

LABORATORY REPRODUCTION STUDIES ON THE
GROUND SQUIRREL, CITELLUS TRIDECIMLINEATUS

by

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INTRODUCTION

During the past six years Dr. G. E. Johnson of the Department of Zoology of the Kansas State Agricultural College has been studying hibernation in the ground squirrel, Citellus tridecemlineatus. For this experimental work the animals used are secured from various localities in central and western Kansas. The animals are captured without injury and shipped by express to the laboratory which they reach in good condition. These shipments are usually received in the early spring and at various times during the summer and fall.

In the laboratory the animals are kept in cages provided for them in the animal house which was formerly one of the government barracks. The building is steam heated in winter and adequately ventilated. Temperature fluctuations are not great and the general conditions are quite constant and uniform. The animals are fed and watered daily and the cages cleaned as requirements demand. The food of the animals naturally differs somewhat from that in nature but the animals eat readily and maintain excellent health and weight. The animals are subject to experimentation throughout the year, and as the building is heated during the cold weather, they usually do not hibernate. Some will go into hibernation for short periods but this

is more of an exception rather than the rule.

Under these conditions it has been observed that these animals do not reproduce in the laboratory. The rats and mice, on the other hand, reproduce freely. Up until this year there has been but one exception to this. In the spring of 1927 a shipment of animals was received of which one pair mated the day of arrival. Living young were subsequently born 28 days later. It is to be noted that these animals had just been received. No reproduction or mating has ever been observed in any of the other animals. In the spring, however, pregnant females are often received which give birth to normal litters. Many of these are raised to maturity.

PURPOSE

The fact that these animals cease to reproduce when brought into the laboratory from the fields opens a new line of investigation. Since any condition, which directly or indirectly influences the organs of reproduction, may be wholly or in part a cause of this unusual behavior of these animals, it seemed desirable to investigate as many factors of possible influence as time and conditions would permit. Knowledge gained from such a study would be valuable to pure science and to its application in animal production and control.

REVIEW OF LITERATURE

An investigation into the literature reveals the fact that very little work has been done directly on this problem. There has been, however, considerable study on other animals concerning various factors which bear directly and indirectly on the present investigation.

It is now well recognized that nutrition and vitamins are very important for normal growth and reproduction. Mathews (1927) covers this field very well in a general way. Vitamin A, first distinguished by McCollum and Davis, is of considerable importance for growth and may have some slight influence on reproduction (Sure, 1928; Evans, 1928a). A deficiency of vitamin B in the male, if the presence of vitamin E is assured does not influence reproduction except through a general weakened physical condition (Evans, 1928b). The findings of Mattill (1927) agree with those of Evans. He finds that rats suffering from a vitamin B deficiency do not show testicular degeneration or an injury to the germinal epithelium. Sterility caused by this agency is believed to be an effect of a loss of vigor and sex expression resulting from a lowered metabolic level. In the female, Parkes (1928) found that a deficiency of vitamin B brings a cessation of oestrus in four weeks with death

resulting some two months later. The injection of oestrin during this period of anoestrus induces oestrus symptoms in accessory organs without causing the ovary to ovulate. He concludes then that anoestrus is caused by an inability of the animal to produce oestrin. Vitamin E has been found necessary for reproduction and its deficiency causes sterility in a different way than other dietary factors (Evans, 1925; Evans and Burr, 1927). In the male this deficiency is manifest by a degeneration of the seminal epithelium which is completed by the 150th day. In the female this deficiency is characterized by an inability to carry the young, which suffer early resorption. An excess of vitamin E does not raise fertility above normal. References to the other vitamins have not been found to have any real bearing on the reproductive functions.

Ultra violet lamps are finding their way more and more into various branches of commercial and scientific work, and some reference in the literature is found relative to their value for reproduction. Saidman (1924) obtained good results after treating 17 cases of amenorrhoea, delayed and painful menses and irregular menstruation in the tuberculous and the atrophy of the ovaries after radiotherapy, with ultra violet light. Leigh-Claire (1927) found that excessive radiation of rats had no ill effect if the eyes were shielded. Czarnicki and Jolly (1928) found injury to the

exposed testicle resulting from radiation with the mercury arc light. The unexposed testicle, however, showed no injury. In relation to the penetration of ultra violet light in living tissue, Anderson and Macht (1928) found 6-10 per cent transmission for wave lengths of 2537-3000 A° and for a depth of 1.2 mm.

It has likewise become firmly established that the pituitary body is intimately related to the genital system and the literature is full of the reports of various investigators. Smith (1927a and 1927b) and Smith and Engle (1927) reported precocious sexual development in rats and mice after pituitary transplantation. Only a few (four to six) transplants were required and sexual maturity was reached as early as the twenty-second day in rats. Males and females both responded to treatment. Smith found that either the whole pituitary or only the anterior lobe would produce these results. Smith and Engle reported superovulation and that the transplants act through the genital organs. Fee and Parkes (1929) found that hypophysectomy one hour after copulation inhibits ovulation which should take place ten or twelve hours later. Hypophysectomy later than one hour does not inhibit ovulation. The active agency is assumed to be the anterior lobe of the pituitary. Transplants of the anterior lobe in early pregnancy causes a termination of the latter by a failure of implantation of

the ovum or an expulsion or resorption of the embryo (Engle and Mermod, 1928). Riddle and Flemion (1928) found that transplants and extracts of the anterior lobe of the pituitary produces increased growth of the testis of immature doves. A less pronounced effect was observed in the female. Erhardt (1928) reported that implants of the anterior lobe of the pituitary brings immature rats into oestrus with hastened follicular and uterine growth. Wolf (1929) induced frogs to ovulate after transplantation of the anterior lobe of the pituitary. Males became activated by the treatment and actual reproduction was obtained.

