STUDIES ON THE REPRODUCTION OF THE GROUND SQUIRREL WITH REFERENCE TO PITUITARY IMPLANTATION, VAGINAL SMEARS AND LIGHT

by

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INTRODUCTION

During the past nine years, members of this laboratory at the Kansas State College of Agriculture and Applied Science have carried on rather extensive research on the

thirteen-lined ground squirrel, Citellus tridecemlineatus (Mitchill), with reference to: hibernation and its relation to reproduction by Johnson (1928, 1929a, 1929b, 1930, 1931) and Johnson and Hanawalt (1930); the effects of pituitary implantation by Johnson and Wade (1931), Foster (1931); determination of the sexual cycle in the female by the vaginal smear method by Coco (1932); and the influence on the reproductive cycle of such controllable conditions as a limited food supply, a high protein diet, and ultraviolet radiations by Johnson and Wade (1931) and Coco (1932).

The work on pituitary implantation has given rather conclusive results in the effect on the female ground squirrel. However the effects of implants on the male have been rather confusing and not at all conducive to drawing definite conclusions. Also among other workers in this same field, the effect of the pituitary gland on the male gonad had been slighted, while attention has been centered on the interrelationship between the hypophysis and the ovaries.

The purpose of this work therefore has been to gain some definite information on the effect of pituitary implants on the reproductive system of the adult male ground squirrel, to fill in some of the gaps in the earlier work on the oestrous cycle of the female as determined by vaginal

smears, and also to carry out more extensive observations on the relation of light to reproduction.

REVIEW OF LITERATURE

The early work of Smith (1926), Smith and Engle (1927) and Zondek and Aschheim (1927) has shown quite clearly that implantation of the anterior lobe of the hypophysis as well as injection of the urine of pregnant women causes marked stimulation of the reproductive system. The effect of such stimulation on the reproductive system of the male has not received as much attention as that of the female (Smith, 1926, 1927; Smith and Engle, 1927; Zondek and Aschheim, 1927, 1928; Allen, 1928; Engle, 1928; Hartman, 1930; Claus, 1931; Johnson and Wade, 1931; Foster, 1931; Coco, 1932; and others).

Evans and Simpson (1926) discovered considerable diminution of testes weights, small tubules but no evidence of degenerative changes in male rats injected with alkaline hypophyseal extracts. Smith (1927) found that ten daily pituitary homeoimplants into immature male rats caused a marked increase in the weight of the genital system exclusive of the gonads. After 17 implants, the weight of the testes of the pituitary implanted animals were almost twice the weight of those of the litter-mate controls. Smith and

Engle (1927) state that the testes of immature mice and rats show a more variable response to implants than do the ovaries, but the remaining organs are increased in weight and physiological activity. In the adult male, aside from a possible increase in libido, no response of the genital system is elicited by the implants. No increase in interstitial tissue and no acceleration of spermatogenesis was observed. Larson, Bergeim, Barber and Fisher (1929) state that the testes of rats receiving anterior pituitary extract show active spermatogenesis but a more than normal amount of degeneration of the germ cells. Weights of testes of injected rats were less than those of the controls. This may be attributed to the proteins injected as Oslund (1926) has shown that injections of this type may lead to degeneration of the seminiferous tissue. Injections of human pregnancy urine produces in the male genital tract, marked hypertrophy of the sex apparatus with the exception of the testis, a marked increase in the interstitial cell mass, but no acceleration of spermatogenesis (Engle, 1929). Injections of the gonad stimulating substance, urinary hebin, according to Moore and Price (1931) causes a marked increase in the size of the prostate glands and seminal vesicles in the male rat. Visible effects upon the testis were restricted to slight increases in interstitial cell tissue. However fresh pitui-

tary homeo-implants have been followed by slight increase in gross measurements of the testis, little or no increase in tubular diameter, little if any acceleration of spermatogenesis and decided increase in the interstial tissue.

At variance with all the results obtained in mammals are the results of Burns (1930). Following implantation of pituitary gland tissue or injection of alkaline extracts into Amblystoma larvae, the most striking effect is upon the male gonads, producing marked precocious enlargement of the testes and advance spermatogenesis including the formation of almost mature spermatozoa.

In male dogs, no hypertrophy of the genital tract is produced by injecting an alkaline extract of the anterior lobe of the hypophysis as performed by Benedict, Putnam and Teel (1930).

Hastened differentiation of the gonads of the albino rat from injecting macerated whole pituitary gland shows itself in both sexes between the tenth and fifteenth day of postnatal life, being more pronounced in the male (Corey, 1930). Parkes and Hill (1930) have found that injection of anterior pituitary preparations into the anoestrous ferret causes far more change in the females than in the males which failed to show either spermatogenesis acceleration or appreciable testis increase. Smith (1930) has found that daily

homeo-implants into hypophysectomized rats will restore the sexually deficient animal to normal or almost normal condition. Engle (1932) produced an increase in testis size which was found to be due to increased tubule growth and increase in interstitial cell mass in monkeys by injection of pregnancy urine and pyridin extraction of powdered sheep pituitary as developed by Fevold, Hisaw and Leonard (1931).

Other rodents commonly used for laboratory work do not give us much information concerning reproduction in the ground squirrel. The guinea pig has an average cycle of 16 days (oestrus lasting about 24 hours) with corresponding changes in the ovaries, uterus, and vagina. These changes are accompanied by characteristic vaginal smears (Stockard and Papanicolaou, 1917, 1919; Selle, 1922). Allen (1922) found the mouse to have a four to six day cycle with characteristic vaginal smears for each phase of the cycle. Long and Evans (1922) found the rat to have a four day cycle with characteristic vaginal smears, accompanied by histological changes throughout the genital tract.

Murphey (1922), Trei and Metzger (1926) in working out the oestrous cycle of the cow; Hartman (1923), the oestrous cycle of the oppossum; and Evans and Cole (1928), the oestrous cycle of the dog, each used the vaginal smear method.

