EFFECTS OF PHOTOPERIOD ON SOME REPRODUCTIVE ORGANS AND ENDOCRINE GLANDS OF YOUNG RAMS

By

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"The great tragedy of Science - the slaying of a beautiful hypothesis by an ugly fact."

T.H. Huxley - Collected Essays, VIII Biogenesis and Abiogenesis

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C H A P T E R I

PHOTOPERIOD EFFECTS ON REPRODUCTIVE ORGAN AND ENDOCRINE GLAND DEVELOPMENT

Section 1 - REVIEW OF LITERATURE

I. THE EFFECTS OF PHOTOPERIOD ON THE REPRODUCTIVE ACTIVITY OF THE EWE

Following Marshall's (1937) observation that ewes transported from one hemisphere to another reversed their oestrous and anoestrous seasons to conform to a new seasonal environment, it was hypothesized that daylength (photoperiod) had some effect on the reproductive activity of the ewe. Sykes and Cole (1944) exposed ewes to an experimental decrease in daylength of 6 hours over a period of 5 weeks. The experiment commenced in the Spring when daylength was 11.5 hours. Breeding occurred earlier than normal and lambs were produced four to five months before the usual time. Criticism of this work arose because of the use of only a limited number of animals, and the fact that the animals were of Rambouillet stock, a breed known to show oestrous activity, under natural conditions, in the Spring and early Summer. These preliminary observations seemed to indicate that the ewe exhibited sexual activity in response to a "short" or "shortening" daylength. From these initial experiments, other observations of natural breeding seasons and more sophisticated photoperiod experiments have followed.

A. Natural breeding seasons

Hammond Jr. (1944), using Suffolk and Suffolk crossbred ewes, found their natural breeding season to extend from mid-Autumn to early Spring. Sexual activity occurred over a period when the daylength was 11.5 hours, or less, and was fairly evenly spaced about the shortest day of the year. Yeates (1949) later confirmed these observations in a control group of ewes from the same flock. Hafez (1952) did an expansive study of the natural breeding seasons of six breeds of ewes in different environments and in specific localities. He concluded that the duration of the breeding season is related to the geographical origin (both latitude and altitude) of the breed. In general, the natural breeding season is shorter as the latitude of origin increases. Following this conclusion, the Blackface Mountain, the Border Leicester and the Welsh Mountain breeds were said

said to have a very restricted sexual season, the Romney Marsh and Suffolk breeds to have a medium length season, and the Dorset Horn breed to have a prolonged season of sexual activity. It was further concluded that complete polyoestrous is reached with a high degree of domestication and also with inhabitance in tropical and equatorial regions. Shelton and Morrow (1965) have made observations on seasonal variation in the sexual activity of Rambouillet ewes. Although there was a peak ovulation rate in early Autumn, and the highest percentage of ewes showed oestrus in early Winter, sexual activity was shown to some degree throughout the entire year. It appears that the effect of seasonal photoperiod on the sexual activity of ewes tends to differ between breeds. In critically evaluating the following experimental work to be reviewed, this fact must be considered.

B. Photoperiod experiments on mature ewes

1. Reversed seasonal light treatment

Yeates (1949) firmly established daylength as having an influence on the sexual activity of the Suffolk ewe. The photoperiodic seasons to which the ewes were exposed were reversed, and consequently the sexual seasons of the ewes were reversed. Yeates proposed that it was not only the length of day, but also the decreasing (day-by-day) light regime which had the stimulatory effect on sexual activity. The onset of oestrus occurred 13 to 16 weeks following the change from increasing to decreasing daily light regimes, and the cessation of sexual activity took place 14 to 19 weeks after the change from decreasing to increasing daylength. Using ewes from this same experimental flock, Hart (1950) confirmed these results, but suggested that decreasing the length of the photoperiod by daily increments was not necessary in order to obtain a stimulatory effect on the onset of oestrus. He stated that a constant light to dark ratio of 1:2 was sufficient for stimulation. Later work has shown reversed seasonal photoperiod to affect Merino (Yeates, 1956b) and Southdown (Thwaites, 1965;

Wodzicka-Tomaszewska et al., 1967) ewes.

2. Continuous light treatment

When Suffolk ewes were exposed to a continuous light regime at the height of their natural sexual season, an inhibitory effect was observed (Hafez, 1951). Ewes ceased cyclic oestrous activity 8 weeks earlier than a control (natural seasonal photoperiod) group. Using Merino ewes, Radford (1961a) was not able to completely suppress sexual activity with continuous light exposure over a period of 3 years even though the ewes were in a continuous light environment from 3 months of age. There was some suppression of oestrus the first year, and greater variability in the length of the sexual season during the second year, but there was no apparent suppression of the occurrence of ovulation. In fact, ewes kept in continuous light of a constant intensity and protected from environmental stresses showed greater sexual activity than ewes in a natural fluctuating photoperiod. Merino ewes have been known to show an onset of oestrous cycles well before the longest day of the year (Watson and Radford, 1955). Ile-de-France ewes have maintained oestrous cycles in continuous light over a period of 3 years (Dauzier and Mauleon, 1962), and Shropshire and Rambouillet ewes kept on continuous light have bred and lambed earlier than ewes kept in continuous dark (Terry and Meites, 1951).

3. Equinoctial light treatment

Radford (1961b) exposed Merino ewes to an equinoctial (12 hour light: 12 hour dark per day) light regime and obtained differing responses, depending upon the pretreatment environmental photoperiod. Animals entering the treatment while natural daylength was decreasing (Autumn) failed to show anoestrus during the first natural Spring; in their second year of treatment, 2 of 5 ewes were anoestrus. Of animals entering the treatment while natural daylength was increasing (Spring), 3 of 5 ewes were anoestrus during the first and second natural Spring seasons. The anoestrus periods were shorter than those periods in the

control ewes. Hafez (1951) had also found pretreatment light environment, due to a "residual effect of light", an important modifying factor.

Southdown ewes treated with an equinoctial light regime lost their seasonal activity after the first year of treatment (Thwaites, 1965). During the second year, oestrous cycles became "sporadic and apparently unrelated to any external environmental factor". The monthly intensity of breeding activity was greatly reduced, but during any month of the year there was some activity by individuals within the group. Other workers observed that when both Peppin Merino and Southdown ewes were treated with an equinoctial light regime for 2.5 years, the annual rhythm of reproductive activity persisted, despite the reversal of thermal seasons (Wodzicka-Tomaszewska et al., 1967).

4. "Hastened" seasonal light treatment

Mauleon and Rougeot (1962) have artificially hastened the annual light rhythm, thus producing two light cycles within a single year. Ewes of the Ile-de-France, Texel, Prealpes du Sud and Limousine breeds exposed to this treatment exhibited two periods of sexual activity each year, coincident with the periods of increasing daily light, and over the time when days had the longest light hours. The authors felt that this unexpected occurrence was due to the interval between the photoperiodic stimulus and the initiation of the sexual cycles, coupled with a shortening of the period of sexual activity caused by increasing the daily light exposure. By increasing daily light more rapidly than normal in the Spring to a normal maximum peak daylength, and then decreasing daily light more rapidly than normal, Mimura (1959) was able to initiate oestrus in treated ewes earlier than in natural light rhythm-control ewes. Symington and Oliver (1966), using tropical ewes, rapidly increased daylength up to 19 hours of light per day following the natural longest day of the year. The incidence of oestrus tended to be greater in ewes subjected to extended daylight than in ewes under the natural photoperiod. Wilson et al., (1961)

have decreased the photoperiod of Rambouillet ewes in the late Spring by 1 hour from each day, and have increased the percentage of animals showing oestrus.

5. Constant light treatment

Early oestrous cycles have been induced in "western ewes" treated with a constant 11 hour photoperiod per day (Means et al., 1960), in Suffolk ewes exposed to a constant 8 hour daylength (Hart, 1950) and in several other breeds under a daily 8 hour light regime (Hafez, 1952). Suffolk and Cheviot ewes treated with a 7 hour daily photoperiod for one month during anoestrous began oestrous cycles within 38 and 56 days, respectively, from the first day of treatment (Fraser and Laing, 1966). Suffolk and Hampshire ewes kept in a constant 6 hour daily photoperiod for 3 years showed a tendency for their periods of sexual activity to become prolonged, and the length of their anoestrum to be greatly shortened following the first year of treatment. Cycles became progressively less regular; however, a cyclic pattern of increasing and decreasing reproductive activity was still apparent (Clegg et al., 1964).

6. Pregnancy and light treatment

Fertility was found to be low in some "short light-induced" oestrus ewes (Means et al., 1960); and some workers have noted lambs born small and weak when mother ewes were brought to oestrus with a "shortened" photoperiod treatment (Yeates, 1949; Mimura, 1959). Yeates (1949, 1956b) observed that the duration of pregnancy in Suffolk and Merino ewes was unaffected by the reversal of the annual light rhythm.

C. Natural seasonal light cycle and the attainment of puberty in immature ewes

Hafez (1952) found the breeding season of ewe lambs to be only a quarter to a third as long as that of adults. Higher sexual performance was associated with birth dates early in the lambing season or with higher growth rates. Ewe lambs born early in the season showed their first oestrus at an older age and heavier live weight than those born later. Lambs born much later in the season

or with retarded growth rates did not attain cyclic sexual activity until the following breeding season. Breed differences for these characteristics were evident. Watson and Gamble (1961) found that Merino ewe lambs born in the Summer tended to have a higher percentage of animals showing oestrus and a higher conception percentage during their first breeding season. However, body weights and ages of the ewe lambs at puberty were greatest in animals born in the Summer, and least in animals born in the Spring. The ewe lambs born in the Spring, being younger and lighter at puberty, had a first sexual season one third the length of the first breeding seasons of animals born in the Summer or Autumn. Here again, the first sexual season was found to be shorter in lambs than in mature ewes (also see Yeates, 1965).

Hammond Jr. (1944) proposed a "threshold of stimulation" theory to explain birth date versus age at puberty discrepancies: "The threshold of stimulation required falls in the lamb as its age increases, until at about 300 days it reaches the adult level. The intensity of stimulation increases as the amount of daylight lessens, and is maximal in, or before, the middle of the breeding season; thereafter, it decreases, and at a greater rate than that at which the threshold for the lamb falls with increasing age - so that the minimum age at first heat comes in the middle of the breeding season, the age being about 180 days; if this age is reached later in the season heat will not occur until the season following when the animal may be 400 days old or more".

D. Photoperiod experiments and the attainment of puberty in immature ewes

Experimentally, younger ewes have been more readily influenced than adults by photoperiod treatments (Hafez, 1951; Kazakov, 1964). Radford (1961a) exposed Merino ewe lambs to continuous light from 3 months of age and noted a tendency toward suppression of the onset of oestrous cycles the first year. Recently, Smith (1967) treated Border Leicester, Southdown and Suffolk ewe lambs, ranging in age from 43 to 110 days of age, with a constant 16 hour daily photo-

period. The lambs began treatment when the natural daily photoperiod was 14 hours, 46 minutes, including civil twilight. A control group remained in the natural photoperiod. The onset of first oestrus was delayed in the experimental group, but not completely suppressed. The control group showed a higher incidence of oestrus, ovulation rate and conception percentage. In the natural photoperiod group, 65% of the ewes conceived at first oestrus, compared with only 33% of the 16 hour daily photoperiod group ewes. Total conception percentage for the entire first breeding season of control and experimental ewes was 83.3% and 37.5%, respectively.

II. THE EFFECTS OF PHOTOPERIOD ON THE REPRODUCTIVE ACTIVITY OF THE RAM

Although seasonal changes in reproductive activity of the ram are not as overtly obvious as oestrous cycles and anoestrum in the ewe, semen characteristics do fluctuate throughout the year. As early as 1937, McKenzie and Berliner noted that there was a larger number of spermatozoa, a greater number of total ejaculates and a smaller absolute and relative number of abnormal spermatozoa in the semen of Shropshire and Hampshire rams during the period from Autumn to Winter. In subsequent studies, many investigators have found seasonal seminal variations in rams of many breeds in several environments.

A. Natural breeding seasons

Most workers have observed a maximum volume of semen ejaculated during the Autumn (Chang, 1941 - Suffolk in England; Kastyak, 1962 - unnamed breed in Poland; Amir and Volcani, 1965 - Awassi and Border Leicester in Israel), yet others find no definite seasonal fluctuations in semen volume (Shukla and Bhattacharya, 1952 - unnamed breed in India; Aslanjan and Lisovaja, 1963 - Askanians in Russia; Amir and Volcani, 1965 - German Mutton Merino, Corriedale, Border Leicester and Dorset Horn in Israel). It has been common for most researchers to equate increased semen volume, as well as increased fructose and

citric acid levels in the seminal plasma, with increased androgen production in the testis, and even further, with increased interstitial cell stimulating hormone (ICSH) secretion from the anterior pituitary gland.

Fructose levels in the seminal plasma of Suffolk, Hampshire and Rambouillet rams in California reached minimum peaks in mid-Spring and maximum peaks in mid-Autumn (Cupps et al., 1960). The investigators feel that the ram's response to changes in the annual light cycle, measured by fructose concentration in the seminal plasma, is more rapid than the response (onset of oestrus?) reported for ewes. Similar results were found in Awassi and Border Leicester rams in Israel, whereas German Mutton Merino, Corriedale and Dorset Horn rams showed only slight variations in fructose concentrations (Amir and Volcani, 1965). Recently, Moule et al., (1966) using Merino, English Leicester and Romney rams in Australia, observed seasonal fructose concentration peaks similar to those noted by the Californian and Israeli workers when their experimental animals were grazed on pasture. When rams were fed on a constant diet without the quality fluctuations of the pasture, there was no seasonal variation in fructose levels in the seminal plasma. When the rams were exposed to a reversed annual light cycle and retained on a constant diet, there was still no indication of seasonal differences.

Spermatozoa total numbers, percentage abnormal and motility, as well as ejaculate respiratory activity have shown seasonal fluctuations favourable to the "decreased daylength - increased reproductive activity" hypothesis (Chang, 1941; Maqsood, 1951; Kastyak, 1962; Amir and Volcani, 1965). Other workers find a nearly reversed occurrence (Shukla and Bhattacharya, 1952). Recently, Lees (1966a) has calculated lag correlations between natural daylength and certain body characteristics and semen properties in the ram to determine time relationships which may exist in the responses of male sheep to daylength changes. Lee's results suggest that testicular activity moves with daylength, and that the ram is therefore a long day breeder, rather than a short day breeder.

Chang (1941) and Maqsood (1951), working with Suffolk rams in England, found a lowered mating desire from early Spring until mid-Summer, as did Pepelko and Clegg (1965), working with Targhee type rams in California and Aslanjan and Lisovaja (1963) using Askanian rams in Russia. Shukla and Bhattacharya (1952) observed no seasonal variation in the libido of an unnamed breed in India.

B. Photoperiod experiments on mature rams

1. Reversed seasonal light treatment

Experimentally, Yeates (1949) was the first to reverse the seasonal daylength of Suffolk rams and observe a reversal in the variations of semen characteristics. Fowler (1962, 1965) noted the same occurrence in Merino rams.

2. "Hastened" seasonal light treatment

Ortavant and Thibault (1956) have condensed the natural annual light rhythm into a six month period beginning on the winter solstice and ending on the summer solstice. At the end of the decreasing light regime, the experimental group of Ile-de-France rams showed increased values of ejaculate volume, total spermatozoa, fructose levels in the seminal plasma and epididymal sperm reserves over animals under normal daylength conditions of the summer solstice. Testicular and seminal vesicle weights were greater in experimental rams.

3. Constant light treatment

Rams exposed to a constant 11 hour photoperiod for 94 days have shown an increase in ejaculate volume and spermatozoa motility, concentration and survival time. There was also an indication that the ability of the spermatozoa to continue progressive motility within the uterine cervix of a ewe was increased (Mihnevic, 1965).

III. THE EFFECTS OF PHOTOPERIOD ON THE PHYSIOLOGY AND HISTOLOGY OF SOME ENDOCRINE GLANDS RELATED TO REPRODUCTION IN THE EWE AND RAM

A. Ovary

Under natural seasonal conditions, follicular atresia within the ovaries of Merino ewes was more evident during the Summer months (Enriquez de Salamanca, 1957-58). Ovulation rate was highest in the Autumn and lowest in the Spring (Hammond Jr., 1944; Averill, 1959; Dutt, 1960).

Some ovarian changes occurring under experimental photoperiods have been briefly reviewed within previous sections.

B. Testis

Suffolk rams have shown arrested spermatogenesis within their testes during the non-breeding season. Atrophic interstitial cells (Leydig cells) were also evident (Maqsood, 1951).

By experimentally treating Ile-de-France rams with "long" (16 hour) and "short" (8 hour) photoperiods, Ortavant (1956) was able to detect differences in the spermatogenic cycle. Using a P^{32} tracer technique, he found the duration of the spermatogenic cycle to be insensible to light treatments. However, photoperiods did affect three definite stages within the cycle: the transformations from Spermatogonia A to Intermediate spermatogonia, from Spermatocyte I in the zygotene stage to Spermatocyte I in the pachytene stage, and in the meiotic divisions.

When an experimental photoperiod was decreased from 16 hours to 6 hours over a period of 3 months, an optimum photoperiod for spermatogenesis in Ile-de-France rams was noted (Ortavant, 1961). Representative animals were withdrawn from the treatment when the photoperiod was 12 hours, 10 hours, 8 hours and 6 hours. Epididymal spermatozoa reserves and testicular weights reached a maximum at an 8 hour photoperiod. However, spermatogenesis progressed most efficiently at a photoperiod of 10 hours when preceded by a 12 hour daylength. When rams were

exposed for approximately 40 days to a constant photoperiod, even the optimal one, the maximum level of spermatogenic activity was never achieved (Ortavant et al., 1964). As a quantitative indicator to the efficiency of the spermatogenic cycle, the number of primary spermatocytes at the leptotene stage in Stage 2 seminiferous tubules were counted and found to be at a peak as soon as the photoperiod dropped to the optimum 10 hours. The investigators suggested that as the total duration of the daily photoperiod is important, the variation of this duration is no less important for optimum spermatogenesis.

Indirect measurements of androgen secretion changes in response to photoperiod changes have been briefly reviewed within previous sections (see Cupps et al., 1960; Moule et al., 1966).

C. Anterior pituitary gland

The relative amounts of gonadotrophins within the anterior pituitary gland of the ewe during the breeding season, as opposed to the anoestrum, are not known conclusively. Some workers have found no differences in gonadotrophin concentrations (Lamond et al., 1959; Hutchinson and Robertson, 1960); others have observed either a slight increase (Robertson and Hutchinson, 1962) or decrease (Kammlade et al., 1952) in concentrations at the beginning of the sexual season. Ewes in an 8 hour photoperiod had a lower anterior pituitary gonadotrophin content than did anoestrous ewes (Allen and Lamming, 1960).

Rams have been treated with a 6 month "hastened" annual light cycle. Representative animals were studied at the maximum (16 hour) and minimum (8 hour) photoperiods. As in similar experiments, testicular weights and epididymal spermatozoa reserves were greatest in rams from the minimum photoperiod. Anterior pituitary gland weights were lightest in the 8 hour photoperiod animals. The content of follicle stimulating hormone (FSH) in these glands was 3 to 4 times greater in the minimum photoperiod rams than in the maximum photoperiod rams. Luteinizing hormone (LH) content was about 2 times greater in the minimum

photoperiod animals. With an additional 48 hours of light given to the 8 hour photoperiod rams, a sharp decrease of FSH and LH levels was induced. When 48 hours of additional darkness was given to the 16 hour photoperiod rams, only a slight and insignificant increase in gonadotrophin content was shown. The authors suggested that while the dark periods may stimulate gonadotrophin production within the anterior pituitary glands of rams, the light periods may affect the discharge of the hormones from the gland (Pelletier and Ortavant, 1964).

D. Thyroid gland

Ewes in a continuous light treatment had a lower thyroid activity than ewes in a continuous dark treatment (Terry and Meites, 1951). The thyroid secretion rates (TSR) of young ewes in constant photoperiods ranging from 4 to 20 hours, while in a continuous temperature of either 50°F or 90°F, were at a minimum when ewes were in a constant 12 hour photoperiod in either environmental temperature. The TSR increased as constant photoperiods were extended toward 20 hours or shortened toward 4 hours. TSR and thyroid epithelial cell heights were positively correlated (Hoersch et al., 1961).

Maqsood (1950, 1951) has found a mild hyperthyroidism in young rams, simulated with thyroxine injections, to be stimulatory to the process of spermatogenesis, which leads to precocious puberty. Injected thyroxine has improved some semen characteristics of heat stressed mature rams (Bogart and Mayer, 1946); and thyroxine implants have increased ovulation rates in mature ewes (Hart, 1958). Other workers have found thyroxine injections or implants to be detrimental to the motility and respiratory activity of spermatozoa (Warwick et al., 1948) and the rate of ovulation (Ross and Lewis, 1958). Yet, some investigators found thyroxine injections to have no effect on semen quality (Brooks and Ross, 1962) or seminal fructose concentrations (Moule et al., 1966). Thyroxine dose level differences probably account for many of the varied results.

Thyroidectomy had no deleterious effects on the oestrous cycle or conception

rate of ewes (Falconer, 1963); however, the same operation on aged ewes reduced the over-all reproductive capacity, with resultant low fertility and small, weak offspring (Brooks et al., 1964). Semen quality of mature rams was unaffected by thyroidectomy (Brooks et al., 1964); however, the same operation on immature rams lead to a low semen quality at maturity (Berliner and Warbritton, 1937).

IV. THE EFFECTS OF TEMPERATURE ON THE REPRODUCTIVE ACTIVITY OF SHEEP

Although photoperiod is considered by many to be the most important environmental factor modifying reproductive activity in sheep, temperature variations appear to be effective secondary modifiers.

A. Temperature and reproductive activity of the ewe

1. Natural seasonal fluctuations

Recently, Lees (1966b), while studying the breeding season of Clun ewes, noted that the mean ambient temperatures between mid-Summer and the time of resumption of cyclic activity were very significantly related. The higher the temperatures, the later the onset of breeding activity began.