Experiments with extracts and hormones of the anterior lobe of the pituitary has established the presence of at least two hormones in that gland (Evans and Simpson, 1925, 1926, 1928; Zondek and Aschheim, 1928; Johnson and Sayles, 1929; Bellerby, 1928). One is instrumental in producing growth and one in hastening sexual maturity. Zondek and Aschheim, Evans and Simpson and others report the maturity provoking hormone in the urine of pregnancy. The latter writers also emphasize the antagonistic action of the two hormones (1928). Reports by other writers follow similar lines.

Feeding experiments with the anterior lobe of the pituitary result in conflicting reports. Marinus (1919) and Goetsch (1916) reported some positive stimulating action on

the genital organs of rats. Lewis and Miller (1913), Evans and Long (1921), and Smith (1923) found no positive influence. Knaus (1926) studied the action of pituitary extracts administered by the alimentary tract. He found no pressor action in the cat when the extracts were administered by mouth, stomach, small intestines or rectum. Some influence was found on the uterine muscles after a latent period when the extracts were given by mouth. There was also some slight absorption from the rectum. Rees and Whitehead (1923) found no destruction of the uterine tone raising action of pituitary extracts by the gastric or intestinal enzymes.

The ovarian and related functions have likewise received considerable attention. Grunert (1927) reported the case of two cows in which oestrus had ceased. An ovary was secured from a younger cow and implanted under the skin of the neck of each cow. Both cows again came into heat and one was bred and became pregnant. Sippel (1924) reported the case of four previously sterile women who became pregnant after receiving ovarian grafts in the abdomen. The implanted ovaries activated their own normal but inactive ovaries. The grafts were secured from other women. Pettinari (1925a) implanted ovaries into a female dog sixteen years old. The dog produced a litter of young fifteen months later. Examination showed regeneration in the pre-

viously senile organs. Runge (1926) made ovarian grafts from young to old rats and the animals showed better health, weight and sexual activity. Castillo (1928) reported the reappearance of the oestrus cycle after ovariectomy in rats, induced by grafting an ovary into the kidney. If the graft does not "take", or if it is later removed, the cycle disappears. Tuffier and Bour (1925) reported the continuance or reappearance of menses with pregnancy possible after intra-uterine implantation of the ovary. Other writers have reported similar cases. Pettinari (1925b and 1926) found that grafts survive best in animals of the same species and where there is a manifest hormone deficiency. Slonaker (1927) found oestrus induced in old albino rats by injection of the follicular hormone. The ovary was not reactivated to ovulation but mating activity was stimulated. Tuisk (1927) injected follicular fluid extracts into castrated and uncastrated mice and induced oestrus for seven days in the former and thirteen in the latter. Doisy, Rolls, Allen and Johnston (1924) gave a method for extracting a hormone from the liquor folliculi. Asdell and Marshall (1927) produced typical prooestrus changes in the dog's uterus during anoestrus by the injection of follicular extracts. Changes were also observed in the vaginal epithelium. Definite oestrus was not produced so it was thought that other factors might be involved in the pro-

duction of oestrus. Golding and Ramirez (1928) made water soluble extracts which caused the vaginae of rats to open before those of the controls, and smears indicated continuous oestrus. In males there was an inhibition of genital growth. Parkes and Bellerby (1926) found that the injection of estrin during the early stages of pregnancy caused the rapid reappearance of oestrus and the termination of pregnancy. In later stages a larger amount was required to terminate pregnancy. In a study of the injection of ovarian extracts Evans and Burr (1926) found that subcutaneous injections are considerably more effective than intraperitoneal injections.

The study of the oestrus cycle by the vaginal smear method has been made in the guinea pig by Stockard and Papanicolaou (1917) and in the rat in detail by Long and Evans (1922). This work has been extended to other animals by other workers as well. However, this method does not apply to all animals. Kunde and Proud (1929) found that in the rabbit the oestrus cycle cannot be followed by the smear method. King (1926) found that in the human the vaginal secretions do not serve as an index of the periodic ovarian and uterine changes.

It has also been definitely shown that conditions and factors acting upon the nervous system may profoundly influence the genital functions. Borries (1929) reported

that 27 per cent of thirty-nine college women at Munich reported menstrual irregularities produced by nervous strain in preparing for examinations. Menstruation returned to normal after the nervous effects disappeared. Lafora (1923) stated that sexual frigidity in women is very often caused by psychic inhibition. Psychoanalysis reveals this and often cures it. Stickel insists that frigidity is rare in healthy women but appears in 50 or more per cent of neurastics. Stieve (1926 and 1927) stated that all civilized nations suffer a decline in population, that savage races tend to die out when brought into civilization and that wild animals usually do not breed in captivity. He explains these conditions as resulting from an environmental deteriorating influence on the gonads. This effect may be more profound in the female due to the type of germatogenesis. He attaches great importance to all of the varied environmental factors which act on the nervous system. Confinement of wild animals usually causes a degeneration of the gonads. War amenorrhea of women is a result of anxiety and worry. A similar degeneration in the testes of criminals is reported. The following quotation is taken from Macomber (1924): "Worry and nervousness in the women are equally important in the derangement of function - the end result of this failure of ovulation is seen in the so-called "retention cyst" ovaries and it is an interesting clinical observa-

tion that such ovaries are seen only in the nervous emotional type of women. Nervous strain then may be an important cause of sterility and its prevention will be valuable in eliminating certain types of sterility."

The following miscellaneous references have bearing on reproduction. Gullickson and Eckles (1927) reported that two calves kept constantly in the dark for two years showed no ill effects from the lack of sunlight. Marshall and Peel (1910) found that excessive fatness may cause sterility in the male and female. Subsequent starving causes a return to normal. Crainicianu (1926) found that calcium may help in ovarian insufficiency. Low environmental temperatures have been found to influence the oestrus cycle of the rat (Lee, 1926). Stone (1926) studied the copulatory response of rats reared in isolation and found that copulation during the first oestrus resulted within a few seconds.

