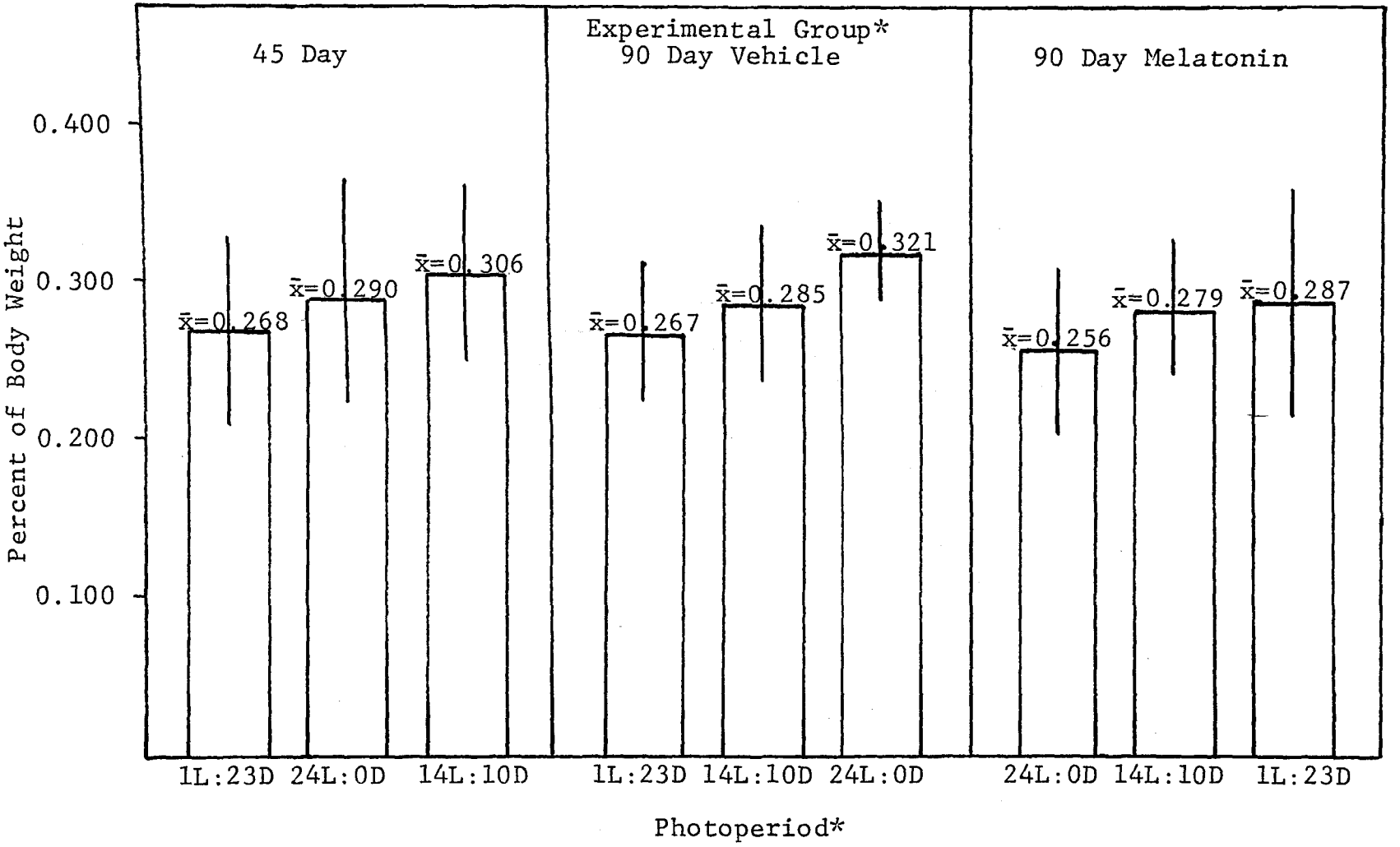

45 Day
Experimental Group*
90 Day Vehicle
90 Day Melatonin
0.400
0.300
0.200
0.100
Percent of Body Weight
x̄=0.268
x̄=0.290
x̄=0.306
x̄=0.267
x̄=0.285
x̄=0.321
x̄=0.256
x̄=0.279
x̄=0.287
1L:23D 24L:0D 14L:10D
1L:23D 14L:10D 24L:0D
24L:0D 14L:10D 1L:23D
Photoperiod*

Figure 13. Mean vas deferens weights (expressed as percents of total body weight) ± Standard Deviation (N=8).

