1947

On the Reproductive Cycle of the Male Columbian Ground Squirrel (Citellus columbianus)

Phil Simon Smith

The University of Montana

ON THE REPRODUCTIVE CYCLE OF THE MALE COLUMBIAN GROUND SQUIRREL,

(*Citellus columbianus*)

by

Phil S. Smith

B.A., Montana State University, Missoula, Montana

Presented in partial fulfillment of the requirement for the degree of Master of Arts.

Montana State University

1947

Approved:

Philip L. Wright

Chairman of Examining Committee

W. P. Clark

Dean of the Graduate School

qt.-2

TABLE OF CONTENTS

INTRODUCTION AND STATEMENT OF PROBLEM

Investigations of various species of hibernating sciurids such as the work of Wells (1935) with *Citellus tridecemlineatus*, of Shaw (1926a) with *Citellus columbianus*, and of Evans and Holdenreid (1943) with *Citellus beecheyi* indicate the onset of sexual maturity after the first period of hibernation, i.e., as yearlings. However, Longhurst (1944) in the prairie-dog, *Cynomys gunnisoni*, in Colorado, finds that subadults (yearlings) produce smaller litters than do adults, and that only one out of 35 adult females but eight out of 20 yearling females failed to produce young. Hamilton (1934) cites similar evidence for woodchucks, *Marmota monax*: one of 44 adult and thirty-two of 43 subadult females failed to produce young and, of forty-five subadult males, only 15 had enlarged testes. In four of the 15 subadults were the testes descended into the scrotum while in four of 38 adults the testes were neither enlarged nor had they descended into the scrotum.

In the spring of 1941 two senior students working under the direction of Dr. P. L. Wright of Montana State University collected 100 or more Columbian ground squirrels, *Citellus columbianus*. Although they did not study the problem

---

All references which are included in the body of this thesis contain the name of the author and the date of publication. Completed references have been placed in alphabetical and chronological order at the end of the thesis.

intensively, it was apparent during the breeding season (which begins shortly after the adult females have appeared from hibernation and continues actively for a period of about three weeks (Shaw, 1924)) that in many small males and females the reproductive organs were sma ll in comparison to those of the larger animals. From 1942 to 1945 Dr. Wright collected nearly 500 ground squirrels and preserved their reproductive organs. In January 1946 I became interested in the problem and during 1946 and 1947 collected 193 specimens of Citellus columbianus.

This paper has been an attempt, based upon the study of nearly 500 animals collected by Dr. Wright and myself (1) to work out criteria for determining the age of animals taken in the field, (2) to describe the reproductive cycle of the males, and (3) to determine the degree of sexual maturity attained by the yearlings and juveniles.

I should like to express my gratitude to Dr. P. L. Wright for his assistance and suggestions and for making available his materials and data; to Peggy A. Smith for her assistance in collecting and preparing the materials for study; and to Mrs. Gordon B. Castle for helpful suggestions.

REVIEW OF RELATED LITERATURE

Citellus tridecemlineatus

According to Moore et al (1934) the thirteen-lined ground squirrel (*Citellus tridecemlineatus*) emerges from hibernation around the first of April, the males about ten days before the females. These authors also indicate that the females come into heat shortly after arriving above ground and their one estrous cycle may last for several weeks if coitus does not occur. That the ground squirrels are sexually inactive from July to December is shown by Coco (1942) and by Moore et al (1934). Coco (1942) indicates there is but a single estrous cycle and divides this into four periods: anestrum, proestrum, estrum (copulation occurs during this period or soon after), and metestrum. The ground squirrel has a duplex uterus and during the anestrous period the vaginal orifice is closed by a cutaneous membrane (Wells and Overholser, 1940). Five to thirteen young (Howell, 1938) are born, after a gestation period of about 28 days, from mid-May to the first of June (Wade, 1927 and Moore et al, 1934). During the post-partum period the reproductive tract undergoes considerable involution although the ovaries increase in size during the four to six weeks of lactation (Moore et al, 1934). Erickson (1940) describes the young squirrels at birth as being without hair or teeth, with red bodies and proportionately large heads; they weigh 3.5 grams each and measure 4.5 to 5.0 cm. in length. That their somatic growth in captivity is rapid is shown by Simmons (1946). They increase in weight from 3 gms. at birth to 160 gms. at 70 days of age. Erickson (1940) indicates that on the 9th day an appreciable amount of fine hair can be seen, the incisors begin to show on the 10th day, the orifice to the ear has formed by the 14th day, on the 24th day the eyes are open and a few days after this the legs are strong enough to support the body weight. Moore et al (1934) show that after weaning the ovaries and all accessories of the adult females regress to a low point and are essentially inactive during the summer and fall. Around January 1 the reproductive tract starts developing and in the spring the older females emerge in a more advanced sexual state than do the yearlings.

At the time of emergence from hibernation the adult males are in full breeding condition while it is highly probable that young adults are the last ones to attain maximal sexual development (Wells, 1935). At the end of hibernation the scrota are dark, become even darker during May, and then pale until no pigmentation remains when the animals return to hibernation in October (Finkel, 1945). Wells (1935) says that the pigmentation of the scrotal skin shows a seasonal rhythm, similar to that of other secondary sex characters.

The genital tract of the male _C. tridecemlineatus_ is more elaborate than that of the gray squirrels (_Sciurus carolinensis_) and of chipmunks (_Tamias striatus_) and one of its most striking features is the enormous bulbar gland which, in an old sexually active adult often measures 20 mm. from end to end and 8 mm. in diameter (Mossman et al, 1932). The tract has large seminal vesicles, a broad muscular urethra which has a series of about 13 pairs of valve-like folds in its lining, and a large mesorchium in which a considerable amount of fat is deposited before hibernation. Other approximate measurements, besides those of the bulbar gland, as indicated by Mossman et al (1932), are given in Table I.

TABLE I

MEASUREMENTS (IN MM.) OF THE REPRODUCTIVE ORGANS AND ACCESSORIES OF THE MALE _C. TRIDECEMLINEATUS_

	Bulbar gland	Testis	Seminal vesicle	Prostate	Muscular urethra	Penis plus bulb
Width	8	8	7	10	5	
Depth	8	9	7	5	4	
Length	20	18	20	8	22	45

Wells (1935) says that body weights in this species cannot be utilized as a griterion for distinguishing immature animals from adults after the young have become 4 or 5 months old, but the reproductive glands offer a reliable complex of criteria for age determination as late as February, since the genital system of young males develop more slowly than those of adults. He also finds that the peak in the function of the reproductive system is reached by most males in either late April or early May. He observed that the entire male reproductive tract begins to regress by June, becomes completely involuted by July or August and is quiescent until December when it again starts to develop. Spermatozoa appear in the seminiferous tubules in January or February. The accessory organs lag behind the testes both in their augumentation and in their involution. He indicates that young males show a lag of from 2 to 3 weeks in the development of spermatozoa and in the development of their accessories.

Numerous experimental studies have been made upon C. tridecemlineatus in order to determine and analyze the factors which operate to produce the sexual periodicity in both the male and female of the species. Wells (1935) felt that unilateral castration produced no observable effects on accessory organs and it failed to affect spermatogenesis, significantly, within periods up to 39 days. He also shows that the whole genital tract of the males is labile and can be stimulated to a functional breeding condition, by administration of gonadotrophic substances during the period of low sexual activity. His results demonstrate that the anterior pituitary shows a seasonal variation in its gonadotrophic potentiality. Wells (1936) says the size of the testes is a reliable index to the hormonal condition of testes and accessories. That raw pregnancy urine, antuitrin-S, bull testes extract, and pregnant mare serum will produce a very great response by the accessory organs is shown by Wells and Moore (1936). They also found that unilateral castration produced some hypertrophy in the remaining testis. Hypophysectomy performed on squirrels during the breeding season causes both the testes and accessories to regress and when it was performed during the aspermatic period the reproductive organs and accessories failed to undergo renewed development at the time when normal controls were showing reproductive activity (Wells and Gomez, 1937). Wells and Zalesky (1940) prevented the seasonal regression of the reproductive organs by maintaining C. tridecemlineatus which were in breeding condition at a constant temperature of 4 degrees Centigrade, but when the low temperature treatments were started during the aspermatic period no more than a slight hastening in the time of appearance of spermatozoa in some experimental animals was caused. Apparently, neither light nor temperature has much effect upon the rate or time of development of the reproductive tract of *C. tridecemlineatus*, as Wells (1935) found that neither increased light periods, constant cold temperature, nor constant darkness produced observable effects upon animals during the period when the gonads were abdominal and the accessories non-functional.