METHODS AND MATERIALS

General

Until recently the thirteen lined ground squirrel of western Kansas was classified as Citellus tridecemlineatus pallidus (Allen). Recently, Howell (1928) has separated this group and called the southern form C. t. arenicola and left the northern form C. t. pallidus. Since C. t. pal-

lidus and C. t. arenicola are found together in western Kansas it seems probable that our ground squirrels may belong to both varieties or an intergradation of the two. This point, however, is of but slight importance in the experimental work at hand.

The nature of these investigations do not permit as carefully controlled experiments as we might wish. We are dealing with a group of animals which when brought into the laboratory from the field cease their normal reproductive functions. To find reasons for this the present experimental work was undertaken. Because of this situation the general facts must first be sought and then examined more carefully by controlling various known factors.

Since there are a large number of animals in the laboratory at all times these animals served in a general way for controls through all of the experiments. In order to be somewhat more specific, a certain few of these animals were watched more closely than the others and were recorded as controls. These animals were chosen more or less at random except that they represent the average laboratory animal in health, weight, and genital development. In each case the females were sexually inactive with the vaginae closed and not swollen. The males were likewise sexually inactive and not scrotal or only slightly so. Any deviation from this principle is noted in the results. It is to be

emphasized that these animals are indicative of all the animals in the laboratory and are included only as an index of the normal laboratory condition.

Practically all of the experimental and control animals had been in the laboratory for six months or a year. A few of the animals used had been in the laboratory only a short time, having been received only this spring. These were used because of specific reasons given later. Observations were made on the experimental and control animals each day or as often as the particular experiments required.

The cages in which all of the animals are kept are of two general types. The larger cages in which most of the animals are kept are made of wood with screen sides, back and door. Some of these have a screen roof as well. These cages are about two feet wide, three feet deep, and three feet high. From one to six animals are usually kept in these cages. Sometimes necessity demands that the cages should be more crowded, but this is avoided if possible because of the mortality from fighting. Small boxes are usually included in which the animals make their nests from the shavings or straw which serves as bedding.

The smaller cages used mostly for smaller groups of experimental animals are made of wire and are about eighteen inches square and twelve inches high. From one to three animals are usually kept in these cages. Ordinarily, paper

serves as bedding in these cages. The animals quickly tear the paper to shreds and make a comfortable nest.

Nutrition

All of the animals in the laboratory receive the same food. There are a few exceptions which will be mentioned under the experiments involved. The following ration has been fed to the animals:

Yellow corn meal	30 per cent
Whole wheat flour	30 per cent
Skim milk powder	30 per cent
Alfalfa meal	4 per cent
Bone meal	4 per cent
Salt	1 per cent
Cod liver oil	1 per cent

This ration was kept mixed except for the cod liver oil which was added to small quantities and then fed to the animals. In addition to this dry food, sprouted oats were fed during the winter months and green feed from the fields during those months when it was obtainable. Water was kept constantly before the animals.

Pituitary Transplants

The ground squirrels chosen for this work had all been in the laboratory for over six months and so had become accustomed to laboratory life. In all cases, the vaginae of the animals used were not open or developed and the

males were not scrotal or only slightly so. Only moderate weight animals were used, the real heavy or real light ones being avoided.

The pituitaries used for transplantation were taken from normal wild gray or albino rats. In a few cases the glands were secured from gonadectomized rats. The wild rats were very difficult to capture and keep and were used only when there was a shortage of the tame animals.

The technic used was simple, direct and fairly rapid and somewhat similar to that of Smith (1927a). Conditions were maintained as sterile as possible, and the instruments were sterilized in alcohol before using. The donor was stunned by a blow at the base of the head and another in the middle of the back. This last was to eliminate the kicking of the unconscious animal. After stunning, the head was quickly severed from the body, the roof of the skull removed, and the brain lifted up. The pituitary body thus exposed in the floor of the cranium was then removed to a small amount of physiological saline solution. The gland was then cut into small pieces with a pair of fine scissors and then sucked up into a small long nosed glass canula which was then kept in a rack until the recipient was prepared. The ground squirrel was quickly etherized and the inner surface of one hind leg washed with 50 per cent alcohol. A small break was made in the skin through which the

canula was inserted for about two inches beneath the skin and the gland together with about 0.5 cc. of the saline solution expelled. The break in the skin was coated with carbolated vaseline. Daily transplants were made alternately in the hind legs for varying lengths of time and the effects studied. The times at which transplants were made are recorded in the table.

To observe the histological effects of transplantation one pair of animals was killed after daily transplantation ranging from one to eight days. The ovaries and base of the uterus were saved from the female and the testes from the male. The tissues were preserved in Bouin's fluid, imbedded in paraffin, cut at ten microns and stained in haematin and eosin (blue).

Ultra Violet Radiation With and Without Pituitary Transplants

To observe the effects of ultra violet light with and without pituitary transplants, a mercury vapor arc lamp was purchased from the General Electric Vapor Lamp Company. The tube first used was of lead glass whose ultra violet transmission is not high. This tube was shortly replaced by one of greater transmission power and this one has been in operation since. This tube transmits all the ultra violet wave lengths of daylight plus some that are not present in natural sunlight. All of the properties are not present,

however, since the ultra violet light from this source is not a white light. This lamp has been shown by Dr. J. S. Hughes, Professor of Chemistry at this College, to cure rickets in chickens by daily radiation of one-half hour. The older tube required about two hours.

The animals radiated were kept in the small wire cages on a table under the lamp. The distance from the lamp to the animals varied from three to four feet depending on the number of cages present. The animals were radiated daily for periods of five or twenty-four hours. Observations were made from early in March through June.

Ovarian Extract Injections

The extract used in these experiments was obtained through the courtesy of Dr. E. R. Frank of the Division of Veterinary Medicine of this College. The method used for the preparation of the extract follows: Beef ovaries were secured and the corpora lutea removed. The remaining material was finely ground in a food chopper, and then mixed with a solution of three parts physiological saline solution and one part absolute ethyl alcohol. One cubic centimeter of this solution was added for each gram of gland tissue. After standing one week the liquid was pressed out, filtered and preserved with an addition of 0.25 per cent formalin.