*See Legend, Figure 1.

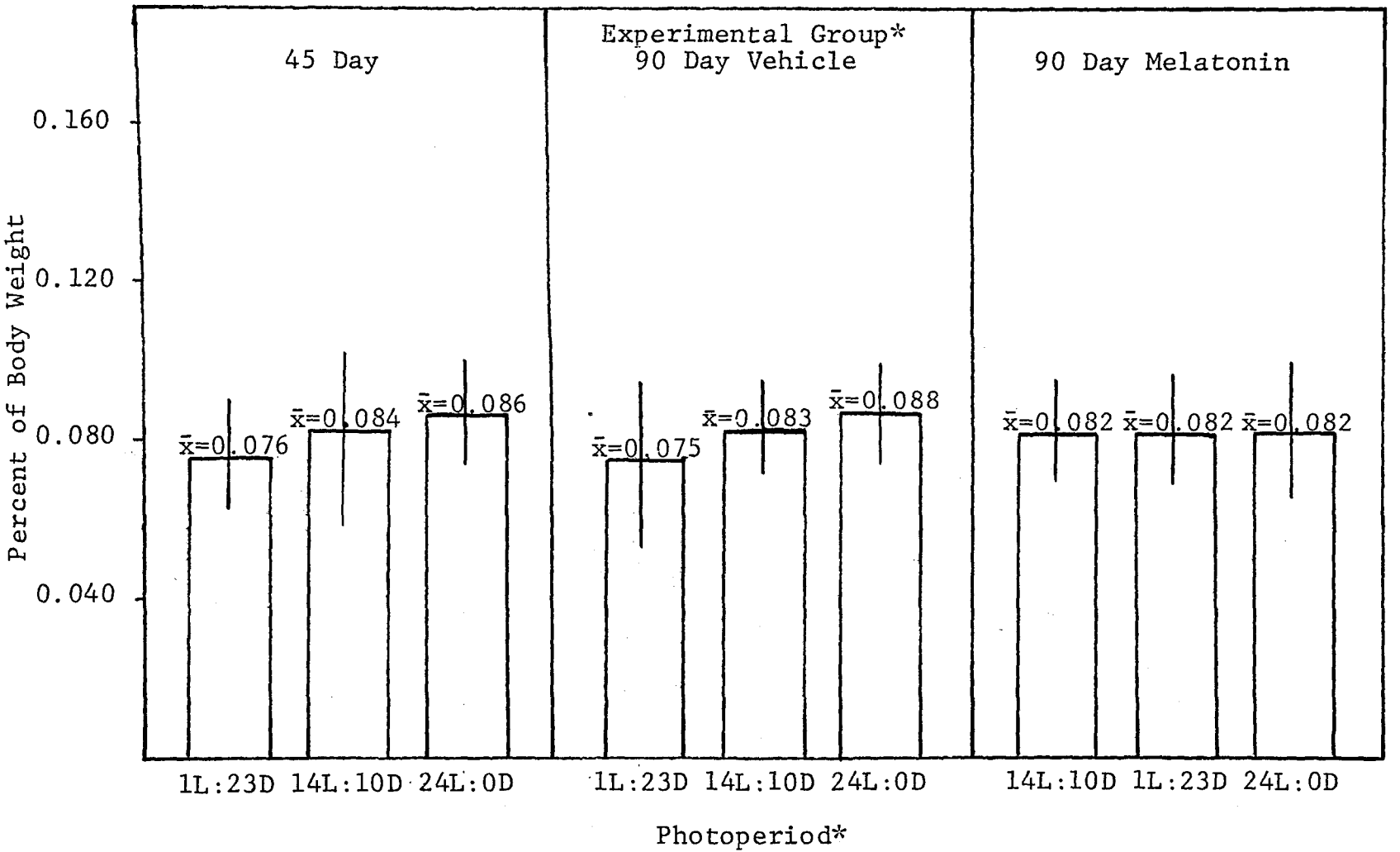

45 Day
Experimental Group*
90 Day Vehicle
90 Day Melatonin
0.160
0.120
0.080
0.040
Percent of Body Weight
x̄=0.076
x̄=0.084
x̄=0.086
x̄=0.075
x̄=0.083
x̄=0.088
x̄=0.082
x̄=0.082
x̄=0.082
1L:23D 14L:10D 24L:0D
1L:23D 14L:10D 24L:0D
14L:10D 1L:23D 24L:0D
Photoperiod*

Figure 14. Mean kidney weights (expressed as percents of total body weight) $\pm$ Standard Deviation (N=8).

Results of Duncan's multiple-range test of mean kidney weights. Means underscored by the same line do not differ significantly at α=0.05.

*See Legend, Figure 1.