Rowan (1929) found that by appropriate manipulation of lighting conditions to which wild Juncos were subjected, it was possible to produce premature recrudesence in the middle of the winter (in the case of testis up to sperm formation). Increased periods of exercise in almost complete darkness produced the same results. It was therefore suggested that light increases in reality afford the birds increased opportunity for exercise. Parkes and Hill (1930), working with the ferret, and Johnson, Foster and Coco (unpublished) with the ground squirrel, tried ultra-violet light radiations without effect. Bissonnette (1932a) treated anoestrous male and female ferrets with light from 200 watt incandescent electric light bulbs for six to six and one-half hours per night. He found that the females came on heat, copulated and became pseudo-pregnant with mammary gland activity. In case of the males, results were less apparent, spermatogenesis being stimulated only to secondary spermatocyte and rare spermatid formation; no spermatozoa were found even at 71 days. It is well to note that he used only a few animals. Marshall (1932) states that bats copulate in the fall and do not ovulate until spring. He suggests that delayed ovulation may be due to comparative absence of light stimulus.

Bissonnette (1932b) found that by subjecting the European starling (Sturnus vulgaris) to filtered light of a

known wave length, spermatogenic activity could be accelerated. By the thirty-third day, red lighted birds showed the greatest acceleration of testis activity, those receiving white light were more active than the controls and green lighted birds were definitely inhibited.

METHODS AND MATERIALS

General

The ground squirrels used for this study were obtained from west-central Kansas. They were of the variety Citellus tridecemlineatus arenicola (Howell), the southern variety recently split off from Citellus tridecemlineatus pallidus (Allen).

Animals were selected as near the same sexual condition as possible. Abnormally fat or thin animals were not used in any of the experiments. The animals were kept in wire cages which were cleaned daily, at which time they were given feed and water.

The temperature in the laboratory was maintained at about 25° to 32° C.

Histological Technique

Allen's modification of Bouin's fluid was used universally as a fixative for tissue of all types and Kornhauser's

(1930) hematin method with eosin-bluish as a counterstain was used. Heidenhain's iron hematoxylin gave excellent results for staining testicular tissue but was abandoned because of the time element involved.

Vaginal Smears

Vaginal smears were prepared daily from laboratory females, by means of a toothpick, the end of which was wrapped with absorbent cotton and dipped in Locke's physiological saline. This was then gently inserted into the vagina and the walls lightly scraped. By this means it was possible to obtain enough cells to make a smear characteristic of the vaginal contents without any harm to the animal. Wright's stain and hematin and eosin-bluish were used as these stained the cells almost instantly.

Implantation

Male animals which were not scrotal and not pigmented were given implants of one rat pituitary daily for from eight to sixteen days. External genital conditions were noted each day at the time of implantation. These experiments were conducted at intervals from October to January and June to August.

Owing to the variation in the sexual condition of dif-

ferent individuals, the technique which had been previously worked out whereby each animal could serve as its own control was followed.

The procedure was as follows: the animal was anaesthetized, a small incision made through the linea alba, the tunic of the right testis pierced with a spear-point needle and a few tubules gently forced out to provide a sample before implantation. Both testes were carefully measured in three dimensions with a pair of calipers. Other animals were treated in the same manner, later to be implanted with macerated brain tissue to determine the effect, if any, of the operation on the gonads.

After allowing three to four days for recovery, implantation was begun. One assistant killed an adult male albino rat, opened the skull dorsally, removed the gland wholly and placed it in a small vial containing Locke's physiological solution heated to about body temperature. The gland was then picked up in a sterile hypodermic syringe. While a second assistant held the animal, the gland was injected subcutaneously along the back being macerated as it was forced through the needle. The entire procedure required from one to two minutes and no anaesthetic was used.

Each animal was implanted for four successive days, and on the fifth was again anaesthetized, the right testis was

removed, measured and prepared for histological study. After eight, twelve or sixteen implants the animal was autopsied and the left testis as well as the Cowper's glands, prostate and seminal vesicles were removed, measured, weighed and prepared for histological examination.

Light

On December 1, 1932, approximately 36 male and 36 female ground squirrels were placed in wire cages, about six animals to each cage. In all cages male and female animals were kept together at all times. Above the cages, especially constructed galvanized tin carriers were suspended; these were built with an extra jacket around the inner one so as to allow no unfiltered light to escape and at the same time allow a current of air to circulate through the lamps preventing them from getting hot enough to crack the glass light filters used.

One group of animals was subjected to red light of wave length .000058 to .000066 cm. produced by placing a 200 watt incandescent electric light bulb in the carrier and fitting it with a Heat Resistant Signal Red Number 243 glass filter furnished by the Corning Glass Works, makers of all the filters used. Data furnished by the company gave us the information that this filter transmitted 10 per cent of

light. The filament of the light was placed at 22 inches from the base of the cage and as the 200 watt bulb in the housing had previously been tested by the Electrical Engineering Department of this college and found to have a candle power of 284 c.p., the illumination at the floor of the cage was computed to be approximately 8.5 foot candles.

For another group, a similar setup was used with the exception that a Heat Resistant Lantern Blue Number 554 filter was used which gave us 8.5 foot candles of light of wave length .0000345 to .000049 cm. as checked by Dr. J. O. Hamilton of the Department of Physics.

A third group received only 1.856 foot candles of wave length .000047 to 000055 cm. It was found impossible to subject these animals to as much light as the others because the only available filter, Heat Resistant Sextant Green Number 401, transmitted only 2 per cent of light.

Another group received 8.5 foot candles of white unfiltered light, and a fifth group received ultra-violet radiations. The apparatus used was a mercury arc ultra-violet lamp. This lamp transmits slightly more ultra-violet light than is found in sunshine and according to Dr. J. S. Hughes of the Chemistry Department of this college, will cure rickets in chickens in one-half hour radiations.