In these experimental animals 1 cc. of this extract was injected subcutaneously in the lower abdomen every third day.

Two types of animals were used in this experiment. One group of six females showed no genital development. Of these six, three had been in the laboratory over one year and the other three had been in the laboratory only about one month, having been recently received from western Kansas. In the second type of animal used, three females were chosen which had been in the laboratory only about a month and which had open vaginae. These females were placed with sexual active scrotal males which had been received at the same time as the females. Daily observations were made as to the sex behavior of these animals. These experiments were begun on June 16.

Ovarian Transplantation

For these experiments four females were chosen which were typical of the laboratory animal. These animals were in excellent health and condition but the vaginae were not open or developed. The experiments were begun June 20.

Young adult albino rats were used for the donor and only normal healthy ovaries were transplanted.

The technic employed was very similar to that used for pituitary transplantation. The donor was stunned with a

blow upon the head and another on the back. The body cavity was then opened and the ovary removed to sterile physiological saline solution in which it was cut into small pieces and sucked up into a fine glass canula which was then placed in a rack while the recipient was made ready. The ground squirrel was prepared and transplanted in the same manner as given under the pituitary transplantation method and will not be repeated here.

The animals were transplanted for four times on every third day. They were kept in the small wire cages under as sterile conditions as possible. The instruments were sterilized with alcohol before using.

Outdoor Cages

In order to observe the effects of placing the animals under more natural conditions it was decided to construct some outdoor underground cages. For this purpose three large packing cases were secured. They were about three feet long, two feet wide, and three feet deep. These boxes were buried underground for about two feet and filled with as loose earth as was available. Small boxes were built into each of two corners with runways leading down to them to serve as underground nests for the animals. The cover of these cages was made of wire. Unfortunately, a severe rain storm flooded the cages, drowning one animal, shortly after

completion. The cages were then dug out and placed in a similar position and condition on top of the ground where they were used throughout the experiment.

Three types of animals were represented in the three cages. In Cage 1 four animals were placed of which one pair had been previously given pituitary transplants for eleven days, and the other pair a similar treatment for thirteen days. The vaginae of both females were open and both males were moderately scrotal. In Cage 2 two pairs of animals were placed which had been previously treated with ultra violet light for thirty days. In these animals the vaginae were not open or developed and the males were not scrotal. In Cage 3 four animals were placed which had received no previous treatment. One pair was quite fat and the male was quite scrotal. The other male was not scrotal and the vaginae of both females were not open or developed.

The food of these animals was similar to that of all the other animals except that there was a 25 per cent addition of wheat germ to the previously used ration. Green feed was given daily.

Because the animals dug freely and extensively in the earth, no careful observations could be made until the animals were dug out and caught at the termination of the experiment. The experiment was begun on June 1 and was continued through that month.

RESULTS

Controls

These results are based on the observations of specific controls from the first of March to the first of July. Six males and eleven females were used and only one of the females had an open vagina. Three of the males were somewhat scrotal. No further genital changes were observed in the females or any sexual interest or development in the males. General observations on the other animals in the laboratory were similar. A few of the females showed a somewhat open and swollen vagina and a larger number of the males became somewhat scrotal. The genital changes, however, did not usually equal the normal condition. No mating or reproduction was observed at any time.

Nutrition

The animals grew well and maintained excellent health and weight. There was no apparent effect of malnutrition of any kind.

Pituitary Transplantation

A general survey of the results are given in Table I, and only a few explanatory remarks will be required. In

general, a swelling of the vagina could be observed at the end of the second day of transplantation. Each day brought additional development until the vagina opened on the day indicated. Further development beyond this is not the rule. In the male, the changes are less clear-cut but the animal begins to be more scrotal at the end of the second day and the height of development is reached from the fifth to the seventh day. After transplantation is discontinued the genitals remain in a relatively constant stage of development. After fifteen or twenty days there is a more or less gradual decline in the prominence of the genitals with a subsequent closing of the vagina and a disappearance of the scrotal condition of the male. No reproduction or mating has resulted from the transplantation. No ill effects have been observed as a result of the pituitary transplants or the technic involved. Temporary swellings sometimes resulted from slight injuries or possibly from minor infection but in only one case was it serious.

The results of the histological study of sections through the ovary, base of the uterus and testis after pituitary transplantation are not clear-cut and definite. From a study of the material at hand, however, the following results may be stated with a fair degree of certainty. Under the influence of the pituitary transplants made, the ovary was stimulated progressively to produce numerous

large and healthy follicles. Atretic small follicles appeared more numerous in the later stages of transplantation. Under the influence of the transplants, the base of the uterus showed oestrus-like changes. The walls became much thicker and the epithelium of the lumen became thicker with an increase in the cell layers. These changes, however, are more quantitative than qualitative and no great difference could be observed in the cellular nature. In the testes there was a uniform absence of spermatozoa and spermatogenesis. A tendency towards regeneration in the germinal epithelium was indicated but indefinite.

Pituitary Transplantation With Ultra Violet Radiation

The results of this work are given in the table and are practically identical to the results from transplantation alone. No distinct or definite influence resulting from the addition of ultra violet radiation. The changes in the genitals of the animals do not differ in any marked degree from those observed from transplantation without radiation.