Other related genera and species

In comparison with the work done upon *C. tridecemlineatus*, little has been done upon other closely related species. Hamilton (1934) studied the rufescent woodchuck (*Marmota rufescens*) in the east. He states that the chucks have been observed in the wild state as early in the year as February 21 and as late as November 15. Some of the subadults (yearlings) breed. The gestation period is approximately 28 days. The average number of young, as derived from embryo counts in 31 gravid females, is 4.07. The young are born from the first week in April up to the middle of May. There is a possibility that woodchucks have several estrous cycles in the spring of each year. The larger, older animals mate earlier than do the younger ones.

Evans and Holdenreid (1943) worked with the Beechey ground squirrel (C. beecheyi) in California and found that the breeding season begins in February and ends around the middle of April. Reduction in size of testes was first noted in May and, by June, they had receded into the abdomen. In mid-October some adult males showed testicular enlargement, and by the end of November the testes had descended in almost every male. They remained large throughout the winter and spring. These animals have a gestation period estimated to be about 30 days. Most of the young are born by the middle of April. Litters average 8.2 young. The young can be distinguished from the adults by weight until the middle of August. There is a period of dormancy among some of the adult animals.

The prairie dog (Cynomys leucurus) is sexually active at the age of one year. The mating season extends from the end of March into April and a rapid reduction in testis size takes place in April. The gestation period is 28 to 33 days. The litter size varies from 2 to 10 with a mean of 5.5 (Stockard, 1929).

Deansely and Parkes (1933) indicate that the female grey squirrel (Sciurus carolinensis) in England does not breed until it is nearly a year old. Two litters a year, one in the spring and one in the summer, may be produced by a single female. The average size of the litters is about 3.6. After the second litter, anestrus sets in and continues throughout the winter. Allanson (1933) says that all young male *S. carolinensis* from the previous year reach puberty by the beginning of April. She indicates that males with fully functional testes may be found throughout the year but it is possible that individual males do not remain continuously in reproductive activity.

The Columbian Ground Squirrel
(*Citellus columbianus*)

According to Shaw (1925a), the time of emergance from hibernation of the Columbian ground squirrels in the region of Pullman, Washington, is rather constant, occurring about March 1 to 15. He indicates that the adult males are the last to go into hibernation in late summer and the first to come out in the spring, with the adult females, young males, and young females following in that order. Very shortly after the females emerge from hibernation, the breeding season begins and continues actively for a period of about three weeks (Shaw, 1924). He indicates (1925d) several estrous cycles during a single breeding period. The time of the breeding season depends primarily upon the elevation (Shaw, 1924). Moore (1937) supports this when he says that there is a delay of one day for each 100 feet rise in elevation in the first appearance of young Columbian ground squirrels from the burrow. His studies include data from Lewiston, Idaho, and from Moiese and Phillipsburg, Montana. That breeding takes place later on north slopes than on south slopes is brought out by Shaw (1925c), who also (1925d) reports a gestation period of 24 days. The average litter size as determined by embryo counts by Shaw (1924) was 5.09, while that given by Howell (1938) was 3.5. A single litter per year is produced. The new born young are naked, blind, and toothless but by the time they are 17 days of age their eyes are beginning to open and by 28 days they are ready to shift for themselves (Shaw, 1918). Shaw (1925d) gives the time of appearance of the incisors as 14 days and they are completely erupted by 19 days. From 21 to 23 days days after birth the eyes open. The reproductive organs of the parous females start to deline after parturition and at estivation the uterine horns are "mere bloodless tubular threads"(Shaw, 1926).

Shaw (1926) describes the testes of all males of this species as being at maximum size, scrotal, and congested with blood upon the emergence of the animals from hibernation. After the breeding season the reproductive organs begin a rapid decline in size and activity and are at the minimum by the time of estivation in late July and early August. Shortly after the start of estivation, the reproductive organs of both males and females begin to develop.

Observations which I have made in the area around Missoula, Montana, have indicated that Columbian ground squirrels emerge from hibernation during the latter part of March and the first part of April. They appear to be most active from April 1 to 15. Probably most of the breeding takes place during this time. Parturition occurs around May 1. Young animals are about 30 days old when they first make their appearance above ground. Estivation (summer hibernation), which merges into true hibernation without an intervening period of activity, largely occurs the latter part of July although, at higher elevations, some animals remain active until almost the middle of August.

MATERIALS AND METHODS

Description of areas in which collections were made

The locations in which collections were made can be divided into two general areas, i.e., the Gold Creek area and the Missoula area. Both are in Missoula county.

The Gold Creek area is in a mountain valley about 29 miles north of Missoula, Montana. The elevation is approximately 4500 feet. The valley lies nearly in a northerly and southerly direction. Collections were made in S. 12, T. 14 N., R. 17 W. and S. 1, T. 14 N., R. 16 W. Through the valley flows Gold Creek, a small mountain stream. Generally, snow starts to accumulate by the middle of October or the first of November and remains until some time the following April. The sides of the valley are heavily wooded but the valley floor is largely meadow land in which timothy (<u>Phleum sp.</u>) is abundant. A farm, owned by Mr. F. Mott, is located about one-half mile upstream from the main bridge. Oats and timothy hay are the main crops. Numerous squirrels were taken from the cultivated fields and from a pasture which almost surrounds the house. They were also collected on a meadow near the bridge. The soil is mainly sandy loam. Of the total animals collected, 69 were from the Gold Creek area.

The Missoula area consists of several parts. The elevation in each instance is approximately 3200 feet. As nearly as could be determined, climatic conditions were similar throughout the Missoula area during 1946 and 1947. Since minor variations exist between the parts, each part will be discussed individually.

O'Brien Creek canyon is in the mountains on the west side of the Bitterroot river. The canyon is about 10 miles from Missoula. Collections were largely made in S. 20 and 29, T. 13 N., R. 20 W. In this area the canyon is approximately one-half mile wide. Nearly all of the level ground was deserted farm land in 1946. Much of it is now being farmed. The north facing slope of the canyon is heavily wooded whereas comparatively few trees are found on the slopes that face south. O'Brien Creek is a small mountain stream. It contained no water during late summer of 1946. The soil in the area is mainly sandy-loam. Types of vegetation on the floor of the canyon consisted largely of _Bromus tectorum_, _Agropyron sp._, and _Poa sp._ and on the less wooded slopes _Bromus tectorum_ and _Agropyron sp._ appeared to dominant. Squirrels were collected on the canyon floor and on the less wooded slopes. Dens were found in all parts of the area. Some were within 6 to 10 feet of the creek channel.

Squirrels were also collected in a pasture in S. 7, T. 12 N., R. 20 W., located about 7 miles south of Missoula, on the east side of the Bitterroot river. The ground is level and of a sandy composition. Vegetation consists largely of Poa sp. A garden, located about 300 feet from the pasture, suffered considerable damage from the squirrels although no dens were found between the garden and pasture. A slow moving stream flows along the west end of the pasture. Vegetation remained green for several weeks after the squirrels had estivated. This entire area was covered with water during the period in 1947 when the Bitterroot river overflowed its banks. No squirrels have been seen in the area since that time.

A third district, included in the Missoula area, is near Avaro, Montana. Most of the collections were made in pasture land and low hills in S. 24, T. 15 N., R. 20 W., and S. 19, T. 15 N., R. 19 W. which lies east of Avaro. The soil is sandy. The vegetation, consisting largely of Phleum sp. (Timothy), and white clover, appeared to be closely grazed throughout the area. Low shrubs appeared to be the dominant type of vegetation on the hills. The sub-story was largely Bromus tectorum. Dens were found throughout the area.

A few animals were collected in other areas than those mentioned, but each area was within 50 miles of Missoula and the environmental conditions were similar to those of the Missoula area. Consequently these animals have been included with those of this area.

Slope of ground apparently has little influence upon the location of dens, as they were found on all slopes up to at least 45 degrees. The type of soil and the density of timber, however, seem to have a definite influence as no dens were found in extremely rocky or heavy clay soil. The majority of the dens were in areas where the soil was sandy and there was no dense timber. A few animals were observed in a brushy, cut-over area near the University Biological Station on the east shore of Flathead Lake. Shaw (1924) specifies the depth, texture and drainage of the soil as being probably the most important factors in determining the location of a den and reports that the squirrels seem to avoid stiff clay hill-tops and low flats. I have found dens in close proximity (within 6-10 feet) to water and also in areas where the animals could not possibly have had access to surface water. The squirrels apparently are able to obtain their water from the vegetation they eat and a direct source is not necessary. Although no stomach analyses were made, it was observed that by far the most food consumed was green vegetation and even animals collected in late March and early April were apparently subsisting on such food. Shaw (1918) indicates that C. columbianus is one of the most vegetarian of all the ground squirrels and, from the time of emergence from hibernation, lives largely upon green succulent vegetation. When this begins to ripen the animals utilize the seed products of grain and plants.