2. Temperature experiments

Experimentally, Yeates (1953) has attempted to inhibit the onset of oestrous cycles in Romney Marsh ewes by exposing them to high (105°F dry bulb, 87°F wet bulb) temperatures for 6 hours per day, five days per week, two months prior to the beginning of the normal breeding season. The onset of oestrous cycles was neither postponed nor inhibited by such a treatment. Dutt et al., (1956) exposed both shorn and unshorn ewes to a 90°F environment just before breeding. Ewes were sacrificed 3 days following breeding. There was a lower percentage of ova fertilized and a higher number of abnormal ova in the unshorn, heat-treated ewes, than in the shorn, heat-treated ewes. Under practical conditions, the effects of delayed shearing of ewes until just before the breed-

ing season have been to increase the survival of fertilized eggs (Inkster, 1959), increase lambing percentage (Inkster, 1959; Whiteman and Brown, 1959), and perhaps increase twinning rate (Whiteman and Brown, 1959).

Dutt and Bush (1955) treated ewes with 45° - 48°F temperatures for one month before the summer solstice. These ewes began oestrous cycles 8 weeks sooner than a control group under natural conditions. McKenzie and Phillips (1933) failed to hasten the onset of breeding activity in ewes when exposing them to a 44° - 48°F environment during a 10 day period in late Summer, as did Warnick et al., (1967) when treating ewes with a 59°F temperature for 48 days from late Spring to early Summer.

Wilson et al., (1961) have cooled Rambouillet ewes from 70°F to 60°F throughout the breeding season, beginning in late Spring, with a resultant increase in the number of ewes lambing.

Pregnancy is affected by high environmental temperatures. With Romney Marsh ewes held in a hot (107°F wet bulb, 92°F dry bulb) environment for 7 hours daily for the last $\frac{1}{3}$ or 2/3 of gestation, there was a decline in the birth-weights and total numbers of lambs born (Yeates, 1953). To obtain similar results in pregnant Merino ewes, very hot (112°F dry bulb, 98°F wet bulb) temperatures were necessary, indicating a breed difference in sensitivity to high temperatures (Yeates, 1956a). Pregnant Hampshire crossbred ewes were treated with a 90°F environment 8 days after breeding, for 16 days; only a group of unshorn ewes had a lower percentage of animals pregnant. Again, shorn ewes were unaffected by the heat treatment (Dutt et al., 1956).

B. Temperature and reproductive activity of the ram

1. Natural seasonal fluctuations

Many workers have found semen quality to be lower during the hottest time of the year (Gunn et al., 1942). Abnormal spermatozoa numbers have risen and total spermatozoa numbers have decreased (McKenzie and Berliner, 1937) and

generally, fertility has been lower (Hulet et al., 1956) during the Summer months.

2. Temperature experiments

Within 13 days of insulating the scrotum, rams were unable to breed ewes and eventually an azoospermatic condition developed. The suggestion from this work is that heat can have its detrimental effect directly on the testis, without necessarily affecting higher physiological systems (Phillips and McKenzie, 1934).

When rams were held in a "hot" environment, there was a degeneration of spermatozoa, a drop in semen volume, a decrease in motility and longevity of spermatozoa, as well as a decrease in total numbers of spermatozoa (Phillips and McKenzie, 1934; McKenzie and Berliner, 1937; Gunn et al., 1942). Dutt and Hamm (1955) treated shorn and unshorn rams with 90°F temperatures during one week in the Winter. Five weeks after the experimental period, spermatozoa motility was lower, percentage abnormal spermatozoa was higher and sperm cell concentration was lower in the semen from unshorn rams. Shorn rams were not affected by this treatment. Within 8 weeks following the treatment, the semen of affected rams returned to normal.

Rams exposed to 45° - 48°F temperatures for one month prior to the Summer solstice showed an increased spermatozoa motility and fertility (Dutt and Bush, 1955). Holding rams in a "cool" environment for the entire Summer increased motility and decreased abnormal numbers of spermatozoa, as well as increased fertility (McKenzie and Colvard, 1938; Dutt and Simpson, 1957). Cooling rams a few degrees below normal for 3 weeks prior to the breeding season initiated a more aggressive sexual behaviour (Whiteman and Brown, 1959).

C. Temperature and thyroid gland activity related to reproduction

Under natural seasonal conditions, the thyroid secretion rate (TSR) is lower in mid-Summer in non-pregnant, non-lactating ewes (Henneman et al., 1955)

and in rams (Griffin et al., 1962). There is a simultaneous decline in spermatozoa concentration and motility, as well as an increase in abnormal spermatozoa in the semen of rams during the summer months (Bogart and Mayer, 1946). Thyroxine injections during this season prevented only the decline in spermatozoa concentration.

Experimentally, ewes held in 50°F temperatures had a TSR 3 times as great as the TSR of animals held in a 90°F environment (Hoersch et al., 1960, 1961); rams treated with 80°F temperatures showed a lowered TSR, as well as a lowered semen quality (Brooks and Ross, 1962).

The fact that mature ewes and rams respond to "short" or "shortening" photoperiods with an increased reproductive activity is generally proven and accepted. To date, however, no studies have been made on the effects of "short" or "shortening" photoperiods on the reproductive development of immature ewes and rams. Correspondingly, a developmental study of the endocrine glands in immature ewes and rams under such photoperiod conditions has not been undertaken.

In the photoperiod experiments previously reviewed, little, if any, regard was given to the effects of uncontrolled, fluctuating environmental temperature, even though some investigators have indicated that this factor is a strong secondary environmental modifier of reproductive activity in sheep. The importance of controlling the environmental temperature to which sheep are exposed in future photoperiod experiments is evident.

In the following experiment the effects of a constant "short" (10 hour) photoperiod on the reproductive and endocrine development of immature rams, exposed to a constant (65°F) environmental temperature, are studied. A 10 hour constant photoperiod was choosen as a treatment because it has been defined by Ortavant (1961) as an optimum stimulatory photoperiod for spermatogenic "efficiency" in mature rams.

Section 2 - MATERIALS AND METHODS

I. ANIMALS

A. Selection and early post-natal treatment

Sixty-five Southdown x Romney ram lambs born within a 20 day period between 8 and 28 September, 1966 on the Massey Sheep Farm were used as experimental animals. Only rams born as singles or same-sex twins with birthweights exceeding 8.0 lbs were choosen.

From birth to 18 days of age the young rams were with their mothers under normal paddock conditions. All animals were tail docked at 13 to 16 days of age.

B. Weaning treatment

As it was necessary for the ram lambs to be weaned at age 28 days when they entered three of the four experimental treatment groups, a "weaning treatment" was carried out. Those animals assigned to a normally treated paddock group (Group 4) did not undergo the "weaning treatment". For a 10 day period, when each of the lambs was 18 to 28 days of age, the following schedule was followed.

The lambs were separated from their mothers for 7 to 8 hours per day (from 9:00 a.m. until 4:00 p.m. or 5:00 p.m.) and held in a well-ventilated, naturally lit shed. During this period, kibbled peas and powdered peas, offered separately and mixed together, were available to the lambs. Water was present. Any detrimental effect of the treatment was noted by body weight changes between 18 and 28 days of age, and any severely affected animal was excluded from the experiment.

II. EXPERIMENTAL GROUPS

A. Photoperiod and temperature treatments

Forty-seven of the ram lambs were transferred from the "weaning treatment" at 28 days of age, and randomly allocated to three experimental groups. Assignment to groups began on 7 October, 1966 (when daylength, including civil

twilight, was 13 hours, 39 minutes and the maximum temperature was 61.3°F and was completed on 22 October, 1966 (when daylength, including civil twilight, was 14 hours, 19 minutes and the maximum temperature was 61.1°F).

Treatments were as follows:

Group	No. of Animals	Daily Photoperiod	Temperature	Nutrition
1	16	constant 10 hours	constant 65°F	SAME FOR GROUPS 1, 2, and 3
2	16	seasonal fluctuation	constant 65°F	
3	15	seasonal fluctuation	seasonal fluctuation	

Group 1 and Group 2 animals were held in "controlled environment" rooms of the Animal Physiology Unit of Massey University. Each room measured 8' x 23' and was illuminated by two 40 watt fluorescent "daylight" bulbs. The temperature was a constant 65°F, and air was circulated through one large light-proof vent at one end of each room. The length of daily photoperiod in Group 1 was controlled by a set time switch to a constant 10 hours. The fluctuating natural seasonal photoperiod of Group 2 was detected and controlled by a photoelectric cell-switch apparatus which switched the room lights on at sunrise and off at sunset.

In each room an elevated wooden grate covered half of the concrete floor, which was cleaned with water daily. The humidity of the rooms was not controlled and at times, rose to high levels following daily cleaning. Some rams showed temporary outward signs of respiratory tract inflammation, caused, presumably, by high humidity and a build up of ammonia gas. These animals were treated with antibiotics. No animal suffered severely, and as the numbers of rams in each room decreased, the problem was eliminated.

Rams in Group 3 were held in one wire pen with a wooden grate floor within a large, well-ventilated, naturally lit room at the Animal Physiology Unit.

The floor space of the pen was similar to the area of each of the "controlled environment" rooms. These animals experienced the natural seasonal fluctuations in photoperiod and temperature and received the same nutrition as rams in Group 1 and Group 2. They were protected from environmental stresses such as wind and rain.

An additional group, Group 4, consisted of 15 ram lambs left with their mothers and raised under normal management practices on the Massey Sheep Farm. These animals were exposed to the natural seasonal fluctuations in photoperiod, temperature and nutrition; they were unprotected from environmental stresses.

B. Nutrition

Group 1, Group 2 and Group 3 rams were given identical feed throughout the entire experiment. For 2 to 3 weeks following the entrance of each ram into an experimental group, kibbled peas, powdered peas and buttermilk powder were fed ad libitum, along with small amounts of cut grass. Following this period, a ration of relatively low quality chopped meadow hay and lawn clippings were fed ad libitum until mid-January 1967, when a better quality chopped meadow hay was available.

Animals in Group 4 were on paddocks of ryegrass and clover with their mothers until early February 1967, when weaning took place.

III. SAMPLING OF EXPERIMENTAL GROUPS

A. Sample age groups

At 28 days of age, at the end of their "weaning treatment", 3 randomly selected animals were sacrificed and autopsied as a means of establishing the pre-experimental status of the tissues to be studied.

Sample groups of 4 rams were sacrificed and autopsied in each of the four experimental groups at ages of 63 and 91 days. Weekly semen samples, obtained by electro-ejaculation and begun at 126 days of age, were taken from the remain-

ing 8 rams in Group 1, 8 rams in Group 2, 7 rams in Group 3 and 7 rams in Group 4 and checked for the presence of spermatozoa. All of the remaining animals in Group 1 and Group 2 were sacrificed and autopsied at 168 days of age; a semen sample was taken from each ram just prior to sacrifice. The remaining rams in Group 3 and Group 4 were used in another experiment.

B. Autopsy procedure

All animals were sacrificed within 3 to 4 hours after the onset of their daily photoperiod. Body weights were taken just prior to sacrifice. Death was achieved by exsanguination. The testes, epididymides, thyroid glands, anterior pituitary glands, pineal glands, adrenal glands and seminal vesicles were dissected and held in sealed jars or plastic bags with a piece of saline moist gauze around them until they were weighed in the laboratory 1 to 2 hours later. Before weighing, all tissues were freed of surrounding fat and connective tissue. The testes and epididymides were weighed on a Welch sliding weight balance, while other tissues were weighed on a Mettler E5 balance.

IV. HISTOLOGICAL TECHNIQUES AND MEASUREMENTS

A. Fixation and embedding

1. Testis and epididymis

A strip of testicular tissue from the central area opposite the cauda epididymis, and a section from the central area of the corpus epididymis were taken from the right testis. Both samples were fixed for 20 to 24 hours in Bouin's fluid with 1% ferric alum added, as recommended by Lillie (1954).

2. Thyroid gland

A transverse section from the posterior area of the left lobe of the thyroid gland was fixed in 10% neutral buffered formalin (pH = 7.0) for 40 to 48 hours.

3. Anterior pituitary gland

A transverse section from the central area of the anterior pituitary gland was fixed in 10% neutral buffered formalin (pH = 7.0) for 40 to 48 hours.

4. Pineal gland

The entire pineal gland was held in Bouin's fluid with 1% ferric alum added for 20 to 24 hours.

5. Adrenal gland

A transverse section from the central area of the right adrenal gland was fixed in 10% neutral buffered formalin (pH = 7.0) for 40 to 48 hours.

6. Seminal vesicles

A transverse section from the posterior area of one lobe of the seminal vesicles was held in 10% neutral buffered formalin (pH = 7.0) for 40 to 48 hours.

Following fixation, sections of tissues were rinsed in several changes of 70% ethanol and held. All fixed and rinsed sections were then dehydrated and embedded with paraffin wax using an Elliott Tissue Processor.

B. Sectioning and staining

All sectioning of embedded tissues was done on an AO Spencer "820" microtome.

1. Testis and epididymis

Twelve micron sections of testis tissue were stained with Heidenhain's iron hematoxylin (Lillie, 1954, p.79) for the study of muclear morphology. No routine sectioning and staining of epididymal tissue was done.

2. Thyroid gland

Ten micron sections of thyroid gland tissue were stained with Heidenhain's iron hematoxylin (Lillie, 1954, p.79) and counterstained with 1% aqueous eosin for the study of general morphology.

3. Anterior pituitary gland

Five micron sections of anterior pituitary gland tissue were stained

by the periodic acid-Schiff (PAS) (Barka and Anderson, 1963, p.73) and Crossmon (Gray, 1954, p.336) methods for differentiation of cell types, as described by Purves (1961, 1966).

4. Pineal gland

Eight micron sections of pineal gland tissue were stained by the chrome alum-hematoxylin-phloxin method (Gomori, 1941) for studies of cytoplasmic inclusions and general morphology, as recommended by Holmgren, et al., (1960).

5. Adrenal gland and seminal vesicles

No sectioning and staining of adrenal gland and seminal vesicle tissue was done.

C. Measurements - qualitative and quantitative

Microscopic observations were made with a Leitz Ortholux microscope.

1. Testis

Stained testis sections were evaluated on the degree of development of the spermatogenic cycle at each sample age group (28, 63, 91 and 168 days) in Group 1 and Group 2 animals only.

2. Thyroid gland

The cell heights of 4 opposing cells in each of 6 randomly selected follicles were measured in thyroid gland sections from Group 1 and Group 2 animals only, on a Reichert "Visopan" projection microscope. This measurement was used as an indicator of relative thyroid gland activity, as in previous work with sheep thyroid glands (Hoersch, et al., 1960, 1961).

3. Anterior pituitary gland

The differentiated cells of the anterior pituitary gland were qualitatively evaluated on the basis of staining characteristics in Group 1 and Group 2 animals only.

V. STATISTICAL ANALYSES OF GRAVIMETRIC AND HISTOLOGIC MEASUREMENTS

Only the data from Group 1 and Group 2 animals was analysed in detail. Group 3 and Group 4 data has been summarized and listed in an appendix.

The data was approached as a two-way classification, one for the two photoperiod treatments and one for the three sample age groups. Means were weighted for disproportionate subclasses using a least-squares method for fitting constants (Harvey, 1960). Sums of squares estimated after fitting constants were analysed for interaction effects. Data which showed no interaction were carried through an analysis of variance using the following mathematical model:

$$Y_{ijk} = \mu + a_i + b_j + e_{ijk}$$

where

Y_{ijk} = the K^{th} observation in the i^{th} A class and the j^{th} B class

μ = overall mean when equal subclass numbers exist

a_i = effect of the i^{th} photoperiod treatment ($i = 1,2$)

b_j = effect of the j^{th} sample age group ($j = 1,2,3$)

e_{ijk} = random errors, assumed to be normally distributed about zero when tests for significance are made.

In this particular set of data, "a" had 2 subclasses (10 hours constant daily photoperiod and seasonal fluctuating photoperiod), while "b" had 3 subclasses (63 day, 91 day and 168 day sample age groups). Both class effects were assumed to be fixed and the restrictions that $a_1 + a_2 = 0$ and $b_1 + b_2 + b_3 = 0$ were imposed. The "F test" was used as a test of significance.

Section 3 - OBSERVATIONS

The main interest of this experiment was a study of the effects of different photoperiods on the development of the reproductive organs and some endocrine glands of ram lambs. The use of the "controlled environment" rooms for Group 1 and Group 2 treatments enabled a study of this interest to be undertaken. In these treatments temperature, another major modifying factor of reproductive and endocrine activity in sheep, was controlled and kept constant. Rams in Group 3 and Group 4 treatments were not in as controlled an environment as Group 1 and Group 2 animals and, therefore, were affected by many environmental variables simultaneously. For this reason, any comparison between the first and second pair of experimental groups for the interpretation of photoperiod effects on reproductive and endocrine activity would be invalid. For the purpose of studying the effects of photoperiod, therefore, only gravimetric and histological data obtained from Group 1 and Group 2 animals at the three experimental sample age groups has been analysed and presented in detail (see TABLE 1). Data collected from Group 3 and Group 4 animals has been listed and briefly summarized in APPENDIX I, while data collected from the pre-experimental 28 day-old rams has been summarized in APPENDIX II.

ANIMALS

During the course of the experiment one ram of Group 1 included in the 91 day-old sample age group made poor body weight gains and was in very poor physical condition at sacrifice; the data collected from this animal were not included in the final analyses. Another animal in the same photoperiod and sample age group fractured its leg one week before its sacrifice. The leg was splinted, and no noticeable physical set-back was observed; the data from this animal were retained. A ram in Group 1 included in the 168 day-old sample age group was found, at autopsy, to be unilaterally cryptorchid; the data collected from this animal were not included in the final analyses.

TABLE 1

EXPERIMENT I – SUMMARY OF ANALYSES OF VARIANCE, WEIGHTED MEANS

	Mean Squares				Weighted Subclass Means					
	Between Sample Age Groups	Between Group 1 and Group 2	Residual		Over-all weighted mean (μ)	μ + Group 1 photo-period effect	μ + Group 2 photo-period effect	μ + 63 day-old age effect	μ + 91 day-old age effect	μ + 168 day-old age effect
degrees of freedom	2	1	26	number of rams	30	14	16	8	7	15
Body weight at 28 days of age	9.83	12.00	23.44		28.5 (lbs)	27.8	29.1	29.2	28.8	27.4
Weight gain	1541.56***	107.49+	25.91		25.1 (lbs)	23.2	27.0	14.1	23.4	37.6
Body weight at sacrifice	1315.59***	196.74*	44.31		53.6 (lbs)	51.0	56.1	43.3	52.3	65.1
Total testes weight	54872.88***	4005.06	1943.03		74.3 (gms)	62.7	85.9	21.8	47.8	153.5
Thyroid gland weight	2.07196***	0.66746	0.23258		2.992 (gms)	2.842	3.142	2.454	3.192	3.330
Anterior pituitary gland weight	0.003901	0.024950***	0.001961		0.280 (gms)	0.251	0.309	0.289	0.293	0.259
Pineal gland weight	0.00003043	0.00033719	0.00012167		0.0409(gms)	0.0375	0.0442	0.0394	0.0431	0.0400
Right adrenal gland weight	0.166240***	0.076339+	0.021647		0.855 (gms)	0.804	0.905	0.748	0.827	0.988
Left adrenal gland weight	0.180259***	0.025294	0.021268		0.895 (gms)	0.866	0.924	0.775	0.880	1.030
Seminal vesicle weight	1.005923	0.188827	0.447633		1.507 (gms)	1.427	1.586	1.157	1.586	1.777

With Interaction

Mean Squares

	Interaction	Residual
degrees of freedom	2	24
Thyroid follicle cell heights	7.68***	1.08

See Text – figure 2 for unweighted sub-subclass[1] cell height means

+ = $P < .10$ * = $P < .05$ *** = $P < .005$

1 – A sub-subclass is defined as any one of the photoperiod treatment groups within any one of the sample age groups.

PHOTOPERIOD TREATMENTS AND SACRIFICE DATES

Text-figure 1 summarizes the photoperiod treatments given to animals of Group 1 and Group 2, and shows the periods during the experiment when sample age group animals were sacrificed.