Ultra Violet Radiation

The results of ultra violet radiations are based on seven males and nine females. No influence could be observed on the genital or general physiological condition

Table I. Pituitary Transplantation Without and With Ultra Violet Radiation

Date	1929	Male	Fe- male	Number of trans- plants	Hours radiated daily	Genital swelling after num- ber of days	Vagina open after number of days	Genital swell- ing very pro- nounced after number of days	Remarks
Nov. 2	998	999	4			2		4	Gradual decline after 15 days
Nov. 19	1112	1110	5			2		4	Male died on 5th day. Vagina swollen after 15 days.
Nov. 28	1114	1113	4			3			Less than usual development. No subsequent change.
1930 Feb. 2	1086	1087	7			2	6	6	No subsequent change of importance
Feb. 8	990	1002	6			2	6	6	Kept separate, put together daily, No mating, no subsequent change of importance.
Mar. 17	1070	1059	6	5		2	3	4	No subsequent change of importance
Mar. 17	1058	1056	6	5		2	5	6	No subsequent change of importance
Apr. 1	1075	1076	5	5		2	2	3	No subsequent change of importance
Apr. 1	1062	1064	5	5		2	3	4	No subsequent change of importance
Apr. 2	1083	1061	5	5		2	3	4	Escaped April 10.
Apr. 29	1090	1091	9	24		2	6	6	No subsequent change of importance
Apr. 29	1092	1093	15	24		2	7	7	No subsequent change of importance
Apr. 29	1100	1101	13	24		2	5	5	No subsequent change of importance
Apr. 29	1102	1103	11	24		2	5	5	No subsequent change of importance

of these animals whether radiated daily for periods of five or twenty-four hours. Observations were made from the first of March to the first of July.

Injections With an Ovarian Extract

The three old animals and the three new animals each injected for four times every third day with the ovarian extract showed no effect whatever. Of the other three females with open vaginae which were placed with males daily, number O-12 was killed accidentally on June 20 when it attempted to escape from the cage. It had been injected twice and had showed no effect or no cooperation at mating. Animal number O-11 under the same conditions was injected three times and showed no genital changes or mating. This animal was exceedingly cross. Animal number O-10 gave birth to a litter of six on June 22 after being injected twice after June 16. That she had been pregnant was not obvious or suspected. The extract apparently had no influence on normal partuition. The young are growing very nicely and the mother is caring for them splendidly. It is to be noted that this female was one of the least wild of the animals worked with.

It should also be mentioned that the males, apparently normally sexually active, attempted or apparently attempted to copulate with the females. In each case, however, they

were savagely repulsed. Female O-11 was the most vicious.

Ovarian Transplantation

The four females receiving ovarian transplants showed no apparent effects on the genital conditions. The transplants were not tolerated well and produced subcutaneous swellings and bunches. These bunches disappeared after a few days and no permanent ill effects were apparent.

Outdoor Cages

The animals in the outdoor cages seemed to revert more nearly to normal living conditions. They spent much of the time in the burrows and would not remain above ground in the presence of humans. They dug extensively in the ground and scarcely used the nest-boxes built for them. They ate well and gained noticeably in weight. The animals in cage 1 gnawed through the cage and escaped. They remained in the immediate vicinity, however, and were all caught in two days. Due to the extensive digging of the animals it was impossible to examine them until the end of the experiment. When they were dug out on June 29, the females of Cage 1 no longer showed open vaginae and the females in the other cages showed no genital changes. The males likewise showed no genital development and none of them were distinctly scrotal. The animals were probably not as wild as when

placed in the cages and showed less of a tendency to bite the hand that had been feeding them.

DISCUSSION

As stated previously a modified cyclic change is observed in the genitals of most of the animals kept in the laboratory. Only a small per cent of the females show changes approaching the normal condition. In the males the per cent is larger but still small. In the female where the vagina swells and opens it is uncertain whether normal oestrus is present or not but the indications are that the oestrus cycle, as it appears, is not entirely normal since no mating is observed with scrotal males. The males and females showing these changes have, of course, just passed through an unnatural winter season in the absence of hibernation. Whether hibernation and its organic changes is necessary to reactivate the reproductive functions is uncertain. That the laboratory males may lose the genital functions is indicated by the lack of spermatozoa and spermatogenesis in the animals studied histologically. Just why the laboratory animal differs from the wild animal in these respects is uncertain but possible explanation is offered later.

If we consider the food fed the laboratory animals it becomes evident that all the vitamins apparently needed for

reproduction and general health are present. As reviewed in the literature a vitamin B deficiency would be manifest by a weakened condition through which any effect on reproduction would act. The laboratory animals show no deficiency of vitamin B since they maintain excellent health and weight. Vitamin B is further assured in the ration by the wheat, corn and alfalfa. Similarly vitamin A deficiency is not apparent and it is adequately supplied in the food by cod liver oil, wheat and corn. The general health of the animals also indicate no vitamin D deficiency. Vitamin D is furnished in the food by cod liver oil, wheat and the green feed. There is no indication of vitamin C deficiency or any effect on reproduction but it is furnished in the ration by the green feed. Vitamin E has been shown in the literature to be very important for reproduction. Our laboratory animals do not reproduce but a deficiency of E is scarcely possible since it is present in the wheat, corn, alfalfa, and green feed. In some of the experiments wheat germ, which is very rich in vitamin E, was added without any influence on the genital condition.

Another important point should also be added. The food fed to the ground squirrels is also fed to the rats and mice which reproduce regularly and raise normal litters. The young weaned animals likewise grew admirably on the diet. It thus appears that in these animals there is no dietary

deficiency and we could logically expect no greatly different dietary requirement in the ground squirrel.

On the basis that the pituitary body and particularly the anterior lobe has been shown to have an intimate relation to the genital system it was thought that pituitary transplants might stimulate the sexually inactive ground squirrel to more or less normal sexual and reproductive functions. This has been but partly realized in the present work. Oestrus-like conditions are produced in the female but that these conditions and changes do not represent the natural or complete oestrus is highly probable since no mating has been observed. The true oestrus cycle is not known so the observations made can not be completely checked. The sections made from the organs of the transplanted animals show that a reaction and change is produced in the genital system but a full study of these effects could not be undertaken in connection with the present work. In the work of Drips (1919) evidence is advanced that the normal period of rut in these animals extends over a period of about two weeks in the spring and that ovulation results after coitus. It is also reported that the corpora lutea persist through the summer. A study of the sections from the laboratory animals in connection with this present work indicates that there may be a difference in genital function between the laboratory confined animal and the wild animal.