Just across the corridor, control animals were kept in semi-darkness, receiving no artificial light but exposed to the daylight in the animal house, which the experimental animals also received.

The lights were all controlled by an automatic time switch which turned them on at 5:00 p.m. and turned them off at 12:00 p.m.

Temperature variations at irregular intervals throughout the time of the experiment may be found by referring to Table 1.

Animals in a single group, each of which was subjected to different lighting conditions were selected as near the same sexual condition as possible.

The animals were all inspected for the first time on December 13, and thereafter once a week until January 26. From that date until May 19, they were examined twice weekly. After that time as most of the animals had returned to the inactive condition, once a week was thought to be sufficient.

RESULTS OF VAGINAL SMEARS

The period from July to December may be said to be the anoestrous period of the laboratory female ground squirrel (Citellus tridecemlineatus). January to June is, generally

Table 1. Temperature Variations in Degrees Centigrade Taken at Irregular Intervals Among the Groups of Light Treated Animals.

Date	Red	Green	White	Blue	Ultra-violet	Control
1933						
Jan. 11	32°	32°			34°	
Jan. 17			32.5°	34°		30°
Jan. 21						
Jan. 27		33°			32.5°	28°
Feb. 2	30°		34°		35°	
Feb. 18	29°				33-°	28-°
Feb. 28		31°	32-°	30.5°	34°	
Mar. 5			32°		34°	32°
Mar. 20	33°		32°		34°	
Mar. 31		32°		31°		30°
Apr. 7	32°			32°	33°	
Apr. 14			32°	33°		31°
Apr. 21		30°			32°	29°
Apr. 29	32°	32°	33°			
May 5		30°		28°	31°	
May 9	34°		31°			30°
May 16		33°	32.5°		33.5°	
June 2		34°			35°	33°
June 12	35°	34°	34°			
June 20	37°			38°		36°
June 24		40°	39°	38°	40°	37.5°

speaking, the period of sexual activity and is accompanied by marked histological changes in the ovaries, uterus and vagina, as well as characteristic changes of the external genitalia (Johnson, Foster and Coco, unpublished). During this active period, daily vaginal smears have been prepared from a group of laboratory females. In this work, we have attempted to use the nomenclature developed by Long and Evans (1922) in their studies of the oestrous cycle of the dioestrous rat.

The ground squirrel has an anoestrous cycle or only one heat period during the year. In most cases we have found a sequence of stages comparable to those found in the rat. However, there is a wide variation in the length of time which the ground squirrel stays in any one stage, in fact, there is so great a variation that to give a definite time limit for any period would be quite misleading, in view of the actual condition existing. Therefore any attempt to divide the oestrous cycle into definite time periods must be an approximation and subject to change after further investigation.

The smear taken as soon as the vagina is fully open is something akin to Long and Evans' Stage 1 in the rat. It is characterized by a predominance of large epithelial cells with small nuclei, however, in no case has the writer

observed a smear of large, nucleated, epithelial cells in which no cornified cells were present, nor conversely, are cornified cells ever predominate without a variable number of large nucleated epithelial cells also present in the same smear. During Stage 1, the walls of the vagina are dry and smooth and a faint pink to pink white in color. It is estimated that Stage 1 lasts approximately from five to twelve days.

Stage 2 has been interpreted as a predominance of cornified epithelial cells without nuclei, though as has been stated, many large nucleated epithelial cells are present. It is thought that the large epithelial cells of Stage 1 give rise to the cornified cells characteristic of oestrum (Stage 2) by a loss of their nuclei. The lips of the vulva are swollen, and the mucosa is white, dry and greatly folded. The length of Stage 2 varies greatly. In one case an animal stayed in oestrum for thirty days; no male was present in the cage. Two experimental female animals were kept with a male ground squirrel but did not show a normal cycle, each smear being characterized by an enormous number of leucocytes. Again an animal may remain in oestrum approximately seven days, going on into Stages 4 and 5 and then returning to Stages 1 and 2 again, giving what might be called a diostrous cycle within the

anoestrous cycle.

Corresponding to Stage 3 in the rat; it is usually found following oestrum that large masses of cells have sloughed off from the walls of the vagina and are present in the lumen. A typical smear of this period shows great masses of large epithelial and cornified cells. The period lasts approximately four days.

Following this the smear is characterized by the invasion of large numbers of polymorphonuclear leucocytes which seem to accumulate around the cornified cells and literally devour or chew them up. This is Stage 4 and is only present a day or so when the smear shows in addition to the leucocytes, cells of a type, which we have called small epithelial cells. These cells are small and rounded and have large nuclei. Small epithelial cells and leucocytosis seem always to be closely associated. This has been called Stage 5; the animal now seems to alternate back and forth between Stage 4 and Stage 5 until the lips of the vulva fuse and the vagina is closed, unless a recurrence of this cycle takes place.

THE EFFECTS OF PITUITARY IMPLANTS ON MALE GROUND SQUIRRELS

The Testes

There is less individual variation among the male ground squirrels than in the case of females, from the standpoint of sexual activity. Most of the adult laboratory males are inactive from about July until December; during this period the testes are small, withdrawn into the abdomen and there is little or no pigmentation in the inguinal region. Males which were in the sexually inactive condition were used for the implantation experiments.

The work in this laboratory of implanting whole pituitary glands from rats into male ground squirrels has been done by Johnson and Wade (1931), Foster (1931) and Coco (1932) in connection with similar work on the female animals. Their results may be summed up briefly: In some cases, a slight degree of enlargement of the testes could be noted after the fifth implant. These measurements were obtained without removing the gonad from the body cavity. Histological examination revealed very evident changes in spermatogenesis which generally consisted in the formation of secondary spermatocytes and spermatids. No spermatozoa were found.