INTERPRETATION OF ANALYSES

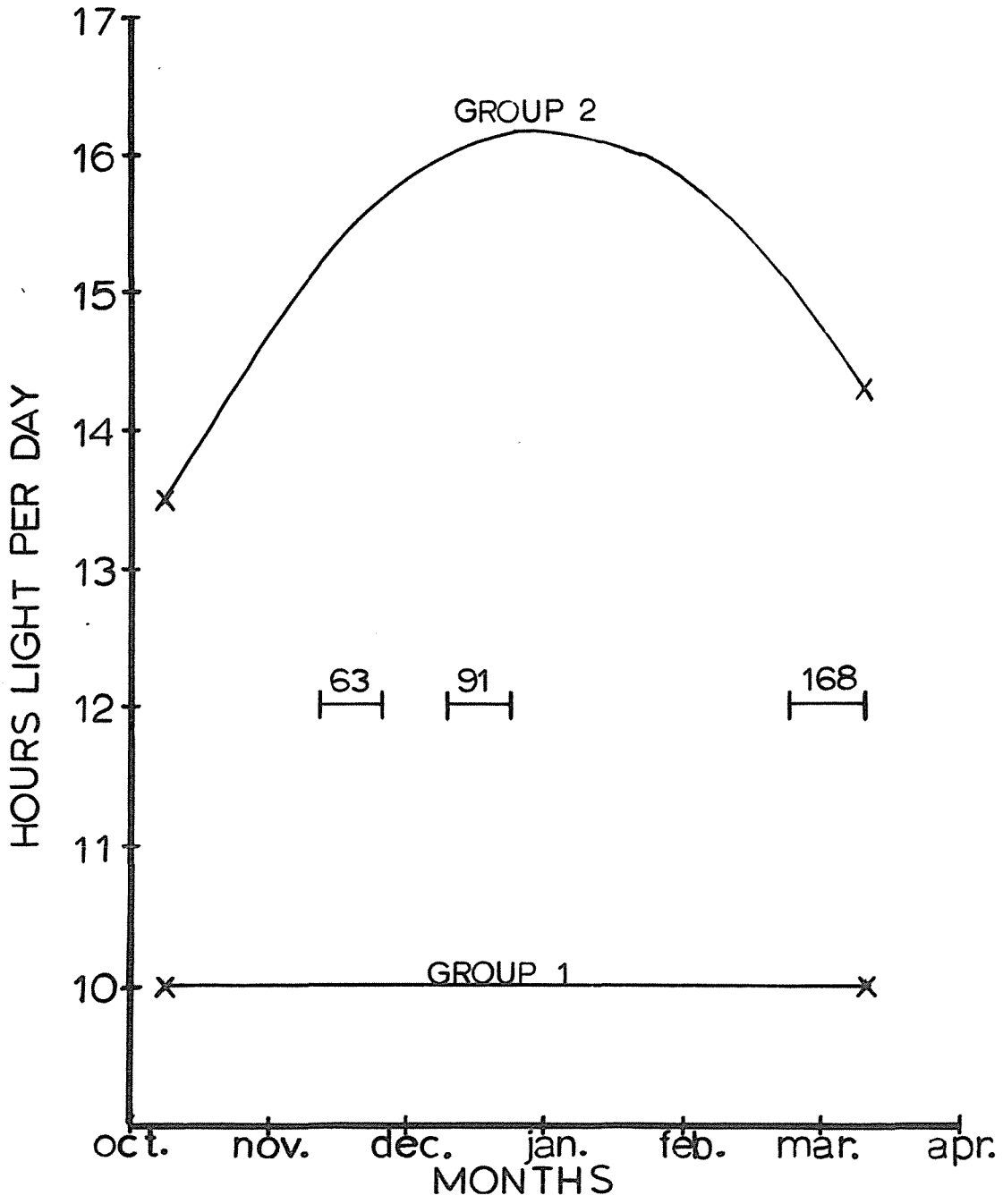
The two way classification of the data needs further definition before the observations are presented. In the following presentation, when reference is made to the significance of differences between sample age groups, the comparisons are made between the weighted means derived from the combination of all values from animals of both Group 1 and Group 2 within each sample age group. Likewise, when Group 1 and Group 2 data are evaluated for significant differences, the weighted means derived from the combination of all values from the three sample age groups within each photoperiod treatment are being compared. Interaction effects between photoperiod treatments and sample age groups are mentioned in the following presentation only when they have been found to be significant ($P < .05$).

I. BODY WEIGHTS

The "weaning treatment" was successful. Within 2 days of entering the "treatment", each ram lamb was eating, to some degree. Only one animal had to be rejected, due to a loss of weight, following the 10 day period. All other ram lambs made body weight gains of from 3.5 to 10.0 lbs with an overall average gain of 6.2 lbs.

A. Weights of 28 day-old ram lambs

The mean body weights of the 28 day-old ram lambs, initially distributed randomly into the three sample age groups of both photoperiod treatments, were not significantly different.



Text - figure 1

Experiment I: Photoperiod treatments for Group 1 and Group 2 rams. Periods when sample age group animals were sacrificed, at 63, 91 and 168 days of age, are indicated.

B. Weight gains

The differences between the mean body weight gains of the three sample age groups were significant ($P < .005$). Rams sacrificed at an older age had higher gains than animals sacrificed at a younger age. Mean gains of Group 1 and Group 2 animals were almost significantly (P almost $< .05$) different. Rams of Group 2 gained more than those of Group 1.

C. Weights at sacrifice

Mean body weights at the three sample age group autopsies were significantly ($P < .005$) different. Older animals had higher body weights at sacrifice. The difference between mean body weights of Group 1 and Group 2 rams was significant ($P < .05$). Group 2 animals weighed more at sacrifice than those of Group 1.

In summary, the rams of Group 2, although having mean body weights not significantly different from mean body weights of Group 1 animals at 28 days of age, gained more weight and had significantly higher mean body weights at sacrifice than rams of Group 1.

II. TESTES

A. Weights

The mean total testes weights, consisting of the combined weights of both right and left testes, were significantly ($P < .005$) different between the sample age groups. Total testes weights increased with the age of the animals. There was no significant difference between the mean total testes weights of Group 1 and Group 2 animals; however, testes of Group 2 rams were heavier than those of Group 1 animals.

B. Histology

The histological observations made of the testis samples from animals of

the one pre-experimental 28 day-old age group and of the three experimental sample age groups are presented in TABLE 2.

In both photoperiod treatments, development beyond the supporting cell and gonocyte stage was first observed in testis samples from rams of the 91 day-old sample age group. At 91 days of age the testes of rams in Group 2 were more advanced in their development of the spermatogenic cycle than were testes of Group 1 animals. At 168 days of age all rams in Group 2 had a complete spermatogenic cycle active in their testes, while all but one ram in Group 1 had the same full cycle of spermatogenic activity.

The interstitial cells in the testes of Group 1 and Group 2 animals showed no striking morphological differences at any sample age group. The morphological changes which took place as the interstitial cells matured, as the age of the sample animals increased, were similar to those described by Albert (1961).

C. Semen samples

A drop of semen taken from samples collected weekly, beginning when each ram was 126 days old, was smeared on a slide and stained by a method recommended by Mayer, et al., (1951). The smear was checked for the presence of any quantity of spermatozoa. At 126 days of age, 6 of the 7 smears made from the semen of rams in Group 1 showed the presence of spermatozoa, while 6 of the 8 smears made from the semen of Group 2 animals showed spermatozoa. By 140 days of age, all but one ram in Group 1 and all rams in Group 2 had spermatozoa in collected semen samples. The ram of Group 1 with no spermatozoa in its semen at 140 days of age was the same animal whose testicular spermatogenic development had reached only the secondary spermatocyte stage at autopsy at 168 days of age. A non-quantitative (eye-appraisal) evaluation of spermatozoa numbers (density) in smears of semen samples, taken just before sacrifice at

TABLE 2

EXPERIMENT I - HISTOLOGICAL OBSERVATIONS OF SPERMATOGENIC DEVELOPMENT

			Number of testis samples within each Sample Age and Photoperiod Group showing development to the stage indicated						
Sample Age Group (days)	Photo-period Group	Total number of testis samples	Supporting cells and gonocytes	Sperma-togonia	Primary sperma-tocytes	Secondary sperma-tocytes	Sperma-tids	Sperma-tozoa	Tubule lumen present
28	Pre-expt'l	3	3						
63	1	4	4						
63	2	4	4						
91	1	3			3				2
91	2	4			1	2	1		3
168	1	7				1		6	7
168	2	8						8	8

168 days of age, showed a good relationship with the subsequent histological observations made of the testicular tissue.

Epididymal weights were not analysed in detail; however, they tended to follow the weight changes of the testes throughout the experiment.

III. THYROID GLANDS

A. Weights

Mean thyroid gland weights were significantly ($P < .005$) different between sample age groups. Older animals had heavier thyroid glands. The difference between the mean thyroid gland weights of Group 1 and Group 2 rams was non-significant, but did approach doubtful significance (P almost $< .10$). Thyroid glands were heavier in Group 2 animals.

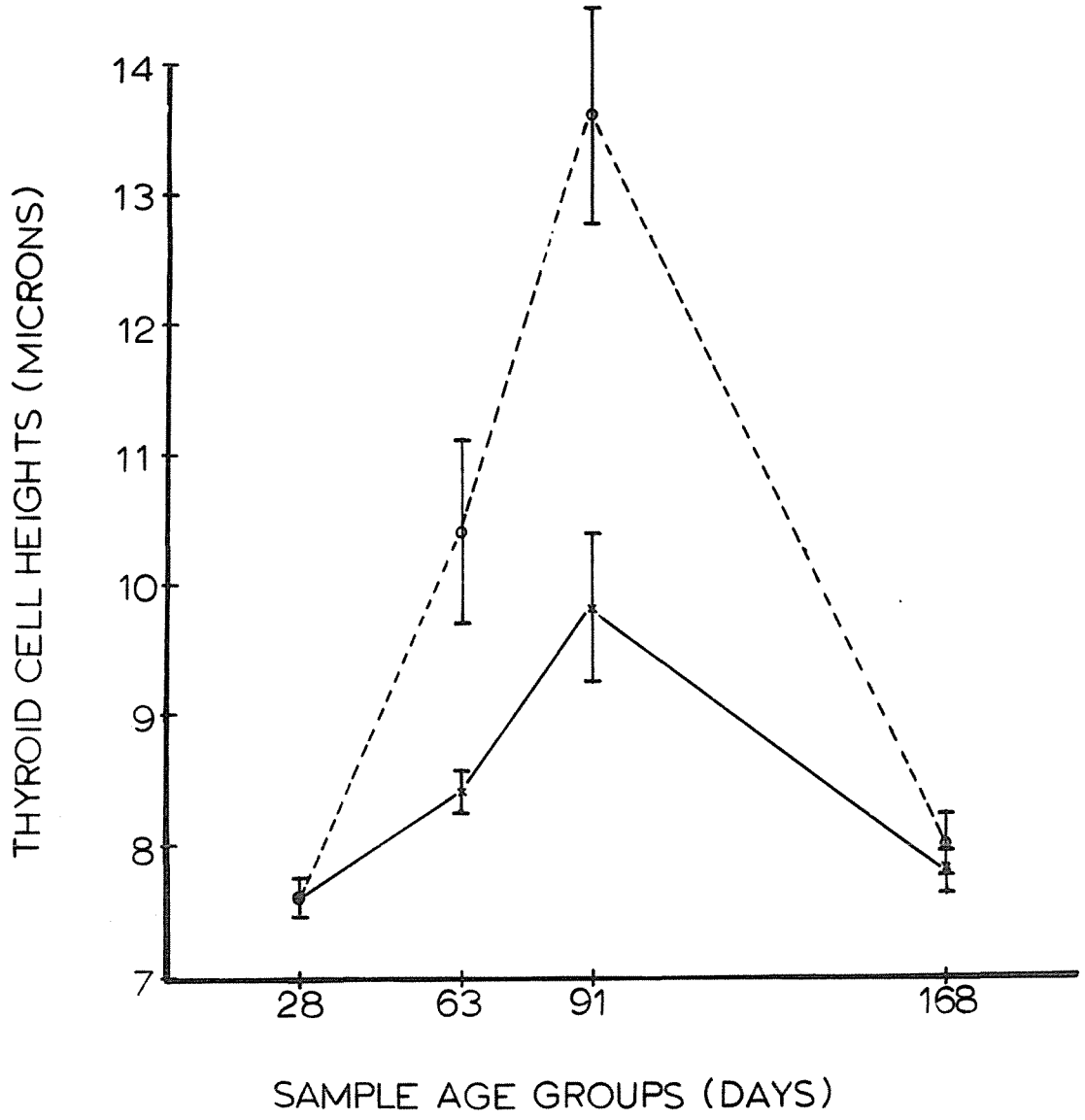
B. Histology

The differences between the mean thyroid gland follicle cell heights of each sample age group and each photoperiod treatment were significantly ($P < .005$) affected by the interaction of both variables. At any one of the sample age groups mean cell heights were greater in Group 2 thyroid glands; however, the magnitude of the differences between the mean cell heights of Group 1 and Group 2 were significantly different at each sample age group. (See Text-fig. 2). Intrafollicular colloid was decreased in the thyroid glands with the greater follicle cell heights.

IV. ANTERIOR PITUITARY GLANDS

A. Weights

Mean anterior pituitary gland weights were not significantly different between the three sample age groups. The difference between mean gland weights of Group 1 and Group 2 animals was significant ($P < .005$). Anterior pituitary glands of Group 2 rams were heavier than glands from Group 1 animals.



Text - figure 2

Experiment I: Unweighted mean thyroid follicle cell heights (± standard errors) of Group 1 (X————X) and Group 2 (0----0) rams of the one pre-experimental (28 day-old) and the three experimental sample age groups.

B. Histology

Because of the extreme regionalism of the hormone producing cells of the mammalian anterior pituitary gland and the lack of precise orientation of the observed sections, no quantitative assessment of specific cell numbers was attempted. Using the PAS technique, a qualitative evaluation of the basophilic cells of the anterior pituitary glands was made. The definitions of sheep anterior pituitary gland cell types used were those given by Clarke and Purves (1960) and Clarke (1961). In each photoperiod treatment, as the ages of the rams increased, the relative number of basophil cells showing granulation appeared to increase. Photoperiod treatment comparisons, especially when the rams were 91 days old, suggested that Group 2 anterior pituitary glands might have had slightly larger and more granular central and peripheral round and oval cells and a greater number of more darkly stained angular cells than glands of Group 1 animals. Similar observations of basophil cells were made using the Crossmon technique. Using this technique, no striking difference in acidophil cell concentration and granulation between photoperiod treatment groups was noted. The variability in concentration and granulation of cell types between the anterior pituitary glands of all animals was great.

V. PINEAL GLANDS

A. Weights

Mean pineal gland weights showed no significant differences between the three sample age groups. Rams of Group 1 and Group 2 had a mean pineal gland weight difference which approached doubtful significance (P almost $<$ .10). Pineal glands of Group 2 animals were heavier than those of rams in Group 1.

B. Histology

The general histology of the pineal glands in rams of each photoperiod group was similar to that described by Jordan (1911, 1921), and was extremely

variable between individual animals. There were no consistent differences in nuclear density, nuclear and nucleolar morphology, nor nuclear and cytoplasmic staining characteristics. Melanin-like granules were seen in the cytoplasm of peripheral cells in some of the lighter glands. No acervuli were noted in any of the pineal glands studied.

VI. ADRENAL GLANDS

Both left and right mean adrenal gland weights were significantly ($P < .005$) different between sample age groups. Adrenal glands were larger in older animals. The difference between mean adrenal gland weights of Group 1 and Group 2 rams was non-significant in the left gland, and of doubtful significance ($P < .10$) in the right gland. The right adrenal glands of Group 2 animals were larger than those of Group 1 rams. In all sample age and photoperiod groups the left tended to be larger than the right adrenal gland.

VII. SEMINAL VESICLES

The differences between mean seminal vesicle weights of the three sample age groups and the two photoperiod treatments were non-significant.

Section 4 - DISCUSSION

A correlative approach to the observations is taken in this discussion, in order that noted differences may be explained through the integrative mechanisms of the endocrine systems.

Anterior pituitary gland weights - photoperiod

The observation that the immature, pubertal rams exposed to "long" daily photoperiods (Group 2) had heavier anterior pituitary glands than animals held in the "short" daily photoperiods (Group 1) is in agreement with observations made of mature rams held in similar, but not equal, photoperiod treatments (Pelletier and Ortavant, 1964). It is interesting to note that the weighted means of the anterior pituitary gland weights are maximum in the 91 day-old, and minimum in the 168 day-old sample age groups (see TABLE 1). Although the weight differences are non-significant, the results show a trend similar to that observed by Macmillan (1967) in developing bulls. The anterior pituitary gland weights of bulls increased gradually until the time when pubertal activity was at a peak; there then was a sharp decrease in weights for a 2 month period, followed by a sharp increase and a continuation of gradual growth. This decrease in gland weight during puberty may also be present in developing rams.

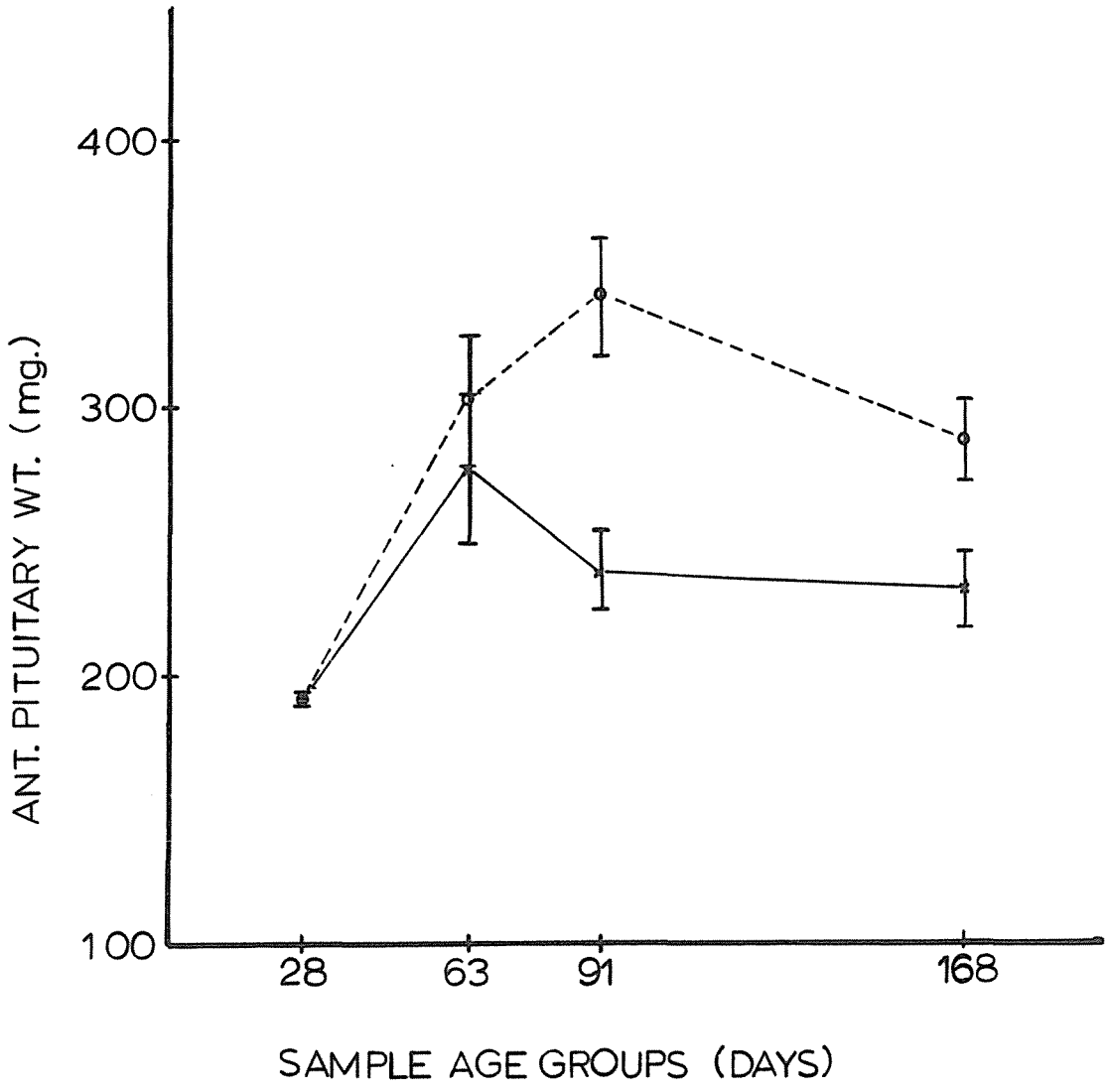
Another possible explanation for this decrease in weight may be that the anterior pituitary gland weights of rams in Group 2 have followed the fluctuation in daily photoperiods; that is, as daily photoperiods reached their maximum length, so pituitary glands reached their maximum weight (see Text - fig.1 and TABLE 1). As the statistical analyses of the weight data combined the values of Group 1 and Group 2 weights to derive the weighted mean for each sample age group (subclass), the unweighted sub-subclass means must be examined to find the degree of fluctuation in Group 1 and Group 2 pituitary gland weights at the three sample ages. When this is done it becomes obvious that

Group 2 gland weights have created the fluctuating maximum-minimum trends, while Group 1 gland weights have been similar in the 91 and 168 day-old sample age groups (see Text - fig.3). It should be remembered that only weight differences between Group 1 and Group 2 glands over all sample age groups were statistically significant, while sample age group weight differences were non-significant.

The weighted mean pineal gland weights also showed fluctuations which appeared to follow the fluctuating daily photoperiods of the Group 2 treatment; although the maximum-minimum weight differences were statistically non-significant, their possible physiological meaning will be discussed in the DISCUSSION of Experiment II.

Anterior pituitary gland histology - photoperiod - sexual development

The increase in the number of cells showing basophilic granulation in the anterior pituitary glands of the rams as their ages increased, has also been noted in the glands of developing male rats (Purves, 1961). Presumably, the changes are indicative of an increased functional activity of the pituitary gland. The functions of the various basophil cell types of the ram pituitary glands can only be presumed from knowledge of their function in laboratory rodents. In rats, the round and oval basophil cells ("gonadotrophs") are known to synthesize the gonadotrophins, follicle stimulating hormone (FSH) and luteinizing hormone (LH); the angular basophil cells ("thyrotrophs") are thought to produce thyrotrophin (TSH) (Purves, 1961, 1966). In the glands of rams in Group 2, the round and oval basophil cells showed slight size and granulation increases over the same types of cells in Group 1 glands, which is indicative, if it is justifiable to apply knowledge of rat pituitary gland cell types to ram glands, of an increased synthesis, and perhaps secretion (or, only a decreased secretion) of gonadotrophins from such glands. Clarke



Text figure - 3

Experiment I: Unweighted mean anterior pituitary gland weights (± standard errors) of Group 1 (X————X) and Group 2 (0----0) rams of the one pre-experimental (28 day-old) and the three experimental sample age groups.

(1961) found the oval cells of anoestrous ewes much more granular than the same cells of oestrous ewes. The photoperiodic conditions during anoestrus would be similar to those of the Group 2 treatment given to rams of this experiment. Although the histological observations made of the anterior pituitary glands of the Group 2 rams and the observations made of glands of ewes in natural seasonal photoperiods seem to be complementary, other photoperiod work with rams related to pituitary gonadotrophin content is contradictory. Using bioassay techniques, Pelletier and Ortavant (1964) have found anterior pituitary glands of mature rams sacrificed during "long" daily photoperiods to contain less gonadotrophins than glands of animals in "short" photoperiods. Concurrently, the testes of rams in "long" photoperiods showed a lowered spermatogenic activity when measured with several histological parameters. In the present experiment the observed histological advancement of spermatogenic activity in the testes of rams in the "long" photoperiod treatment (Group 2) over the testes of rams in the "short" photoperiod treatment (Group 1) lends support to the histological indication that Group 2 pituitary glands may have had a higher content of gonadotrophins than Group 1 glands, assuming that a higher pituitary gonadotrophin content is necessary for greater gonadal activity.