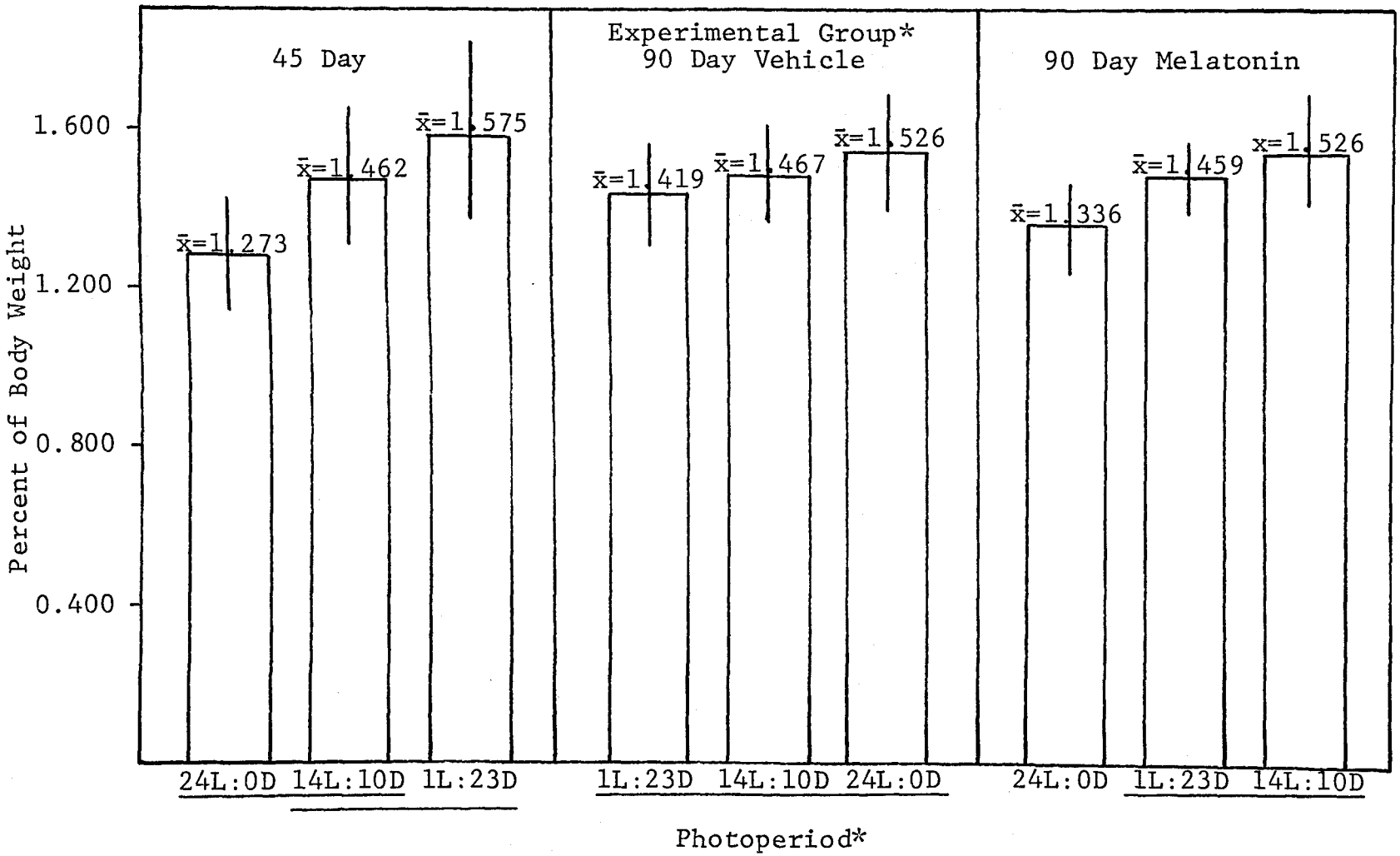

45 Day
Experimental Group*
90 Day Vehicle
90 Day Melatonin
1.600
1.200
0.800
0.400
Percent of Body Weight
x̄=1.273
x̄=1.462
x̄=1.575
x̄=1.419
x̄=1.467
x̄=1.526
x̄=1.336
x̄=1.459
x=1.526
24L:0D 14L:10D 1L:23D
1L:23D 14L:10D 24L:0D
24L:0D 1L:23D 14L:10D
Photoperiod*

Figure 15. Mean pituitary gland weights (expressed as percents of total body weight) $\pm$ Standard Deviation (N=8).

Results of Duncan's multiple-range test of mean pituitary gland weights. Means underscored by the same line do not differ significantly at α=0.05.

*See Legend, Figure 1.

