THE EFFECT OF CONSTANT LIGHT OR DARKNESS ON THE THYROID GLAND OF THE SHEEP AND ON THE ESTROUS CYCLE

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THE EFFECT OF CONSTANT LIGHT OR DARKNESS ON THE THYROID GLAND OF THE SHEEP AND ON THE ESTROUS CYCLE

By

William Anderson Terry

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INTRODUCTION

Certain areas of the science of animal breeding present an increasing number of practical problems in physiology. This is especially true when the breeder desires to change the natural mating season of a species. He finds that phases of endocrine function, which as yet are little understood, may play an important part in the seasonal rhythm of sexual activity in some species.

The function of the various endocrine glands have been described in considerable detail by many workers. However, some of the environmental factors which may enhance or inhibit the functions of the endocrines have not been nearly so well investigated. Although considerable work has been done in the areas of temperature and nutrition, the light factor has not been held constant because of failure to recognize the profound influence it might have on the animal's endocrine system. As a result, some of the evidence obtained might be questioned in view of information now available.

To illustrate this point, breeders and research workers have long theorized that summer heat is the limiting factor in sperm production in the ram. Berliner and Warbritton (1937) were among those who advanced this hypothesis. They noted the low fertility of rams in the summer and suggested a relation to low thyroid activity because of the well established effects of heat in depressing thyroid function. It now seems highly possible, in view of recent experimental evidence (Yeats 1949), that the increased length of daylight during the summer may also play a role in reducing thyroid activity, and hence partially account for the summer sterility in sheep. Photogenic influences on reproductive activity have been demonstrated by several workers (Rowan 1931, Brissonnette 1932, and others) but until recently little or no effort was made to determine the effects of light on the thyroid.

Breeders have attempted to produce two lamb crops a year by various means, chiefly through the use of gonadotropic hormones. However, only limited success has been obtained even in the most recent trials. Since this method has not proven very practical, it seemed worthwhile to investigate further the effects of light on thyroid and reproductive function in the ewe. The primary purpose of the present work, therefore, was to determine whether light or darkness influences the thyroid activity and breeding performance of sheep. It was hoped that the knowledge thus obtained might lead to more practical methods for inducing breeding activity in sheep during the spring and summer months.

REVIEW OF THE LITERATURE

Effects Of Light On Thyroid Function

While the literature abounds with references concerning the effects of light on gonadal function of birds and mammals, very little work seems to have been done on the relation of the thyroid to photogenic influences.

Most of the material available on the subject has been published in recent years, for it has long been assumed that heat and cold are the major external factors in thyroid regulation. Mills (1918) showed that high external temperature diminished the activity of the thyroid glands in rabbits as judged by both morphological and growth rate changes. Conversely, he found that a low external temperature increased the rate of growth in rabbits. The numerous experiments of other investigators of both earlier and later dates have quite firmly established the fact that the thyroid responds to thermal influences.

The following studies, made of light effects on the thyroid, have been far less uniform. They seem to indicate that in different species opposite thyroid responses may result at times when the animals are subjected to the same conditions.

Dempsey (1943) studied the histological changes of the thyroids of twelve female rats, six of whom had severed pituitary stalks. They were kept under continuous light for one month. He measured the change in cell height of the thyroids to determine the secretion of thyrotropic hormone by the anterior pituitary.

He reported that the rats under continuous light showed a reduction in thyroid cell height. He found the same histological changes in normal rats subjected to heat for an equal period. Simultaneous exposure to both light and cold resulted in constant estrus in the female rat. He assumed that these results were due to altered pituitary function caused in part by the exposure to constant light.

Dempsey (1943) further noted that all the results obtained, except the effect of cold, could be produced in the rats with severed pituitary stalks. This, of course lead to the assumption that the external stimulus of light reached the anterior lobe of the pituitary by a route other than the neural pathways through the stalk.

Puntriano and Meites (1950) investigated the effects of continuous artificial light or darkness for a twenty-eight day period on thyroid activity in 242 Rockland mice of both sexes. Four experiments were conducted with females and three with males. Thyroid activity was measured by determining (a) the weight increase of the thyroids following injections for ten days of a constant dose of thirouacil, (b) the normal weight changes of the thyroids of the mice kept under light or darkness, and (c) the uptake by the thyroid of a singly-injected tracer dose of radioactive iodine administered sixteen hours prior to sacrifice.

Continuous light induced the following changes in the thyroids of male and female mice, respectively, as compared to controls maintained under the light conditions prevailing in the animal room. There was a decrease in thyroid weight of 11.30 and 12.37 percent; a reduction in thyroid response to thiouracil of 32.89 and 31.04 percent, and a reduction of 44.00 and 44.00 percent in thyroid uptake of radioactive iodine. The above are average changes based on a 100 gram body-weight basis.

Continuous darkness induced the following average changes, which are also on a 100 gram body-weight basis. As compared to the controls there was an increase in thyroid weight of male and female mice, respectively, of 17.24 and 18.71 percent; increases in thyroid weight of 22.70 and 30.43 percent in response to thiouracil, and increases of 41.40 and 57.43 percent in thyroid uptake of radioactive iodine.

In a second group of experiments the effects of continuous light or darkness for twenty-eight days were determined on the gonadal function of 186 young female rats. Three experiments were carried out on Rockland and Carworth rats. During the last four days of the experiment each rat was injected with a constant dose of pregnant mares' serum. The results were as follows: under continuous light the Rockland rats showed average weight increases of ovaries and uterus in response to injections of pregnant mare's serum of 23.72 and 39.37 percent above the control. Continuous darkness produced no significant change in gonadal response to pregnant mares' serum.

Neither continuous light nor continuous darkness appeared to influence the response of the ovaries and uterus to pregnant mares' serum in the Carworth rats. From this they concluded that light did not induce changes in this strain of rats. They offered the hypothesis that continuous light increases the production of FSH by the pituitary and decreases the production of thyrotropic hormone.

Stein and Carpenter (1943) reported on the behavior of the green salamander when exposed to normal daylight in the fall for forty days, and again to artificial light for 150 days. Under normal fall light the salamander showed an increase in thyroid activity as compared to the control which was kept in darkness. When exposed to artificial light the thyroids showed a greater degree of stimulation. The controls kept in darkness had much less active thyroids. They found, however, that low temperature had a greater stimulating power than light. It was concluded from the result that light and temperature both effect the thyroid of the green salamander. It will be noted that the reaction of the thyroid of the salamander was just the opposite to mice (Puntriano and Meites, 1950) when exposed to light.

Kleinpeter and Mixner (1937) determined the effects of wave length (quality and quantity) of light on the thyroids of baby chicks. The chicks were kept under artificial illumination for fourteen-day periods. Controls were maintained under normal light conditions. While they found that increased light increased the thyroid activity slightly, they could find no relationship between the wave length and thyroid function.

Reineke and Turner (1947) studied the seasonal variation in the thyroid hormone secretion of the chick. They found that in both sexes thyroid secretion reached a maximum level in October and November. It declined during February and early March. In the latter part of March it declined further and remained at a low level until the following August. During the fall thyroid secretion rose again to the levels observed during the previous year, indicating a definite rhythm of activity throughout the season. It seems that the increasing light in the spring may have had some influence on thyroid activity.

Turner (1948) found that old White Leghorn hens were stimulated to increased egg production in the summer months if thyroprotein was fed. He could find no beneficial effects from feeding thyroid hormone in the winter months. From this he concluded that thyroxine secretion in the White Leghorn was already at maximum levels during the winter months. He also noted that an increase in gonadotropic hormone was shown by the enlarged combs of the birds fed thyroprotein. This decline in thyroid secretion rate in the summer may indicate the possibility that light as well as increased temperatures influence the thyroid of the fowl.