As may be seen by Figure 1, the temperatures averaged below normal during the months from May through August of 1946. Figure 2 shows the amount of precipitation during the same period to be below normal with the exception of July when it was above. Figure 1 and Figure 2 were plotted from data formulated by the Weather Bureau, U.S. Department of Agriculture.

Materials

Four hundred and ninety-three ground squirrels were collected during the period from 1942 to 1947. Of these 239 were males and 254 were females, a sex ratio of 51.5 percent females to 48.5 percent males. No effort was made to be selective in making the collections. The sex ratio

FIGURE 1

AVERAGE MONTHLY TEMPERATURES NEAR MISSOULA, MONTANA FOR SPRING AND SUMMER OF 1946 COMPARED WITH THE AVERAGE FOR 67 YEARS

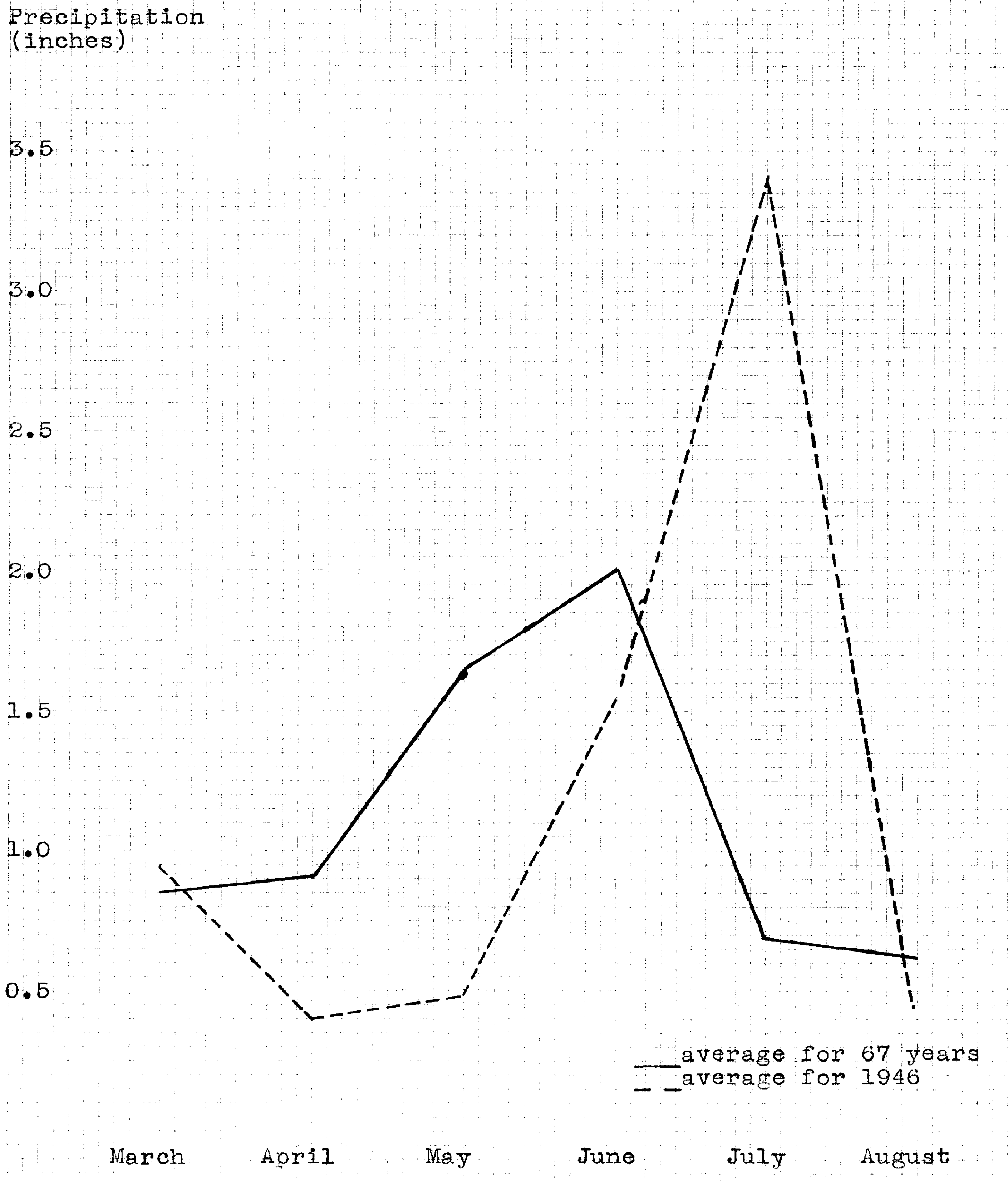


FIGURE 2

AVERAGE MONTHLY PRECIPITATION NEAR MISSOULA, MONTANA FOR SPRING AND SUMMER OF 1946 COMPARED WITH THE AVERAGE FOR 67 YEARS obtained differs considerably from that found by Shaw (1924) in which, of 28 squirrels, 60 percent were females and 40 percent males, and from that of Foster (1911) in which, of 545 squirrels examined, 69 percent were females and 31 percent males. Shaw's numbers, however, are too small to be of real value.

The body weights, reproductive organs and a few skulls were available from the 300 squirrels collected by Dr. Wright from 1942 to 1945, and much of this material and data has been included in this thesis. Since I was interested in developing a method whereby the age of animals could be determined in the field, not only the reproductive organs but also the skulls and the right femur from each animal of those collected in 1946 and 1947 were saved. In a very few instances the reproductive organs were not in condition for studying because they had been ruined by shooting or by desiccation. In the late summer of 1946 three juvenile females and one juvenile male, their age being determined by pelage and size, were live-trapped and placed in the laboratory. In April, 1947, five yearling females and two yearling males were also placed in the laboratory for study. Body size and appearance indicated that these animals were yearlings and later examination of the teeth and femurs of two of them supported the indications.

Methods

The animals were collected either by shooting with a .22 calibre rifle or by the use of size No. 0 or No. 1 steel traps. Smaller traps than this are not satisfactory as even with the larger traps some of the animals managed to escape. When traps were used they were checked as often as possible, usually every three or four hours except when they were left overnight and then they were checked the following morning before 8:00 A.M. Animals found dead in the traps were discarded after they had been weighed and their skulls removed. With the exception of those retained for observation in the laboratory, all trapped animals were killed immediately, weighed and dissected.

Field dissection

The animals were weighed on a fulcrum balance. They were then checked for disease by examining the liver and spleen for the presence of necrotic areas. The glands on the inner side of the legs were checked for swelling. In only two instances were there any indications of necrosis and in both cases animals were discarded which had been collected in these areas but which had not been dissected. A later examination of squirrels from these areas failed to show any indication of disease. At no time were animals found with swollen glands.

Upon completion of this check, the reproductive organs, a portion of the scrotal skin, and, in the majority of cases, the left femur, were removed and placed in AFA. The formula for AFA is as follows: 50 parts water, 30 parts 95% alcohol, 10 parts glacial acetic acid, and 10 parts formalin.

In order to determine the amount of fusion of the epiphysis to the diaphysis of the femur at a time when the animal was being dissected, the right leg was bent so the knee joint was forced inward until either the ligaments gave way, breaking the femur from the tibia, or the epiphysis of the femur broke from the diaphysis. The femur and skull were then removed from the carcass and retained for future study.

Definition of terms

In this thesis, juveniles are defined as those animals which have not passed through a hibernation period, yearlings are those which have passed through one hibernation period, and adults, those which have passed through more than one. When reference is made to the femurs, those of which the epiphysis broke cleanly from the diaphysis are <u>not fused</u>;

those where either part of the diaphysis or part of the epiphysis broke off, leaving the remainder, are defined as being partly fused; while those in which the femur remained intact are fused. The nature of the parietal-frontal suture is classified as follows: open---when a clearly distinguishable suture near the mid-dorsal line is present; partly-closed---when partial fusion has taken place but a line of demarcation still exists; closed---when fusion is complete and no line of demarcation between the parietals and frontals remains. In animals in which the latter condition is present the median occipital keel is usually strong.

Since some animals were killed when their stomachs were distended with freshly eaten food and others had empty stomachs, it is apparent that a considerable amount of variation in the recorded body weights would result from this factor alone. Accordingly, some animals were first weighed intact, and then reweighed after the stomach and intestines had been removed. This was done in order to secure a correction value which could be applied to obtain the real body weight when the animals were weighed with these digestive organs removed. Since no record of the amount of food in the digestive tracts of the animals taken early in the study was kept, it was impossible to apply the correction.

The weights as recorded, then, include the weight of the food in the digestive tract.