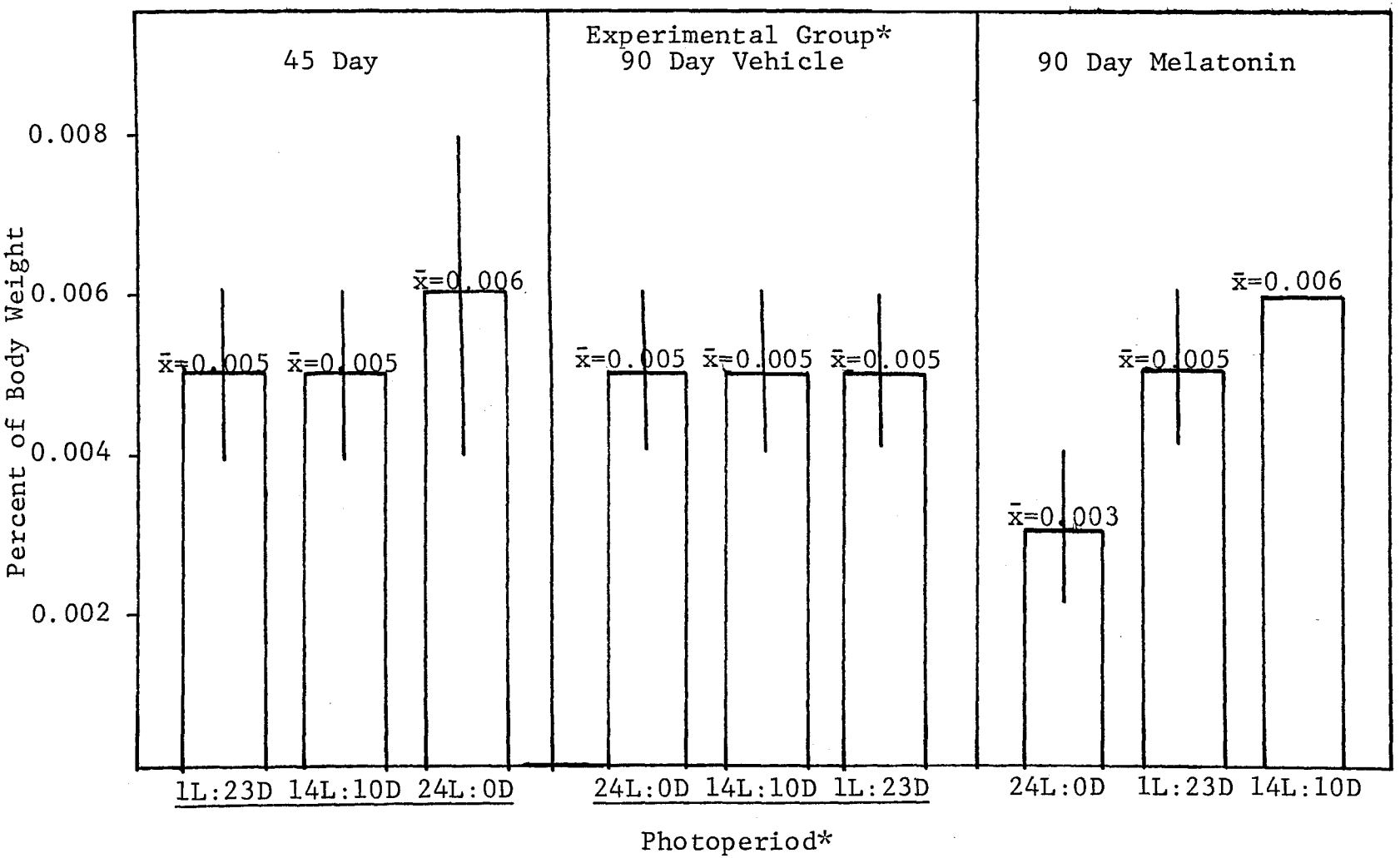

45 Day
Experimental Group*
90 Day Vehicle
90 Day Melatonin
0.008
0.006
0.004
0.002
Percent of Body Weight
x̄=0.005
x̄=0.005
x̄=0.006
x̄=0.005
x̄=0.005
x̄=0.005
x̄=0.003
x̄=0.005
x̄=0.006
1L:23D 14L:10D 24L:0D
24L:0D 14L:10D 1L:23D
24L:0D 1L:23D 14L:10D
Photoperiod*

Figure 16. Mean thyroid gland weights (expressed as percents of total body weight) ± Standard Deviation (N=8).

Results of Duncan's multiple-range test of mean thyroid gland weights. Means underscored by the same line do not differ significantly at α=0.01.

*See Legend, Figure 1.