Berliner and Warbritton (1937) commented on the low fertility of rams during the summer months and suggested that it might be due to low thyroid activity resulting from the high summer temperatures. Their hypothesis was at least partially confirmed by Bogart and Mayer (1946). These investigators noted that high summer temperatures caused a decline in spermatogenesis in the ram. They found that when thyroprotein or thyroxine was given to the rams during the summer months, breeding capacity was restored to near normal levels. All these workers attributed the hypothyrodism observed to the high summer temperature. However, it has been demonstrated several times (Cole and Miller, 1935, and Yeats, 1949) that ewes can be induced to breed in the spring and summer if the amount of light received each day is gradually reduced by artificial means. It seems logical to assume that the ram would be influenced by the same conditions. Therefore, light may be an important factor in the summer decline in ram fertility.

Effects Of Light On Gonad Function

A great deal of evidence has been accumulated showing that light exerts an effect on the reproductive cycle, especially in avian species. No extensive review of this tremendous amount of literature is contemplated here since such reports are already available in other papers. Yeats (1949) gives a very complete summary of the progress made in determining light influenced, sexual changes in birds and mammals. However, a brief survey is indicated by the nature of the experiment being reported.

Rowan (1929) observed that in the junco changes took place in the testes just prior to its northward migration in the spring. The interstitial tissue and germinal cells of the testis and ovaries reached maximum development during migration, and reproductive ability was thus assured by the time the northern breeding grounds were reached. He found a second period of gonad interstitial activity in the fall. This hyperfunction of the gonads seemed to occur just before and during migration.

Later Rowan (1931) artificially altered the daylight period of several migratory species. He found that by doing this he could cause a bird to come into full song and breeding condition during the winter months. From the observations he had made Rowan (1931) postulated that the migration of birds is stimulated by the hormone elaborated in the interstitial tissue of the testis and ovary, its elaboration being caused by the increase or decrease in normal daylight.

Van Oordt and Danste (1939) observed similar results when they studied the reaction of the greenfinch to light and darkness. Birds in full song (May) were placed in darkness and killed at varying intervals. While in the dark both the testes and ovaries decreased in size and spermatogenesis ceased. The birds also began to molt, a process which usually takes place in August. The greenfinches were then exposed to increased light in August, after being in the darkness. Their gonads initiated spermatogenesis and increased in size. Song was restored.

Benoit (1936, 1937) observed that ducks ceased to breed when the eyes were removed. However, when artificial light was directed into the eye sockets by quartz rods, breeding capacity was restored.

The reports on avian response to light led to work designed to show whether mammals were influenced in the same manner or degree. Brissonette (1932) first showed a similar increase in gonad response to light in the ferret. Marshall (1940) also subjected female ferrets to different degrees of light intensity by placing them at various distances from a 1000 watt light bulb. He found the response (acceleration of the estrus cycle) was correlated somewhat with the light intensity. Morgan (1949) observed the same results when he studied the female opossum's reaction to light in its non-breeding season. He found that the increase in the size of the reproductive tract was directly proportional to the amount of light received from an artificial source.

Sykes and Cole (1944) attempted to alter the breeding season of eight ewes, five of which were of Rambouillet breeding. The other two ewes were of the English breeds, one being a Southdown, the other a Hampshire. The sheep were placed under artificial light in March and the lighting was increased gradually until three hours had been added to the normal day. Light was then decreased in one hour steps during late March, April, and early May.

Services by the rams turned with the ewes were observed in the case of five of the ewes, and five ewes gave birth to normal healthy lambs. The Hampshire ewe did not breed. One of the two Rambouillet ewes who failed to lamb was suspected of having aborted. These lambings occurred about five months previous to the usual time. However, none of the ewes had lambed the previous year so direct comparison could not be made.

Yeats (1949) demonstrated that the sexual season of the sheep, which normally breeds in the fall, could be successfully reversed by altering the light conditions. Using two groups he placed one on increasing light in the spring while the other was subjected to decreasing increments of light each day. He found that if the decreasing light treatment was continued about two months the ewes showed estrus and accepted the rams. Normal lambs were born in the fall during the usual season of mating. He noted that while temperatures ranged in the high summer levels the sheep on decreasing light showed no depression of sexual activity. Conversely, when the group on decreasing light was changed to increasing light in the fall (normal breeding season) no estrus cycle was shown and the sheep refused to breed, although a group on the normal decreasing level of light bred as expected. He therefore concluded that decreasing light in the fall of the year, rather than the onset of cooler weather (as had been widely suspposed), acted as the stimulus for breeding.

Once the fact had been established that light influenced the breeding season of birds and mammals, investigators attempted to determine the manner in which that influence was transmitted to the gonads. All of the evidence gathered to date points to a primary effect on the anterior pituitary.

Whitaker (1940) demonstrated that the white-footed mouse can show no cyclic sexual activity after it has been blinded by removal of the eyes. Mice kept in constant darkness also showed a reduced and non-cyclic sexual activity. If light of low intensity (one foot-candle) was used, breeding took place throughout the year and the mice did not go into anestrum. Low temperature did not effect this constant estrus.

Fiske (1939) observed that female rats kept in constant light showed long periods of estrus and diestrus. Other groups of rats kept in constant darkness were found to display a long period of metaestrus. When the pituitaries of rats under constant light were assayed they were found to contain large amounts of FSH hormone. Those of the rats kept in constant darkness showed high concentrations of luteinizing hormone. Adult males kept in constant darkness had larger pituitaries and gonads as compared to males under constant light.

Truscott (1944) showed that sexual maturity in rats was delayed after the optic nerve was severed, even though constant lighting was used. In normal rats constant lighting was found by both Truscott (1944) and Fiske (1939) to hasten the advent of sexual maturity.

Pomerat (1942) stated that the pituitaries of rats kept in constant darkness for one and one-half months resembled those of young castrated females. This condition persisted for as long as a year if the animals were kept in darkness, but the changes became less pronounced.

There are many mammals and fowl whose sexual cycle is not influenced by light. These are primarily of tropical origin. The guinea pig was reported by Dempsey (1934) to be uneffected by light changes. Since it normally lives where the days are twelve hours long throughout the year, this is not surprising.

In summary, it can be seen that many animals and birds of the temperate zone exhibit cyclic sexual activity. This activity of the gonads has been shown repeatedly by many workers to be influenced by seasonal changes in the daily level of light. The light is conducted to the pituitary through the eyes causing that gland to increase its secretion of gonadotropic hormones.

Thyroid, Pituitary, And Gonad Interrelations

It has been well established that there is an interrelationship between the endocrine glands of the body. The relation of the thyroid to the gonads has been studied by many workers, and the results furnish ample proof that altered thyroid condition causes a change in gonadal function.

In 1923 Jaap reported on the effects of feeding dessicated thyroid tissue to Mallard drakes. This species normally shows an increase in testis size in the late winter and spring. He noted that an increase of as much as ten times over the controls could be induced with the thyroid treatment at this season. He attributed this increase to the higher metabolism of the birds which resulted in greater loss of testis hormone from the body. This loss, he felt, would allow greater elaboration of gonadotropic principle by the anterior pituitary lobe, causing gonad stimulation and increased spermatogenesis.

Cole (1925), another early investigator, stated that a group of five to eight year old fowls underwent rejuvenation in feathering and increased in egg production during a period of thyroid hormone feeding.

Van Horn (1933) investigated the effect of excessive amounts of thyroid substance on the physiological activity of estrogen. He reported that approximately three times as much estrogen was necessary to induce artificial estrus in castrated white female rats after they had been placed in a hyperthyroid condition. He further stated that when hypophyseal implants from hyperthyroid female adult rats were implanted in immature females, they caused an increase in gonad stimulating power of the hypophyses ranging from 15 to 63 percent.