In animals implanted with anterior pituitary tissue a very striking increase in testis size is noted which equals

that found in normal animals completely scrotal. This increase in length and diameter of the gonad is very evident by the fourth successive day of implantation. The average size of the left testis of the experimental animals before implantation was about 10 x 5 x 5 mm. After four pituitary implants the average size of the same organ was found to be 14 x 6 x 6 mm. In those animals which were killed after a total of eight implantations the average size of the left testis was 16 x 8 x 7 mm. Those receiving 12 implants, the gonads were found to average 16 x 7 x 7 mm. In those animals autopsied after 16 consecutive days of implantation, the average size of the left testis was found to be 15 x 8 x 8 mm. For data on the individual animals, refer to Table 2.

The reason for this increase in testis size was found to be an enlargement of the seminiferous tubules (Table 2). Histological examination revealed little if any hypertrophy of the interstitial tissue.

The control animals, receiving the same treatment as the experimentals, but being implanted with brain tissue showed no increase in testis size with the exception of one animal No. 131, which showed a slight increase in the size of the left testis. Histological examination of this testis showed that the animal was probably entering the

Table 2. Effects of Daily Implants of Rat Pituitaries into Male Ground Squirrels.

Number of animal	Date begun	Size of Testes After Implantations — Number of Implants: 0	4	8	12	16	Weight of animals in gms. after implantations — Number of Implants: 0	4	8	12	16	Weight at autopsy in mg.: Prostate	Cowper's	Seminal vesicles	Diameter of Tubules in Micra — Number of Implants: 0	4	8	12	16
	1932	R13x7x6	15x8x7																
188 Exp.	Oct.20	L13x7x6	14x7x6			17x9x9	188	145			108	30	20	72	120	147			149
		R 8x6x5	14x5x5																
1713 Exp.	Oct.28	L 8x5x4	12x7x6		14.5x8x7		171	155		144		32	35	135	102	127		144	
		R12x6x5	12x6x5																
131 C	Nov.11	L12x6x5	12x6x5			14x8x7.5	131	129			127	9	8	20	144	131	143		
		R11x5x5	14x6x6																
1785 Exp.	Nov. 3	L12x6x5	14x6x6	17x8x7			178	157	147			44	30	130	121	136	161		
		R10x4x4																	
1205 Exp.	Nov.12	L10x5x5	10x5x5		13.5x7x6.5		120	112			97	25	30	50	97	135			130
		R 9x5x4	14.5x7x6																
150 Exp.	Nov.12	L 9x5x5	14x6x5		14.5x7.5x6.5		150	141		138		20	35	70	98	147		152	
		R12x6x6	12.5x6x6.5																
176 C	Nov.27	L12x7x6	13x7x6		12.5x7x5.5		176	170		153		10	15	12	104	113		109	
		R9x5x4	13x7.5x7																
141 Exp.	Nov.20	L10x7x4	14.5x7.5x6			16x7x9.5x9	141	110			89	55	140	270	106	158			167
		R10x5x5	13x6.5x5																
147 Exp.	Nov.12	L10x5x5	16x7.5x7		21x7.5x7		147	128		118		20	40	100	101	123		137	
		R12x5x5	15x7.5x7																
151 Exp.	Nov.25	L11x5x4	15x7.5x7.5	16.3x8.5x7			151	137	126			25	60	95	96	152	147		
		R12x5x4	15x7.5x6																
1515 Exp.	Nov.27	L12x6x5	14x8x6.5		14.5x8.5x7		151	151		129		33	42	64	112	121		125	
		R13x6x5	16x8.5x7																
172 Exp.	Dec. 5	L12x7x6	16x8.5x8	16.5x9x8			172	175	170			35	30	60	114	136	144		
		R10x6.5x6	10x6.5x6																
1165 C	Dec. 5	L11x6x6	11x6x6	11x6x6			116	126	128			12	12	15	110	106	120		
	1933	R5x3x2.5	11x5x4																
1695 Exp.	June 13	L5x3x3	11x4.5x4	11x6x5			109	109	117			35	38	77	71	101			126
		R10x4x4	11x4x4																
1696 Exp.	June 13	L9x4x4	11x5x4		15x7x7		113	111		120		35	25	75	61	100		123	
		R7x3x3																	
1697 C	June 13	L7x3.5x3	7x3.5x3.5		9x4x3		101	92		117		23	5	25	63	65			

normal sexually active period.

Upon microscopic study of the testes of the anterior pituitary implanted animals, very striking changes are noted. Sections of the right testes of the animals before implantation present a rather definitely uniform picture. In no case did the tubules possess lumina. Each tubule is lined with a definite layer of spermatogonial cells around the outside while the center is filled with great numbers of spireme stages of primary spermatocyte cells. In a very few cases, metaphase and anaphase stages of the first maturation division were found. In a limited number of the tubules before implantation, a few secondary spermatocytes were found.

After four pituitary implants the picture has definitely changed. A large lumen is present in most of the tubules, though in some, a lumen is just beginning to appear. The walls of the tubule are still quite thick and composed of the ever present layer of spermatogonial cells around the edge. Inside of these are numerous spireme stages of primary spermatocytes and an increased number of metaphase and anaphase stages of the first maturation division. Next to the lumina of the tubules are numerous secondary spermatocytes, both in resting stages and in active division. Spermatids are found rarely in some of the tubules.

Histologically, the seminiferous tubules of animals receiving eight, twelve or sixteen implants present a characteristic appearance. The tubules have greatly enlarged, and are almost entirely cleaned out of germinal and intercellular tissue. In some cases, the lumina of the tubules are enormous, being entirely free of germinal tissue and only the basement membrane remaining. In others a thin wall may remain composed of a single line of spermatogonial cells, and in some cases secondary spermatocytes and spermatids.

Control animals did not show an increase in tubule size, except in the case previously mentioned, comparable to the pituitary implanted animals. By autopsy, a small lumen was present in some of the tubules, spermatogonial and primary spermatocytes were abundant.

In no case did implantation produce scrotality or cause excessive pigmentation in the inguinal region.