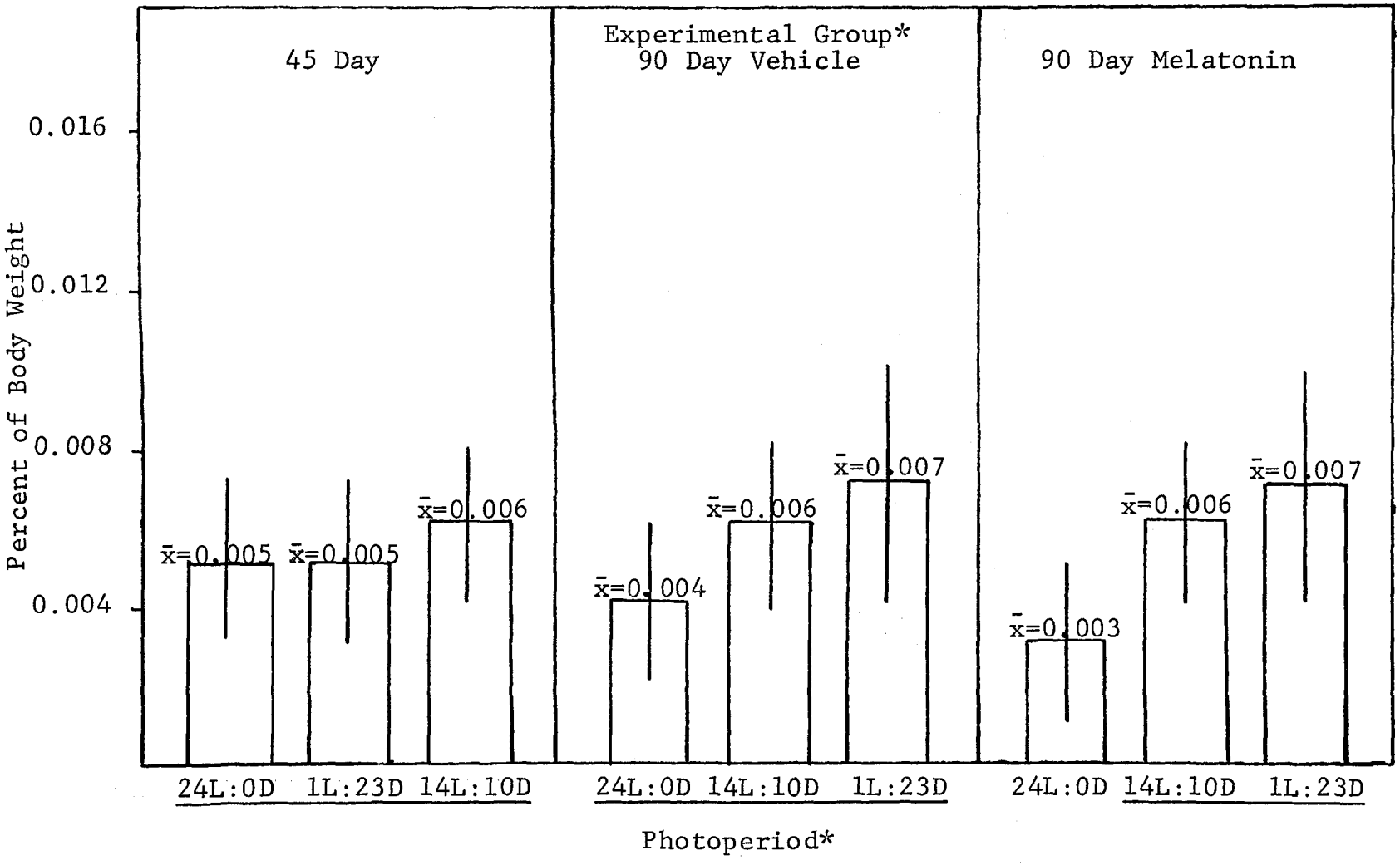

45 Day
Experimental Group*
90 Day Vehicle
90 Day Melatonin
0.016
0.012
0.008
0.004
Percent of Body Weight
x̄=0.005
x̄=0.005
x̄=0.006
x̄=0.004
x̄=0.006
x̄=0.007
x̄=0.003
x̄=0.006
x̄=0.007
24L:0D
1L:23D
14L:10D
24L:0D
14L:10D
1L:23D
24L:0D
14L:10D
1L:23D
Photoperiod*

Figure 17. Mean adrenal gland weights (expressed as percents of total body weight) ± Standard Deviation (N=8).

*See Legend, Figure 1.