Meites and Chandrashaker (1949) studied the effects of induced hyper- and hypothyroidism on the response of the gonads to a constant dose of pregnant mares' serum in immature male rats and mice. They produced hypothyroidism by feeding 0.1 percent thiouracil in the ration for four to twenty day periods, and hyperthyroidism by feeding thyroprotein in various concentrations for ten day periods. They found that thiouracil or thyroprotein, when fed alone, had no effect on the weight of the seminal vesicles and coagulating glands. When pregnant mares' serum was injected, the response of the seminal vesicles and coagulating glands was inhibited by all except the lowest levels of thyroprotein in the rat, while in the mouse gonadotropic response was increased by all except the highest concentrations of thyroprotein. Thiouracil increased the gonadotropic response in rats by as much as 300 percent, while the response in mice was reduced by as much as 73 percent.

This work was repeated with female immature rats and mice by Johnson and Meites (1950) and the same results were obtained. They concluded that young rats secreted more than an optimal amount of thyroid hormone whereas young mice secrete less than an optimal amount of thyroid hormone.

Reineke, Bergman, and Turner (1941) studied eight thyroidectomized male kids for lactogenic, thyrotropic, and gonadotropic hormones. They found that both lactogenic and thyrotropic hormones were present in normal amounts in the cretinous pituitaries. The gonadotropic hormones were low and the testes were lighter than those of the controls.

P'an (1940) reported that the gonadotropic content of the pituitary of normal and castrate rats and rabbits was decreased after thyroidectomy. Evans and Simpson (1929) reported that the gonad-stimulating ability of the anterior pituitary was increased in hyperthyroid rats, while the glands from hypothyroid rats were less effective than the controls.

Chu (1944) found that in thyroidectomized rabbits the ovaries contained many more large follicles than normal controls. He stated that the hypophysises of the thyroidectomized rabbits were free of ovulating hormone, whereas the follicle stimulating hormone was considerably increased.

While there are apparent contradictions in some of the works cited, the fact stands out that the thyroid does influence the gonads directly. It also causes the gonadotropic secretion of the pituitary to change markedly. The pituitary, thyroid, and gonads are thus seen to be so interrelated that any alteration in one causes a secondary effect in the others.

PROCEDURE

Method Of Handling The Sheep

The sheep used were from the Michigan State College Experimental Flock. This group is composed of purebred ewes of several breeds, with the Rambouillet predominating in numbers. The flock has been used in part for lamb and wool production but its main function is to serve for experimental research of various types. Its size is maintained by shifting into it the poorer types from all the college flocks. The sheep in the experimental group are not culls, however, in the usual sense of the word, but rather the poorer individuals from an excellent college flock.

A group of about fifty ewes was available from which to select the animals needed. However, those of Dorset breeding were eliminated since they were considered undesirable in an experiment where out-season breeding was one of the factors to be considered. Other sheep, some of which were bred, and others of excessive age, were also eliminated. When all the undesirables had been culled out there remained only thirty-two ewes from which to select. From these, twenty-four were picked on the basis of breed, age, weight, and previous lambing history.

The ewes finally selected for the work were of Rambouillet and Shropshire breeding. It was realized that the Rambouillet ewe sometimes breeds in the summer months and might show less reaction to light and darkness than the English breeds. Their inclusion was necessary since they were the only breed available in sufficient numbers.

TABLE I

SHEEP RECORDS ON THE EWES USED, SHOWING THEIR LAMBING HISTORY FOR THE TWO YEARS PRECEDING THE EXPERIMENT

Ewe No.	Breed	Weight	Age	Lambed in 1950	Lambed in 1949
		Control			
215	Shropshire	---	3	-------	March 17
27	Shropshire	118	5	Apr. 1	March 8
541	Shropshire	119	2	-------	Yearling
555	Shropshire	119	2	Apr. -	Yearling
589	Rambouillet	139	-	Jan. 19	Yearling
691	Rambouillet	99	Yr.		
591	Rambouillet	130	2	Nov. 28	Yearling
574	Rambouillet	113	2	Jan. 23	Yearling
277	Rambouillet	124	3	Jan. 22	--------
		Constant Darkness			
502	Shropshire	120	2	Mar. 14	Yearling
526	Shropshire	136	2	Mar. 10	Yearling
517	Shropshire	126	2	Apr. 5	Yearling
590	Rambouillet	129	2	Nov. 24	Yearling
584	Rambouillet	---	2	Jan. 19	Yearling
162	Rambouillet	---	-	-------	--------
684	Rambouillet	107	Yr.		
145	Rambouillet	128	9	Jan. 7	Jan. 26
		Constant Light			
558	Shropshire	115	2	Jan. 8	Yearling
546	Shropshire	115	2	Mar. 8	Yearling
232	Shropshire	123	3	Mar. 12	Apr. 7
92	Rambouillet	117	-	Apr. 16	Feb. 26
176	Rambouillet	114	6	Jan. 22	Jan. 27
679	Rambouillet	114	Yr.		
585	Rambouillet	127	2	Mar. 13	Yearling
575	Rambouillet	128	2	Jan. 24	Yearling

The age and weight were considered very important. No valid comparison could be made of thyroid activity if the experimental animals were of several extremes of age. The weight was held as nearly equal for the groups as possible in order to simplify the calculations of the results.

The ewes were divided into three groups with five Rambouillets and three Shropshires in each group. They were then designated as a control group, a constant light group, and a constant darkness group. A Shropshire ewe (No. 215) entered the control group pen from the pasture through an unlatched gate and was allowed to remain for the rest of the experiment. Her results are averaged in with the rest of the control sheep.

The selection of rams was very limited. Only a small number were available for use and most of these were found to be in a low state of fertility when their semen was examined. Two Hampshires and one Shropshire were finally chosen. They all showed good libido and the semen check indicated that they were capable of breeding any ewes which might come in heat.

A fourth ram was introduced into the experiment on August 10th. This is the last Hampshire listed in Table II. A routine semen check on this date showed the Shropshire used originally to be completely sterile and he was therefore replaced.

Semen checks were made on all the rams several times during the course of the summer to determine the level of their fertility. The

TABLE II

BREED, AGE, AND SEMEN QUALITY OF THE RAMS USED

Ram No.	Breed	Age in Years	Semen Quality Scale from I to V
734	Hampshire	Yearling	V (June 29)
77	Hampshire	5	IV (June 29)
Chatman	Shropshire	2	V (June 29)
---	Hampshire	-	IV (Aug. 10)

semen was collected by the artificial vagina method, which was found to be very satisfactory. It was judged on motility, volume, concentration, and amount of abnormal spermatozoa present. The semen was then rated on an arbitrary scale from one to five; one being considered extremely poor and five excellent.

Quarters Used For The Sheep

A basement type barn was selected which had three doors opening on the north side of the structure. A floorplan of this basement is given in Figure 1. It can be readily seen from this plan that plenty of air and light were available in all parts of the structure.

Three pens were partitioned off in the basement. Each was accessible from the outside by one of the doors, and each was equipped with a door in the ceiling through which hay could be dropped from the mow overhead.

The first pen was used for the control group. The two large doors at opposite ends of the pen were left open at all times. The sheep were restrained by low gates across these exits. Plenty of normal daylight and good ventilation were thus assured. Except for the fact that they were confined to dry feed, these ewes were under almost exactly the same conditions as the sheep on pasture.

The center pen was lightproofed by covering the walls with tar paper. However, due to the rough nature of the structure absolute exclusion of light was not obtained. It is possible that the stray rays of light may have influenced the activity of the thyroids of this group.

A small lightproof ventilator was installed in the darkened pen after the experiment had been in progress for approximately forty-five days. Before this ventilator was installed a strong ammonia smell was noticeable and the humidity was somewhat high. This condition markedly decreased once the fan was installed. No respiratory trouble was noticed among the sheep, however.