The greater number of heavily stained angular basophils in Group 2 ram pituitary glands, especially in the 91 day-old sample age glands, may be indicative of an increased TSH content. These differences are seen at the same time that thyroid gland weights are heavier and thyroid gland follicle cell heights are greater in Group 2 animals. There appears to be a positive relationship between the staining characteristics of the angular basophil cells of the ram pituitary gland and the activity of the ram thyroid gland.

Thyroid gland activity - photoperiod - sexual development

There are several possible explanations as to why the activity of the

thyroid glands, as measured by follicle cell heights, varied so greatly in the rams between sample age groups. The fluctuations in cell heights of thyroid glands in Group 2 rams (see Text - fig.2) may find explanation in the simultaneous fluctuations of the daily photoperiods to which the animals were exposed. Hoersch et al., (1960, 1961) found that in young ewes, thyroid secretion rates, to which thyroid follicle cell heights were shown to have a strong positive correlation, increased as daily photoperiod increased or decreased from a length of 12 hours per day. In Group 2 animals, when daily photoperiods were at a maximum, follicle cell heights were also at a maximum (see Text - fig.1 and 2); however, the cell heights in glands of rams held in a constant daily photoperiod of 10 hours (Group 1) also showed fluctuations throughout the experiment (see Text - fig.2).

Puberty is known to increase the demand for thyroid hormones in the body and consequently, increase the activity of the thyroid gland (Ham and Leeson, 1961). The increased cell heights in both photoperiod groups of rams may be due to this increased thyroid hormone demand; and, the fact that testes samples from Group 2 rams appeared to be more advanced in their spermatogenic development (and therefore, more advanced in their pubertal development?) than samples from Group 1 animals at 91 days of age (see TABLE 2) may explain the greater apparent thyroid gland activity noted in this group. By 168 days of age rams of both photoperiod groups had nearly completed their sexual development and their thyroid cell heights were almost equal.

The nutrition of the experimental rams may have accentuated the response of the thyroid glands to the metabolic demands of puberty. As the feed given during most of the experiment was of poor quality, it is entirely possible that only a minimal, if not subminimal, quantity of iodine was available to the animals for utilization in thyroid hormone synthesis. Several rams in Group 3, which had been given the same feed as Group 1 and Group 2 animals,

had very heavy thyroid glands, which gave some indication of the existence of goiterous conditions (see APPENDIX I). If the suggestion that a limited source of iodine was available to the rams of Group 1 and Group 2 is correct, the increase in thyroid gland activity noted was merely an effort to keep a minimal quantity of thyroid hormones in the circulation; however, the threshold of that necessary minimum amount may have been raised due to pubertal demands. This possibility casts doubt on the idea that there may have been a mild hyperthyroidism induced by the "long" photoperiod treatment, followed by an over-supply of thyroid hormones in the circulatory system.

If the creation of a mild hyperthyroid condition in rams of Group 2 due to a lengthened daily photoperiod is assumed, a reinterpretation of the observations made of the reproductive organs and endocrine glands can be undertaken. The condition could have been stimulated by an increased "thyrotrophin releasing factor" synthesis and release from the hypothalamus, which, in turn, increased TSH synthesis and release in the anterior pituitary gland. Indeed, an increase in darkly stained angular "thyrotrophs" was seen in Group 2 pituitary glands. An increase in thyroid gland activity should then be noted, as it was. Maqsood (1950, 1951) has shown that a mild hyperthyroid condition, simulated by the injection of small doses of thyroxine, has caused precocious puberty in immature rams. He suggested that the greater thyroid hormone concentrations in the circulation may have their effect by stimulating an increase in pituitary gonadotrophin synthesis and release, or by facilitating the utilization of gonadotrophins through an ability to metabolically condition target organs. The round and oval basophilic "gonadotrophs" of Group 2 anterior pituitary glands did show some indication (increased granulation) of increased activity, and a more advanced testicular spermatogenic activity was evident in rams of Group 2 at 91 days of age.

It is interesting that none of the weighted mean seminal vesicle weights

were significantly different between Group 1 and Group 2 treatments, nor between sample age groups. As seminal vesicle development and growth depend, to a great extent, upon androgen stimulation (Barraclough, 1967), these results indicate that androgens, either from the testes or the adrenal glands, were not being secreted in large quantities, which indicates further, perhaps, a low secretion of LH from the anterior pituitary glands of all rams during the experimental period.

Body weights - endocrine gland activity

The significant ($P < .05$) difference between the mean body weights at sacrifice and the almost significant (at $P < .05$) difference between the mean weight gains of Group 1 and Group 2 rams and the corresponding changes noted in the activity of the thyroid glands, are in agreement with previous work. Hoersch et al., (1960, 1961) found a positive correlation (almost significant at $P < .05$) of 0.24, whereas Singh et al., (1956) found a positive correlation of 0.81 (significant at $P < .01$) to exist between body weight gains and thyroid secretion rates in young ewes. The rams of Group 2 made greater gains, weighed more at sacrifice and had follicle cells of greater height in their thyroid glands than did Group 1 animals.

The obvious question as to which variable was altered first is applicable to all of the observations in this experiment. For example, anterior pituitary weights were heavier in the same rams that made greater body weight gains and weighed more at sacrifice; is it because their pituitary weights were greater, that their body weights were also greater, or is the converse true? It is because of this interpretive uncertainty that no covariance analyses were done to correct reproductive organ and endocrine gland weights and measurements for body weights at sacrifice. There is the possibility that some other factor common to each or all of the studied variables is exerting simultaneous inhibitory or stimulatory effects.

Perhaps the assumed increase in thyroid hormone levels in the circulation of rams in Group 2 have potentiated and/or facilitated the effects of other hormones on their target organs. Growth hormone is known to have little effect on body development without the presence of thyroid hormones (Rall et al., 1964). In humans, hyperthyroidism has been shown to cause adrenal cortical hypertrophy by increasing the rate of reduction of adrenal steroid hormones in liver cells, and therefore, increasing the stimulus to the anterior pituitary gland to secrete more adrenocorticotrophin (ACTH) (Rall et al., 1964). The increased weights of the right adrenal glands of Group 2 rams (see TABLE 1) may be a mild symptom of this acute clinical change. The left adrenal gland is known to be larger than the right in sheep (Sisson and Grossman, 1959; May, 1964), and this fact has found further support in the results of this experiment. It is therefore possible that any further stimulation by an increased ACTH level in the blood may affect the smaller of the two glands to a greater extent, due to a greater ratio of stimulative ACTH to stimulated target organ tissue. An increased turnover of glucocorticoids may have lead to an increased carbohydrate metabolism, which, in turn, may have lead to the observed increase in body weights.

Conclusion

The results of this experiment seem to favour an "increased photoperiod-increased reproductive development and endocrine activity" hypothesis. Perhaps it would be more correct to implicate the "short" and constant photoperiod treatment as an inhibitor to reproductive development and endocrine activity. This general conclusion is opposed to the widely held ideas that "short" photoperiods (10 hours per day) are stimulatory to the reproductive activity of mature rams (Ortavant et al., 1964). Thyroid gland secretory activity has been implicated as a possible intermediate factor affecting the gravimetric and histologic differences noted in animals of the two photoperiod groups.

C H A P T E R II

PHOTOPERIOD EFFECTS ON PINEAL GLAND ACTIVITY

Section 1 - REVIEW OF LITERATURE

The function of the pineal body in mammals has been questioned for centuries. Herophiles of Alexandria, around 300 B.C., thought the pineal body to function as a sphincter controlling the "stream of thoughts" within the brain, while Galenos of Pergamon (Circa 130-200 A.D.) questioned this explanation and thought the body to function as a lymph gland. Descartes (1596-1650) claimed the pineal to be the seat of the soul. Even as late as 1907, De Cyon believed the pineal body to regulate the flow of cerebrospinal fluid into the Aqueduct of Sylvius. Interest lapsed until the beginning of this century, when Marburg associated the pineal with an endocrine function relating to the development of the reproductive organs (Kitay and Altschule, 1954a; Ariens Kappers and Schade, 1965). Within the last decade, extensive research has shown the pineal body to function as an endocrine gland in some laboratory animals.

To uncover the endocrine functions of the pineal gland, investigators have used standard techniques of endocrine research: ablation of the gland, replacement therapy with an extract of the gland in an effort to prevent the syndrome following ablation, and the isolation and purification of the stimulating secretory products of the gland. Most experimental work has been done with laboratory rodents, specifically, rats and hamsters; the extent to which this review will show endocrine function for the pineal gland of all mammals will, therefore, be limited.

I. THE RELATIONSHIP OF THE PINEAL GLAND AND DAILY PHOTOPERIOD TO THE SIZE OF THE REPRODUCTIVE ORGANS AND ENDOCRINE GLANDS

A. Effects of pinealectomy

1. Female reproductive organs

Following pinealectomy, an increase in ovarian weights has been noted by most workers (Kitay, 1954; Simonnet et al., 1954; Wurtman et al., 1959; Wurtman et al., 1960; Wurtman et al., 1961; Thieblot and Blaise, 1965),

whereas some have found no increase (Gittes and Chu, 1965; Hoffman and Reiter, 1966; Wragg, 1967). Likewise, some investigators have found pinealectomy to increase uterine weights (Thieblot and Blaise, 1965; Hoffman and Reiter, 1966); others have not (Wurtman et al., 1960; Wurtman et al., 1961; Wragg, 1967). Removal of the pineal gland results, at times, in an early vaginal opening in immature female rats (Simonnet et al., 1954; Thieblot and Blaise, 1965) and an increased incidence of oestrus in mature animals (Chu et al., 1964; Gittes and Chu, 1965), and at other times not (Kincl and Bengiano, 1967; Wragg, 1967). Pinealectomy had no effect on mammary gland development (Mishkinsky et al., 1966).

2. Male reproductive organs

At times, there has been an increased testicular weight (Thieblot and Blaise, 1963, 1965; Hoffman and Reiter, 1965a, 1965b; Kincl and Bengiano, 1967), and at other times, no increase has been observed (Roth, 1964, 1965) following pinealectomy. More often, seminal vesicle and ventral prostate size in males is increased following such an operation (Thieblot and Blaise, 1963, 1965; Roth, 1964, 1965; Kincl and Bengiano, 1967).

3. Endocrine glands

Pituitary gland weights sometimes increase (Wurtman et al., 1959), as do thyroid gland weights (Scepovic, 1963; Moreau, 1964), although others find no pituitary gland weight increase following pinealectomy (Roth, 1965; Wragg, 1967). Adrenal gland size in pinealectomized animals has, at times, increased (Wurtman et al., 1959; Wurtman et al., 1960; Wurtman et al., 1961; Hoffman and Reiter, 1966), and at other times, been unaffected (Hoffman and Reiter, 1965a; Roth, 1965; Wragg, 1967).

B. Effects of daily photoperiod

1. Continuous photoperiod

The effects of continuous light exposure are similar to the effects

produced by pinealectomy in the laboratory animals studied. Fiske (1941) found that a continuous light regime increased the weights of the ovaries, uteri, testes and seminal vesicles, as well as the weights of the pituitary glands of female and male rats. A hastened vaginal opening in immature females and an increased incidence of oestrus in mature animals was also noted, and has since been confirmed (Ifft, 1962; Wurtman et al., 1964b; Wurtman et al., 1967). Subsequent work has confirmed the weight increases of the ovaries (Wurtman et al., 1960, 1961; Fiske et al., 1960; Wurtman et al., 1964b; Wurtman et al., 1967) and uteri (Wurtman et al., 1961; Wurtman et al., 1964b), in addition to noting an adrenal gland weight increase (Wurtman et al., 1960, 1961) following exposure to continuous light. Perhaps the most important simultaneous observation has been that there is a pineal gland weight decrease in animals exposed to a continuous light regime (Wurtman et al., 1960, 1961; Fiske et al., 1960, 1962; Quay, 1961; Wurtman et al., 1964b, 1964c).

2. Short photoperiod

Hamsters exposed to a continuous dark environment or a short photoperiod (1 hour light: 23 hours dark per day) have shown ovarian atrophy (Reiter and Hoffman, 1966; Reiter et al., 1966a), uterine atrophy (Reiter et al., 1966a) and testicular atrophy (Hoffman and Reiter, 1965a, 1965b; Reiter and Hoffman, 1966). Other workers found no ovarian (Hoffman and Reiter, 1966) or adrenal (Hoffman and Reiter, 1965a, 1965b) weight changes following such a treatment. Male rats matured at a slower rate when kept in a continuous dark environment (Fiske et al., 1960). Female rats blinded at 90 days of age showed reduced ovary, uterus, pituitary gland and adrenal gland weights, as well as a decreased incidence of oestrus (Hoffman, 1967). Pinealectomy has prevented the atrophy of the gonads in blinded male and female hamsters (Reiter and Knigge, 1967; Reiter, 1967).

C. Effects of pineal gland extract injections and pineal gland transplants

The observation that the pineal gland lost weight when laboratory rodents were in a continuous light environment caused investigators to hypothesize that this was an indication of a decreased activity of the gland. In hopes of finding an active secretory product of the gland, pineal gland extracts have been prepared, usually from a bovine source, as described by Altschule (1957), and injection experiments have been carried out.

Ovarian and adrenal gland hypertrophy induced by the exposure of female rats to continuous light has been decreased with injections of bovine pineal extract (BPE) (Kitay and Altschule, 1954b; Wurtman et al., 1959; Wurtman et al., 1961). The uterine hypertrophy and decrease in pineal gland weight of such animals was neither inhibited nor enhanced by BPE (Wurtman et al., 1961). The prolonged oestrus normally observed in "middle aged" female rats was inhibited with BPE, and an anoestrous state initiated. The injections decreased ovarian weight greatly, and to a lesser extent, decreased uterine and adrenal gland weights (Meyer et al., 1961). Immature female rats had a delayed vaginal opening, as well as a decrease in the normal increase in the incidence of oestrus following a continuous light treatment, when injected with EPE (Moszkowska and des Gouttes, 1962; Moszkowska, 1963). The injections did oppose the normal pineal weight decrease in these experimental animals.

Pineal gland extracts have decreased seminal vesicle and ventral prostate gland weights (Reiss et al., 1963a; Roth, 1965), as well as the testicular hypertrophy of pinealectomized male rats (Thieblot and Blaise, 1965).

Extract injections have inhibited the gonadal compensatory hypertrophy normally seen in unilaterally ovariectomized female rats (Reiss et al., 1963a) and unilaterally castrated male rats (Moszkowska, 1963). The compensatory hypertrophy of the testis of unilaterally castrated male hamsters was inhibited in a short (1 hour light: 23 hour dark per day) daily photoperiod, provided

that a precastration short photoperiod was given. Pinealectomized animals or animals given a long (16 hours light: 8 hours dark per day) daily photoperiod prior to unilateral castration showed marked compensatory hypertrophy. This work suggests that the activity of the pineal gland may alter pituitary-gonad hormonal inter-relationships (Hoffman and Reiter, 1965b).

Some workers suggest both a stimulatory and an inhibitory effect of pineal gland extracts, depending upon the age of the experimental animal (Reiss et al., 1963a).

Isogenic pineal gland transplants into the leg musculature of pinealectomized female rats reduced the increased incidence of oestrus normally seen in such animals. Subsequent removal of these transplants returned the animals to a high incidence of oestrus (Gittes and Chu, 1965). The transplantation of pineal glands into the kidney capsule of pinealectomized, blinded male hamsters had no effect upon the size of the testes (Reiter and Knigge, 1967).

To summarize the reviewed information to this point, the early work seemed to indicate that the presence and activity of the pineal gland was inhibitory to the reproductive organs and some endocrine glands. Removal of the pineal gland released the inhibition, and affected tissues increased in size. The length of the daily photoperiod appeared to regulate pineal gland activity: long photoperiods inhibited and short photoperiods stimulated activity of the gland. The injection of pineal gland extracts reversed the effects of the release of inhibition caused by pinealectomy or long photoperiods. The next logical step in pineal gland research was to isolate and purify the active secretory product(s) of the gland.

II. PINEAL GLAND PHYSIOLOGY AND HISTOLOGY RELATED TO DAILY PHOTOPERIOD

A. Physiology and histology

A substance found in high concentration in the pineal gland was isolated from bovine material by Lerner et al., (1958) and called melatonin because of its ability to oppose the darkening effect of melanophore stimulating hormone (MSH) in frog skin. Following this observation, other workers found a specific enzyme, hydroxyindole-O-methyl transferase (HIOMT), that catalyzed the O-methylation of N-acetyl-serotonin to melatonin in the pineal gland (Axelrod and Weissbach, 1961). In mammals, HIOMT, and therefore the synthesis of melatonin, occurs only in the pineal gland. Wurtman et al., (1963b, 1964c) have shown HIOMT activity within the pineal gland to vary with the environmental light regime. Pineal glands of rats in continuous dark had an HIOMT activity 10 times greater than glands of animals kept in continuous light and twice as strong as glands of animals in a normal diurnal photoperiod. From this and later work it was proposed that light inhibits HIOMT activity, and therefore, inhibits melatonin production (Wurtman et al., 1967). Quay (1963) and Synder et al., (1965) have shown a diurnal rhythm of serotonin, a precursor of melatonin, to exist in the pineal glands of rats. The maximum concentration of serotonin was at midday, the minimum during the night. An early onset of photoperiod caused an early rise in serotonin levels and an early succession of light caused an immediate drop of levels in the pineal gland. Later work has shown melatonin and HIOMT concentrations within the pineal gland to have diurnal rhythms inverse to the rhythm of serotonin (Quay, 1964; Axelrod et al., 1965).

Histologically, the pineal parenchyma cells of rats showed an altered morphology when animals were exposed to different light environments. Rats kept in continuous dark had pineal glands whose parenchyma cells were larger and whose cytoplasm was more granular than the cells of animals kept in

continuous light. The pineal parenchyma cells of the continuous dark group had oval, piriform and triangular nuclei showing prominent nucleoli, with highly irregular and much indented nuclear membranes. The cells of the continuous light group had more consistently oval nuclei, which showed less prominent nucleoli with smoother walls. Cells in the pineal glands of the continuous dark group were 30% larger than the cells in the glands of the continuous light group, as estimated from nuclei density measurements (Roth et al., 1962). The mean nuclear diameters of pineal parenchyma cells of hamsters in a short photoperiod showed increases over the nuclear diameters of long photoperiod animals (Hoffman and Reiter, 1965a). Mogler (1958) stated that the nuclear diameters of the pineal parenchyma cells and the interstitial cells of the testis vary inversely with photoperiodic changes.

Injections of pineal extracts decreased the granular density of the nuclei and cytoplasm, as well as reduced the number and size of nucleoli of rat pineal parenchyma cells. Cell size was slightly reduced (Holmgren et al., 1960).

These histological observations have been related to pineal gland secretory activity. An increase in cytoplasmic basophilic granules in pineal cells of continuous dark-treated rats was indicative of an increase in RNA content. This increased RNA content may indicate an increased synthesis of nucleoprotein and protein which may relate to the increased enzyme synthesis (HIOMT?) necessary for the endocrine activity of the pineal gland (Roth et al., 1962).

B. Effects of melatonin injections

Melatonin has been taken up by all tissues, to some extent, following injection. The pineal gland itself held 40 times the concentration in the blood plasma, the ovary and iris-choroid layer of the eye held 10 times the plasma levels, and other endocrine glands and peripheral nerves held from

3-5 times the concentrations in the plasma. When rats were kept in continuous light, the ability of the pineal gland and ovary to take up melatonin decreased, which suggests that some of the effects of light upon the rat gonad might be mediated by changes in the rates of synthesis and release, or in the peripheral actions and physiologic deposition of melatonin (Wurtman et al., 1964a, 1964d).

Some workers have found melatonin injections to decrease ovary weights (Wurtman et al., 1963a; Chu et al., 1964; Adams et al., 1965; Narang et al., 1967), yet others have found no effect on the ovaries (Ariens Kappers, 1962; Tilstra and Prop, 1963; Ebels and Prop, 1965), testes (Ariens Kappers, 1962) or uteri (Wurtman et al., 1963a; Ebels and Prop, 1965). Although no spermatogenic degradation was observed, Ariens Kappers (1962) did find that seminal vesicle and ventral prostate gland size decreased with injections of melatonin. Some investigators have found a stimulatory effect of melatonin injections on the weights of the ovaries, testes, seminal vesicles and ventral prostate glands of rats (Thieblot et al., 1966a, 1966c). Melatonin injections have delayed vaginal opening in immature female rats (Wurtman et al., 1963a; Adams et al., 1965).

Although the incidence of oestrus of female rats in a normal diurnal light regime could be decreased with melatonin injections (Wurtman et al., 1963a; Chu et al., 1964), melatonin could not inhibit the onset of continuous light-induced persistant oestrus (Wurtman et al., 1963a), but could depress the established persistant oestrus of mice (Chu et al., 1964) and rats (Wurtman et al., 1963a) in continuous light. Some workers found that melatonin had no effect on the persistant oestrus condition of rats, induced by continuous light (Ebels and Prop, 1965). The persistant oestrus caused by pinealectomy in rats was depressed with melatonin injections (Chu et al., 1964).

Thyroid gland weights decreased following melatonin injections (Baschieri

et al., 1963), as did pituitary gland weights (Narang et al., 1967).

Injections of serotonin had no effects on the weights of the ovaries or uteri (Wurtman et al., 1963a) or the incidence of oestrus (Wurtman et al., 1963a; Chu et al., 1964), but increased adrenal gland weights (Wurtman et al., 1963a) in rats.

All of the experimental work with melatonin injections has been confounded for comparisons by the varied dose levels used in the investigations.

III. PATHWAYS OF RESPONSE OF THE PINEAL GLAND TO DAILY PHOTOPERIOD

Pineal gland activity has been shown to change in response to variation in photoperiod. The secretory product of pineal gland activity then influences the reproductive organs and some endocrine glands. The pathways by which photoperiod variations reach and influence the activity of the pineal gland will be reviewed in this section.