Laboratory procedure

<u>Skulls and femurs</u>. The skulls and femurs were boiled in water for varying periods depending upon the apparent age as determined by body size of the animal. The larger animals were assumed to be the older. Seven minutes of boiling were allowed for the largest, three for the smaller, and one to two minutes for those which were known to be juveniles. After boiling, the flesh was removed from the skulls and femurs. They were then bleached in hydrogen peroxide.

In order to obtain sufficient contrast between the dentine and enamel for the purpose of photography it was necessary to stain the teeth. The staining method used was as follows: All debris was picked from the upper right row of teeth. This tooth row is used in identification of the stages discussed later in the paper. The skulls were then placed in Acid-fuchsin, heated to just below the boiling point, for three minutes. Upon removal from the stain they were destained in 5% HCl-alcohol until the enamel was white. Following this the skulls were placed in hydrogen-peroxide for four hours, after which they were taken from the peroxide and the staining procedure repeated except that the larger skulls were left in the stain for six minutes. Upon removal from the stain the second time they were partially destained in 3% HCl-alcohol, washed in water and allowed to dry. The method stains the dentine red and leaves the enamel without stain.

It is possible to differentiate enamel from dentine in unstained teeth by pricking with a sharp needle since the dentine can be easily dented.

Male reproductive organs. The male reproductive organs studied consisted of the testes, epididymides, vasa deferentia, and, when possible, the seminal vesicles. No attempt was made to make use of the baculum or its weight as Bond (1941) was unable to form any conclusions concerning either.

After removing the extraneous material from the testes and epididymides the organs were individually removed from the AFA, rolled on paper towelling to remove the excess liquid, and weighed on a torsion balance. The weight was measured in milligrams. The combined weight of the paired organs was taken except when one had been destroyed or damaged and then the weight of the remaining one was doubled.

Some error was possibly introduced by doing this but it could not be avoided.

__Microtechnique.__ After fixation, one testis, caput epididymis, vas deferens, and a portion of the scrotal skin of each animal from a majority of the 1946-47 series and from a portion of those collected by Dr. Wright were sectioned at 8-10 micra and stained in Erlich's hematoxylin and eosin. In some instances orange-G dissolved in clove oil was used in combination with the hematoxylin and eosin.

__Female reproductive organs.__ The female reproductive organs were checked for evidence of pregnancy or estrus as indicated by uterine scars, embryos, copulation plugs, and the presence or absence of the vaginal membrane. One of the ovaries of 1-f, a captive female which was killed with ether at the time when the vaginal swelling was decreasing, was serially sectioned and stained by means of the above technique.

__Methods of taking measurements.__ Average diameters of the seminiferous tubules for most testes as well as for the diameters of the tubes in a large portion of the caput epididymides were calculated. Ten measurements of the diameters of tubules chosen at random were made from slides of each testis or epididymis. An ocular micrometer was used for making the measurements. In each case the smallest diameter was measured to avoid unduly large measurements due to the coiling of the tubes. Where possible the height of epithelial cells and thickness of muscle layers were obtained by taking the average of ten measurements from each slide. When it was not possible to obtain an average, as of the epithelial height in the vas deferens, care was taken to use only measurements from true cross sections. The diameter of nuclei was obtained by averaging twenty-five measurements from each slide. Except when it has been necessary to use decimals where very small measurements, such as diameters of nuclei, were taken, all averages have been calculated to the nearest whole quantity.

GROUPING OF ANIMALS BASED UPON TOOTH WEAR, BODY WEIGHTS, CLOSURE OF THE PARIETAL-FRONTAL SUTURE, AND FUSION OF THE EPIPHYSIS OF THE FEMUR

Superficial examination of the skulls and teeth of specimens collected indicated a considerable variation in the amount of tooth wear. A more intense examination was made and the skulls from both male and female animals were separated into ten stages (Plates I, II, and III). These stages were based upon tooth succession and the comparative amounts of dentine and enamel showing on the occlusial surface of the upper right tooth row.

Unknown conditions related to problem.

Although there are probably many conditions related to this problem which are not known, two appear to be of primary importance.

The first is the effect upon tooth wear of the variation in length of the dormant period. It has not been possible to determine definite dates of hibernation, although the records taken show that in the immediate vicinity of Missoula, squirrels have been observed as early as March 12. On July 22, 1946, although no squirrels were observed, there was evidence of activity on the part of the animals as was shown by fresh dirt at the entrance of the dens. No squirrels were seen and no evidence of activity was observed after this date in this vicinity. On August 13, 1946, in the Gold Creek area, one squirrel was seen. On August 20, Mr. F. Mott, a farmer in the area, said he had seen very few during the past few days. On September 1, no evidence of recent activity was to be observed. No observations were made on the ground squirrels in the Gold Creek area during the early summer because of road conditions. Mr. Mott reported that they had come from hibernation between the 10th and 20th of April in 1947. Shaw (1925a) gives the time of coming from hibernation for Columbian ground squirrels at Pullman, Washington, as being from March 1 to March 15, and the time of estivation as the latter part of July and the first part of August, depending on the weather. Moore (1937) indicates that the time of going into estivation and coming from hibernation of the Columbian ground squirrels is in direct relation to the elevation. These findings correlate closely with present observations, although it is possible that the date when the majority of squirrels come from hibernation in this area is closer to the period from March 10 to March 30, than from March 1 to March 15 as is indicated by Shaw (1925a).

The second condition which is not known is the effect of bacterial action or other possible causes of wear on the teeth during the dormant period. Sarnat and Hook (1942), who investigated the relative changes which occur in teeth during hibernation of the thirteen-lined ground squirrel, say there was no change to be observed in roentgenograms and gross examination of the skulls, femurs, incisors, and bone structure of animals subjected to hibernation. They further concluded that all stages of tooth development, namely, growth, calcification, eruption, and attrition are relatively retarded during hibernation and that this is in accord with the marked depression of the general metabolism during hibernation. With this as a reference and, as no evidence of decay has been found in any of the teeth examined, this condition may be considered as of little importance for the purposes of this study.

Description of the individual stages.

All description of the various stages is made with reference to the upper right tooth row. In individual animals a comparison of the occlusial surfaces of the teeth in the upper right with those in the upper left tooth rows shows little, if any, variation in the amount of wear. The terminology of Howell (1938) for the various portions of the teeth has been used in this paper. The stages are described as follows:

Stage I. With lacteal premolars. Molars may, or may not, have erupted (Plate I, fig. 3).

Stage II. Permanent premolars, which show no wear, have replaced the lacteal premolars. Slight wear on M1, and M2, especially on the protocone; dentine, if showing at all, only in the region of the protocone. M3 showing little or no wear (Plate I, fig. 4).

Stage III. Pm4 showing wear on protocone, metacone, and paracone and Pm3 showing wear on the single ridge. Worn areas on all molars extending from protocone to paracone and to metacone with small amount of dentine appearing on cones and in small areas on the ridges between the cones (Plate I, fig. 5).

Stage IV. Premolars showing wear, with dentine showing on protocone of Pm4. Molars worn to show dentine plainly on protocone, metacone, and paracone and extending along the ridges between the cones (Plate I, fig. 6).

Stage V. Dentine showing on protocone, paracone, and metacone of Pm4 and along ridge of Pm3. Protocone, paracone, and metacone of M1, M2, and M3 worn to the same level, when viewed from the side, as the ridges between the cones (Plate II, fig. 7).

<u>Stage VI</u>. Dentine showing on about 1/3 of the occlusial surface of M1, M2, and Pm4. Worn areas often extend down sides of ridges to parastyle, mesastyle, and metastyle. About 1/3 of the occlusial surface of M3 showing wear (Plate II, fig. 8).

<u>Stage VII</u>. Protocone, paracone, and metacone of molars and Pm4 worn nearly to the level of the styles. Dentine showing on about 1/2 of tooth surface of molars and 1/3 that of Pm4. Height of protocones at nearly the same level as that on paracone and metacone (Plate II, fig. 9).

<u>Stage VIII</u>. Dentine showing on approximately 2/3 of occlusial surface of molars, 1/2 that of Pm4 and 1/3 of Pm3. The surface of the molars is cupped and almost smooth. Pm4 is similar to the molars (Plate II, fig. 10).

<u>Stage IX</u>. All teeth practically smooth on the occlusial surface. Dentine showing on 3/4 of the surface of all teeth (Plate III, fig. 11).

<u>Stage X</u>. Surface of all teeth smooth with little enamel showing. The styles form only slight indentations, if they form any at all (Plate III, fig. 12).