The third pen was maintained under a condition of constant light. This was effected by using two ordinary household lightbulbs of 150 watts each. These lightbulbs were located on the ceiling about one-third the distance in from either end of the room. They were allowed to burn for the entire time the experiment was in operation, and thus insured that the sheep were never in darkness at any time. No reflectors were used on the lights and in some corners the intensity was rather low. Hurdles were used to force the sheep into the better lighted areas of the pen so that they were constantly subjected to the

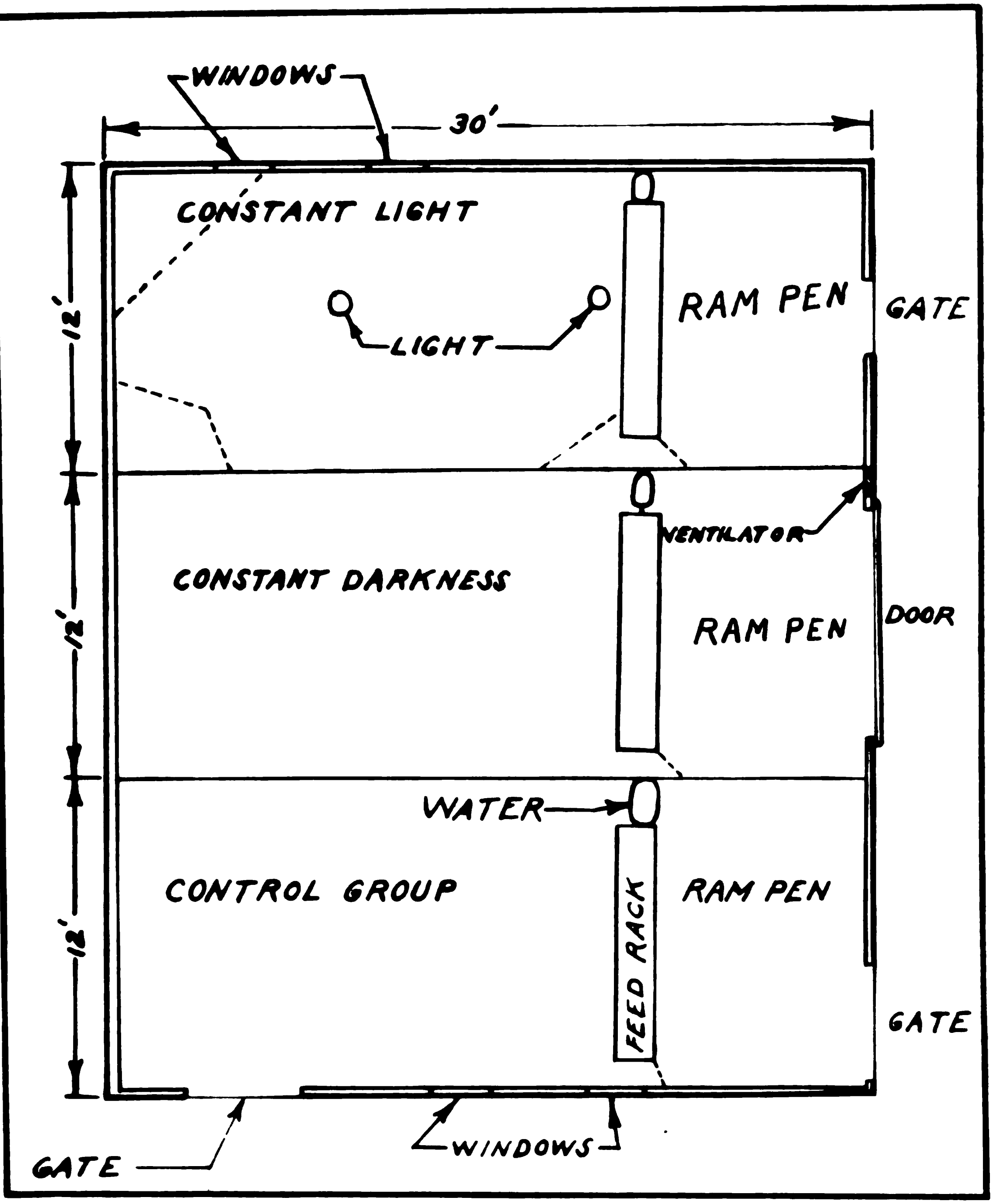

Figure 1. Floorplan of the sheep quarters. Note that all the pens except the one in constant darkness were well supplied with light and air. Forced ventilation was provided in the dark pen. The ewes were housed on one side of the feed racks and the rams on the other. Feed was stored in the loft overhead. The dotted lines in the lighted pen indicate hurdles placed to force the sheep into the better lighted area of the room.

light. The windows in this pen were open at all times to give ventilation for the quarters and to take advantage of the normal light during the day. Except for the light the ewes were kept exactly as were those in the control group.

The three groups were placed in their respective pens on the 22nd day of June. They were released on August 29th when the experiment was terminated. Temperature measurements were made in the three pens daily. The temperature was never found to vary over three degrees from one pen to another. The pen in constant darkness averaged about 1^{o} C. higher than the control pen.

Equipment And Feeding

A large feed rack, accessible from both sides, was placed in each of the pens. This rack was of sufficient size to hold a day's supply of feed and long enough to allow the entire group to feed from one side without crowding. Fresh hay was placed in this rack each morning after the unpalatable residue of the previous day was removed and spread for bedding. Water was piped to each pen from a nearby well.

The pens were cleaned each day as much as seemed necessary, and were completely bedded once each week. A phenothiazine and salt mixture was kept in each pen at all times to control internal parasites. The entire barn was sprayed once during the summer with a D.D.T. water base solution to reduce the fly population.

Since the conditions, except for lighting, were held constant for all the groups the sheep were not turned out on pasture at any time during the experiment. They depended entirely on new hay for their ration. This hay was, for the most part, a bromegrass, clover, and timothy mixture. The sheep were always supplied with all of it they could clean up.

All of the sheep were weighed at the start of the experiment and again about forty-five days later to determine any change in condition. Table III shows the results of these weight checks. It will be noted that there were no significant weight changes in any of the groups.

TABLE III

THE GAIN OR LOSS OF THE GROUP IN WEIGHT OVER MOST OF THE COURSE OF THE EXPERIMENT. AS WILL BE NOTED THERE WAS LITTLE CHANGE

	Group Weight On June 22	Group Weight On August 10	Pounds Gain or Loss
Control	961 lbs.	961 lbs.	0
Constant Darkness	982 lbs.	1010 lbs.	+28
Constant Light	953 lbs.	949 lbs.	-4

Checking The Breeding Activity

It was originally planned to check the estrus dates occurring in each ewe while the experiment was in progress. The brisket of each ram was kept well marked with grease and ochre, and any activity by the rams was observed when they were turned with the ewes in the evening. (The rams were kept penned separate from the ewes during the day.) Any service marks appearing on the ewes' rumps in the morning were recorded when the rams were removed. This plan was finally abandoned after a trial period because the results were so unreliable. Several workers have used this method of checking oestrus and have reported excellent results. In this experiment, however, the results were highly unreliable at best. It was found that when the ewes were closely penned with an active ram too many false markings were observed to allow any reasonable emphasis to be placed on those ewes who were marked during the night.

In view of the lack of reliability which could be placed in the service marks, it was decided to determine breeding activity by recording all lambs dropped within 147 days after the close of the experiment. Ewes who lambed within this period were presumed to have been bred while under the experimental conditions.

Since the fertility of the rams declined as the experiment progressed into the summer they were rotated from pen to pen during the last month of the trial in an effort to keep a fertile ram with each ewe most of the time. This rotation was made once every two days and as a result no data was gathered on the effect of light on spermatogenesis or thyroid activity in the ram.

Checking Thyroid Activity

The ewes were checked for thyroid activity by injecting a tracer dose of I^{131} on the morning of August 21st, sixty-two days after the experiment was started. The exact amount of radio-activity in the iodine used was computed by the formula

$$N = N_o e^{-\lambda t}$$

where N_o = origional activity

e = base of natural logarithm

λ = decay constant of the iodine

t = time

A dosage of one microcurie of iodine per pound of body weight was injected subcutaneously in the rear flank.