The present work, however, is not extensive enough to settle this point. Just how much influence laboratory confinement may affect the power of pituitary transplants to induce oestrus and reproduction in these animals is uncertain. That nervous functions may enter in will be considered later.

Ultra violet radiations have been shown to have considerable therapeutic value and influence on reproduction so it was hoped that some effect of radiation on reproduction in the ground squirrel might be obtained either alone or in connection with pituitary transplants. Ultra violet radiations are assumed to be able to correct the ill effects of the absence of sunlight. On the other hand, it is uncertain if sunlight or its substitute may have any great bearing on reproduction and especially in these animals since normal reproduction in nature results soon after the animals awake from hibernation through the winter in the complete absence of sunlight. Shaw (1926) also reports that the Columbian ground squirrel spends but a relatively short part of its life above ground and that most of the preparations for reproductive functions are performed below ground. Copulation in these animals likewise results shortly after the animals come out of hibernation.

The attempts to reactivate the ovary of the ground squirrel by an ovarian approach was also without effect. Dr. E. R. Frank, through whose courtesy the extract used was secured, has used this extract successfully in cattle. The extract was administered to the ground squirrels in dosages relatively 200 times as great as used in cattle and no effects were observed. Treatment over a longer period of time or at more frequent intervals might possibly be more effective in the ground squirrel. The uncertainty of this is probably better expressed when it is recalled that no influence was seen on animals with either the open or closed vaginae.

The ovarian transplants were tried with the hopes of producing an effect similar to that expected by ovarian extract injections. Auto- or homeotransplants of the ovary in the cow and in the human, it is recalled, were found to "take" and become instrumental in the reestablishment of the ovarian functions with pregnancy possible or resulting. The fact that our transplants were from different species might account for the fact that they did not "take" but would not entirely account for the fact that no influence at all was observed. We could still expect an influence since the hormones in the glands should be absorbed by the tissues of the recipient and the action should be similar to an injection of an ovarian extract and effective ovarian extracts

are not necessarily used in the same species from which they are prepared.

The question as to whether aptrodisiacs might be effective is uncertain. An approach by this method was not tried, however, since the therapeutic dose is very close to the lethal dose. Also the mode of action of these drugs does not involve any stimulating action on the gonads. To be assured of any result then by these agents one must first be assured that the animals involved are otherwise sexually normal.

The outdoor cages offer perhaps the nearest simulation to the natural life of the ground squirrel and under these conditions the captive animal might well be expected to revert to a natural reproductive cycle. Reproduction or the necessary reproductive changes were not observed in the animals placed in our outdoor cages but several factors may be involved. Conditions of the weather and of the ground made it undesirable and almost impossible to make underground cages in the location available until late spring. Difficulty due to heavy rains was experienced in this respect even after June first. Ground squirrels in nature are not found in such unfavorable locations but normally make their burrows where weather conditions do not affect them. Placing the cages, filled with earth, on top of the ground may solve this experimental difficulty as it did in

this present work. In cages of this type there is always a certain amount of artificiality and disturbance which may affect the truly normal behavior of the animal. Another factor of possible influence which was mentioned before is that of natural hibernation on the normal reproductive cycle. It is possible that there is some stimulus involved in hibernation which is functional in reactivating the reproductive functions in the spring. If the outdoor cages were made so that the animals could be kept in them over winter different results might be obtained.

In the literature it has been shown quite conclusively that various factors acting through the nervous system may inhibit or suspend the genital function. Because the attempts made in these experiments failed to reactivate the genital system of the ground squirrel when the same procedure proves successful in other animals points to a possible and probable nervous inhibition in these animals. Certain factors involved and observations made in the laboratory tend to support this view. In working with these animals one of the most obvious points of interest is the fact that these animals never become tame or permit any familiarities. The old saying "Absence makes the heart grow fonder" is literally true in these animals. They are always wary and will not submit willingly to be touched or handled. When cornered and caught they invariably scold

and bite. Gloves are practically a necessity in handling these animals. One point of interest here is the fact that the older animals are the most vicious while many of the new animals can be handled without danger of being bitten. An interesting incident occurred this spring when a new shipment of animals was received. One animal was unusually tame and would readily permit being handled. Because of this the animal was taken to the office in hopes that it would become still tamer. The opposite effect, however, resulted, and each day it grew wilder and wilder so that it had to be taken back and placed with the others.

Observations of a similar nature were made this spring on wild rats. The supply of albino rats available for pituitary transplants was limited and wild gray rats were captured for this purpose but with considerable difficulty. When placed in cages, these animals would fight violently so that but one or two could be kept safely in a cage. If these animals were kept for several days a number of them would invariably die from no apparent cause other than nervousness. Some of these wild rats, however, were fairly sociable, and were not very vicious or wild when caught. Some of these, both male and female, were placed with white rats of the opposite sex and left for about two months. No reproduction or mating was observed. Placing male and female wild rats in the same cage likewise produced the same

results. A pregnant female, which was captured, gave birth to young while in captivity but as usual preferred to eat them rather than raise them. Wild males not in captivity will mate with albino females in oestrus as was proved by a case in the laboratory when a female escaped from her cage.

Since nervous influences, as reported in the literature, can disturb the menstrual functions of women, cause testicular degeneration in criminals and produce sterility in many captive wild animals it seems possible and highly probable that a nervous inhibition is responsible for the altered genital functions of the ground squirrel and the latter's failure to respond to stimulants found effective in other animals.

The mechanism of this inhibition, if this is the true solution, is uncertain but it is probably indirect and acting through the endocrine system. It is furthermore uncertain if larger dosages of the hormones of the endocrine organs such as the pituitary body or the ovary can override the inhibitory influence of the nervous functions. The true cause of these abnormal genital conditions probably lies in a complex linkage of the nervous and endocrine systems acting toward a common end.