PLATE I

Figure 3

Stage I
(mag. x 7)

Figure 4

Stage II
(mag. x 7)

Figure 5

Stage III
(mag. x 7)

Figure 6

Stage IV
(mag. x 7)

PLATE II

Figure 7

Stage V
(mag. x 7)

Figure 8

Stage VI
(mag. x 7)

Figure 9

Stage VII
(mag. x 7)

Figure 10

Stage VIII
(mag. x 7)

PLATE III

Figure 11

Stage IX
(mag. x 7)

Figure 12

Stage X
(mag. x 7)

Discussion of stages

Stage I. On May 30, 1946, a male and a female showing the characteristics of Stage I were collected in the Missoula area. The lacteal premolars and the first molar had erupted and were functional. Covering the occlusial surface of M2 was a cartilaginous layer, whereas M3 was hidden by a thin layer of bone.

By June 7 the second molar had erupted and was functional in all juvenile animals collected, and by June 12, the third molar had erupted but was not functional. By June 20 all molars were functional. Not until July 16 was an animal collected in which the permanent premolars had erupted. The majority of young animals showed the characteristics of Late Summer Stage II by July 18. On July 26 the only animal in Stage I collected after July 18 was taken in the Gold Creek area. The lacteal premolars fell out as the skull of this animal was being prepared.

In four males born and raised in the laboratory, M1 had erupted by the time the animals were 30 to 31 days old, M2 had erupted by 38 days and M3 by 45 to 46 days.

From the above it is apparent that the permanent molars erupt at intervals of about a week, whereas, the permanent premolars do not erupt until about a month later. At this time Pm3 and Pm4 erupt almost simultaneously. Since some animals were still in Stage I in July and, it is during this month that the animals in the Missoula area enter estivation, and, since all stages of tooth development are severely retarded during hibernation (Sarnat and Hook, 1942), we might expect to find some squirrels emerging from hibernation in the spring while still in Stage I. No animal in this stage was collected in the early spring, however, and it is therefore probable that all squirrels have progressed to Stage II before estivation starts.

No evidence which would indicate a sexual difference in the time of eruption of the teeth was found.

A comparison of the upper right tooth row of the two wild juveniles collected May 30 with that of the 30 to 31 day old squirrels, raised in the laboratory, indicates that the two wild animals were about 30 days of age.

As can be seen from Table II, a rapid increase in weight is indicated for both males and females from the time of emergence of Stage I squirrels until Stage II is reached.

Of thirty squirrels in Stage I, the parietal-frontal suture was not-closed in 23 and partially-closed in 7.

TABLE II

SUMMARY BY STAGE AND MONTH OF BODY WEIGHT, CLOSURE OF PARIETAL-FRONTAL SUTURE, AND FUSION OF EPIPHYSIS TO THE DIAPHYSIS OF THE FEMURS.

		MALES									FEMALES							
				Parietal frontal suture			Fusion of femurs					Parietal frontal suture			Fusion of femurs		
Stage and Month		No.	Ave. wt. gms.	C	PC	O	F	PF	NF	No.	Ave. wt. gms.	C	PC	O	F	PF	NF
	M	1	80	-	-	1	-	-	1	1	75	-	-	1	-	-	1
I	J	10	268	-	2	8	-	-	9	7	243	-	-	7	-	-	7
	J	1	256	-	1	-	1	-	--	10	296	-	4	6	2	-	7
	A	5	356	-	4	-	-	-	5	3	303	-	3	-	-	-	3
	M	1	320	-	1	-	-	-	1	1	269	-	1	-	-	-	1
II	J	-	---	-	-	-	-	-	-	-	---	-	-	-	-	-	-
	J	7	371	-	3	4	-	-	7	11	349	-	7	4	-	-	11
	A	1	335	-	-	1	-	-	1	1	324	-	-	1	-	-	1
	M	-	---	-	-	-	-	-	-	3	289	3	-	-	-	-	3
	A	14	330	4	9	1	2	-	11	15	301	2	6	1	1	-	10
	M	9	440	5	3	-	-	-	9	3	366	1	-	-	-	-	3
III	J	-	---	-	-	-	-	-	-	2	401	2	-	-	-	-	--
	J	-	---	-	-	-	-	-	-	3	301	-	3	-	1	-	2
	A	1	546	-	1	-	1	-	-	1	378	-	1	-	-	-	--
	M	2	482	2	-	-	2	-	-	-	---	-	-	-	-	-	--
	A	9	411	9	-	1	4	4	1	4	379	3	1	-	-	3	1
	M	3	473	2	1	-	-	2	1	6	455	4	2	-	-	6	--
IV	J	1	420	1	-	-	-	1	-	1	562	1	-	-	-	1	--
	J	4	503	4	-	-	2	-	-	4	522	2	-	-	2	-	--
	A	1	523	-	1	-	1	-	-	-	---	-	-	-	-	-	--
	M	1	487	1	-	-	1	-	-	-	---	-	-	-	-	-	--
	A	6	448	6	-	-	4	2	-	2	443	2	-	-	-	1	1
	M	1	612	1	-	-	1	-	-	4	463	4	-	-	3	1	--
V	J	-	---	-	-	-	-	-	-	1	484	1	-	-	-	1	--
	J	-	---	-	-	-	-	-	-	1	510	-	-	-	1	-	--
	A	2	677	2	-	-	-	2	-	-	---	-	-	-	-	-	--
	A	3	437	3	-	-	3	-	-	3	449	3	-	-	1	1	1
	M	1	591	1	-	-	1	-	-	3	575	3	-	-	2	1	--
VI	J	1	712	1	-	-	1	-	-	2	465	2	-	-	-	-	--
	J	-	---	-	-	-	-	-	-	3	533	3	-	-	3	-	--
	A	1	519	1	-	-	1	-	-	1	532	1	-	-	1	-	--

(Continued next page)

TABLE II, Continued

Stage and month		MALES No.	MALES Ave. wt. gms.	Parietal Frontal suture C	PC	O	Fusion of femurs F	PF	NF	FEMALES No.	FEMALES Ave. wt. gms.	Parietal frontal suture C	PC	O	Fusion of femurs F	PF	NF
VII	A	3	503	3	-	-	3	-	-	1	378	1	-	-	-	1	-
	M	-	-	-	-	-	-	-	-	3	492	3	-	-	3	-	-
	J	-	-	-	-	-	-	-	-	1	461	1	-	-	1	-	-
VIII	A	5	469	5	-	-	5	-	-	2	453	2	-	-	1	1	-
	M	1	580	1	-	-	1	-	-	1	544	1	-	-	1	-	-
IX	A	2	476	2	-	-	2	-	-	1	448	1	-	-	1	-	-
	M	-	-	-	-	-	-	-	-	1	520	1	-	-	1	-	-
X	J	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	J	1	682	1	-	-	1	-	-	1	590	1	-	-	1	-	-

The epiphysis was fused to the diaphysis of the femur in two females and one male. A distinct layer of cartilage was present at the point of fusion in each of these. This layer is not found in more completely fused femurs of late stages. The failure of the femurs to break in these three animals was probably due to relatively weak ligaments of the knee region rather than a true fusion of the epiphysis to the diaphysis as occurs in older animals.

Late Summer Stage II. The first two females which showed the characteristics of this stage were collected on July 16, 1946. The first male was taken on July 20 of the same year. Both males and females in this stage were found in collections until the time of estivation.

By the time the Stage I males had attained Stage II characteristics they had acquired an average weight of 371 gm. The females had an average weight of 349 gm. The weights of the males and females in August suggest a decrease in weight just before estivation commences, but this may be due to thinner, lighter, animals remaining active after the heavier ones had estivated.

Table II shows the parietal-frontal suture to be either not-closed or partly-closed. No correlation between the date of collection and the amount of closure of the sutures was obtained.

All femurs removed from animals in this stage showed the epiphysis not fused to the diaphysis.

As was indicated among animals in Stage I, no observable differences were found to exist between males and females with respect to the factors discussed except in body weight, the males being heavier (Table II).

<u>Late Summer Stage III</u>. The squirrels seem to be less active and more difficult to approach as the summer progresses. The number of animals collected in June, July, and August was therefore less than the number obtained earlier in the year. That the females are more active than the males during July is indicated by Table III. This difference is highly significant as $\chi^2 = 9.52$ (χ^2 for P of .01 being 6.635) (Snedecor, 1940:6-12)

Animals in Late Summer Stage III did not appear in collections until after the middle of July. Of the five animals in this stage collected in 1946, three females were taken from the Missoula area in July and the remaining two from the Gold Creek area in August.

In all skulls in this stage the parietal-frontal suture was partly-closed.

The epiphysis of the femur was fused to the diaphysis in two individuals and not-fused in three. Both fused femurs had a distinct layer of cartilage between the epiphysis and the diaphysis.