The iodine collected by the thyroid was measured by counting the radioactivity over the gland. A Geiger-Muller type ionization tube attached to a portable amplifier and recording circuit was used for counting. The tube was used with an aluminum shield in place so that only gamma photons were measured. All counting was done while holding the tube directly against the skin over the thyroid isthmus. The wool had been clipped from the necks of all the animals before the I^{131} was administered so that the tube could be placed as close to the thyroid gland as possible.

The radiation was counted over the gland for a four minute period. This length of time seemed to give a reasonably accurate count. Several of the ewes in each group were recounted after the group had been checked and the results were always within one or two percent of the origional count. The background count was determined for each ewe at the time the thyroid count was made and this figure was subtracted from the thyroid count.

Six counts were made on each of the groups at intervals of twenty-four hours to determine the level of iodine collection in the gland. The counts were made at four, twenty-four, forty-eight, seventy-two, ninety-six, and one-hundred-ninety-two hours.

Statistical Analysis Of Data

All of the data obtained on thyroid function was treated statistically. The standard error of the group means was computed by the formula

$$S.E. = \sqrt{\frac{d^2}{n(n-1)}} .$$

The significant differences between the means were determined by the formula

$$S.D. = \frac{m_1 - m_2}{\sqrt{E_1^2 + E_1^2}} .$$

The regression lines for the plotted curves of thyroid activity were also computed using the formula

$$y = a + b \log x,$$

where

$$b = \frac{\Sigma y \log x - \frac{(\Sigma y)\ (\Sigma \log x)}{n}}{\Sigma (\log x)^2 - \frac{(\Sigma \log x)^2}{n}}$$

in which y = counts per minute

$\log x$ = the log of the time

n = number of cases.

a is found by the formula

$$a = \bar{y} - b\,\overline{\log x} \quad .$$

σ_e, the standard error of estimate for the lines, was computed by the following formula:

$$\sigma_e = \sqrt{\frac{\Sigma y^2 - \frac{(\Sigma y)^2}{n} - b\left[\Sigma (y \log x) - \frac{(\Sigma y)\ (\Sigma \log x)}{n}\right]}{(n-2)}} \quad .$$

The significant differences between the regression lines was computed by using the formula

$$T = \frac{b_c - b_1}{\sqrt{\sigma_{b_1}^2 - \sigma_{b_2}^2}}$$

where σ_{b_1} is obtained by the formula

$$\sigma_{b_1} = \frac{\sigma_e}{\sqrt{\Sigma (\log x)^2 - \frac{(\Sigma \log x)^2}{n}}} \quad .$$

RESULTS

Effects Of Light On Thyroid Function

It can be seen in Figure 2 that a consistent difference exists between the thyroid function of the three groups. The group kept in constant darkness averaged a higher count than the controls, while in the group kept in constant light less iodine was collected by the thyroids as compared to the controls. The averages given in Table IV show, however, that when the standard error is taken into consideration the apparent difference tends to disappear at some of the time intervals measured.

TABLE IV

THE AVERAGE COUNT FOR EACH GROUP COMPUTED ON THE BASIS OF AVERAGE COUNTS PER HUNDRED POUNDS OF BODY WEIGHT. THE STANDARD ERROR OF ESTIMATE IS ALSO GIVEN

Time in Hours After Injection	Average Counts Per Minute ± the Standard Error		
	Control	Dark	Light
4	234.9 ± 34.1	283.1 ± 30.6	176.1 ± 42.5
24	685.1 ± 60.2	762.3 ± 72.8	578.0 ± 106.8
48	762.3 ± 69.8	1013.3 ± 83.7	604.2 ± 149.7
72	871.2 ± 101.8	964.5 ± 104.6	610.1 ± 169.1
96	976.5 ± 96.5	990.9 ± 86.4	743.0 ± 34.9
192	591.7 ± 93.4	647.9 ± 49.4	491.1 ± 144.3

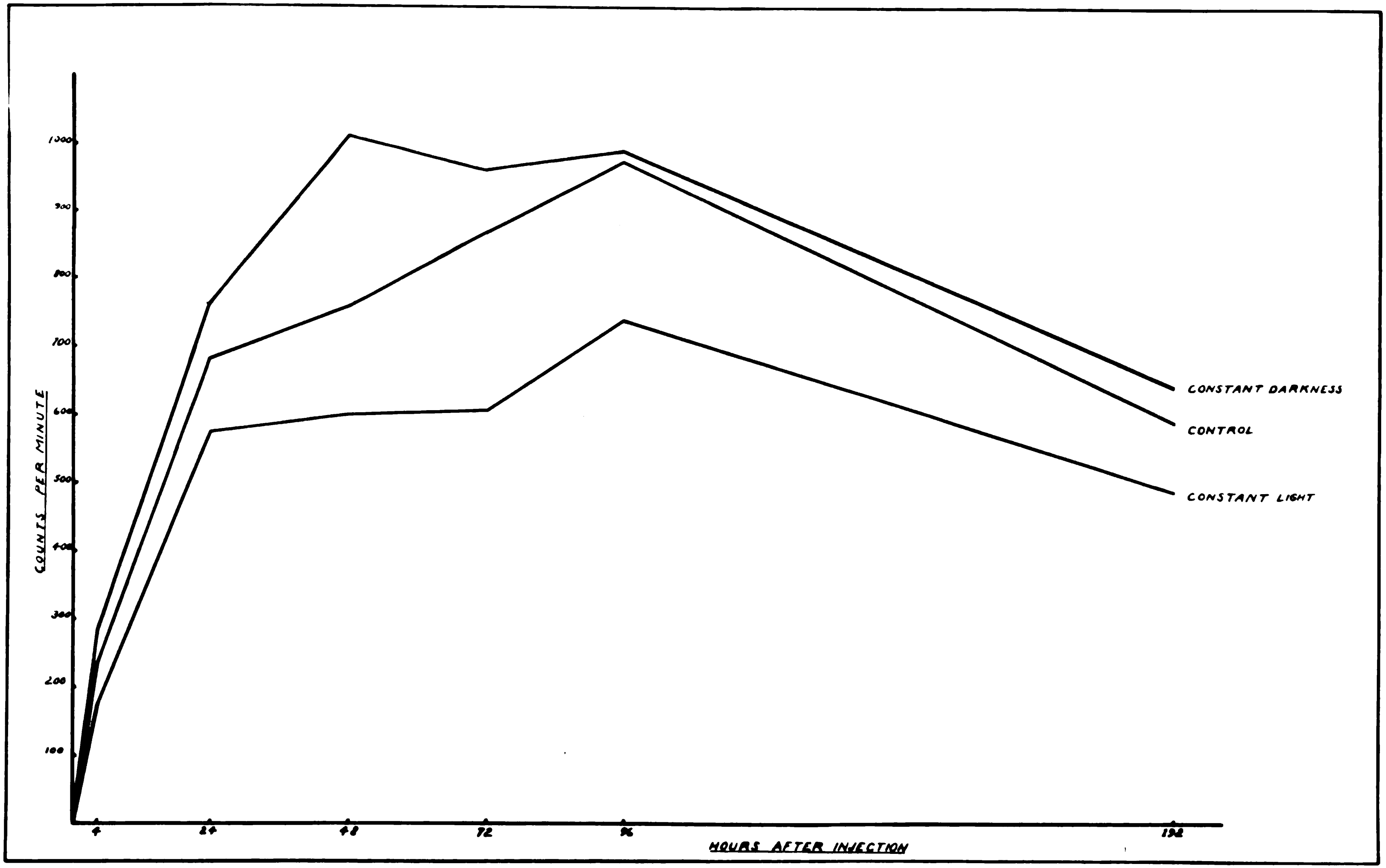

FIGURE 2. GRAPH OF EACH GROUP'S THYROID COUNT FOR 192 HOURS AFTER INJECTION WITH I^{131}.