As early as 1937, Browman found that the removal of the eyes of rats eliminated the effects of continuous light on the weights of the ovaries and uteri as well as the incidence of oestrus. Much later, Quay (1961) found that severing the optic tract had a similar effect. After removal of the eyes, there was a complete loss in the capacity of the pineal gland to respond to a variation in photoperiod, as shown by a lack of variation in pineal gland weights and a lack of fluctuation in pineal HIOMT activities (Wurtman et al., 1964b, 1964c). Bilateral enucleation of female and male hamsters has caused a decrease in the size of the ovaries, uteri, testes, seminal vesicles and adrenal glands. Pinealectomy and superior cervical ganglionectomy have eliminated this response to enucleation (Hoffman and Reiter, 1965a; Reiter and Hester, 1966; Reiter and Hoffman, 1966; Reiter and Knigge, 1967). In rats also, removal of the superior cervical ganglion has eliminated the effects of photoperiod on the weights of the pineal glands, ovaries and uteri, as well as on the incidence

of oestrus and levels of pineal HIOMT (Wurtman et al., 1964b, 1964c).

The diurnal rhythm of serotonin content in the pineal gland has been terminated with a superior cervical ganglionectomy (Fiske, 1964; Synder et al., 1965). Although normal rats in continuous light showed no diurnal rhythm of serotonin content in the pineal gland, the rhythm persisted in continuous dark. Blinded rats had a diurnal rhythm of pineal serotonin, whether in continuous light or dark (Synder et al., 1965). This work suggests that the controlling mechanism for the pineal serotonin rhythm is extrinsic to the pineal gland, and communicated from the central nervous system via sympathetic fibers to the superior cervical ganglion. The sympathetic nervous system has also been implicated in the regulation of the diurnal rhythm of pineal HIOMT levels and melatonin synthesis. Unlike the serotonin rhythm, however, the diurnal variations in melatonin synthesis are not endogenous, but are directly influenced by external lighting which is relayed to the pineal gland via the superior cervical ganglion (Axelrod et al., 1965).

Recent work has shown lesions which transect the medial forebrain bundle of the lateral hypothalamus to abolish the effects of photoperiod variations on the reproductive organs and endocrine glands, and the changes in pineal weight and HIOMT activity (Axelrod et al., 1966; Wurtman et al., 1967). Only one of the inferior accessory optic tracts had to be intact to maintain a normal pineal HIOMT response to continuous light, even when both of the primary optic tracts and superior accessory optic tracts had been severed (Moore et al., 1967).

There is some evidence that in newborn rats there is a nonretinal pathway of light to the pineal gland, as young blinded animals still maintain a high level of serotonin within the pineal gland when exposed to an additional photoperiod (Zweig et al., 1966).

IV. THE RELATIONSHIP OF PINEAL GLAND ACTIVITY AND DAILY PHOTOPERIOD TO THE PHYSIOLOGY AND HISTOLOGY OF ENDOCRINE AND NEUROENDOCRINE SYSTEMS

A. The response of the pineal gland to altered endocrine gland activity

The weight changes of pineal glands in response to photoperiod variations have been unaffected by hypophysectomy, gonadectomy, adrenalectomy or thiouracil feeding of rats (Quay, 1961; Fiske et al., 1962; Wurtman et al., 1964c). The HIOMT activity response to photoperiod change has also been unaffected by hypophysectomy or ovariectomy (Wurtman et al., 1964c). Hypophysectomy, gonadectomy, thyroidectomy and adrenalectomy have had no effect on the diurnal serotonin rhythm of the pineal gland (Synder et al., 1965).

Using a P^{32} uptake technique as an assessment of metabolic activity, the pineal gland has been studied under various experimentally altered physiological states. Hypophysectomy increased pineal P^{32} uptake (Reiss et al., 1963b), as did male and female gonadectomy (Borell and Orstrom, 1947). Some workers found no effect of ovariectomy on pineal P^{32} uptake in rats (Brewer and Quay, 1958). Injections of oestradiol and progesterone left the pineal gland unaffected metabolically (Brewer and Quay, 1958) and volumetrically (Quay and Levine, 1957).

B. The response of endocrine and neuroendocrine systems to altered pineal gland activity

1. Hypothalamic neuroendocrine system

Histologically, there have been differences noted in the amount of stained "neurosecretory material" in areas of the hypothalamus when rats were treated with experimental photoperiods and/or experimental alterations in pineal gland secretory status. The supraoptic nuclei of male and female rats housed in continuous light showed a greater neurosecretory activity when compared with animals in an equinoctial or continuous dark photoperiod. No changes were noted in the activity of the paraventricular nuclei (Fiske and Greep, 1959). Conversely, some workers found that when the supraoptic nuclei

are active the activity of the pineal gland is depressed (Miline, 1960). In contrast to these observations, other investigators have noted that, following pinealectomy, there is an increase of "neurosecretory material" in the paraventricular nuclei and paraventricular-hypophyseal tracts, with no alterations in the supraoptic nuclei (Bugnon and Moreau, 1961b; Moreau, 1964). Cells of the paraventricular nuclei have shown an hypertrophy and an increase in neurosecretory activity following injections of bovine pineal gland extracts (Aron et al., 1961; Miline, 1963). Other workers noted no change following extract injections (Bugnon and Moreau, 1961b).

There was a 3 fold increase in the oxytocic activity of the paraventricular nuclei of adult male dogs following pineal gland extract injections. (Milcou and Pavel, 1960). The investigators suggested that this increase was due to a blocking of the release of oxytocin caused by the extract, and further related oxytocin to gonadotrophin release from the anterior pituitary gland. If their hypothesis is accepted, the "antigonadotrophic function" of the pineal gland can be explained as an inhibiting action upon the release of hypothalamic releasing factors.

2. Anterior pituitary gland

Immature male and female rats treated with continuous light for 8 weeks showed a higher circulating follicle stimulating hormone (FSH) level in the blood than animals confined to continuous dark. Circulating luteinizing hormone (LH) was higher in the continuous dark-treated females, whereas males showed no differences between photoperiod treatments. Likewise, anterior pituitary gland FSH content was higher in animals exposed to continuous light, and anterior pituitary LH content was higher in continuous dark-treated females, with males again showing no LH concentration differences (Fiske, 1941). Underfed female rats held in continuous light for 10 days showed no changes in anterior pituitary gland FSH or LH concentrations (Piacsek and Meites, 1967).

When melatonin was injected into immature female rats for 4 weeks, a decrease in pituitary gland weight and an increase in LH levels was noted. There was an inhibition of an ovarian weight increase and a delayed vaginal opening. The increased pituitary gland LH concentration of ovariectomized female rats was not further increased by daily administration of melatonin. The investigators, therefore, suggested that while melatonin may exert a delaying influence upon hypophyseal-gonadal maturation, it does not augment the increase in pituitary LH induced by ovariectomy (Adams et al., 1965).

Pineal gland extract injections inhibited the stimulatory action of human chorionic gonadotrophin (HCG) and human menopausal gonadotrophin (HMG) on female mouse uterine weight (Soffer et al., 1965). The stimulatory actions of the gonadotrophins on the seminal vesicles and ventral prostate glands of immature male rats were also eliminated with extract injections. The levels of gonadotrophins in the circulation and in the anterior pituitary gland have been diminished following extract injections (Thieblot and Blaise, 1963). Incubation, in vitro, of anterior pituitary tissue with pineal gland extracts caused a decrease in FSH secretion (Moszkowska, 1963, 1965). Melatonin injections did not have the same inhibitory effects on the stimulatory actions of gonadotrophins as did pineal gland extract injections (Soffer et al., 1965); however, melatonin did limit the number of rats ovulating spontaneously, or in response to an exogenous source of gonadotrophin under normal diurnal light conditions (Psychoyos, 1966). The active pineal glands of blinded female hamsters neither prevented completely the action of exogenous gonadotrophins on the ovaries, nor the action of oestrogen on the uterus (Reiter, 1967).

Wurtman et al., (1961) suggested that because photoperiod changes alter both ovarian and uterine weight, and pinealectomy affects only the weight of the ovary, the influence of the pineal gland upon gonadotrophins is primarily upon FSH secretion, whereas light seems to influence the secretion of both FSH and LH.

Histologically, pinealectomy has caused an increase in the number of eosinophilic cells and a decrease in the number of chromophobic cells (Simonnet et al., 1954; Theiblot and Blaise, 1963), as well as an hypertrophy and vacuolization of gonadotrophic cells (Bugnon and Moreau, 1961a, 1964; Moreau, 1964) in the anterior pituitary glands of male and female rats. Pinealectomy increased the granulation of pituitary thyrotrophic cells. All of the above observations were reversed following pineal gland extract injections.

From their work with hamsters, Reiter and Hester (1966) concluded:

> "... the lack of a hypertrophic response by the pituitary glands of blinded or dark exposed hamsters with atrophic gonads suggests that pineal substances may be acting at the hypothalamic or pituitary level to restrict growth of the hypophyses and possibly to modify endocrine functions."

3. Thyroid gland

Feeding goitrogenic thiouracil to rats caused no pineal gland weight change, nor did different photoperiod treatments affect the degree of thyroid hypertrophy in these animals (Fiske et al., 1962). The thyroid glands of thiouracil-fed female hamsters treated with a short photoperiod hypertrophied less than those of similarly fed animals treated with a long photoperiod; however, the response of the thyroid glands in either photoperiod treatment was not affected by the removal of the pineal gland. Pinealectomy did prevent the normally observed regression of the uteri following thiouracil feeding (Reiter et al., 1966a, 1966b).

Following pinealectomy, female rats showed an 11.8% increase in thyroid secretion rate (TSR), as well as a slightly increased feed consumption (Ishibashi et al., 1966). Another report suggested a decreased TSR following pinealectomy in rats, although histologically, cellular hypertrophy was evident (Moreau, 1964). For the first 2 months after pinealectomy in rats, hypertrophy and hyperplasia of thyroid follicular cells and colloid resorption was detected. Following this period, the thyroid glands had a polymorphous structure

with the follicles being different sizes and shapes in many adenomatous formations (Scepovic, 1963).

Melatonin injections in moderate doses decreased the TSR by 16% in female rats. Feed consumption was also lowered (Ishibashi et al., 1966). With large injected doses of melatonin, the TSR of male rats decreased 95.2% and the thyroid gland weight decreased 23.5% (Baschieri et al., 1963). The effectiveness of melatonin in lowering TSR was shown to be reduced as the age of the animal increased (Narang et al., 1967). When melatonin was given to thiouracil-fed animals, there was no more decreased activity in the thyroid gland, although gland weights were lower than those of animals treated with thiouracil only (Baschieri et al., 1963). Other workers found melatonin injections, in large doses, to increase, histologically, the activity of the thyroid glands of young rats (Thieblot et al., 1966b). Most workers have postulated that melatonin has an effect on thyroid function, perhaps by inhibiting thyroid stimulating hormone (TSH) synthesis and/or release from the anterior pituitary gland, or perhaps by inhibiting the action of TSH on the thyroid gland. Miline (1963) felt that the correlative activity of the thyroid and pineal glands is under the control, regulation and co-ordination of the paraventricular nucleus of the hypothalamus.

The length of daily photoperiod affects the secretory activity of the pineal gland in laboratory rodents. Melatonin is one of the major products of this secretory activity, and is considered by many workers to be the physiologically active pineal hormone. "Short" or continuous dark photoperiods stimulate melatonin synthesis in the pineal glands of laboratory rodents. Melatonin in the circulatory system then, through still undefined pathways, depresses some reproductive organ and endocrine gland activities.

Investigators have found that mature rams respond to "short" photoperiod

treatments with increased reproductive activities. No work has yet been reported examining the effects of daily photoperiod on the activity of the pineal glands of rams. Nor have the effects of melatonin injections on reproductive and endocrine activities of rams been studied. The following experiment is meant to provide preliminary information on these topics.

Section 2 - MATERIALS AND METHODS

I. ANIMALS

The 14 Southdown x Romney rams remaining from the previous experiment (7 rams from Group 3 and 7 rams from Group 4) were used as experimental animals. Their ages at the beginning of this experiment ranged from 166 to 185 days. The treatment of the rams prior to their use in this work has been described.

II. EXPERIMENTAL DESIGN

A. Photoperiod treatments

The rams were randomly distributed into two "controlled environment" rooms, with assurance that equal numbers of animals from each of the two groups of the previous experiment were represented in melatonin injected sub-groups of this experiment. The "controlled environment" rooms and their maintenance have been described. Individual 3' x 4' wire pens with wooden slate floors were provided for each animal. The rams could see each other, but no heavy contact was possible through the wire divisions between pens.

The animals, with the wool trimmed from around their eyes, entered the rooms and were exposed to the natural daily photoperiod and a constant 65°F temperature for 2 days prior to the beginning of the experimental photoperiod treatments. The two photoperiod treatment groups were: Group 1, 7 rams exposed to 4 hours light: 20 hours dark per day, and Group 2, 7 rams exposed to 20 hours light: 4 hours dark per day. The environmental temperature for Group 1 and Group 2 was a constant 65°F throughout the experiment. The day before the rams entered the experimental rooms the daylength (including civil twilight) was 14 hours, 10 minutes, and the maximum temperature was 80.9°F. The experiment was begun during the early Autumn.

On the day that experimental photoperiod treatments began (DAY 0), each ram was weighed and a semen sample was taken with an electroejaculator and checked

for the presence of spermatozoa. On the thirteenth day of treatment (DAY 13) body weights of all animals were again taken.

B. Melatonin injections

On the fourteenth day of photoperiod treatments (DAY 14) subcutaneous injections (in the neck region) of melatonin began for 4 randomly selected rams ("experimental" rams) within each photoperiod group. The remaining 3 rams within each group were injected daily with an equal volume of the melatonin diluent ("control" rams). Five mg. of melatonin (Sigma Chemical Co., U.S.A.) dissolved in 5 ml. of 1% alcoholic sterile saline were given to each "experimental" ram daily for 28 days. Injections were given $\frac{1}{2}$ to $1\frac{1}{2}$ hours after the onset of the daily light period. The melatonin solution and diluent were held in darkness within a refrigerator.

Body weights of all animals were taken the day of the last injection, (DAY 41) and the rams were sacrificed and autopsied the following day.

C. Nutrition

The nutrition was the same for all animals during the entire experiment. A pelleted feed composed of lucerne meal, barley meal and wheat husks, with added vitamins and minerals was fed ad libitum, and from DAY 13 to DAY 41 the daily intake of each animal was measured to the nearest 0.1 lb. Water was always available.

III. AUTOPSY PROCEDURE

Each ram was sacrificed by exsanguination just before the onset of its photoperiod on DAY 42. The anterior pituitary gland was quickly dissected from the sella turcica, weighed on a Mettler balance and rapidly frozen within a glass stoppered test tube held in carbonic ice. A block of brain tissue containing the hypothalamus was dissected and held in a sealed jar with a piece of saline-moist gauze until being fixed. The testes, epididymides, thyroid gland,

pineal gland, adrenal glands and seminal vesicles of each ram were dissected, held and weighed in the same manner as in the previous experiment.

IV. TISSUE TREATMENTS

A. Lypholization of anterior pituitary glands

After 48 hours in carbonic ice, all rapidly frozen anterior pituitary glands were lypholized. Following drying, the powdered tissue was held in glass stoppered test tubes within a dessicator kept in a dark room at 5°F.

B. Fixation and embedding

The blocks of brain tissue containing the hypothalami were fixed in 10% neutral buffered formalin (pH = 7.0) for 40 to 48 hours, followed by several rinses in 70% ethanol, then embedded in paraffin wax.

Samples of the testes, epididymides, thyroid glands, pineal glands, adrenal glands and seminal vesicles were prepared, fixed and embedded in paraffin wax in the same manner as in the previous experiment.

C. Sectioning and staining

1. Hypothalami

Transverse frontal sections were taken in a "semi-serial" fashion through the anterior areas of the hypothalami. Sectioning commenced just anterior to the optic chiasma and terminated where the infundibular stalk began. Five 10 μ sections were mounted on a slide, with the next ten 10 μ sections being discarded, followed by the mounting of the next five 10 μ sections, and so on throughout the anterior areas of the hypothalami studied.

Mounted sections were stained by the chrome alum-hematoxylin-phloxin method described by Gomori (1941). General morphology was studied, and a qualitative evaluation of "neurosecretory material" in the supraoptic and paraventricular nuclei of the hypothalami was made.

2. Other tissues

All other tissues were sectioned and stained in the same manner as in the previous experiment, with two exceptions: the testes were sectioned at 10 μ instead of 12 μ, and additionally, pineal gland sections were stained for ribonucleic acid (RNA) with pyronin Y in acetate buffer at pH = 4.4 (Barka and Anderson, 1963, p.100).

V. MEASUREMENTS

A. Histological

1. Testes

The diameters of 25 seminiferous tubules within the stained testes sections of each ram were measured on a projection microscope. Primary spermatocytes in the leptotene stage of the spermatogenic cycle were counted in 5 Stage 2 tubules of each ram testis section at a magnification of 250X. These measurements were used as an index of spermatogenic activity and "efficiency", as described by Ortavant et al., (1964).

2. Thyroid glands

Thyroid gland follicle cell heights were measured in the same manner as described in the previous experiment.

3. Pineal glands

The numbers of nuclei in a standard grid area were counted in 10 randomly selected fields at a magnification of 250X within pineal gland sections of each animal. This measurement was used as an indicator of relative cell size, to be applied as an index of cell activity, after Roth et al., (1962).

B. Bioassays of anterior pituitary gland hormones

1. Luteinizing hormone (LH)

The concentrations of LH in the lypholized anterior pituitary glands were measured with a modified rat ventral prostate gland assay technique (Greep

et al., 1941). Twenty to twenty-eight day old hypophysectomized male Sprague-Dawley rats were injected subcutaneously once a day for four days with an accumulative total dose of either 1.0 mg. or 2.0 mg. dried anterior pituitary gland tissue. Tissue was suspended in sterile saline and injected in a 0.5 ml. volume to each rat each day. Solutions were held in a refrigerator.

The animals were killed with ether on the fifth day, and the ventral prostate gland was dissected out and weighed to the nearest 0.1 mg. on a Roller-Smith torsion balance. The sella turcica of each rat was checked macroscopically for the presence of pituitary gland tissue, and if tissue was present, the data collected from that animal was discarded.

2. Thyroid stimulating hormone (TSH)

The concentrations of TSH were measured with a thyroid I^{131} depletion technique described by Bates and Cornfield (1957) and Bates and Condliffe (1960). Pituitary tissue from all rams within each of the two injection groups within each of the two photoperiod treatment groups was pooled, and bioassays were done on the resulting four samples. Day-old cockrels were injected with 1.0 μc of I^{131} diluted in 0.2 ml. distilled water. Immediately after injection, a one minute radioactivity count was taken with a scintillation counter over the injection site. Twenty-four hours later, a one minute count was taken over the thyroid gland of each chick, and the percentage absorption of I^{131} was calculated. An injection of 8 μg. L-thyroxine and 0.5 mg. methyl thiouracil was then given to each chick in a volume of 0.2 ml. distilled water, followed by an injection of 0.2 mg. or 0.4 mg. dried anterior pituitary tissue suspended in 0.2 ml. distilled water. After another 24 hours, a final one minute count was taken over the thyroid gland of each chick and by allowing for natural radioactive decay and background counts, a corrected final radioactivity count was calculated. All injections were done subcutaneously in the inner thigh. Chicks were starved during the entire bioassay, but water was always available.

VI. STATISTICAL ANALYSES

A. Gravimetric and histologic measurements

A two-way subclass classification, similar to that employed in Experiment I, was used for the analysis of the gravimetric and histologic measurements. A mathematical model for the analysis of variance has been given. In this experiment a_i = the effect of the "control" and "experimental" injection treatments ($i = 1, 2$) and b_j = the effect of the photoperiod treatments ($j = 1, 2$). The assumptions and restrictions made on Experiment I data were imposed in this analysis.

Covariance analyses were done on weighted means and adjusted subclass means were calculated. The mathematical model for such analyses was

$$Y_{ijk} = \mu + a_i + b_j + \beta x_{ijk} + e_{ijk}$$

where β is the slope of the common regression line in the population and x_{ijk} is the deviation of any X value from the total mean.

The "F test" was used as a test of significance for both the analyses of variance and covariance. All computations were done by an IBM 1620 computer.

B. Bioassays

1. Luteinizing hormone (LH)

A covariance analysis of ventral prostate gland weights on the logarithms of the dose levels was done to check the validity of the assumption of parallelism between all dose response lines.

Following the above verification, an analysis of variance of ventral prostate gland weight data was carried out using heirarchal classifications for handling numbers of samples and sub-samples of unequal sizes, as suggested by Snedecor (1956).

2. Thyroid stimulating hormone (TSH)

Due to the great variability of the initial absorption of radio-

activity, a covariance analysis correcting final for initial radioactivity counts was done. If the differences between the adjusted final counts of the dose level groups within the sub-subclass groups were non-significant, no further analyses were carried out, as this non-significance would render the assay invalid.

The "F test" was used as a test of significance in all analyses of bioassay data.

Section 3 - OBSERVATIONS

STATISTICAL ANALYSES

As in Experiment I, a two-way classification of the collected data was used for the statistical analyses of this experiment. One subclass comparison was that between data from the "control" and "experimental" rams of both Group 1 and Group 2 photoperiod treatments, while the second comparison was between Group 1 and Group 2 data from both "control" and "experimental" animals. Summaries of the analyses of variance with weighted subclass means and the analyses of covariance with adjusted subclass means are given in TABLE 3 and TABLE 4, respectively. Only covariance analyses with total regression coefficients with $P < .10$ are presented. Interaction effects have been mentioned in the text only when they have been found to be significant ($P < .05$).

When the word "mean" is used in the following presentation it will be assumed that its reference is to a weighted or adjusted mean derived from a variance or covariance analysis, respectively.