TABLE III

SUMMARY BY MONTH OF MALES AND FEMALES COLLECTED

	Males						Females					
	Mar.	Apr.	May	Jun.	Jul.	Aug.	Mar.	Apr.	May	Jun.	Jul.	Aug.
Skulls available	3	47	17	14	11	6	3	27	23	13	31	3
Not available	4	67	55	9	6	0	1	66	54	14	19	0
Totals	7	114	72	23	17	6	4	93	77	27	50	3

It is doubtful if many squirrels develop beyond Stage III before they hibernate for the first time. Since numerous animals in Stages II and III were found in collections during April (Table II), it may be assumed that they are of the same age group as those which estivated the preceeding summer when in the same stages. They will be discussed as Spring Stages II and III.

Spring Stage II. The first animals collected in the spring in Stage II were shot on April 13, 1946. The average body weight of both males and females during April was considerably less than for animals of the same stage collected in July (Table II), the weight apparently being lost during hibernation. Since no animals in this stage were collected after May 3, an advance in tooth wear from Spring Stage II to Spring Stage III was indicated.

The parietal-frontal suture of all animals in this stage was partly-closed.

In all cases the epiphysis was not fused to the diaphysis.

As in previously discussed stages, males weighed, as an average, more than females (Table II).

Spring Stage III. March 27, 1946 was the earliest date animals in Spring Stage III were collected. During March 1946, three females were collected, the average weight being 283 gm. On April 3, 1946 the first male was taken. The first three males had an average weight of 392 gm. No males in this stage were collected after May 30 and no females were taken after June 3.

Table II shows that closure of the parietal-frontal suture was not complete in all animals in Spring Stage III.

Of thirty-nine femurs from animals in this stage 34 were not-fused and 5 were fused. These 5, however, showed a clear line of separation at the point of fusion between the epiphysis and diaphysis and fusion was not complete.

An examination of the data (Table II) indicates that animals in Late Summer Stage II were more numerous than those in Late Summer Stage III while the opposite was true for the Spring Stages II and III. It is possible that some tooth wear does occur during hibernation and the majority of animals in Late Summer Stage II emerge the following spring as Spring Stage III. Another possibility is that squirrels failed to estivate as early in 1945 as they did in 1946. No evidence is available, however, which supports either possibility.

Late Summer Stage IV. As no animals in Stage III were to be found among collections from June 3 until in July, it was necessary to assume that the teeth of the animals in Spring Stage III had been so worn that they had the appearance of Stage IV by the first part of June. In this thesis, therefore, Stage IV animals collected during and after the latter part of May are considered to be in the same age group as the II's and III's taken earlier in the spring. The animals in Stage V which were taken after the latter part of July have also been included in this group as it is probable that at least some of the previously mentioned Stage IV squirrels could have advanced to this stage while those in Stage V, in

June, may have advanced to Stage VI.

Two males taken on May 24, 1946, which were included in this group, weighed 445 and 500 gm. respectively. One female taken May 25 and weighing 422 gm. was also included.

As may be observed in Table II, the parietal-frontal suture was closed in all but one of the animals of this stage.

Of seven animals in this stage, 4 showed the epiphysis partly-fused, and 3 fused. When complete fusion of the epiphysis was found in animals of this stage, examination showed that complete calcification of the cartilaginous layer had not occurred.

Late Summer Stage V. A female, weighing 510 gm., taken on July 26, and two males with an average weight of 677 gm., taken August 6, were the only animals obtained in this stage. In each of these the epiphysis was fused to the diaphysis of the femur and the parietal-frontal suture was closed.

During the latter part of July and the first part of August, animals in Stage IV and V estivate and the following spring probably appear in the same stages. Therefore, animals in Stages IV and V which were collected before May 24 will be considered as Spring Stages IV and V.

Spring Stage IV. Squirrels in this stage were among the first to be taken in the spring. In 1946 a male, weighing 524 gm. was shot on March 26 and, on March 18, 1947, a male weighing 489 gm., was collected. Of the first three males, none weighed less than 425 gm. The first three females weighed 446, 276, and 378 gm. respectively. The average weight for males in April was 409 gm. and, for females, 367 gm.

The parietal-frontal suture was closed in 12 skulls, partially-closed in 3, and open in 1.

As the parietal-frontal suture was closed in all animals of stages older than Spring Stage IV it cannot be used as an aid in determining the ages of animals older than this.

In addition to a sexual difference in body weights there is another possible variation. None of the femurs from females in Stage IV, taken before May 24, showed complete fusion while those from 6 of thirteen males did.

Spring Stage V. Animals in this stage appeared in collections early in April but it is entirely possible that many of them emerge from hibernation before this.

The average weight of six males in April was 448 gm.

Two females collected during the same month weighed 461 and 424 gm. respectively. During May four females, averaging 463 gm., and one male, weighing 612 gm. were taken.

As in the Spring Stage IV, in addition to the weight difference, the fusion of the femur shows a possible sex variation. Table II shows that in the males, 2 femurs were partially-fused and 6 fused, while in the females, 1 was not-fused and 9 partially-fused. This variation might be caused by the effects of pregnancy as 9 of ten females were pregnant or had been pregnant that summer.

Stage VI. Squirrels in this stage were found in collections during every month from March through August. A total of six males and twelve females was collected. A comparison of the average weights of males and females (Table II) indicates that males are the heavier. A male which weighed 712 gm. was the largest collected. It was taken on June 20, 1946. It is probable that few animals in this area attain a much greater weight. Shaw (1925e) gives the maximum weight of 830 gm. for a male C. columbianus collected near Pullman, Washington.

A possible sexual difference, other than weight, is the apparent delay in the fusion of the femurs in the females. Whereas all femurs were fused in males in stages from the May

Stage V through Stage X, complete fusion of the femurs of the females did not occur until Stage IX (Table II).

<u>Stages</u> <u>VII</u> - <u>X</u>. That there is a decrease in the numbers of animals in stages from and including VII to X is indicated by Table II. A total of eight Stage VII, nine Stage VIII, four Stage IX, and two Stage X animals were collected.

A tendency for ground squirrels (<u>C. columbianus</u>) to show a decrease in body weight as old age is approached has been observed among those in the Missoula area by the author. This is also indicated by Shaws' (1925b) data on <u>C. columbianus</u> and by Linduska (1947) in <u>Marmota monax</u>.

Division of animals into age groups.

The following discussion will be concerned with dividing the animals into juveniles, yearlings, and adults based upon the previously discussed factors.

<u>Juveniles</u>. As has been stated before, juveniles are those animals which have not passed through a hibernation period. Animals in Stage I certainly belong to this group as it is unlikely that any of the lacteal teeth are retained until the time of estivation. Furthermore, animals in this stage are only to be found during the period extending from

the latter part of May until the middle of July.

Although Stages II and III are found in collections at two periods during the summer, it is probable that those taken during the months of June, July, and August have progressed to this stage directly from Stage I without an intervening hibernation period. As can be seen by Figure 13, the average combined weight of the Late Summer Stage II and III is greater than that for the same Spring stages. No over-lapping of the body weights of animals in these stages with the other stages is found.

Hall (1926) indicates that closure of the parietal-frontal suture is complete before the first period of hibernation in Citellus beecheyi. This is apparently not so in C. columbianus since no evidence is found of complete closure of this suture in animals of Stages I, II, and III which were taken after the first of June. It seems reasonable to assume that all animals in Stages I, II, and III, which are collected after the first of June, are juveniles and they will be considered as such in this thesis.

Yearlings. Numerous animals in Stages II and III were taken in the spring which were similar in all respects, except for being lighter in weight, to the animals in the same stages which were collected in the late summer (Table II).