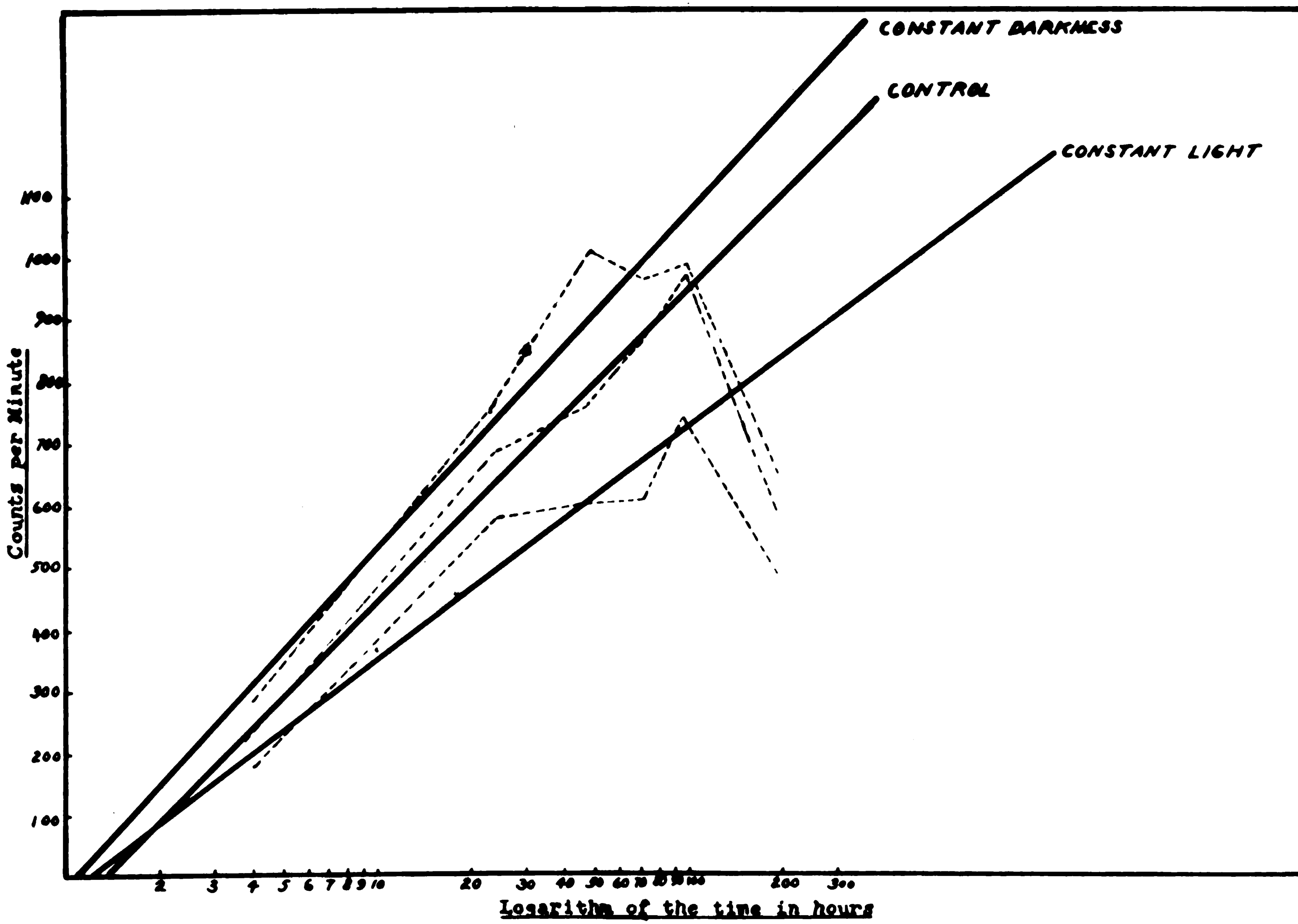

Figure 3. Straight lines fitted to the curves of thyroid activity for each of the groups. The significant differences between the lines was found to be low.

Statistical analysis showed the constant-darkness group to be significantly higher than the control at forty-eight hours after injection, as can be seen in Table V. The group under constant light had a significantly lower thyroid activity than the control, ninety-six hours after injection. It should also be noted that the constant-light group had a significantly lower thyroid activity than the constant-darkness group in 50 percent of the checks that were made after injection with the I^{131}. The individual counts made on each ewe over the entire ninety-six hours are given in the appendix.

TABLE V

THE SIGNIFICANT DIFFERENCES OBSERVED BETWEEN THE THREE GROUPS AT THE SIX TIME INTERVALS WHEN CHECKS WERE MADE

Hours after Injection	Significant Differences: Control Group – Light Group	Control Group – Dark Group	Light Group – Dark Group
4	1.10	1.05	2.04*
24	0.89	0.82	1.42
48	0.95	2.30*	2.85*
72	1.18	0.10	1.25
96	2.54*	0.72	3.80*
192	0.24	0.53	1.03

* Significant differences

The regression lines computed for each of the curves are plotted in Figure 2. The significant differences between the lines were computed by the T-score method. A reference to Table VI will show that the values obtained were too low to allow the thyroid differences for the entire time measured after injection to be called significant. However, the difference between the light and dark groups of 1.756 borders on significance.

Two methods of statistical analysis have thus been applied to the data obtained on thyroid function. The thyroid uptake of iodine was shown to be significantly different at several time intervals after the injection of I^{131} as compared to the control. A significant difference in rate of iodine collection was shown in 50 percent of the observations when the constant-darkness group was compared to the constant-light group.

TABLE VI

THE STATISTICAL DIFFERENCES BETWEEN THE REGRESSION LINES AS COMPUTED BY THE T-SCORE METHOD

Differences Between		
Control and Constant-Darkness	Control and Constant-Light	Constant-Light and Constant-Darkness
0.571	.406	1.756

Effect Of Light On Breeding Activity

In these groups of sheep constant light advanced the breeding season while constant darkness had little effect. The data were not conclusive enough to treat statistically. A summary of the results obtained from

TABLE VII

BREEDING RESULTS. THE SHEEP LISTED AS HAVING LAMBED ARE ASSUMED TO HAVE BEEN BRED WHILE THE EXPERIMENT WAS IN PROGRESS
(Based on 147 day gestation period)

Ewe No.	Breed	Lambed 1951	No. Lambs	Lambed 1950	No. Lambs	Lambing Date Advanced
			Control Group			
215	Shropshire					
27	Shropshire			Apr. 1	1	
541	Shropshire					
555	Shropshire			Apr. --	1	
589	Rambouillet	Jan. 9	2	Jan. 19	2	No
691	Rambouillet	Jan. 13	1	Yearling		
591	Rambouillet	Jan. 23	1	Nov. 28	1	No
574	Rambouillet	Jan. 26	2	Jan. 23.	1	No
277	Rambouillet					
			Constant Darkness			
502	Shropshire	Jan. 20	1	Mar. 14	2	Yes
517	Shropshire			Apr. 5	1	
526	Shropshire			Mar. 10	1	
590	Rambouillet	Dec. 10	2	Nov. 24	1	No
584	Rambouillet					
162	Rambouillet	Jan. 23	1			?
684	Rambouillet			Yearling		
145	Rambouillet			Jan. 7	2	
			Constant Light			
232	Shropshire	Jan. 16	1	Mar. 12	1	Yes
546	Shropshire	Jan. 19	1	Mar. 8	1	Yes
558	Shropshire	Jan. 14	1	Jan. 8	1	No
92	Rambouillet	Jan. 25	2	Apr. 16	1	Yes
176	Rambouillet	Jan. 14	2	Jan. 22	1	No
679	Rambouillet			Yearling		
585	Rambouillet	Jan. 4	2	Mar. 13	1	Yes
575	Rambouillet			Jan. 24	1	

breeding the ewes is presented in Table VII. In the control group four ewes dropped lambs within 147 days after the experiment terminated. Three ewes lambed of the group kept in constant darkness. In the group exposed to constant light six out of the eight ewes lambed.