The Accessory Glands of Reproduction

Implantation of anterior pituitary glands into male ground squirrels causes a marked hypertrophy of the accessory glands of reproduction and increase in weight when compared with those of control animals autopsied at the same time (Table 2).

The ducts of the prostate glands of the inactive control animals are entirely closed; in a few, small lumina may show but in most cases the site of the duct is marked by a small compact nest of darkly staining secretory cells inclosed in more or less concentric rings of more lightly stained, widely dispersed non-secretory cells. Pituitary implanted animals however show a distinct change in the gland; the lumina of the acini are evident, the ducts being lined with a rather low cuboidal epithelium.

Heller (1932) found that Cowper's glands of the rat react to sex hormone conditions too slowly and are too variable to be used as an assay method. Though no attempt has been made to use the glands of the ground squirrel for assay, it is found that the Cowper's glands respond quickly to implantation, and this response is more marked than that shown by either the prostate or the seminal vesicles. In the sexually inactive controls, the bulbo-urethral glands are quite small, the secretory ducts are lined with a low cuboidal epithelium whose cells are very little larger than the nuclei. The average height of the secretory epithelium is about 7 micra. In contrast, Cowper's glands of the experimental animals have enlarged enormously; the acini spaces are distended with secretion, secretory epithelium has become greatly proliferated and averages from 16 to 18

micra in thickness. The tall columnar cells are quite characteristic and have their nuclei located near the basal ends of the cells.

The seminal vesicles of the experimental animals showed an enormous increase in size and weight over the controls as well as marked histological changes. The glands of the control are characterized by rather small ducts lined with a low cuboidal epithelium about 7 to 9 micra in thickness. After implantation with pituitary glands the ducts have enlarged and are filled with secretion; the epithelial cells have assumed the columnar appearance and the layer averages about 20 micra in width. The eccentric nuclei appear slightly elongated and are located near the basal ends of the cells.

THE EFFECTS OF LIGHT ON THE REPRODUCTIVE CYCLE

For information concerning the cycle of any one animal or the average condition of any single group of animals subjected to one type of light, reference is made to Tables 3 and 4.

As will be noted by a study of Table 3, there was very little variation among the differently treated groups of males. Most of the animals at the beginning of the experiment were sexually inactive, some perhaps were slightly

Table 3. Sexual Condition of Male Animals Subjected to Artificial Light as Observed at Bi-weekly Intervals

No.	Dec. 1	Dec. 13	Jan. 5	Jan. 19	Feb. 3	Feb. 17	Mar. 3	Mar. 17	Mar. 31	Apr. 14	Apr. 28	May 12	May 27	June 16	June 24	Average sexual activity of group
Red Light																
674	0*	0	2	4	5	5	-	-	-	-	-	-	-	-	-	
627	0	0	2	4	6	6	6	6	6	3	2	0	0	0	0	
635	0	0	2	3	6	3	-	-	-	-	-	-	-	-	-	
542	0	6	6	6	6	5	4	3	3	3	2	0	0	0	0	
669	0	0	2	4	5	5	6	6	6	3	0	0	0	0	0	
Ave.	0	1	3	4	6	5	5	5	5	3	1	0	0	0	0	2.7
Green Light																
670	1	2	2	4	6	6	6	6	6	6	5	5	3	0	0	
538	0	2	5	5	6	6	6	6	6	6	5	5	-	-	-	
672	1	2	2	2	2	4	-	-	-	-	-	-	-	-	-	
678	0	2	5	2	4	5	5	6	6	6	3	1	0	0	0	
649	0	1	5	-	-	-	-	-	-	-	-	-	-	-	-	
Ave.	0	2	4	3	5	5	6	6	6	6	4	4	2	0	0	3.7
White Light																
623	1	4	5	6	6	6	6	5	5	5	3	0	0	0	0	
637	0	0	0	4	5	6	6	6	6	6	6	3	2	2	3	
642	1	2	6	6	6	6	6	6	6	3	1	0	0	0	0	
671	0	2	4	4	6	4	5	6	6	6	6	2	2	0	0	
379	0	4	5	5	5	6	6	6	5	4	1	1	1	1	0	
Ave.	0	2	4	5	6	6	6	6	6	5	5	1	1	1	0	3.7
Blue Light																
539	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	
667	0	0	2	3	4	6	6	6	6	6	6	6	1	1	0	
650	0	2	2	3	4	3	4	6	6	6	6	6	5	5	3	
631	1	2	5	4	4	5	5	5	5	6	3	1	1	1	0	
648	1	1	4	3	4	3	3	3	3	3	1	2	2	0	0	
Ave.	0	1	2	3	4	4	5	5	5	5	4	4	3	1	0	3.2
Ultra-Violet																
131	1	5	5	5	5	5	6	6	3	0	0	0	0	0	0	
666	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	
651	0	0	4	5	5	5	5	5	5	-	-	-	-	-	-	
643	1	0	4	5	5	5	5	1	0	0	0	0	0	0	0	
640	0	2	5	5	5	5	6	5	3	2	2	0	2	0	0	
Ave.	0	2	5	5	5	5	6	4	3	1	1	0	1	0	0	1.7
Controls																
537	0	0	4	4	5	6	6	6	6	5	5	5	3	0	0	
668	0	0	0	2	5	5	5	5	5	1	1	1	0	0	0	
647	1	4	4	5	-	-	-	-	-	-	-	-	-	-	-	
632	0	2	2	2	2	2	3	3	3	3	3	3	3	0	0	
639	0	0	0	2	6	-	-	-	-	-	-	-	-	-	-	
Ave.	0	1	2	3	4	5	5	5	5	3	3	3	2	0	0	2.8

0, No sexual activity. Testes small and in abdomen.
1, Testes medium and in abdomen
2, Testes medium; animal partly scrotal.
3, Testes medium; animal partly scrotal. Inguinal region slightly pigmented.
4, Testes large; animals scrotal and slightly pigmented.
5, Testes large; animal scrotal and deeply pigmented.
6, Testes very large, animal scrotal and deeply pigmented.
-, Animal dead.