A sub-subclass is defined as any one of the injection treatment groups within any one of the photoperiod treatment groups. When reference is being made to an unweighted mean of a sub-subclass it will be stated.

ANIMALS

All rams, except one, entered and proceeded through the experiment with no outward signs of physical "stress". One ram in the "control" Group 2 sub-subclass group began to show signs of nasal inflammation and blockage on DAY 30. This animal had slightly laboured breathing from this day until the termination of the experiment on DAY 42. The data from this animal was retained in the final analyses, as the feed intakes and body weight gains of the animal showed no severe set-backs; and the weights and histology of the animal's adrenal glands and other endocrine glands gave no indication of any extraordinary metabolic "stress".

TABLE 3

EXPERIMENT II - SUMMARY OF ANALYSES OF VARIANCE, WEIGHTED MEANS

	Mean Squares					Weighted Subclass Means				
	Between "control" and "expt'l"	Between Group 1 and Group 2	Interaction	Residual		Overall weighted mean (μ)	μ + "control" effect	μ + "expt'l" effect	μ + Group 1 effect	μ + Group 2 effect
degrees of freedom	1	1	1	10	number of rams	14	6	8	7	7
DAY 0 body weight	15.1810	410.1562*	8.3700	61.0521		73.3 (lbs)	72.1	74.2	67.7	78.6
DAY 13 body weight	12.8710	442.0015**	7.5010	59.9271		78.4 (lbs)	77.3	79.2	72.5	83.9
Weight gain from DAY 13 to DAY 41	40.0238+	6.0952	1.5238	11.1167		13.8 (lbs)	11.8	15.3	14.2	12.9
Feed intake from DAY 13 to DAY 41	177.9400	4.1171	45.1600	100.7460		98.1 (lbs)	93.9	101.1	97.0	98.1
DAY 41 body weight	98.2900	344.2871+	15.7800	86.9360		92.1 (lbs)	89.1	94.4	86.8	96.8
Pineal gland weight	597.3950	0.8859	1269.4000	508.7708		60.6 (mg)	53.1	66.3	60.0	59.5
Pineal gland nuclei density	6060.0000	8816.0047+	7241.7000	2361.5500		308.4 nuclei field	284.3	326.4	330.7	280.0
Wet anterior pituitary gland weight	0.00188000	0.00259286	0.00709790	0.00878570		0.431 (gm)	0.444	0.421	0.419	0.446
Dry anterior pituitary gland weight	22.8800	20.0238	486.8800	542.7670		99.4 (mg)	100.8	98.3	98.3	100.8
Thyroid gland weight	0.06701000	10.16127700+	2.69267000	2.81834900		6.907 (gm)	6.827	6.967	6.036	7.758
Thyroid follicle cell heights	24.3808***	28.5036***	6.5608*	0.9768		Significant interaction: see Text - figure 6 for unweighted sub-subclass means				
Total testes weight	205.1000	18.9342	8.2000	5423.3160		354.6 (gm)	359.0	351.3	356.3	354.0
Seminiferous tubule diameters	132.1200	1109.8284+	165.2400	331.5920		188 (μ)	185	191	197	179
Leptotene cells in Stage 2 tubules	0.7740	779.1621**	120.3620	68.6750		42.8 cells tubule	42.5	43.0	50.3	35.2
Total epididymides weight	20.7200	99.0535	18.5340	76.4104		43.9 (gm)	42.5	45.0	41.1	46.4
Seminal vesicle weight	5.573200	0.000457	0.751630	6.274676		7.655 (gm)	6.927	8.202	7.558	7.570
Total adrenal gland weight	0.00170500	0.03363167	0.01665800	0.18037700		2.534 (gm)	2.547	2.524	2.486	2.585
Right adrenal gland weight	0.00095200	0.00078867	0.00957100	0.03839070		1.221 (gm)	1.212	1.229	1.213	1.228
Left adrenal gland weight	0.00533800	0.02440848	0.00092000	0.05360620		1.313 (gm)	1.335	1.296	1.273	1.358

+ = $P < .10$ * = $P < .05$ ** = $P < .025$ *** = $P < .005$

TABLE 4

EXPERIMENT II - SUMMARY OF COVARIANCE ANALYSES, ADJUSTED MEANS

				Mean Squares				Adjusted Subclass Means			
		Regression coefficient	± Standard error	Residual	Between "control" and "expt'l"	Between Group 1 and Group 2	Inter-action	"Control"	"Expt'l"	Group 1	Group 2
	degrees of freedom	1		9	1	1	3				
X	Y										
DAY 0 body weight	DAY 41 body weight	1.097456***	0.1562	14.893606	31.022360	8.054440	0.617430	90.4 (lbs)	93.5	92.9	90.9
DAY 13 body weight	"	1.132592***	0.1366	11.181706	33.507900	15.898240	0.747430	90.3 (lbs)	93.5	93.3	90.5
DAY 13 body weight	Feed intake from DAY 13 to DAY 41	0.702770+	0.3631	79.054244	114.581800	93.500600	73.803200	94.7 (lbs)	100.6	101.1	94.2
DAY 41 body weight	Wet anterior pituitary gland weight	0.005385+	0.0028	0.006961	0.008409	0.001720	0.003880	0.461 (gm)	0.408	0.448	0.421
"	Dry anterior pituitary gland weight	1.301244+	0.7110	439.515240	280.953800	277.141000	280.346800	104.8 (gm)	95.3	105.4	94.7
"	Total adrenal gland weight	0.031019**	0.0111	0.107476	0.109306	0.110169	0.062526	2.642 (gm)	2.453	2.654	2.442
"	Right adrenal gland weight	0.014214**	0.0052	0.023141	0.010883	0.039779	0.023384	1.256 (gm)	1.196	1.290	1.162
"	Left adrenal gland weight	0.016836**	0.0061	0.032182	0.051738	0.017471	0.009285	1.387 (gm)	1.257	1.364	1.280
"	Total epididymides weight	0.083584**	0.0281	42.802657	32.924450	106.387760	16.520890	42.2 (gm)	45.3	40.9	46.5

+ = P < .10 ** = P < .025

* = P < .05 *** = P < .005

I. BODY WEIGHTS

A. DAY 0 weights

There was no significant difference between the mean body weights of the "control" and "experimental" rams at DAY 0. There was, however, a significant ($P < .05$) difference between weights of Group 1 and Group 2 animals. Rams of Group 2 were heavier than those of Group 1 at DAY 0.

B. DAY 13 weights

As might be expected, the significances of mean weight differences at DAY 13 followed those of the DAY 0 analysis. There was no significant difference between "control" and "experimental" mean weights, but there was a significant ($P < .025$) difference between Group 1 and Group 2 measurements. Again, Group 2 animals were heaviest.

C. Weight gains from DAY 13 to DAY 41

The difference between mean weight gains of "control" and "experimental" rams was of doubtful significance ($P < .10$). "Experimental" rams gained more weight than "control" animals. There was no significant difference between Group 1 and Group 2 mean weight gains.

D. Feed intakes from DAY 13 to DAY 41

The differences between mean feed intake values of "control" and "experimental" rams and between the values of Group 1 and Group 2 animals were non-significant.

E. DAY 41 weights

At DAY 41 the difference between mean body weights of "control" and "experimental" rams was non-significant, and the Group 1 - Group 2 difference was of doubtful significance ($P < .10$). Again, Group 2 rams were heavier than Group 1 animals.

F. Covariance analyses

As would be expected, there was a very significant ($P < .005$) positive regression of DAY 41 body weights on DAY 0 and DAY 13 body weights. Following adjustment, there were no significant differences between DAY 41 mean body weights of "control" and "experimental" or between Group 1 and Group 2 rams. The regressions of body weight gains from DAY 13 to DAY 41 on DAY 0 and DAY 13 body weights were non-significant.

The positive regression of feed intakes on body weights at DAY 13 was of doubtful significance ($P < .10$), while the regressions of feed intakes on body weights at DAY 0 and DAY 41 were non-significant, as was the regression of body weight gains from DAY 13 to DAY 41 on feed intakes.

II. PINEAL GLANDS

A. Weights

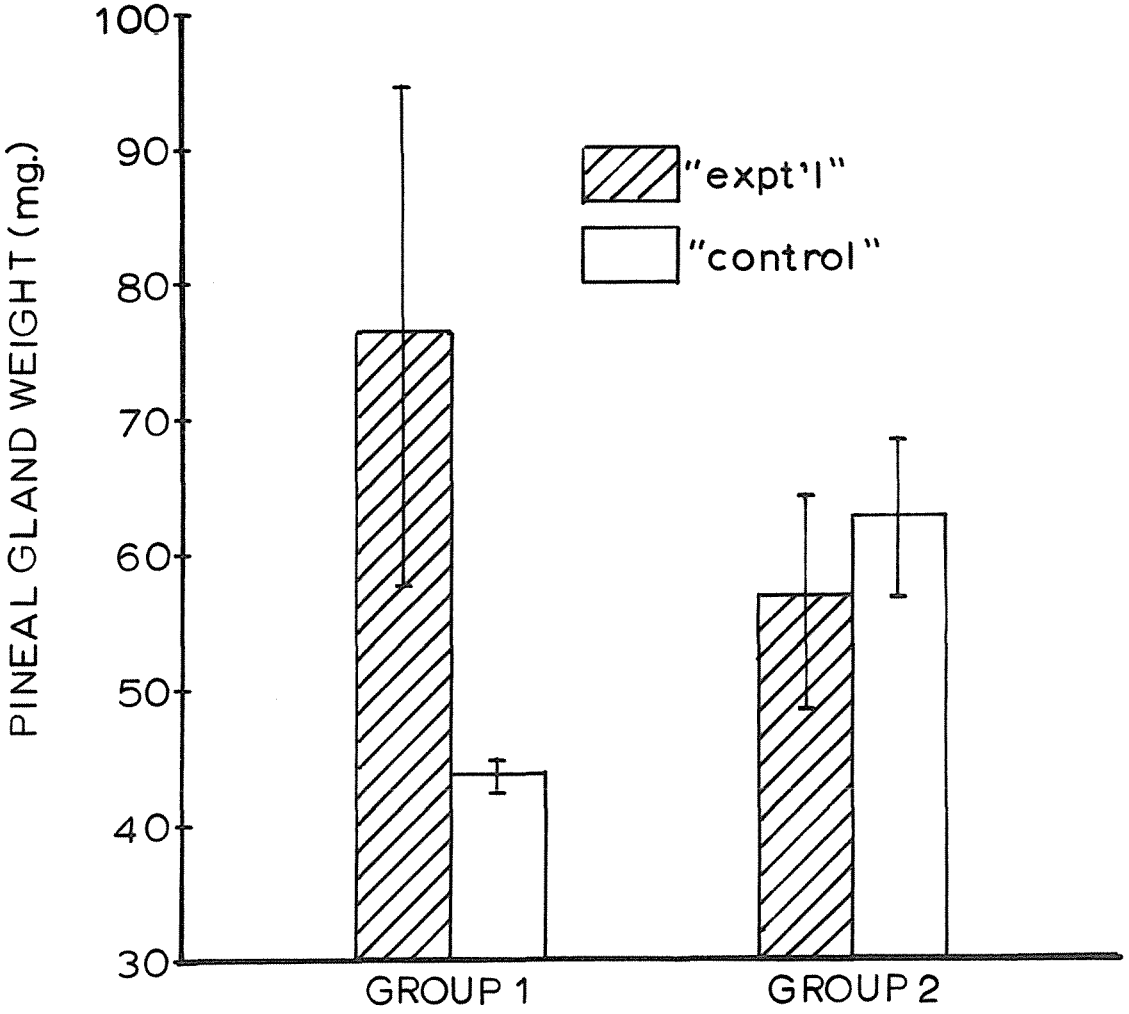
The difference between the mean pineal gland weights of "control" and "experimental" rams was non-significant, as was the difference between Group 1 and Group 2 values.

Text-figure 4 illustrates the unweighted sub-subclass mean pineal gland weight values and their standard errors. A relatively lower unweighted mean weight value for "control" Group 1 sub-subclass pineal glands will be noted in this illustration.

The regression of pineal gland weights on DAY 41 body weights was non-significant.

B. Histology

The difference between mean values of nuclei density in glands of "control" and "experimental" rams was non-significant; however, there was a difference of doubtful significance ($P < .10$) between values of Group 1 and Group 2 animals. The density of nuclei was lower in Group 2 pineal glands.



Text - figure 4

Experiment II: Unweighted mean pineal gland weights ($\pm$ standard errors) of "experimental" and "control" rams of Group 1 and Group 2.

A relatively lower nuclei density value was noted in glands from "control" rams of Group 2. An illustration of the sub-subclass unweighted mean nuclei density values and their standard errors is presented in Text - figure 5.

The regression of nuclei densities on pineal gland weights was non-significant.

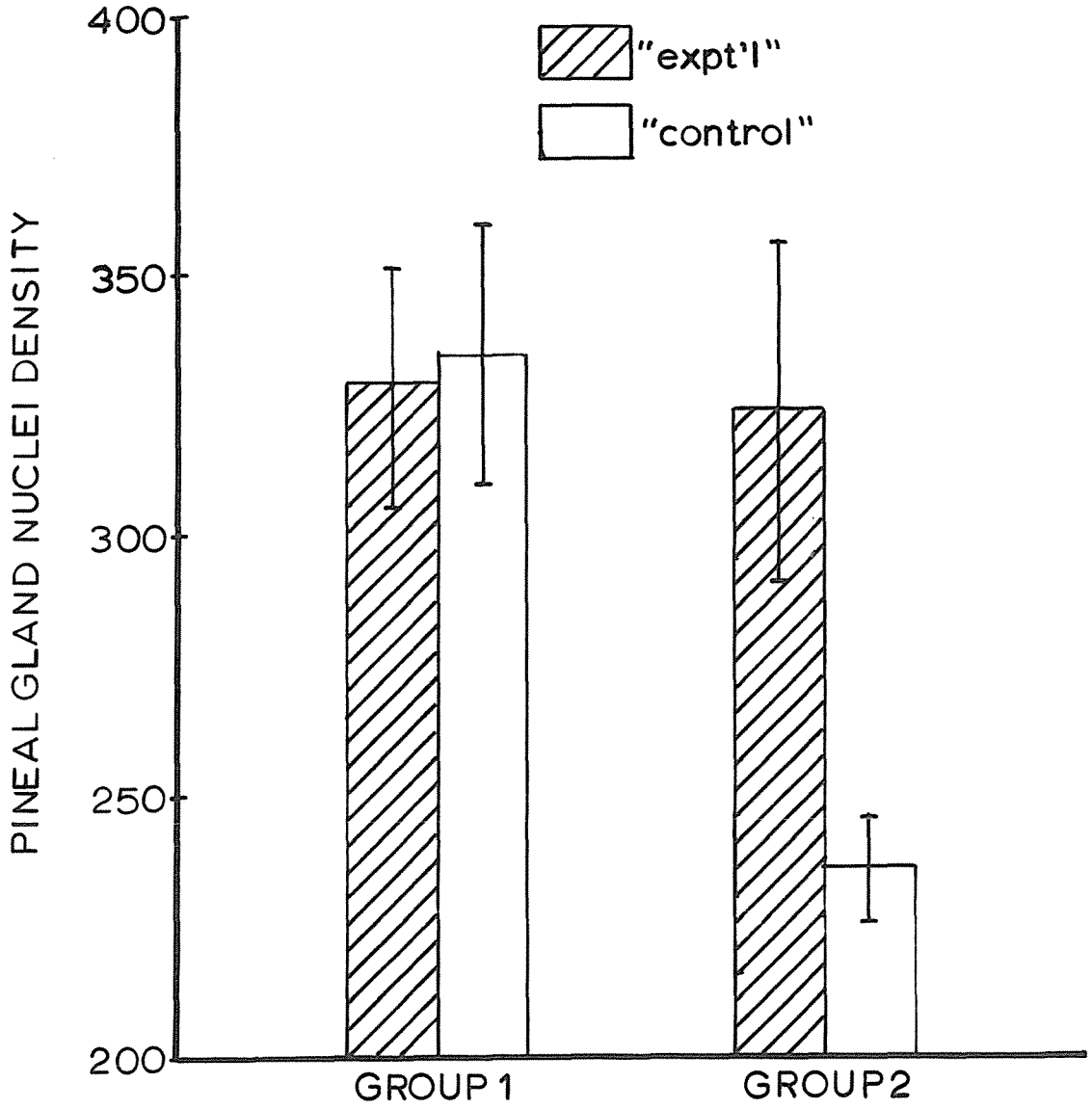
Pineal histology was similar to that described by Jordan (1911, 1921) for sheep pineal tissue. No consistent differences were noted between the nuclear or cytoplasmic staining characteristics of the pineal parenchyma cells in glands from animals of the four subclass or four sub-subclass groups. There was some indication that the nuclei in glands of Group 1 and "experimental" Group 2 rams were slightly smaller and more irregular in shape; and more "pyknotic-like", or possibly neuroglial, nuclei were noted in glands of these groups of animals in comparison with the histology of "control" Group 2 ram pineal glands. PLATE 1 illustrates these differences, as well as those quantitative differences noted in nuclei densities.

There were no stain concentration differences noted between subclass or sub-subclass groups when pineal gland sections were examined for RNA with pyronin Y.

III. HYPOTHALAMI

No consistent differences were noted between the morphologic and staining characteristics of the supraoptic nuclei in animals of the four subclass or four sub-subclass groups.

Differences were evident between the paraventricular nuclei (PVN) of the sub-subclass groups of animals (see PLATE 2). In hypothalami of "control" and "experimental" animals of the Group 1 photoperiod treatment, most of the PVN cells showed an hypertrophied cytoplasm containing a great amount of granular, Gomori-positive "neurosecretory material" (NSM). The nuclei of these



Text - figure 5

Experiment II: Unweighted mean pineal gland nuclei density values ($\pm$ standard errors) of "experimental" and "control" rams of Group 1 and Group 2.

Density values are nuclei per standard field at 250X.

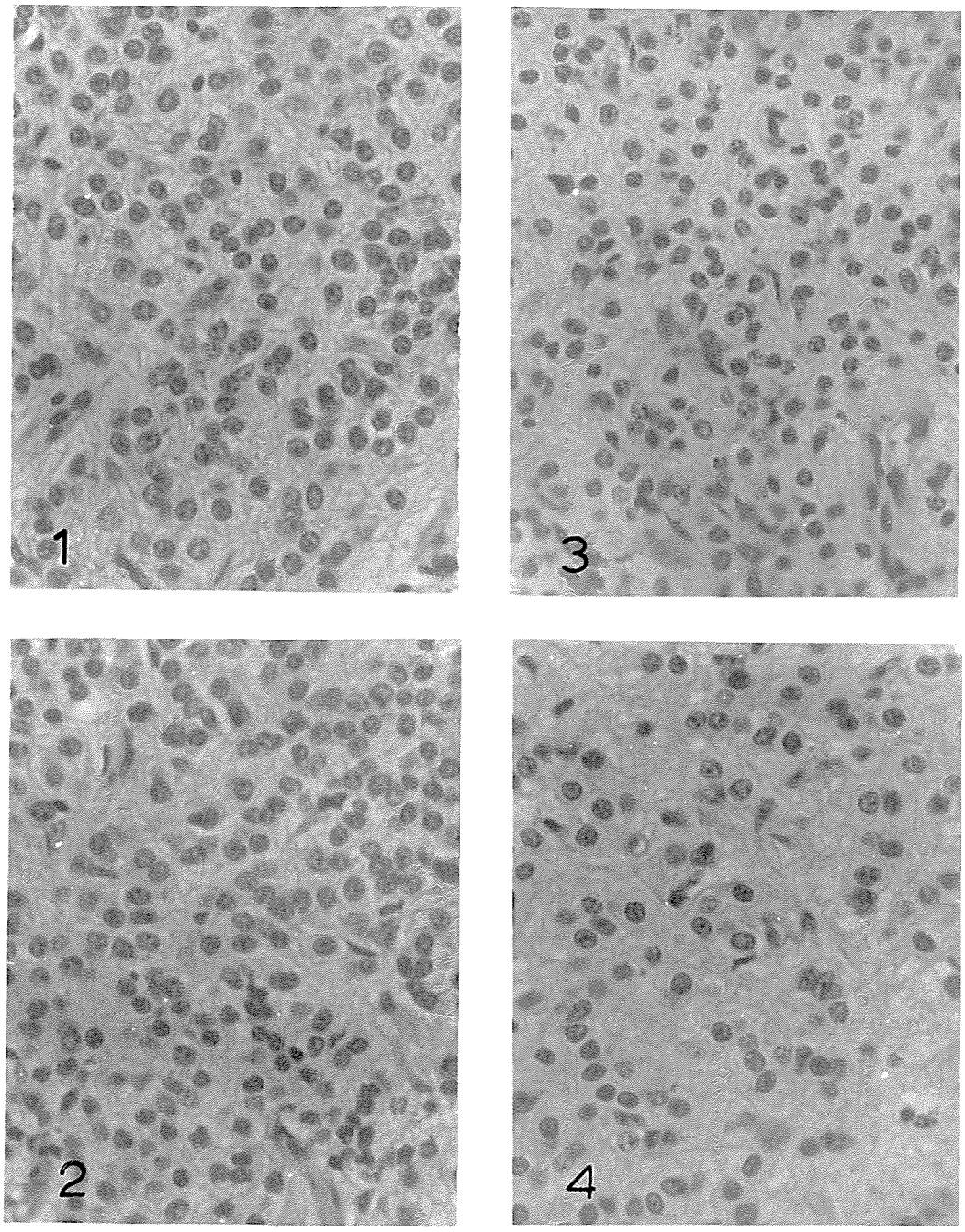


Plate 1

Experiment II: Sample pineal gland histology of rams of the Group 1 "experimental" (1) and "control" (2) and the Group 2 "experimental" (3) and "control" (4) subgroups (approx. 380X).

cells were rounded, and distinct nucleoli generally occupied a central position. In the PVN of "experimental" Group 2 rams there was a smaller number of cells showing an hypertrophied, granular cytoplasm. Nuclei appeared slightly smaller and less uniform in shape, with nucleoli being centrally located. The histology of the PVN cells of the "control" Group 2 animals showed the most striking deviation from the histology of the other three sub-subclass groups. In two of the three animals in this group, very few of the PVN cells showed any NSM in their cytoplasm; nuclei were very irregular in shape (some appeared "atrophic"), and more nuclei had nucleoli occupying a peripheral, rather than a central position. The one animal of this group not showing such histology had acquired a nasal inflammation and blockage 12 days prior to sacrifice, which lead to a laboured breathing.