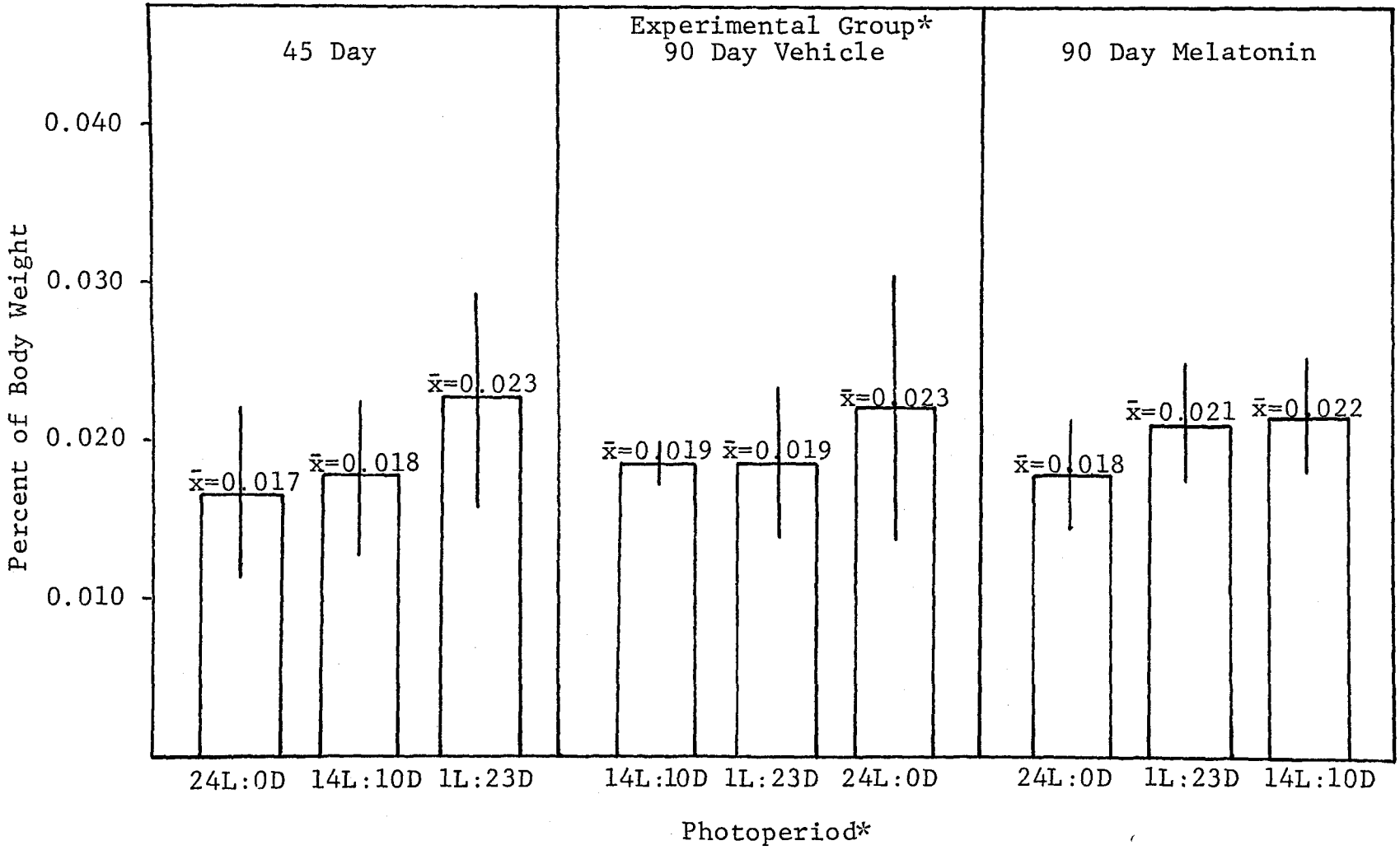