Table 4. Sexual Condition of Female Animals Subjected to Artificial Light as Observed at Bi-weekly Intervals.

No.	Dec. 1	Dec. 13	Jan. 5	Jan. 19	Feb. 3	Feb. 17	Mar. 3	Mar. 17	Mar. 31	Apr. 14	Apr. 28	May 12	May 27	June 16	June 24	Average sexual activity of group
Red Light																
654	0*	0	0	0	0	2	4	2	1	0	0	0	0	0	0	
600	0	0	1	1	4	4	2	1	1	0	0	0	0	0	0	
619	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	
624	0	0	0	0	1	1	3	3	3	3	0	0	0	0	0	
544	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Ave.	0	0	0	0	1	1	2	2	1	0	0	0	0	0	0	½
Green Light																
540	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
675	0	0	0	0	0	2	4	4	4	3	3	1	0	0	0	
645	0	0	0	0	1	1	2	1	4	4	4	4	2	2	1	
677	0	0	2	2	2	2	2	0	0	0	0	0	0	0	0	
628	0	0	0	0	0	2	3	3	3	2	3	4	4	2	0	
656	0	0	0	0	1	4	5	4	4	3	1	0	0	0	0	
Ave.	0	0	1	1	1	2	3	2	3	2	2	2	1	1	0	1½
White Light																
625	0	1	1	1	2	5	3	2	2	3	1	1	1	1	0	
655	0	0	0	0	1	2	4	4	3	3	4	4	4	4	3	
615	0	0	1	1	1	1	3	3	4	5	3	4	5	3	2	
541	0	0	0	1	2	2	4	4	4	3	1	0	0	0	0	
658	0	0	0	0	0	1	4	4	3	4	3	4	4	4	3	
Ave.	0	0	0	1	1	2	4	3	3	3	2	3	3	2	1	2
Blue Light																
644	0	0	0	0	0	0	0	1	2	1	1	1	0	0	0	
543	0	1	2	3	2	3	-	-	-	-	-	-	-	-	-	
661	0	0	0	0	0	0	1	2	2	1	4	3	1	0	0	
602	0	1	1	1	1	3	4	4	4	-	-	-	-	-	-	
659	0	0	0	0	0	0	0	1	2	4	5	3	2	0	0	
618	0	1	0	0	0	1	1	4	5	4	4	4	0	0	0	
Ave.	0	1	1	1	1	1	1	2	3	3	4	3	1	0	0	1 4/7
Ultra-Violet																
616	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
210	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
665	0	0	0	0	2	4	litter'	0	0	0	0	0	0	Torpid	Torpid	
633	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	
657	0	0	0	2	1	1	0	0	0	0	0	0	0	0	0	
601	0	0	0	2	2	4	4	4	3	3	3	0	0	0	0	
Ave.	0	0	0	1	1	2	1	1	1	1	1	0	0	0	0	9/14
Controls																
662	0	0	0	0	0	0	1	5	4	-	-	-	-	-	-	
620	0	0	0	0	1	1	1	1	2	5	2	2	0	0	0	
545	0	0	0	0	0	0	0	1	2	4	5	4	4	2	2	
613	0	0	0	0	0	0	1	1	2	4	5	4	4	1	1	
653	0	0	0	0	1	2	4	4	3	2	1	1	1	0	0	
676	0	0	1	0	2	3	3	3	4	4	3	2	2	0	0	
Ave.	0	0	0	0	1	1	2	3	3	4	3	3	2	1	1	1 5/7

*0, No sexual activity. Vulva not swollen.
2, Vulva slightly swollen and partly open.
4, Vulva swollen; walls of the vagina white and dry and the vaginal mucosa greatly folded (estrum).
-. Animal dead.
1, Vulva slightly swollen but not open.
3, Vulva swollen; walls of the vagina pink and dry.
5, Presence of large masses of cellular debris in the vaginal lumen (cheesy stage).
', Litter of four born on Feb. 23.

farther advanced than others as the table shows. Attention is called to the fact that successive dates on the table are approximately two weeks apart, allowing ample time for considerable sexual development between any two successive dates used, and which accounts for what appears to be too abrupt a change in some cases. There were no males, which did not show sexual development at some time during the experiment. In almost every case the animal increased in sexual activity until it was recorded as "completely scrotal". This period of highest activity is seen to fall in the majority of cases from the middle of February to about the middle of April. An average of all of the groups together shows approximately the first part of March to be the time of greatest sexual activity. Previous observation of laboratory males have shown that the peak of the sexual cycle is normally reached during the months of April and the first of May. While in contrast, by the close of April, the gonads of the light treated animals are definitely regressing, the peak having been passed. By the end of June, practically all animals are again at the sexually inactive stage. The animals treated with red light, which according to Bissonette (1932b) should have the greatest accelerating effect had reached the inactive condition as early as the middle of May.

The figures in the right hand columns of Tables 3 and 4 indicate the height of sexual activity reached by a whole group during the entire period of the experiment.

The female light treated ground squirrels were kept with the male animals at all times. They show slightly greater individual variations and differences between the groups as a whole than the males, which, however, is not enough to appear significant with available data. The females starting in every case at the sexually inactive stage, showed a gradual sexual development which reached oestrum approximately during the period from March 1 until April 14. Taken as a whole, it is seen that the male animals reached the point of maximum development approximately two weeks earlier than the females, which agrees with previous observations of laboratory animals.

Only about 11 per cent of the light treated female animals including the controls escaped any sexual activity, while past observations of laboratory animals indicate that only about 50 per cent of the females normally show any sexual development during the breeding season. Two of these inactive animals were subjected to ultra-violet radiations, and two other animals of this same group showed a slight swelling of the vulva and then regressed, without the vagina ever having opened.