SUMMARY AND CONCLUSIONS

1. The ground squirrel, Citellus tridecemlineatus does not continue the normal functions of sexual activity under usual laboratory conditions.
2. The diet fed the animals contains all the vitamins which have been found to be necessary for the reproductive functions.
3. Pituitary transplants from rats, with or without ultra violet radiation, do not reactivate the sexually inactive animal but do stimulate the genital systems to a condition resembling normal development.
4. Ovarian transplants, ovarian extract injections, ultra violet radiations, and keeping animals in outdoor cages caused no apparent effect on the genital functions under the conditions of the experiment.
5. The inhibition of the normal genital functions and the reason for the failure of the attempts made to reactivate the animals probably lies in a complex linkage of the endocrine and nervous systems.

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LITERATURE CITED

- Anderson, Wm. T., and David I. Macht
 1928. The penetration of ultraviolet rays into live animal tissue.
 Amer. Jour. Physiol., 86:320-330.
- Asdell, S. A. and F.H.A. Marshall
 1927. On the effect of the ovarian hormone in producing prooestrous development in the dog and rabbit.
 Proc. Roy. Soc. (London) B 101:185-192.
 Biol. Absts., 1:840. 1927.
- Bellerby, C. W.
 1928. Relation of anterior lobe of pituitary to reproductive organs.
 Lancet, 1:1168-1169.
- Borries, Kara von
 1929. Zur Frage der biologischen Wirkungen des Frauenstudiums.
 Arch. Rass. u. Ges. Biol., 22:51-54.
 Biol. Absts., 4:1392. 1930.
- Castillo, E. B. del
 1928. Reappearance and character of oestrus cycle after ovarian grafting in castrated white rats.
 Compt. rend. Soc. de biol., 99:1501.
 Biol. Absts., 4:1084. 1930.
- Crainichianu, A.
 1926. Calcium in treatment of ovary insufficiency.
 Presse Méd., 34:545.
 J.A.M.A., 86:2004. 1926. (Abstract)
- Czarnecki, E. and J. Jolly
 1928. Action des Rayons ultra-violets sur le testicule.
 Compt. rend. Soc. de biol., 98:380-383.
- Doisy, E. A., J. O. Rolls, Edgar Allen and C. G. Johnston
 1924. The extraction and some properties of an ovarian hormone.
 Jour. Biol. Chem., 61:711-727.

Drips, Della

1919. Studies on the ovary of the spermophile with special reference to the corpus luteum.
Amer. Jour. Anat., 25:117-184.

Engle, E. T. and Camille Mermod

1928. The effect of daily transplantation of the anterior lobe on the course of pregnancy in the rat and mouse.
Amer. Jour. Physiol., 85:518-526.

Erhardt, Karl

1929. Hypophysen-Vorderlappen und Genitale.
Münchener Med. Wochenschr, 75:785-786.
Biol. Absts., 3:1743. 1929.

Evans, H. M.

1925. Anti-sterility vitamin, fat soluble E.
Science, 61:519-520.
- 1928a. Effects of inadequate vitamin A on sexual physiology of females.
J. Biol. Chem., 77:651-654.
- 1928b. Effect of inadequate vitamin B upon sexual physiology in the male.
J. Nutrition, 1:1-21.

Evans, H. M. and Geo. O. Burr

1926. Increased efficacy of subcutaneous when compared with intraperitoneal administration of the ovarian hormone.
Amer. Jour. Physiol., 77:518-521.

Evans, H. M., Geo. O. Burr and T. L. Althausen

1927. The antisterility vitamin, fat soluble E.
Mem. Univ. California, 8:1-176.
Biol. Absts., 2:1560. 1928.

Evans, H. M. and J. A. Long

1921. Effect of feeding the anterior lobe of the hypophysis on the oestrus cycle of the rat.
Anat. Rec., 21:62.

Evans, H. M. and M. E. Simpson

1925. Characteristics of the anterior hypophyseal hormones.
Anat. Rec., 29:356.

- Evans, H. M. and M. E. Simpson
1926. Effects of anterior hypophyseal extracts on the male.
Anat. Rec., 32:206.
1928. Antagonism of growth and sex hormone of the anterior hypophysis.
J.A.M.A., 91.
- Fee, A. R. and A. S. Parkes
1929. Studies on ovulation. I. Relation of the anterior pituitary body to ovulation in the rabbit.
Jour. Physiol., 67:383-388.
- Goetsch, E.
1916. Influence of pituitary feeding upon growth and sexual development.
Bull. of Johns Hopkins Hosp., 27:29-50.
- Golding, George T. and F. T. Ramez
1928. Ovarian and placental hormone effects in normal immature albino rats.
Endocrinology, 12:804-812.
- Grunert, C. H.
1927. Absence of estrum corrected by ovary transplantation.
Veterinary Medicine, 22:112-113.
- Gullickson, T. W. and C. H. Eckles
1928. The relation of sunlight to growth and development of calves.
Jour. Dairy Sci., 10-87-94.
Biol. Absts., 2:227. 1928.
- Howell, A. H.
1928. Descriptions of six new North American ground squirrels.
Proc. Biol. Soc. of Washington, December, 1928.
- Johnson, G. E. and E. D. Sayles
1929. The effect of daily injection of bovine anterior pituitary extract upon the developing albino rat.
Physiol. Zool., 2:285-301.