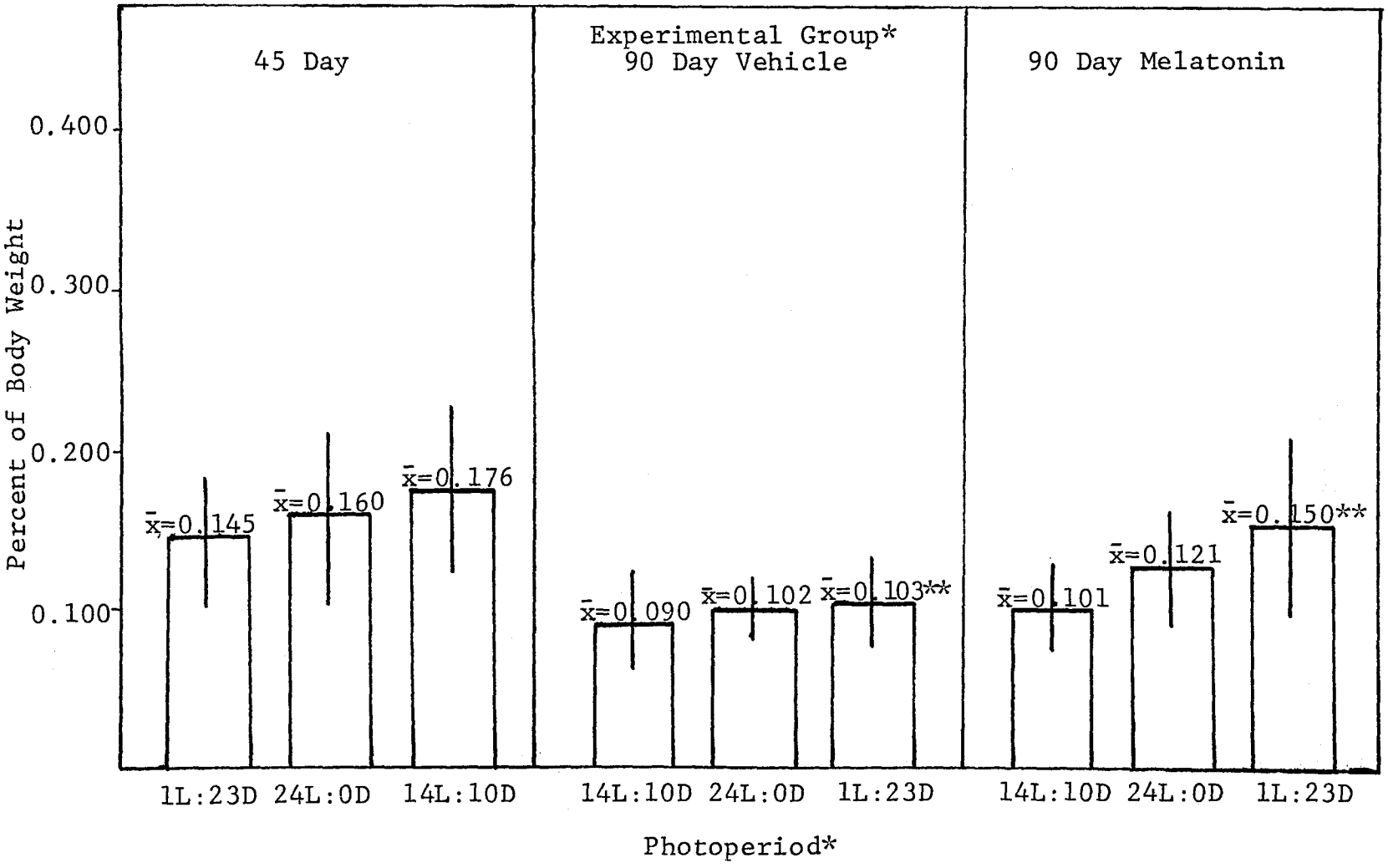