The PVN of all Group 2 rams contained some cells of a distorted nature, with the appearance of polymorphic nuclei and enlarged, heavily-stained nucleoli, surrounded by heavily-stained, but less granular "atrophic-like" cytoplasms. These "distorted cells" were more prevalent in "control" animals of this photoperiod treatment group (see PLATE 2).

IV. ANTERIOR PITUITARY GLANDS

A. Weights - wet and dry

The differences between mean weights of both wet and dry anterior pituitary glands of "control" and "experimental" rams, and Group 1 and Group 2 animals were non-significant.

The positive regressions of both wet and dry gland weights on DAY 41 body weights were of doubtful significance ($P < .10$).

B. Relative hormone concentrations

As no LH and TSH standard preparations were available for absolute concentration and total pituitary potency values to be estimated from the

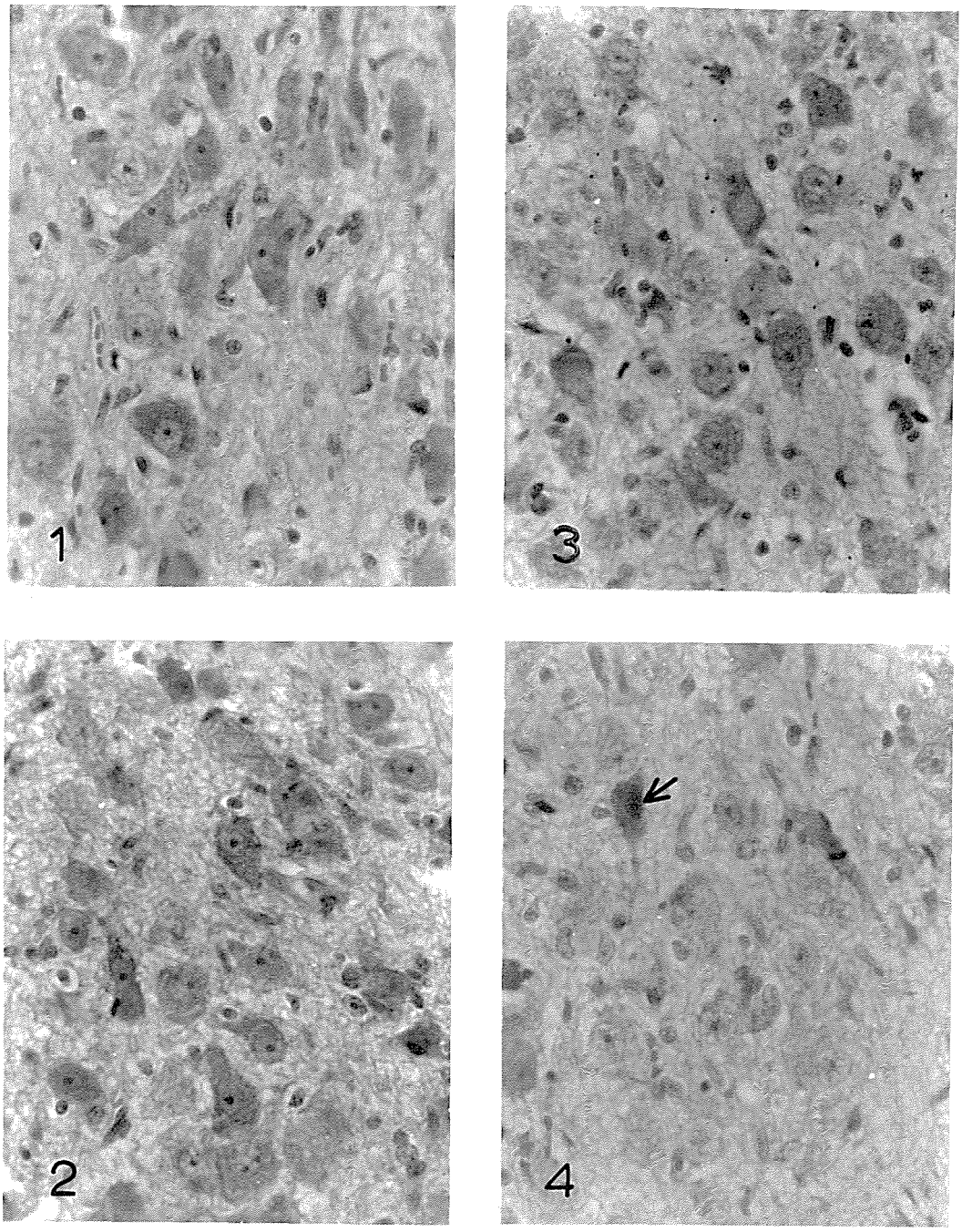


Plate 2

Experiment II: Sample paraventricular nucleus histology of rams of the Group 1 "experimental" (1) and "control" (2) and the Group 2 "experimental" (3) and "control" (4) subgroups. Arrow indicates "distorted cell" (approx. 380X).

bioassays, only relative values of the assay responses have been compared between the four sub-subclass groups.

1. Luteinizing hormone (LH)

Regressions of the ventral prostate gland weights on the log-doses of the pituitary gland material given. the assay rats were not significantly different between sheep within sub-subclasses or between sub-subclasses of sheep within the total. There was no statistical reason, therefore, to consider that the dose-response regression lines were not parallel.

Subsequent analyses of variance showed that prostate gland weights in rats given higher doses of sheep pituitary material were significantly ($P < .005$) greater than gland weights in lower dosed rats. There was no evidence of pituitary LH concentration differences between sheep of the four sub-subclass groups or between sheep within the sub-subclass groups. A summary of the analysis is presented in APPENDIX III.

2. Thyroid stimulating hormone (TSH)

Following the covariance analysis, which adjusted final for initial radioactivity counts in both dose level groups of assay chicks, between the four sub-subclass groups of pooled sheep pituitary tissue no significant final count differences were noted. No further analyses were done. Due to a lack of greater responses to greater doses of pituitary material, this bioassay cannot be considered valid.

There was some indication that the depletion of radioactivity was greater in those chicks injected with "control" Group 2 pooled pituitary tissue, but the variability between responses of individual chicks was extremely large; therefore, no definite statement can be made. An increased radioactivity depletion would, in a valid assay, indicate a greater TSH concentration in the assayed pituitary tissue. A summary of the analysis is presented in APPENDIX IV.

V. THYROID GLANDS

A. Weights

The difference between mean thyroid gland weights of the "control" and "experimental" rams was non-significant, whereas there was a difference of doubtful significance ($P < .10$) between Group 1 and Group 2 gland weights. Thyroid glands of Group 2 animals were heavier than those of Group 1 rams.

The regression of thyroid gland weights on DAY 41 body weights was non-significant.

B. Histology

The analysis of thyroid follicle cell height measurements showed there to be a significant ($P < .05$) interaction effect between injection and photoperiod treatments. Unweighted sub-subclass mean cell heights and their standard errors are presented in Text - figure 6. Cell heights were greatest in thyroid glands of "control" Group 2 rams, and least in glands of "experimental" Group 1 animals. Glands with the greater cell heights had greater depletions of intrafollicular colloid (see PLATE 3).

The regression of cell heights on thyroid gland weights was non-significant.

VI. TESTES

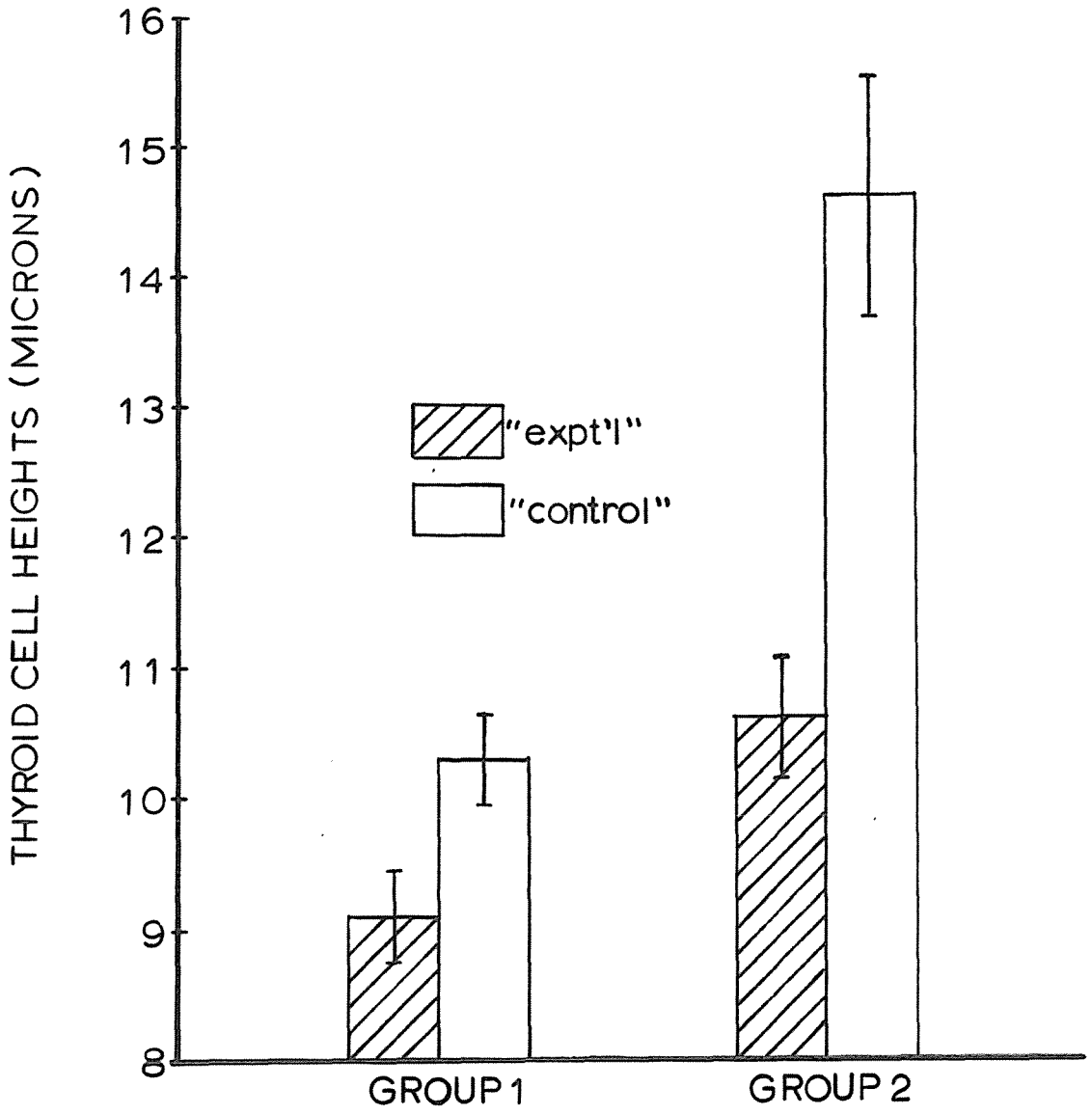
All semen samples contained spermatozoa on DAY 0.

A. Weights

The differences between mean total testes weights of "control" and "experimental" rams and between Group 1 and Group 2 animals were non-significant, as was the regression of total testes weights on DAY 41 body weights.

B. Histology

The difference between mean seminiferous tubule diameters of "control" and "experimental" animals was non-significant, whereas the Group 1 - Group 2



Text - figure 6

Experiment II: Unweighted mean thyroid follicle cell heights ($\pm$ standard errors) of "experimental" and "control" rams of Group 1 and Group 2.

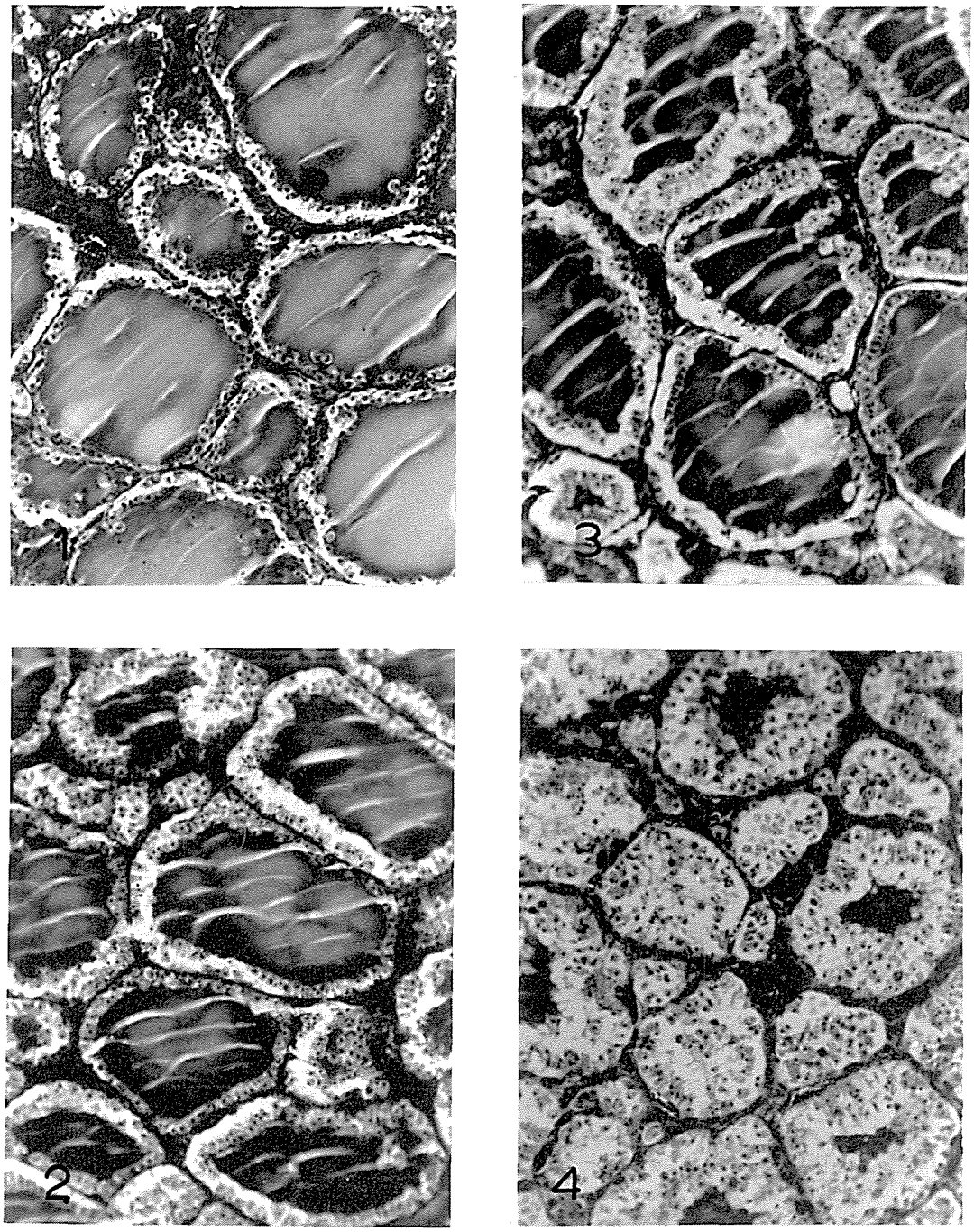


Plate 3

Experiment II: Sample thyroid gland histology of rams of the Group 1 "experimental" (1) and "control" (2) and the Group 2 "experimental" (3) and "control" (4) subgroups (approx. 120X).

difference was of doubtful significance ($P < .10$). The tubules in testes of Group 1 rams were of greater diameter.

The regression of tubule diameters on total testes weights was non-significant.

The difference between the mean number of leptotene primary spermatocytes in Stage 2 seminiferous tubules in testes of "control" and "experimental" rams was non-significant; however, the difference between Group 1 and Group 2 mean measurements was significant ($P < .025$). There were more leptotene cells in tubules of Group 1 rams. Contrary to what might have been expected, the regression of leptotene cell numbers on seminiferous tubule diameters was non-significant, as was the regression of cell numbers on total testes weights.

No striking histological differences were noted between the interstitial cells in the testes of rams of the four subclass or the four sub-subclass groups.

VII. EPIDIDYMIDES

No significant differences were noted between the mean total epididymides weights (the combined weights of the left and the right epididymis) of the four subclass groups.

The positive regression of total epididymides weights on total testes weights was significant ($P < .025$).

VIII. SEMINAL VESICLES

There were no significant differences between the mean seminal vesicle weights of the four subclass groups. The regression of seminal vesicle weights on total testes weights was non-significant.

IX. ADRENAL GLANDS

When analyses of variance were done on the right, left, and a total of the

right and left adrenal gland weights, no significant differences were noted between the mean weights of the four subclass groups.

The positive regressions of right, left and total gland weights on DAY 41 body weights were significant ($P < .025$).

Section 4 - DISCUSSION

Prior to evaluating the results of this experiment, it should be emphasized that there were relatively few animals in each of the treatment groups and that the length of the experiment was comparatively short. The photoperiod treatments were extreme and "unnatural", but were necessarily so due to the preliminary, exploratory nature of the study. When choosing the injection treatments two major assumptions were made: that melatonin is the major secretory product of the ovine pineal gland, and that, assuming this gland to be functional in sheep, melatonin is the secretory substance with some physiological activity. Melatonin injections would then be expected to create a state of "hyperpinealism" in the treated rams. Although melatonin has been isolated from bovine pineal glands (Barchas and Lerner, 1964), to this author's knowledge no such isolation from ovine glands has been reported.

The use of gravimetric and histologic measurements for interpreting the physiological states of the studied endocrine glands was limiting. It was hoped that the bioassays of some of the pituitary gland hormones would contribute supplementary information which would increase the value of an interpretative analysis centered on weights and histology only.

As the statistical analyses were based on a small number of animals, evident "trends" in differences between treatment groups may not have always been "statistically significant" ($P < .05$). In the OBSERVATIONS and DISCUSSION sections of this presentation, so-called "doubtfully significant" ($.05 < P < .10$) differences have been mentioned and discussed when it was felt that their "trends" were contributory to the interpretative analyses.

Pineal glands - weights and histology

Although no significant differences were noted between mean weights or nuclei density values of the pineal glands from rams of the four subclass groups, discussion of the unweighted mean weights in combination with the unweighted

mean nuclei density values of pineal glands of the four sub-subclass groups of animals (see Text - fig. 4 and 5) is helpful in interpreting the metabolic activities of the glands.

The unweighted mean weight of the pineal glands in rams of the diluent-injected ("control"), "short" photoperiod (Group 1) sub-subclass group was lower than the unweighted mean gland weights in animals of the other three sub-subclass groups. Applying the reasoning of investigators of laboratory rodents that pineal glands of lesser weights are less active (Wurtman et al., (1964b), rams of the diluent-injected, "short" photoperiod treatment group had pineal glands of a lesser activity than did rams of the other three sub-subclass groups. In Experiment I the pineal glands of animals in the shorter photoperiod treatment were also of a lesser weight (non-significant) than glands of the longer photoperiod treatment rams.

The unweighted mean nuclei density values of glands of animals of the diluent-injected, "long" photoperiod (Group 2) sub-subclass group were lower than the unweighted mean values in rams of the other sub-subclass groups. In the statistical analysis of the subclass nuclei density values, values were lowest in glands of the "long" photoperiod treatment animals ($P < .10$), and there was some indication of an interaction effect ($P < .10$). The lower values in glands of animals in the diluent-injected, "long" photoperiod sub-subclass group were undoubtedly the major cause of these statistical trends. If the definition of pineal gland activity based on nuclei density values observed in laboratory rodents (that the lower density glands are of higher activity due to cytoplasmic hypertrophy [Roth et al., 1962]) is applied here, the glands of the diluent-injected, "long" photoperiod rams were the most active, while glands of the animals in the other three sub-subclass groups had similarly lesser activities.

By combining the activity estimating parameters of weight and nuclei density, the pineal glands of a single sub-subclass group of rams can be defined as being

the most active. Glands of the diluent-injected, "short" photoperiod rams had nuclei density values similar to those of glands of the melatonin injected ("experimental") animals in both the "short" and the "long" photoperiod treatments, but had lower weights. These glands must, therefore, have had a lower number of total cells. Correspondingly, glands of the diluent-injected, "long" photoperiod animals had the same weights as those of the glands of the melatonin injected rams of both the "short" and the "long" photoperiod treatment groups, but had lower nuclei density values. These glands must also have had a lower number of total cells. It appears that the melatonin injection treatments may have induced an increase in the total number of cells, and therefore, an increase in weight in the pineal glands of animals in both the "short" and the "long" photoperiod treatments. Histologically, however, no striking differences were noted between the mitotic activities of the parenchyma cells in the pineal glands of the rams of the subclass or sub-subclass groups. Following melatonin injections into chickens, pineal gland weights have increased (Singh and Turner, 1967); and pineal extract injections have opposed the normal pineal weight decrease in rats exposed to continuous light (Moszkowska and des Gouttes, 1962; Moszkowska, 1963).

The total number of cells in the pineal glands of the diluent-injected rams of both photoperiod treatment groups was probably similar; however, this statement would be difficult to prove conclusively. If this were true, the weight differences noted between the glands of animals (diluent-injected only) in the "short" and "long" photoperiod treatments could be due primarily to the hypertrophy of the cells (due to an increased metabolic activity) in the glands of rams in the "long" photoperiod treatment group.

The nuclear histology of the pineal parenchyma cells in glands of the diluent-injected rams was not completely comparable with observations that have been made of the nuclear histology in pineal glands of rats exposed to "short"

and "long" photoperiods. Nuclei in ram glands of greater weight and lower nuclei density were slightly larger and round or oval in shape (see PLATE 1), whereas nuclei in rat glands of a similar relative gravimetric and histologic description, although also being larger in size, were polymorphic in shape with greatly indented nuclear membranes (Roth et al., 1962). Nuclear diameters were greatest in hamster pineal glands of high weight and low nuclei density (Hoffman and Reiter, 1965a).