- King, Jessie L.
1926. Menstrual records and vaginal smears in a selected group of normal women.
Carnegie Inst. Washington Publ., 363:79-94.
- Knaus, H. H.
1926. The action of pituitary extract administered by the alimentary canal.
Brit. Med. Jour., 1:234-235.
Biol. Absts., 4:422. 1930.
- Kunde, M. M. and F. Proud
1929. The ineffectiveness of vaginal smears in predicting the oestrus cycle in the rabbit.
Amer. Jour. Physiol., 88:446-452.
Biol. Absts., 4:405. 1930.
- Lafora, G. R.
1923. Sexual frigidity in women.
Siglo Med., 72:1053.
J.A.M.A., 82:251. (Abstract)
- Lee, Milton O.
1926. Studies on the oestrus cycle in the rat. The effect of low environmental temperatures.
Amer. Jour. Physiol., 87:246-253.
- Leigh-Claire, Joan Leigh
1927. The effect of excessive radiation with ultra violet light upon the growth of rats.
Biochemical Journal, 21:208-210.
- Lewis, Dean D. and J. L. Miller
1913. The relation of hypophysis to growth and the effect of feeding anterior and posterior lobe.
Arch. Int. Med., 13:137.
- Long, J. A. and H. M. Evans
1922. The oestrous cycle in the rat and its associated phenomena.
Mem. Univ. California, 6:1-148.
- Macomber, Donald
1924. Prevention of sterility.
J.A.M.A., 83:678-683.

- Marinus, C. J.
1919. Effect of feeding pars tuberalis and pars anterior propior of bovine pituitary glands upon the early development of the white rats. Amer. Jour. Physiol., 49:238-247.
- Marshall, F. H. A. and W. R. Peel
1910. Fatness as a cause of sterility. J. Ag. Sci., 3:383-389.
- Mathews, Albert P.
1927. Physiological Chemistry. William Wood and Company, New York. 4th Ed.
- Mattill, H. A.
1927. The relation of vitamins B and E to fertility in the male rat. Amer. Jour. Physiol., 79:305-315.
- Parkes, A. S.
1928. The nature of the anoestrous condition resulting from vitamin B deficiency. Quart. Jour. Exper. Physiol., 18:397-401. Biol. Absts., 3:1860. 1929.
- Parkes, A. S. and C. W. Bellerby
1926. Studies on the internal secretions of the ovary. II. Effects of injection of oestrous producing hormone during pregnancy. Jour. Physiol., 62:145-155.
- Pettinari, V.
1925a. Regeneration of ovaries from ovarian grafts. Compt. rend. Soc. de biol., 92:1294.
1925b. The sexual hormones in the grafting of genital tissue. Rev. Franc d'endocrinol., 3:163. J.A.M.A., 85:1010. (Abstract)
1926. La greffe ovarienne et ses applications a'la therapie humaine. Gynec. et Obstet., 13:19-40. Biol. Absts., 2:972. 1928.

- Rees, Maurice H. and Richard W. Whitehead
 1923. Effect of digestive enzymes on pituitary extract action.
 Amer. Jour. Physiol., 65:90-100.
- Riddle, O. and Florence Flemion
 1928. The role of the anterior pituitary in hastening sexual maturity in the ring doves.
 Amer. Jour. Physiol., 87:110-123.
- Runge, S.
 1926. Essais de transplantation des glandes sexuelles chez les animaux domestiques.
 Compt. rend. Soc. de biol., 94:1210-1212.
- Saidman, J.
 1924. Ultra violet rays in ovarian insufficiency.
 Bull. Acad. de méd., 92:938.
 J.A.M.A., 83:795. (Abstract)
- Shaw, Wm. T.
 1926. A short season and its effect upon preparation for reproduction by the Columbian ground squirrel.
 Ecology, 7:136-139.
- Sippel, P.
 1924. Pregnancy after ovary grafting.
 Zentralbl. f. Gynäk., 48:15.
 J.A.M.A., 82:667. (Abstract)
- Slonaker, J. R.
 1927. The effect of the follicular hormone on old albino rats.
 Amer. Jour. Physiol., 81:325-335.
 Biol. Absts., 2:1590. 1928.
- Smith, C. S.
 1923. The alleged effects on body growth and gonad development of feeding pituitary gland substance to normal white rats.
 Amer. Jour. Physiol., 65:277-281.
- Smith, P. E.
 1927a. The induction of precocious sexual maturity by pituitary homeotransplants.
 Amer. Jour. Physiol., 80:114-125.

- Smith, P. E.
 1927b. Genital system responses to daily pituitary transplants.
 Jour. Exper. Biol. and Med., 24:337-338.
- Smith, P. E. and E. T. Engle
 1927. Induction of precocious sexual maturity in mouse by daily pituitary homeo and hetero transplants.
 Proc. Soc. Exper. Biol. and Med., 24:561-562.
- Stieve, H.
 1926. Unfruchtbarkeit als Folge unnatürlicher Lebensweise.
 Grenzfragen des Nerven und Seelenlebens, Heft. 126. 52 p., 20 fig. J. Bergmann, Munich.
 Biol. Absts., 2:1601. 1928.
- 1927 Die Abhängigkeit der Keimdrüsen vom Zustand des Gesamtkörpers und von der Umgebung
 Naturwissenschaften, 15:951-963.
 Biol. Absts., 3:1776. 1929.
- Stockard, C. R. and G. N. Papanicalaou
 1917. The existance of a typical oestrous cycle in the guinea pig with a study of its histological and physiological changes.
 Amer. Jour. Anat., 22:225-284.
- Stone, Calvin P.
 1926. The initial copulatory response of female rats reared in isolation from age of twenty days to age of puberty.
 Jour. Comp. Psych., 6:73-83.
 Biol. Absts., 1:67. 1926.
- Sure, Barnett
 1928. Dietary requirements for fertility and lactation: The vitamin A content of wheat oil.
 Jour. Ag. Res., 37:93-99.
- Tuffier and Bour
 1925. Pregnancy after ovary grafting or transposition.
 Presse Méd., 33:1073.
 J.A.M.A., 85:1010. (Abstract)

Tuisk, Robert

1927. Protracted oestrus induced by ovarian extracts.
Jour. Physiol., 63:180-186.

Wolf, Opal Marie

1929. Effect of daily transplants of anterior lobe
of pituitary on reproduction of frog (Rana
pipiens Sherber).
Proc. Soc. Exper. Biol. and Med., 26:692-693.

Zondek, B. and Aschheim, S.

1928. Das Hormone des Hypophysenvorderlappens.
Klin. Wchnschr., 7:831-835.