This might indicate a possible inhibitory effect were it not for the fact that one other animal in the same cage underwent a normal cycle and the remaining animal bore a litter of four young on February 23. This, of course, is by no means significant. However it is quite interesting because it is only the fifth time since work began with the ground squirrel that a litter of young has been conceived in the laboratory, from animals that have been in the laboratory over winter.

DISCUSSION

One of the most striking things about the sexual cycle of the female ground squirrel is the extreme variation shown among the different animals in regard to the degree of sexual activity shown during the breeding season and the length of time which an animal remains in any one stage. In fact as has been previously pointed out, only about 50 per cent of the female laboratory animals show any sexual activity whatsoever. As the laboratory ground squirrel does not normally reproduce, it has been impossible for us to observe whether the female will actually mate during oestrus and if so, whether such a mating would be fertile.

Generally speaking, the injection of various sex stimulating extracts of the anterior pituitary into male animals

has resulted in a slight increase in testis size which has been attributed to an increase in the interstitial tissue, while whole gland implantations have apparently not produced this effect; though some workers have observed this hypertrophy of the interstitial cell tissue following implantation (Moore and Price, 1931). Microscopic examination of the enlarged left testis of a pituitary implanted ground squirrel reveals no hypertrophy of this interstitial tissue, but a marked increase in tubular diameter was evident.

In sharp contrast to the results of most other workers, spermatogenesis was accelerated to the formation of secondary spermatocytes and spermatids. Implantation invariably produced lumina in the tubules where before implantation none were present.

In no case did the implanted male animals become scrotal and no spermatozoa were present in the tubules. Moore and Oslund (1923) found that the testes of a normal ram removed from the scrotum to the peritoneal cavity uninjured, would lose its germinal epithelium and become a typical cryptorchid testis. Also external wrapping of the scrotum for 80 days resulted in the loss of all spermatozoa and considerable degeneration of the tubular epithelium. Moore and Quick (1924) report that experimentally induced cryptorchid testes in rats, rabbits, and guinea-pigs suffer

a degeneration of the germinal epithelium after retention in the body cavity for some seven to eight days. It is thought that failure to induce scrotality in the male ground squirrel may account for the absence of spermatozoa in the tubules. To the writer's knowledge, such characteristic cleaning out of the seminiferous tubules after implantation as was observed has not been previously described as a result of pituitary implants. It is suggested that here again failure to cause the descent of the testes into the scrotum may explain this phenomenon.

Groups of thirteen-lined ground squirrels subjected to artificial light of known wave length showed no significant variations in sexual activity. Bissonnette (1932a) states that sexual cycles in female ferrets are conditioned largely, if not completely, by light rations in the visable part of the spectrum, however males are much less so and require some other conditioning factors for complete fertility. It is well to note that Bissonnette used only six animals. It is not at all impossible that normal variation may account for the differences he observed between the experimental animals and the controls. Although the present work definitely denies the results obtained by Bissonnette, it is thought that further data should be obtained involving such conditions as temperature and exercise before definite

conclusions may be drawn.

CONCLUSIONS

1. Vaginal smears showed: (a) Proestrum to be characterized by the presence of large nucleated squamous epithelial cells combined with a few cornified epithelial cells. Average time, five to twelve days. (b) Oestrum, by the presence of cornified cells with few large nucleated epithelial cells. Great variation in time. (c) Early metoestrum by masses of cornified cells, and large epithelial cells. Average time, four days. (d) Late metoestrum by the invasion of polymorphonuclear leucocytes. Average time one day. (e) Anoestrum by leucocytosis and small epithelial cells.

2. Pituitary implants from adult male albino rats produced a marked increase in testis size, a marked increase in tubule diameter but little or no increase in interstitial cell tissue.

3. Spermatogenesis was accelerated to the formation of secondary spermatocytes and spermatids. No spermatozoa were found.

4. Implantation produces marked increase in weight, secretory function and average height of the secretory epithelium of the Cowper's glands, prostate, and seminal vesicles.

5. In no case did the implanted animals become scrotal nor show the characteristic pigmentation of the inguinal region.

6. Artificial light produced no significant variations among the differently treated groups of animals.

ACKNOWLEDGMENTS

I wish to express my sincere thanks and appreciation to Doctor George E. Johnson for his kindly advice and assistance in the choice and study of this problem. I am also indebted to other members of the department of Zoology for their advice and criticism.

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EXPLANATION OF FIGURES

Fig. 1. A section through the right testis of ground squirrel No. 188 before implantation. X300. Note especially the primary spermatocyte spiremes.

Fig. 2. Same as Fig. 1, after four pituitary implants. Small lumen present, lined with secondary spermatocytes and spermatids. X300.

Fig. 3. A section through the left testis of animal No. 188 after 16 pituitary implants. X300. Note especially the large lumen and the rather thin germinal epithelium present.

Figure 1.

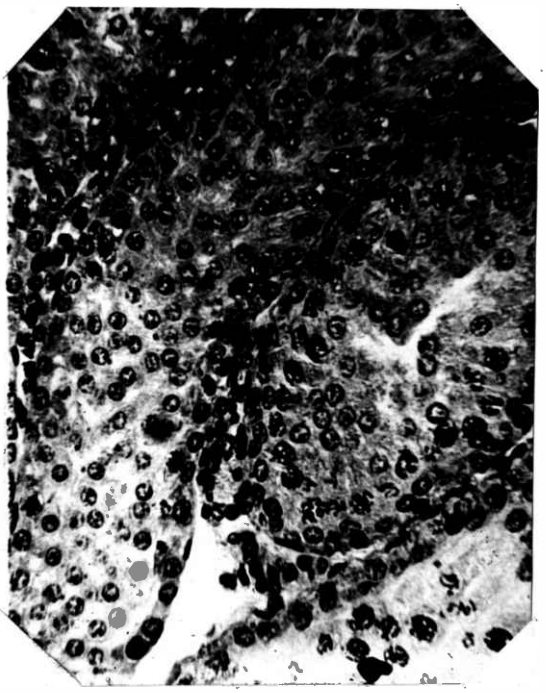

Figure 2.