There were no differences noted between the cytoplasmic staining characteristics of the pineal glands in rams of the subclass or sub-subclass groups. In work with rats, heavier glands with lower nuclei densities had a more basophilic granular cytoplasm (possibly indicating more RNA) than did glands of lighter weights and higher densities (Roth et al., 1962). As the staining technique for RNA used in the present experiment is known to be delicate and variable, group differences may not have been detected.

For the purpose of the discussion to follow, a definition of the relative secretory activities of the pineal glands in rams of the four sub-subclass groups must be made. Glands of animals receiving melatonin injections in both photoperiod treatment groups, due to their high nuclei density values and indicative nuclear morphology, must be considered as having low metabolic activities. It will be assumed that exogenous melatonin has inhibited the metabolic activities of the pineal glands, while increasing total cell numbers (see above) in these glands. Indications are, from other endocrine gland observations to be discussed, that if there is a physiologically active pineal gland metabolite secreted in the ram it may be some substance (or substances) other than melatonin, and injected melatonin may have suppressed the synthesis and secretion of this substance. Assuming this to be true, melatonin injections, instead of creating a condition of "hyperpinealism", appear to have induced a condition of "hypopinealism" in treated rams. In some work with rats,

injections of other pineal gland metabolites have been found to be more potent physiologically than melatonin injections (McIsaac et al., 1964). Considering now only pineal glands of diluent-injected animals in the two photoperiod groups, a definition of their relative activities can be based on interpretative definitions applied in laboratory rodent work. The glands which are heaviest with the lowest nuclei densities are, therefore, most active; these glands were in rams of the "long" photoperiod treatment group. In rams, it appears as if "long" photoperiods stimulate and "short" photoperiods inhibit pineal gland metabolic activity. In laboratory rodents, the opposite effect of "short" and "long" photoperiods on gland activity has been found (Kitay, 1967). In chickens, however, pineal gland weights and melatonin synthesis, as measured by HIOMT activity, were greatest in glands of animals held in constant light (Axelrod et al., 1964). For the remainder of the discussion, the pineal glands of the diluent-injected, "long" photoperiod animals will be considered the most active, while glands from melatonin-injected, "long" photoperiod, diluent-injected, "short" photoperiod, and melatonin-injected, "short" photoperiod rams will be considered, in order, progressively less active.

Pineal glands - hypothalami

The rams with pineal glands of greatest activity had very little "neurosecretory material" (NSM) in the paraventricular nuclei (PVN) of their hypothalami (see PLATE 2). The animals with pineal glands whose activities were inhibited and/or suppressed had hypertrophied PVN cells with large amounts of NSM. In rats, pineal gland extract injections have induced an hypertrophy and increased NSM content in the PVN (Aron et al., 1961; Miline, 1963).

Although it is probable that both oxytocin and vasopressin are synthesized and secreted from the PVN, many workers believe the major product of PVN activity to be oxytocin (Olivecrona, 1957; Nibbelink, 1961; Cross, 1966). The material of the PVN cells, stained by the Gomori method (1941) used in the

present experiment, may either represent the synthesized hormone itself or its carrier substance necessary for hormone transport to the posterior pituitary gland; which ever it may be, the degree to which it is stained gives some indication of relative neurosecretory activities (Bern and Knowles, 1966). The increase of NSM in the PVN may represent either an increased synthesis and secretion or only a decreased secretion of oxytocin. For the purpose of this discussion, the increased NSM represents an increased synthesis and release of oxytocin from the PVN. It therefore appears that concurrent with an increase in ram pineal gland activity, there is a decrease in the synthesis and secretion of oxytocin from the PVN; and vice versa, an inhibition of pineal activity increases PVN synthesis and secretion of oxytocin. Rats reared under photoperiod conditions which inhibited their pineal gland activities had increased oxytocin contents in their hypothalami at sacrifice (Fendler, 1967). In contrast to these results, injections of pineal gland extracts into dogs increased the oxytocic activities of their PVN (Milcou and Pavel, 1960).

The one ram in the group of animals with the greater pineal gland activities that did have some NSM in its PVN had been "stressed" by a nasal infection prior to sacrifice. An increased adreno-corticotrophic hormone (ACTH) secretion from the pituitary gland and an increased secretion of adrenal-corticord hormones from the adrenal glands might be expected under such circumstances; however, there was no gross indication from the weights and histology of the adrenal glands that this did occur. ACTH and cortisone have been known to activate the cells of the PVN in adrenalectomized rats (Voitkevich et al., 1966).

Pineal glands - hypothalami - endocrine gland activities

Facts and opinions are extremely varied (see D'Angelo, 1964 and Davidson, 1966), but some workers believe oxytocin to have characteristics of gonadotrophin and thyrotrophin hypothalamic releasing factors. Injected oxytocin

has depleted anterior pituitary gland stores of luteinizing hormone (LH) (Fraschini et al., 1963) and increased the gonadotrophin content of the urine (Martini et al., 1958); furthermore, oxytocin has increased the weights of the testes in rats (Shibusawa et al., 1955) and rabbits (Armstrong and Hansel, 1958).

Following the exposure of female rats to photoperiod conditions stimulatory to pineal gland activity (Fiske, 1941) or following melatonin injection treatments (Adams et al., 1965), there was a decrease in pituitary gland weights and an increase in pituitary LH stores. A reduction in reproductive organ activity occurred as a result of these treatments.

Although there were pineal gland activity and PVN oxytocin content differences between sub-subclass groups of rams in the present experiment, no pituitary gland weight or LH concentration differences were noted. The sex of the animals may be a reason for a lack of response, as male rats have shown less pituitary gland and reproductive organ responses than female rats to the altered status of pineal gland secretion (Fiske, 1941; Motta et al., 1967). No significant weight differences were observed between testes or seminal vesicles of rams in the four subclass groups; however, seminiferous tubule diameters and leptotene cell numbers in the tubules were greater in the subclass group of animals with the lesser pineal gland activity and the greater oxytocin synthesis and secretion. It appears that perhaps an increased synthesis and secretion of oxytocin, made possible by a reduction of inhibition due to a lowered pineal gland secretory activity, may have stimulated an increased production and release of gonadotrophins from the anterior pituitary gland (although this was probably not LH, it may have been follicle stimulating hormone [FSH]), which, in turn, stimulated an increased testicular activity and spermatogenic "efficiency". An alternative interpretation would be that the increased circulatory oxytocin may have had a direct stimulatory effect on the

testes of the rams (Fitzpatrick, 1966).

The PVN has been localized by some workers, using lesion and electro-stimulation techniques, as an area responsible for the synthesis and secretion of a "thyrotrophin releasing factor" (Greer and Erwin, 1954; de Jong and Moll, 1965; Van Rees and Moll, 1967) or as an area which "filters" thyroid hormones working to regulate the hypophyseal "feed-back" mechanism controlling thyro-trophin release from the pituitary gland (Purves, 1960). Oxytocin injections have elevated the thyroid stimulating hormone (TSH) level in the blood (Fraja and Martini, 1953).

Melatonin injections have elevated plasma TSH levels and lowered pituitary TSH levels in young rats. Thyroid weights and DNA contents were greater in these animals (Panda and Turner, 1968). The responses of the pituitary and thyroid glands to the melatonin injections were similar to responses noted following goitrogen injections. The authors postulated that melatonin acted as a goitrogen by inhibiting the synthesis of thyroid hormones, which thereby reduced the inhibition of TSH release from the pituitary gland via the hypothalamo-hypophyseal-thyroid "feed-back" mechanism.

The bioassay for TSH in the pituitary glands of the rams was of question-able validity, but did give some indication that there was a higher TSH concentration in glands of animals in the sub-subclass group which also had the greater pineal gland activity and the lesser amount of oxytocin in the PVN. The thyroid follicle cell heights were greatest in this group of rams (see Text - fig. 6).

If the hypothesis is correct that the physiologically active secretion of the ram pineal gland is some substance other than melatonin, and that in the present experiment the injection of melatonin has actually inhibited the pro-duction and release of this substance, an interpretation may be given to the above results using the aforementioned experimental findings. The greater

amount of pineal gland secretory substance in the circulation of the diluent-injected, "long" photoperiod rams may have increased an inhibition of thyroid hormone synthesis in the thyroid gland, as does melatonin in rats. This would then lower the level of circulating thyroid hormones, which could have caused an "exhaustion" of the PVN cells in their effort to produce a greater amount of "TSH-releasing factor" (perhaps oxytocin) which now would be in greater demand. Indeed, there were "atrophic-like, exhausted" cells, with little NSM in the PVN of this group of animals. In rats, increased thyroid hormone levels in the blood have been associated with increased NSM in the cells of the PVN (Akada, 1959). The indication that this group of rams may have had a greater pituitary TSH content might be expected if "TSH-releasing factor" secretion did increase (although, by Panda and Turner [1968] such an increase would be interpreted as a decreased "TSH-releasing factor" secretion and a decreased TSH secretion). Assuming, then, an increase in circulating TSH, the observed greater cell heights (and the tendency towards greater weights) in the thyroid glands of this group of rams would be expected. The thyroid cell heights became progressively lower as the pineal gland activities of the sub-subclass groups of animals became progressively lower; these changes were accompanied by an increase in PVN-NSM.

The lowered thyroid secretion rates (TSR) noted in rats following melatonin injections (Ishibashi et al., 1966; Narang et al., 1967) matches well the recent finding that melatonin has a goitrogenic action on the thyroid gland (Panda and Turner, 1968). Similarly, the finding that melatonin induced an histological hyperactive appearance in the thyroid glands of young rats (Thieblot et al., 1966b) is in agreement with the normal histological response of the gland to goitrogenic materials.

The interpretations given the histology of the thyroid glands of the diluent injected rams of this experiment are in disagreement with TSR studies of ewes exposed to the same "short" and "long" photoperiod treatments as the rams.

Hoersch et al., (1961) found these ewes to have similar TSR, while the interpretation of the histological responses in the rams is that animals in the "long" photoperiod group had lower TSR than those rams held in the "short" photoperiod.

Another possible intermediate factor between pineal gland secretory activity and thyroid gland activity may be melanocyte stimulating hormone (MSH). Melatonin injections have decreased (Kastin and Schally, 1967), while pinealectomy has increased (Kastin et al., 1967) pituitary MSH concentrations. Destruction of the PVN (and only the PVN) has caused a decrease in the pituitary MSH content of rats (Taleisnik et al., 1967). The investigators felt that melatonin, and perhaps other pineal gland secretions, oppose the action of an hypothalamic "MSH-release inhibiting factor" (MIF) and allow the release of MSH from the pituitary gland (thereby decreasing the pituitary MSH content). Pineal gland secretions may also be inhibiting the production of MIF or chemically binding MIF and rendering it physiologically ineffective. Presumably, the destruction of the PVN eliminates an area where MIF is synthesized and secreted.

MSH injections have increased the TSR in rabbits (Courrier and Cehovic, 1960), guinea pigs (Cehovic, 1962) and mice (Werner et al., 1964). MSH is not considered to be a "TSH-releasing factor" because it has no effect on TSH production in pituitary gland incubates and it has its thyroid stimulating effect even in hypophysectomized mice (Yamazaki et al., 1963).

If it is assumed that the greater follicle cell heights in the thyroid glands of rams with the greatest pineal gland secretory activities were not due to an effort just to hold a minimum level of circulatory thyroid hormones, but were the result of an overstimulation and an indication of an overproduction of thyroid hormones, a new interpretation may be formulated from the results applying the "facts" given in the two previous paragraphs. The greater secretion of pineal substances may have increased the opposition to the production

or effective activity of MIF in the PVN. The NSM (perhaps representing MIF) in the PVN of these animals was, indeed, decreased. An increased opposition to MIF would be expected to cause a lowered MSH concentration in the pituitary and an increased concentration of MSH in the blood. This increased circulatory MSH might then be expected to increase the TSR of the thyroid glands and induce the increase in follicle cell heights noted in the glands of rams in this sub-subclass group.

The possibility must be mentioned that the peripheral utilization of thyroid hormones may have been changed through some other physiological system in response to the treatments, and that this change may have, in turn, stimulated the gravimetric and histologic changes noted in the thyroid glands; however, as no striking and consistent body weight and feed intake changes (possible gross indicators of thyroid hormone utilization changes) occurred between the sub-class or sub-subclass groups of rams, this possibility must be considered a less likely interpretation for the results of this experiment.

If the theory that the increased pineal gland secretory substance in the rams of the "long" photoperiod subclass group has acted as a goitrogen is correct, an explanation of the simultaneous decrease in seminiferous tubule diameters and leptotene cell numbers may be attempted. Hypothyroid rams have been known to have a slower reproductive development and a lesser spermatogenic "efficiency" than euthyroid and slightly hyperthyroid animals (Maqsood, 1950, 1951). If the rams of the "long" photoperiod group are considered to be in a hypothyroid condition (due to the goitrogenic action of the pineal substances inhibiting thyroid hormone synthesis and secretion), the smaller seminiferous tubules and lower number of leptotene cells in this group of animals may be a response to this condition. Thyroid hormones are presumed to have some direct stimulatory or permissive effect on testicular tissue.

Summary and conclusions

In summary, no definite or conclusive statements can be made concerning the secretory and functional activity of the pineal gland in the ram. As the definitions of pineal gland secretory activity have been based solely on gravimetric and histologic measurements, the interpretations made from these measurements must be considered of questionable value. These interpretations have, however, provided the basis for a correlative discussion relating possible pineal gland activity changes with simultaneous neuroendocrine and endocrine changes. If, indeed, the pineal gland of the ram is variably responsive to different photoperiods, it appears from this experiment that a "long" photoperiod is stimulatory, while a "short" photoperiod is inhibitory to its secretory activity. If a substance (or substances) is then produced and released from the gland in response to a stimulatory photoperiod, its major physiological role in the regulation of other neuroendocrine and endocrine systems may be as an affector of thyroid gland activity, either directly or indirectly via the paraventricular nucleus of the hypothalamus and the anterior pituitary gland. Depending upon whether the pineal substance acts directly or indirectly on the thyroid gland, this substance may be inhibitory or stimulatory, respectively, to thyroid hormone synthesis. Testicular responses to photoperiod treatments may be regulated by pineal gland secretory activity via the hypothalamo-hypophyseal system, but it is, perhaps, more likely that the simultaneous thyroid gland activity and presumed thyroid hormone secretion changes have a more direct effect on testicular responses.

There was some indication that melatonin may not be the physiologically active pineal gland secretory substance in the ram, if, indeed, the pineal gland and its possible secretions are at all functional in the ram.

GENERAL SUMMARY

Two experiments were designed to study the effects of "short" and "long" photoperiods on some reproductive organs and endocrine glands of rams held in constant temperature environments. There were, however, differences between the designs of the experiments, as well as differences in the materials and methods employed to study the effects of the experimental treatments. In Experiment I the "short" photoperiod treatment was a constant 10 hours per day, while in Experiment II this treatment was a constant 4 hours per day. The "long" photoperiod treatment in Experiment I fluctuated with the natural seasonal light cycle from about 13 hours, 30 minutes at the beginning of the experiment to a peak of about 16 hours, 10 minutes, followed by a decrease to about 14 hours, 5 minutes at the end of the treatment period, while the "long" treatment of Experiment II was a constant 20 hours per day. At the beginning of the experiments rams were 28 days (Experiment I) and 166 to 185 days (Experiment II) of age; photoperiod treatments were imposed for 140 days (Experiment I) and 42 days (Experiment II), and the feeds given to the rams of the two experiments were different (low quality hay - Experiment I, high quality pellets - Experiment II). All rams were exposed to a constant environmental temperature of 65°F.

In spite of the dissimilarities in treatments, there were some consistent similarities in response to the "short" and "long" photoperiods of both experiments. Differences in thyroid follicle cell heights and thyroid gland weights were the most consistent similar responses noted between "short" and "long" photoperiod-treated animals of both experiments. Cell heights and gland weights were greater in rams exposed to "long" photoperiods. The possible physiological interpretations of these observations and their relationship to other noted differences in the treated animals have been discussed. Pineal gland weights were also greater (non-significant) in rams held in the "long"

photoperiod treatments; in Experiment II this observation was considered, along with information collected on pineal parenchyma cell nuclei density and the effects of melatonin injections on pineal histology, in an effort to define possible secretory activity in the glands. A hypothesis that "long" photoperiods are stimulatory to pineal gland activity in rams was outlined.

Some dissimilar and contradictory results were noted between the "short" and "long" photoperiod-treated rams of the two experiments. Greater body weight gains were made by "long" photoperiod-treated animals in Experiment I, while no difference in gains was noted between rams of the two photoperiod treatments of Experiment II. Anterior pituitary gland weights were greater, and histology was most "active" in appearance in glands of "long" photoperiod-treated rams of Experiment I, while no weight or luteinizing hormone (LH) concentration differences were noted between treated animals of Experiment II. The relatively short exposure of the rams to the "short" and "long" photoperiod treatments of Experiment II may account for the lack of any perceptible differences in the above mentioned measurements. Histologically, the testes of rams exposed to the "long" photoperiod treatment of Experiment I appeared to mature more rapidly, while a lesser spermatogenic "efficiency" was noted in testes of animals held in the "long" photoperiod treatment of Experiment II.

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APPENDICES

APPENDIX I

DATA FROM RAMS OF GROUP 3 AND GROUP 4 - EXPERIMENT I

GROUP 3	Body weight at 28 days (lbs)	Weight gain (lbs)	Body weight at sacrifice (lbs)	Total testes weight (gms)	Thyroid gland weight (gms)	Anterior pituitary gland weight (gms)	Pineal gland weight (gms)	Right adrenal gland weight (gms)	Left adrenal gland weight (gms)	Seminal vesicle weight (gms)	Total epididymides weight (gms)
63 day-old SAMPLE AGE GROUP	27.7	18.3	46.0	31.8	2.800	0.381	0.0288	0.850	0.921	1.115	8.7
	21.0	13.0	34.0	18.7	1.596	0.329	0.0117	0.757	0.803	1.056	7.3
	REMAINING TWO RAMS MADE VERY POOR BODY WEIGHT GAINS, NOT INCLUDED										
$\bar{X}$	24.4	15.7	40.0	25.3	2.198	0.355	0.0203	0.804	0.862	1.086	8.0
91 day-old SAMPLE AGE GROUP	24.3	31.2	55.5	70.0	7.489	0.253	0.0409	0.889	0.883	3.015	12.4
	31.2	32.3	63.5	107.7	2.996	0.334	0.0453	0.815	0.919	2.180	16.8
	30.6	35.9	66.5	70.0	5.612	0.386	0.0326	1.172	1.315	2.000	17.7
	33.6	27.4	61.0	68.2	3.131	0.325	0.0304	0.902	0.954	1.215	12.3
$\bar{X}$	29.9	31.7	61.6	79.0	4.807	0.325	0.373	0.945	1.018	2.103	14.8
GROUP 4	27.3	14.7	42.0	21.7	1.557	0.246	0.0349	0.715	0.739	0.675	5.5
63 day-old SAMPLE AGE GROUP	27.7	18.3	46.0	30.3	2.720	0.218	0.0255	0.673	0.694	2.254	8.5
	30.7	22.3	53.0	33.2	2.156	0.318	0.0399	0.814	0.879	1.428	7.6
	36.3	21.2	57.5	43.8	1.951	0.298	0.0192	0.673	0.787	2.431	9.4
$\bar{X}$	30.5	19.1	49.6	32.3	2.096	0.270	0.0299	0.719	0.774	1.697	7.8
91 day-old SAMPLE AGE GROUP	23.2	37.8	61.0	66.5	3.261	0.247	0.0256	0.799	0.818	3.108	15.1
	31.8	25.2	57.0	82.9	2.230	0.349	0.0372	0.756	0.800	2.224	13.7
	29.9	36.1	66.0	91.7	1.980	0.338	0.0406	0.876	0.879	3.553	16.2
	22.3	22.7	45.0	35.8	2.407	0.223	0.0561	0.960	1.015	1.076	9.9
$\bar{X}$	26.8	30.5	57.3	69.2	2.470	0.289	0.0399	0.848	0.878	2.490	13.7

APPENDIX II

DATA FROM RAMS OF THE PRE-EXPERIMENTAL, 28 DAY-OLD AGE GROUP - EXPERIMENT I

	Body weight at sacrifice (lbs)	Total testes weight (gms)	Thyroid gland weight (gms)	Anterior pituitary gland weight (gms)	Pineal gland weight (gms)	Right adrenal gland weight (gms)	Left adrenal gland weight (gms)	Seminal vesicle weight (gms)
	30.6	6.6	1.148	0.205	0.0391	0.537	0.622	0.855
	32.2	7.7	1.752	0.178	0.0240	0.504	0.461	0.721
	25.5	5.0	1.651	0.190	0.0202	0.528	0.534	0.509
$\bar{X}$	29.4	6.4	1.517	0.191	0.0281	0.523	0.539	0.695

APPENDIX III

LUTEINIZING HORMONE BIOASSAY, SUMMARY OF ANALYSES OF VARIANCE - EXPERIMENT II

	d.f.	Mean Square			d.f.	Mean Square
Sub-subclass groups	3	98.59$^{n.s.}$		Doses in sheep	14	94.95***
Sheep in sub-subclass groups	10	90.21		Rats in doses in sheep	103	11.84

*** = $P < .005$ n.s. = non-significant

APPENDIX IV

THYROID STIMULATING HORMONE BIOASSAY,
SUMMARY OF ANALYSIS OF COVARIANCE ADJUSTING FINAL
FOR INITIAL RADIOACTIVITY COUNTS - EXPERIMENT II

Source of variance	d.f.	Regression coefficient	Mean Square
Total	46	0.7611	
Between dose groups over all sub-subclass groups	7	0.8193	195,491 n.s.
Within dose groups over all sub-subclass groups	39	0.7569	256,819

n.s. = non-significant