45 Day
Experimental Group*
90 Day Vehicle
90 Day Melatonin
0.040
0.030
0.020
0.010
Percent of Body Weight
x̄=0.017
x̄=0.018
x̄=0.023
x̄=0.019
x̄=0.019
x̄=0.023
x̄=0.018
x̄=0.021
x̄=0.022
24L:0D
14L:10D
1L:23D
14L:10D
1L:23D
24L:0D
24L:0D
1L:23D
14L:10D
Photoperiod*

Figure 18. Mean thymus weights (expressed as percents of total body weight) $\pm$ Standard Deviation (N=8).

Results of Student t test of mean thymus weights. Means that are asterisked (**) differ significantly at α=0.05.

*See Legend, Figure 1.


45 Day
Experimental Group*
90 Day Vehicle
90 Day Melatonin
0.400
0.300
0.200
0.100
Percent of Body Weight
x̄=0.145
x̄=0.160
x̄=0.176
x̄=0.090
x̄=0.102
x̄=0.103**
x̄=0.101
x̄=0.121
x̄=0.150**
1L:23D
24L:0D
14L:10D
14L:10D
24L:0D
1L:23D
14L:10D
24L:0D
1L:23D
Photoperiod*

VITA

Margaret Ann Shugart was born on May 2, 1952, in Ranson, West Virginia. She graduated from Henrico High School in Richmond, Virginia, in 1970 and attended Westhampton College receiving her Bachelor of Science Degree in May, 1974. After graduation, she taught mathematics at Tuckahoe Middle School and then became a mathematician at the Life Insurance Company of Virginia. In January, 1978, she began graduate work in biology at the University of Richmond and obtained her Masters Degree in August, 1980. While there, she was initiated into Beta Beta Beta Honorary Biological Society. She will attend The School of Medicine of the Medical College of Virginia upon graduation.