Figure 3.

Fig. 4. A section through the seminal vesicles of control animal No. 1165. X50.

Fig. 5. A section through the seminal vesicles of ground squirrel No. 1713 after 12 pituitary implants. X50.

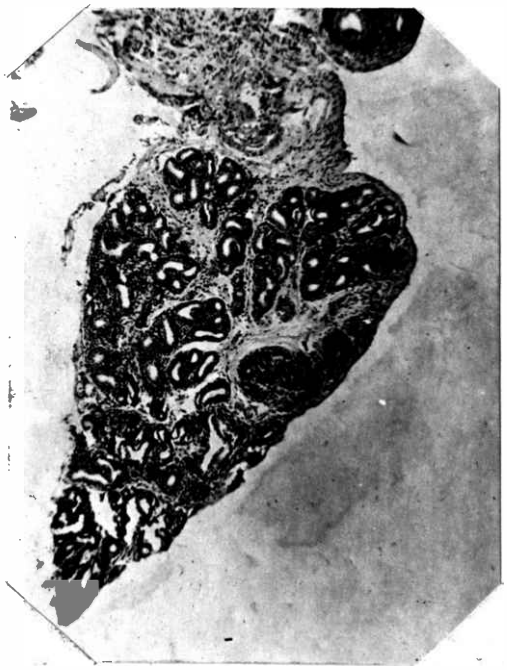

Figure 4.

Figure 5.

Fig. 6. A section through the prostate gland of control animal No. 1165. X50.

Fig. 7. A section through the prostate gland of ground squirrel No. 1713 after 12 pituitary implants. X50.

Figure 6.

Figure 7.

Fig. 8. A section through the Cowper's gland of control animal No. 1165. X50.

Fig. 9. A section through the Cowper's gland of ground squirrel No. 1713 after 12 pituitary implants. X50.

Figure 8.

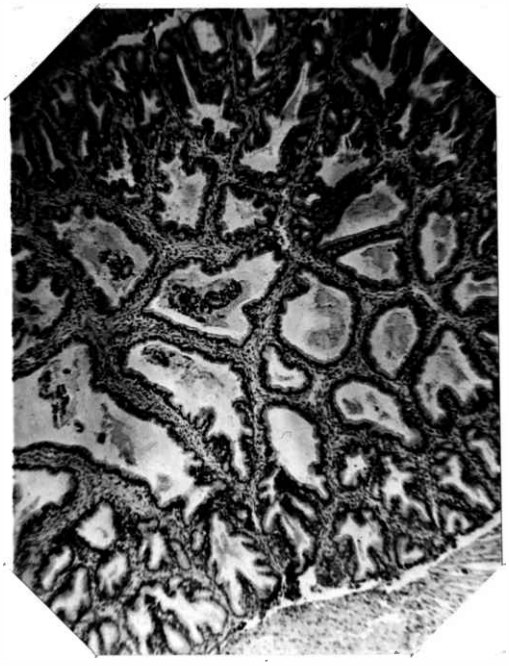

Figure 9.

Fig. 10. A photomicrograph from the vaginal smear of a ground squirrel, showing the large epithelial cells and few cornified cells of proestrum. X300.

Fig. 11. A photomicrograph from the vaginal smear of a ground squirrel, showing the cornified cells and few large epithelial cells of oestrum. X300.

Fig. 12. A photomicrograph from the vaginal smear of a ground squirrel, showing the numerous leucocytes and small epithelial cells of anoestrum. X300.

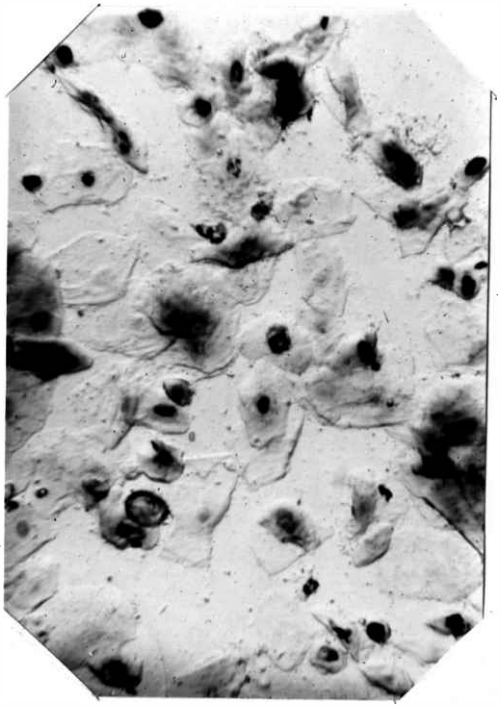

Figure 10.

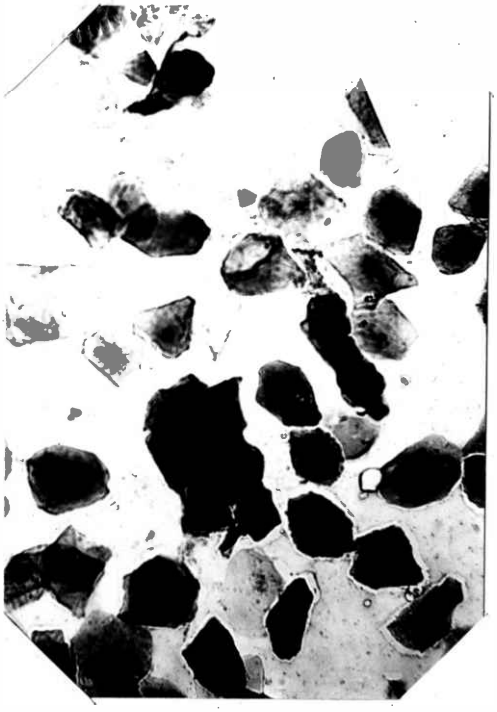

Figure 11.

Figure 12.