In an examination of the lambing date for 1951 as compared to 1950 (Table VII) the following points are noted. The four ewes of the control group who lambed were all of Rambouillet breeding. The lambing date of none of these ewes was advanced over the previous season. None of the four Shropshires in the group dropped lambs earlier than usual. There was only one Shropshire among the three ewes which were bred in the group kept in constant darkness. This lone Shropshire was also the only one of the group which bred early. All of the Shropshires exposed to constant light were bred, and all except one lambed earlier than usual as compared to their previous lambing history. A total of four ewes in this group showed an advancement in breeding date.

DISCUSSION

The evidence obtained in this experiment shows that thyroid activity was altered. It is clear that the thyroids of both experimental groups acted in a particular and different manner whether compared to the control or to each other. Since the only known variable was the amount of light used it must be concluded that the differences were a direct result of the increased or decreased light.

There was not, however, a consistent statistical difference at all the time intervals when measurements were made. This is an apparent contradiction to what would logically be expected. Certain errors in experimental procedure, most of which were unavoidable, may have had a direct bearing on this uneven result. First, the number of thyroid measurements made after the iodine was injected was very small. It can be seen by inspecting Figure 2 that the curves of activity were plotted from only six points spread over an interval of more than a week. This was very undesirable. A greater number of points would undoubtedly have smoothed out the curves and reduced the possibility of experimental error, which could exert such a large influence in a few counts. It is quite probable that another experiment designed to allow more frequent checks would show a much higher significant difference between the experimental groups and the control. In the present work lack of the necessary labor to handle the sheep precluded the possibility of making more than one count in a day.

Second, it is possible that the Rambouillet is not as strongly influenced by light as are the English breeds. It is well known to be a sporadic summer breeder. This might have resulted in eratic thyroid function under experimental conditions of the type used. This supposition was not borne out, however, when the results of the radioactive count of the Rambouillet and Shropshire ewes were computed separately. No significant difference was found, although the Shropshires were slightly more active in their collection rate of iodine. It should be remembered in this comparison that several of the Rambouillets ewes were quite old as compared to the Shropshires. This might well cause a reduction in their thyroid activity and result in a somewhat lower count when compared to the Shropshires. Also, the number of Shropshires used was not great enough to allow any valid conclusions to be drawn from their results. Since the calculations showed little visible breed variation, and in view of the above considerations, it was concluded that no difference in thyroid activity could be shown between the two breeds in this work. The data used in the calculations was not presented because it adds nothing to the main theme of the work. The breeds compared were represented by an unequal number of member and of wide differences in age and such calculations could not be valid and would be only of academic interest.

The data gathered in this work on sheep thyroid activity is in close agreement with similar work done by Puntriano and Meites (1950) on rats and mice. While these workers obtained a highly significant difference between their experimental groups and the control they were only able to make one count of activity since the animals were sacrificed and the glands removed for observation under a Geiger-Muller counter in the laboratory. This procedure, while an excellent method, of course precludes the possibility of making a series of observations on the same group. A direct comparison of the present results with theirs is therefore impossible. It would seem, however, that the sheep in this experiment were influenced in the same manner by constant light or darkness. A similarity in gonad response could also be expected and this was borne out to a small degree.

In conclusion then, it can be said that ewes of Rambouillet and Shropshire breeding show increased thyroid activity during the summer months when subjected to total darkness for a period of sixty-eight days. It can also be stated that when ewes of Rambouillet and Shropshire blood are placed under constant light for an equal period a reduction in thyroid activity takes place. It is quite likely that other breeds of sheep would give the same results under similar conditions.

Breeding

The breeding results do not present any clear-cut information, but there are indications which would make further study of the problem highly interesting. In the control pen four ewes were bred, all of which were of Rambouillet breeding. A study of their lambing dates of the previous year shows that none of them bred earlier than usual. This control compares quite favorably with the ewes of the same breed who were on pasture during the summer. A rough comparison showed approximately the same number of lambs were dropped by each group in the period being studied.

It is interesting to note also that Sykes and Cole (1944) reported five ewes bred while under an artifically decreasing level of light in the spring. They kept no control but in this present work almost as many (four) ewes were bred in the untreated control group. This throws the work they reported open to considerable doubt, especially when the ewes of the same breed gave comparable results on pasture during the summer when they were allowed to run with the ram.

The group on constant darkness appeared to be uneffected in their breeding cycle. Three of the ewes were bred but only one of these was advanced in her breeding date over the previous year. Just why the one ewe (a Shropshire) should be effected cannot be explained unless it were due to some endocrine unbalance which might or might not be related to the darkness.

The most breeding occurred in the group exposed to constant light. It is interesting, to say the least, that all of the Shropshires in this group produced lambs. It is also regretable that more ewes of this breed were not under experimental conditions, since three ewes are hardly enough from which to draw conclusions. Despite the fact that only four ewes bred early it does appear that constant light may have exerted a positive influence on the breeding dates of this group. This is the type of gonad response obtained by Puntriano and Meites (1950) when they exposed rats to constant light, and Fiske (1939) reported very similar results with groups of rats.

These results, if the interpretation is a true one, are in direct opposition to the results obtained by Yeats (1949) who found that breeding in sheep was influenced by decreasing light. However, there is no reason at present to believe that decreasing light and constant illumination could not exert the same effect. Further investigation of this possibility would be highly desirable.

In conclusion, the hypothesis is offered that constant darkness has no influence on the sexual cycle of the sheep, while constant light causes estrus and breeding. Such a possibility is well worth more extensive investigation and an acceptable proof would add considerably to the present concept of the ewe's endocrine function.

SUMMARY

1. The effects of continuous light or continuous darkness on the thyroid glands of fifteen Rambouillet ewes and ten Shropshire ewes was determined. The sheep were subjected to experimental conditions for a period of sixty-nine days between June 22nd and August 29th. The effects of such lighting on breeding activity were also studied.

2. The sheep were placed in three groups of five Rambouillets and three Shropshires each. They were designated a control group, a constant light group, and a constant darkness group. A ram was placed with each group to check oestrus activity in the ewes. The thyroid activity was measured by administering a tracer dose of radioactive iodine. The iodine collection in the thyroid was determined by counting with a Geiger-Muller type tube placed directly over the thyroid isthmus, and in contact with the skin.

3. The sheep under continuous light collected somewhat less iodine in the thyroid than did the control. Sixteen per cent of the observations made were significantly lower than the control when measured statistically.

4. Sheep under continuous darkness showed a higher iodine collection by the thyroid. Sixteen per cent of the observations were significantly higher than the control group.

5. The thyroid iodine collections of the constant-darkness group were higher than those of the constant-light group in 50 per cent of the observations.

6. It was concluded that constant light decreases thyroid activity in the ewe, while constant darkness increases thyroid activity.

7. The following breeding results were obtained. Lambing dates were not advanced for any of the ewes in the control pen. One ewe lambed earlier than usual in the pen where constant darkness was inforced. Four ewes lambed early in the group under constant light.