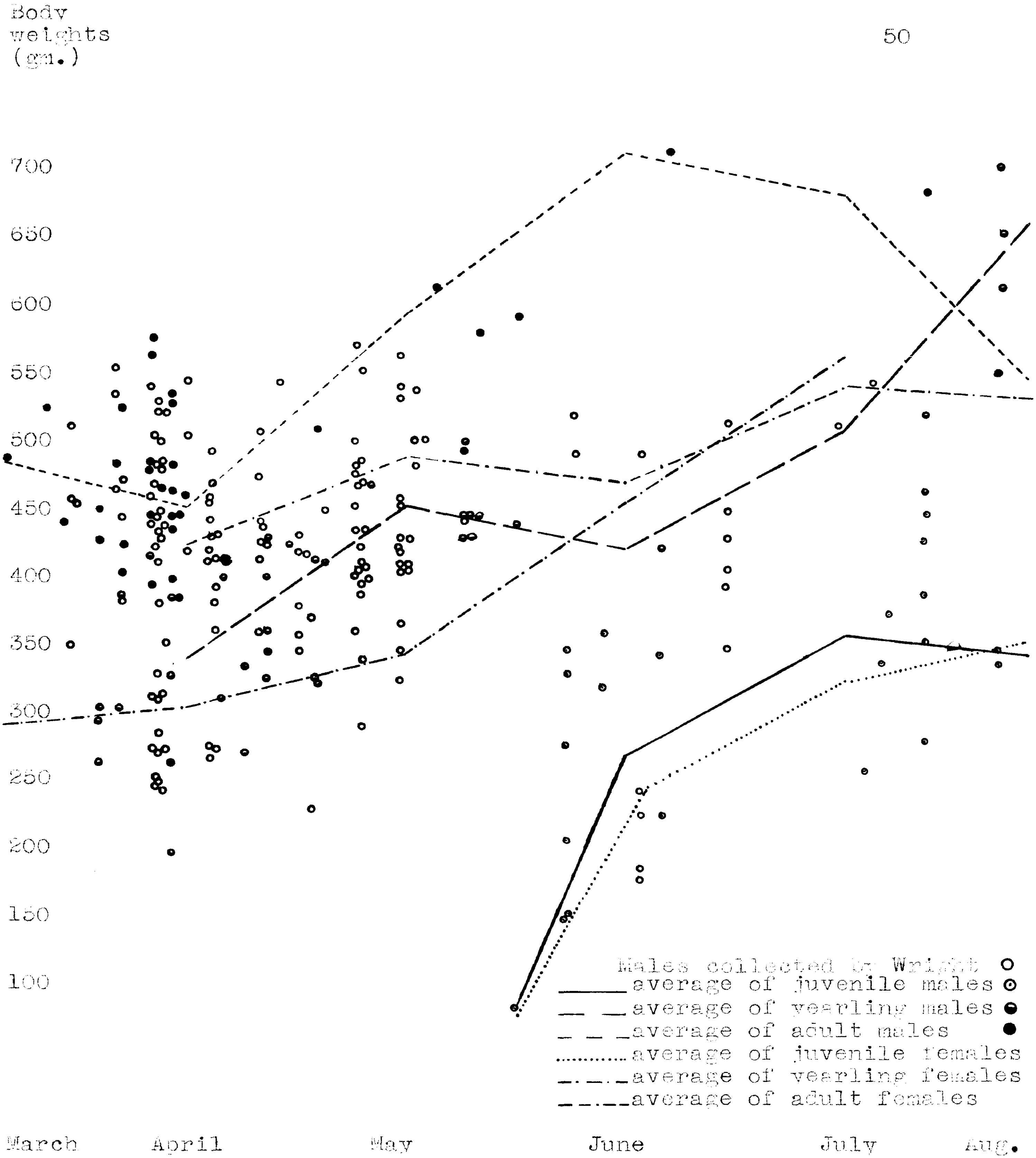


FIGURE 13

DISTRIBUTION OF BODY WEIGHTS

Since there are no observable gross changes in the skulls, femurs, and bone structure and, since tooth development is relatively retarded during hibernation in C. tridecemlineatus (Sarnat and Hook, 1942), it is probable that the animals in the Spring Stages II and III are those which have recently ended their first period of hibernation and may be assumed to be yearlings.

The latter part of May found Stages II and III disappearing from collections. As tooth wear progresses continuously it seems apparent that these animals have entered Stage IV, and possibly Stage V, by this time. Therefore, the animals in Stage IV, at least, which were taken after the first of June may be considered to be yearlings and in Table V and VI this has been done. For similar reasons it is probable that the animals in Stage V by the latter part of July were also yearlings and they have been so considered.

Adults. For reasons similar to those given above it may be assumed that the animals in Spring Stages IV and V have recently passed through a second hibernation period and may, therefore, be classed as adults. It is highly probable that animals in the stages above V are adults.

Discussion

While the previously discussed data indicate fairly definite limits in relation to the age groups, it is realized that some overlapping might occur; for example juveniles might reach Stage IV before entering estivation. Such possibilities will be discussed later in connection with the reproductive organs.

An examination of Figure 13 shows two groups during March and April between which there is little overlap in body weights. The lower weight group is considered to c comprise the yearlings and the other group to be the adults on a basis of tooth wear, closure of the parietal-frontal suture, and fusion of the epiphysis to the diaphysis of the femur. By June, the overlap has become so large that it is impossible to distinguish yearlings from adults by weights alone. A third group, the juveniles, becomes evident at about the time the yearlings and adults merge. The group of juveniles remains well defined throughout the remainder of the summer on the basis of weight alone. In each of these groups the average weights of the females are consistently lower than those of the males. It is also indicated that a slight loss of weight is suffered by the animals immediately after they emerge from hibernation. They soon begin to gain weight, however, and this gain is steady except that the adult females apparently stop gaining weight during May and June. Since this is the period when the females are nursing their young it is probable they would show little gain in weight.

Table II indicates that fusion of the femurs is not complete in all the males until Stage VI. In the females, it is not complete until they have reached Stage IX.

The closure of the parietal-frontal suture is apparently complete within a short time after the yearlings emerge from hibernation.

It has been indicated that there is a delay in the fusion of the epiphysis to the diaphysis of females as related to the aging of adults, based upon the amount of tooth wear.

In order to obtain data on the maximum life span, it would be necessary to tag or mark a large number of juveniles and then retrap in the area year after year, releasing the animals after capture, until the marked animals disappear from the population. This type of study has been made by Evans and Holdenried (1943) in California with C. beecheyi and they found that some animals lived at least three years.

The study was not carried on over a long enough period to determine whether any animals might live longer than that. As has been shown in the foregoing section of this thesis, the two-year old specimens of C. columbianus obtained in the spring are in Stages IV and V. These stages represent only moderate tooth wear. How much time is required for animals to progress through the following five stages of tooth wear, we can only guess. If the later stages of tooth wear succeed one another at the same rate as the earlier ones are shown to do, animals in Stages IX and X might very well be at least four years old. Shaw (1925b) kept a captive animal for four years.

From the above data it seems apparent, by considering tooth wear, amount of fusion of the epiphysis to the diaphysis of the femur, closure of the parietal-frontal suture, and body weights as related to the dates when the animals were collected, that juvenile, yearling, and adult C. columbianus can be distinguished with reasonable certainty.

MALE REPRODUCTIVE ORGANS AND CYCLE

General morphology of the male reproductive tract

The male reproductive tract of C. *tridecemlineatus* had been described by Mossman et al (1932). Some of their data have been included in the discussion of related literature which is found earlier in the thesis. A gross examination of the reproductive tract of an adult male C. *columbianus* and a comparison of Plate IV with photographs of the reproductive tract of C. *tridecemlineatus*, (Mossman et al, 1932) and Table I with Table IV indicates the two tracts are essentially similar. The only difference observed is that the reproductive organs and accessories of C. *columbianus* are larger than those of C. *tridecemlineatus*. The larger tract shown in Plate IV was taken from an adult male near the end of the breeding season (April 28) and it had started to regress. The smaller tract in Plate IV was taken from a yearling animal collected a day later.

The testis of an adult male in full breeding capacity measures 27x14x15 in length, width, and depth respectively and the pair weighs 2323 gm. This animal was collected on March 18, 1947, near the beginning of the breeding season and probably regression had not commenced.

The male reproductive organs and cycle will be discussed in reference to the age groupings previously determined. Numerous animals had been collected by Dr. Wright, for which all of the aforementioned data were not available but for which data on the reproductive organs were available. Because of this, these animals were not considered with respect to tooth wear, fusion of the femurs, or closure of the parietal-frontal suture but will be included in the discussion of the reproductive cycle.