8. The hypothesis was offered that constant light as well as decreasing levels of light may influence the ewe to breed.

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APPENDIX

TABLE I

INDIVIDUAL DATA ON THE RADIOACTIVE COUNT OF EACH EWE THROUGH THE FIRST FIVE OBSERVATIONS MADE OF THYROID ACTIVITY. THE GROUP AVERAGES, AND THE AMOUNT BY WHICH EACH EWE'S COUNT DIFFERED FROM THE MEAN OF THE GROUP ARE ALSO GIVEN

Ewe No.	Total Counts/Min.	Counts Per 100 Lbs.	Difference From Mean	Group Mean
		Control (Four Hour Check)		
27	200	183.5	51.4	234.9
541	520	412.7	177.8	
215	255	212.5	22.4	
555	335	270.2	35.3	
691	420	385.3	150.4	
589	215	149.3	85.6	
574	255	214.3	20.6	
277	170	136.0	98.9	
591	200	148.2	86.7	
		Constant Darkness (Four Hour Check)		
502	360	318.6	35.4	283.2
517	325	266.4	16.8	
526	290	204.2	79.0	
145	430	286.7	3.5	
162	120	96.8	186.4	
584	300	245.9	37.3	
590	440	341.1	57.9	
684	385	356.4	73.2	

TABLE I - Continued

INDIVIDUAL DATA ON THE RADIOACTIVE COUNT OF EACH EWE THROUGH THE FIRST FIVE OBSERVATIONS MADE OF THYROID ACTIVITY. THE GROUP AVERAGES, AND THE AMOUNT BY WHICH EACH EWE'S COUNT DIFFERED FROM THE MEAN OF THE GROUP ARE ALSO GIVEN

Ewe No.	Total Counts/Min.	Counts/Min. Per 100 Lbs.	Difference From Mean Count of Group	
Group III (Four Hour Check)				
558	325	295.5	119.4	
585	150	114.5	61.6	
176	460	403.5	227.4	
575	90	69.8	106.3	Group Mean
679	70	58.3	117.8	762.4
92	180	156.5	19.6	
232	285	254.5	78.4	
546	215	182.2	6.1	
Group I (24 Hour Check)				
27	850	779.8	94.7	
541	1440	1142.9	457.8	
215	710	591.7	93.4	
555	850	685.5	0.4	Group Mean
691	810	743.1	58.0	685.1
589	795	552.1	133.0	
574	490	411.8	273.3	
277	640	512.0	173.1	
591	1000	740.7	55.6	
Group II (24 Hour Check)				
502	920	814.2	59.1	
517	1070	877.0	166.6	
526	925	651.4	110.9	
145	745	496.7	265.6	Group Mean
162	465	375.0	387.3	762.3
584	975	799.2	36.9	
590	1215	941.9	179.6	

TABLE I - Continued

INDIVIDUAL DATA ON THE RADIOACTIVE COUNT OF EACH EWE THROUGH THE FIRST FIVE OBSERVATIONS MADE OF THYROID ACTIVITY. THE GROUP AVERAGES, AND THE AMOUNT BY WHICH EACH EWE'S COUNT DIFFERED FROM THE MEAN OF THE GROUP ARE ALSO GIVEN

Ewe No.	Total Counts/Min.	Counts/Min. Per 100 Lbs.	Difference From Mean Count of Group	
		Group III (24 Hour Check		
585	425	324.4	253.6	
176	1335	1171.1	593.1	
575	285	220.9	357.1	
679	625	520.8	57.2	Group Mean
92	700	608.7	30.7	578.0
232	800	714.3	136.3	
546	1000	847.5	269.5	
558	660	600.0	22.0	
		Group I (48 Hour Check)		
27	1070	981.7	219.3	
541	1100	873.0	110.7	
215	740	616.7	145.7	
555	1330	1072.6	310.2	Group Mean
691	825	756.9	5.5	1013.3
589	1260	875.0	112.6	
574	605	508.4	253.9	
277	575	460.0	302.4	
591	935	692.6	69.8	
		Group II (48 Hour Check)		
502	1300	1150.4	137.1	
517	1205	987.7	25.6	
526	1330	936.3	76.7	
145	950	633.3	380.0	Group Mean
162	730	588.7	424.6	1013.3
584	1365	1118.9	105.5	
590	1320	1023.3	9.9	
684	1285	1189.8	176.5	

TABLE I - Continued

INDIVIDUAL DATA ON THE RADIOACTIVE COUNT OF EACH EWE THROUGH THE FIRST FIVE OBSERVATIONS MADE OF THYROID ACTIVITY. THE GROUP AVERAGES, AND THE AMOUNT BY WHICH EACH EWE'S COUNT DIFFERED FROM THE MEAN OF THE GROUP ARE ALSO GIVEN

Ewe No.	Total Counts/Min.	Counts/Min. Per 100 Lbs.	Difference From Mean Count of Group	
		Group III (48 Hour Check)		
585	480	366.4	237.8	
176	1835	1609.7	1005.4	
575	285	220.9	383.3	
679	655	545.8	58.4	Group Mean
92	690	600.0	4.2	604.2
232	555	495.5	108.7	
546	905	766.0	162.7	
558	685	622.7	18.5	
		Group I (72 Hour Check)		
27	895	821.1	50.1	
541	1870	1484.1	612.9	
215	770	641.7	229.5	
555	1175	947.6	76.4	Group Mean
691	1100	1009.2	137.9	871.2
589	990	687.5	183.1	
574	1075	903.4	32.2	
277	570	456.0	415.2	
591	1300	963.0	91.8	
		Group II (72 Hour Check)		
502	1075	951.3	13.2	
517	1335	1100.7	136.1	
526	1065	750.0	214.5	
145	1168	778.7	185.9	Group Mean
162	610	491.9	472.6	964.2
584	1090	893.4	71.1	
590	1325	1027.1	62.6	
684	1360	1259.3	294.7	

TABLE I - CONTINUED

INDIVIDUAL DATA ON THE RADIOACTIVE COUNT OF EACH EWE THROUGH THE FIRST FIVE OBSERVATIONS MADE OF THYROID ACTIVITY. THE GROUP AVERAGES, AND THE AMOUNT BY WHICH EACH EWE'S COUNT DIFFERED FROM THE MEAN OF THE GROUP ARE ALSO GIVEN

Group III
(72 Hour Check)

Ewe No.	Total Counts/Min.	Counts/Min. Per 100 Lbs.	Difference From Mean Count of Group	
585	640	488.6	121.6	
176	1400	1228.1	617.0	
575	480	372.1	238.1	
679	415	345.8	264.3	Group Mean
92	990	860.9	200.7	610.2
232	435	388.4	221.8	
546	855	724.6	114.4	
558	935	850.0	239.8	

Group I
(96 Hour Check)

Ewe No.	Total Counts/Min.	Counts/Min. Per 100 Lbs.	Difference From Mean Count of Group	
27	680	623.9	32.1	
541	1580	1253.9	662.3	
215	490	408.3	183.4	
555	790	637.1	45.4	Group Mean
691	345	316.5	275.2	591.7
589	590	409.7	181.9	
574	635	533.6	58.1	
277	390	312.0	279.7	
591	1050	777.7	186.1	

Group II
(96 Hour Check)

Ewe No.	Total Counts/Min.	Counts/Min. Per 100 Lbs.	Difference From Mean Count of Group	
502	885	769.6	121.6	
517	550	450.8	197.1	
526	635	447.2	200.8	
145	1085	723.3	75.4	Group Mean
162	635	504.1	143.9	647.9
584	695	569.7	78.3	
590	1045	810.1	162.1	
684	535	495.4	152.6	

TABLE I - Continued

INDIVIDUAL DATA ON THE RADIOACTIVE COUNT OF EACH EWE THROUGH THE FIRST FIVE OBSERVATIONS MADE OF THYROID ACTIVITY. THE GROUP AVERAGES, AND THE AMOUNT BY WHICH EACH EWE'S COUNT DIFFERED FROM THE MEAN OF THE GROUP ARE ALSO GIVEN

Group III
(96 Hour Check)

Ewe No.	Total Counts/Min.	Counts/Min. Per 100 Lbs.	Difference From Mean Count of Group	
585	285	217.6	273.5	
176	1680	1473.7	982.6	
575	335	259.7	231.4	
679	350	291.7	199.4	Group Mean
92	630	547.8	56.7	491.1
232	445	397.3	93.8	
546	740	627.1	136.0	
558	485	440.9	50.2	