TABLE IV

MEASUREMENTS, IN MILLIMETERS, OF THE LARGER REPRODUCTIVE TRACT SHOWN IN PLATE IV

	Bulbar gland	Testis	Seminal vesicle	Prostate	Muscular urethra	Penis plus bulb	Penis
Width	20	9	9	8	5		6
Depth	13	8	7	5	4		5
Length	22	17	25	9	24	54	32

Juveniles

When juveniles first appear above ground about May 30, their reproductive organs are extremely small (Table V). The increase in size that occurs during June is probably due to normal somatic growth. Shortly after the first of July the

TABLE V

SUMMARY OF MALE DATA BY AGE GROUPS

Age	No.	Total animals	No. sectioned	Body Weight: Ave.	Body Weight: Min. and Max.	Testes: Weight Ave.	Testes: Weight Min. and Max.	Testes: Tubule Dia. Ave.	Testes: Tubule Dia. Min. and Max.	Testes: Capsule Ave. thickness	Testes: No. with sperm	Epididymides: Weight Ave.	Epididymides: Weight Min. and Max.	Epididymides: Tubule Dia. Ave.	Epididymides: Tubule Dia. Min. and Max.	Epididymides: Epith. Ave. Ht.	Epididymides: Cilia Ave. Ht.	Epididymides: Capsule Ave. thickness	Epididymides: No. with sperm	Vasa Deferentia: Ave. Dia.	Vasa Deferentia: Epith. Ave. Ht.	Vasa Deferentia: Cilia Ave. Ht.	Vasa Deferentia: Long. Muscle Ave. thickness
Juveniles	M	1	1	80	---	24	----	40	---	22	0	30	----	43	---	11	?	33	0	154	40	?	37
	J	10	10	268	145 359	62	29 87	54	48 57	22	0	58	45 79	49	32 63	13	2.2	16	0	185	40	?	39
	J	8	8	356	256 448	92	76 139	55	51 69	22	0	55	36 71	34	28 41	13	2.2	15	0	148	31	?	32
	A	2	2	341	335 346	86	73 99	56	54 57	23	0	47	46 48	43	33 53	14	2.8	17	0	132	29	?	30
Yearlings	A	19	14	338	195 428	721	186 2742	104	75 129	35	2	196	70 475	77	41 134	20	3.0	14	0	238	45	5.8	55
	M	12	5	451	324 500	176	141 237	67	60 70	33	0	106	65 128	49	43 60	15	2.2	19	0	184	31	3.5	43
	J	1	1	420	---	137	----	54	---	31	0	95	---	53	---	11	2.2	20	0	154	29	4.4	40
	J	4	2	508	461 517	333	----	73	73 74	34	0	88	77 98	54	43 64	13	2.2	18	0	158	22	3.3	40
	A	3	3	659	623 701	900	831 944	116	114 117	31	0	111	92 143	45	40 52	12	6.6	19	0	195	37	11.0	40
Adults	M	3	3	484	439 524	4913	4531 5523	180	172 185	23	3	1192	1109 1303	153	147 165	33	17.2	15	3	368	44	13.6	88
	A	28	21	451	256 574	2459	199 4462	152	71 197	26	19	863	90 1365	149	40 217	32	12.1	22	19	333	36	8.4	64
	M	3	3	594	580 612	246	194 323	64	61 66	88	0	174	97 213	50	41 57	14	3.0	39	0	227	21	5.8	59
	J	1	1	712	---	309	----	77	---	83	0	175	----	59	---	17	2.2	44	0	220	33	11.0	44
	J	1	1	682	---	1202	----	107	---	39	0	203	----	53	---	17	2.2	44	0	242	33	5.5	44
	A	1	1	549	---	854	----	101	---	33	0	83	----	42	---	13	2.2	37	0	158	48	11.0	41

rate of enlargement decreases (Fig. 14 and 15). Variations in the growth rate of the organs and accessories will now be discussed.

Testes. When juveniles make their first appearance above ground their paired testes weights are about 24 mg., as shown by the weight of the testes from a single animal which was collected May 30. The testes are abdominal and turgid. No evidence of spermatogenic activity is to be found (Plate V, fig. 17). The tubules have an average diameter of 40 u and are lined with Sertoli cells. The lumen is apparently filled with what Allanson (1932), who worked on the ferret, calls "a cytoplasmic syncytium of Sertoli cells". A few nuclei are to be found scattered through the syncytium. Intertubular tissue is abundant. Cells and nuclei vary from oval to round in shape. The average diameter of 25 rounded nuclei is 5.5 u. Several measurements of the thickness of the tunica albuginea at the thinnest part give an average of 22 u. Blood vessels are few in number and small.

By the time the young animals attain Stage II the testes have increased in weight to between 76 and 139 mg., the average for July being 92 mg. At this time there is little change in the appearance of sections from those of

Stage I, the major difference being the increased size of the Stage II testes. The tubules average 55 μ in diameter and no spermatogenic activity can be observed (Plate V, fig. 18). In a few instances, cell division, which appears to be of Sertoli cells, may be seen in the seminiferous tubules of animals of this age. A comparison of fig. 17 with fig. 18, (Plate V) indicates a relative decrease in the amount of interstitial tissue with the enlargement of the tubules. A slight increase in the size of nuclei is indicated by an average diameter of 6.3 as against 5.5 μ found in early juveniles. There is no discernable change in the thickness of the tunica albuginea.

An examination of sections of testes from a Late Summer Stage III animal shows no variation from the above. Apparently, therefore, the testes of juveniles are in the above condition at the time of hibernation.

A number of animals collected by Dr. Wright, have testis weights which group rather closely to the median for animals of this age (Fig. 14). As these testes are histologically similar to those which were collected in 1946 and 1947, it is probable they are also juveniles.

Epididymides. The paired epididymis weights of Stage I squirrels, when they emerge the first time, is approximately

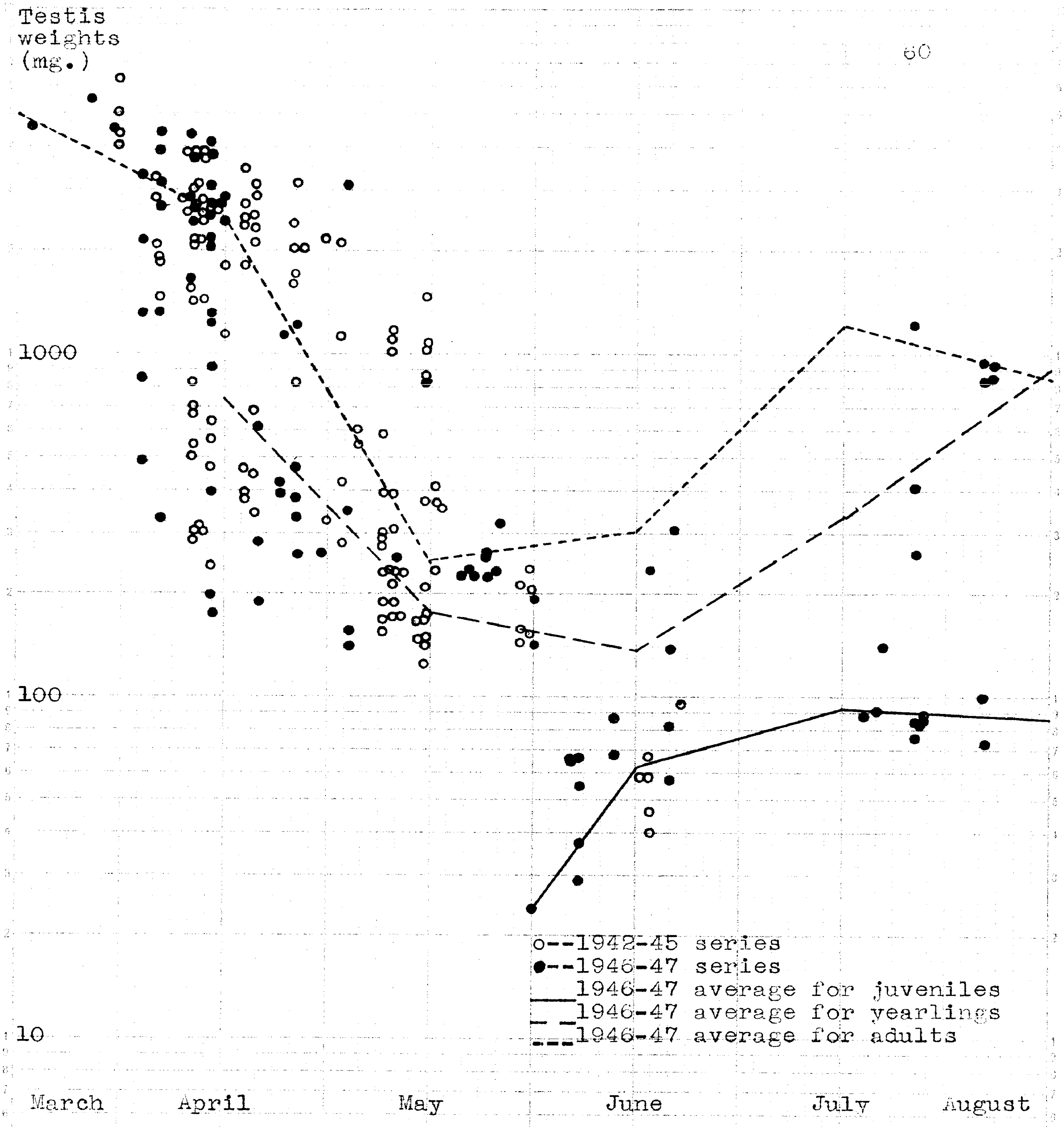


FIGURE 14

DISTRIBUTION OF TESTIS WEIGHTS

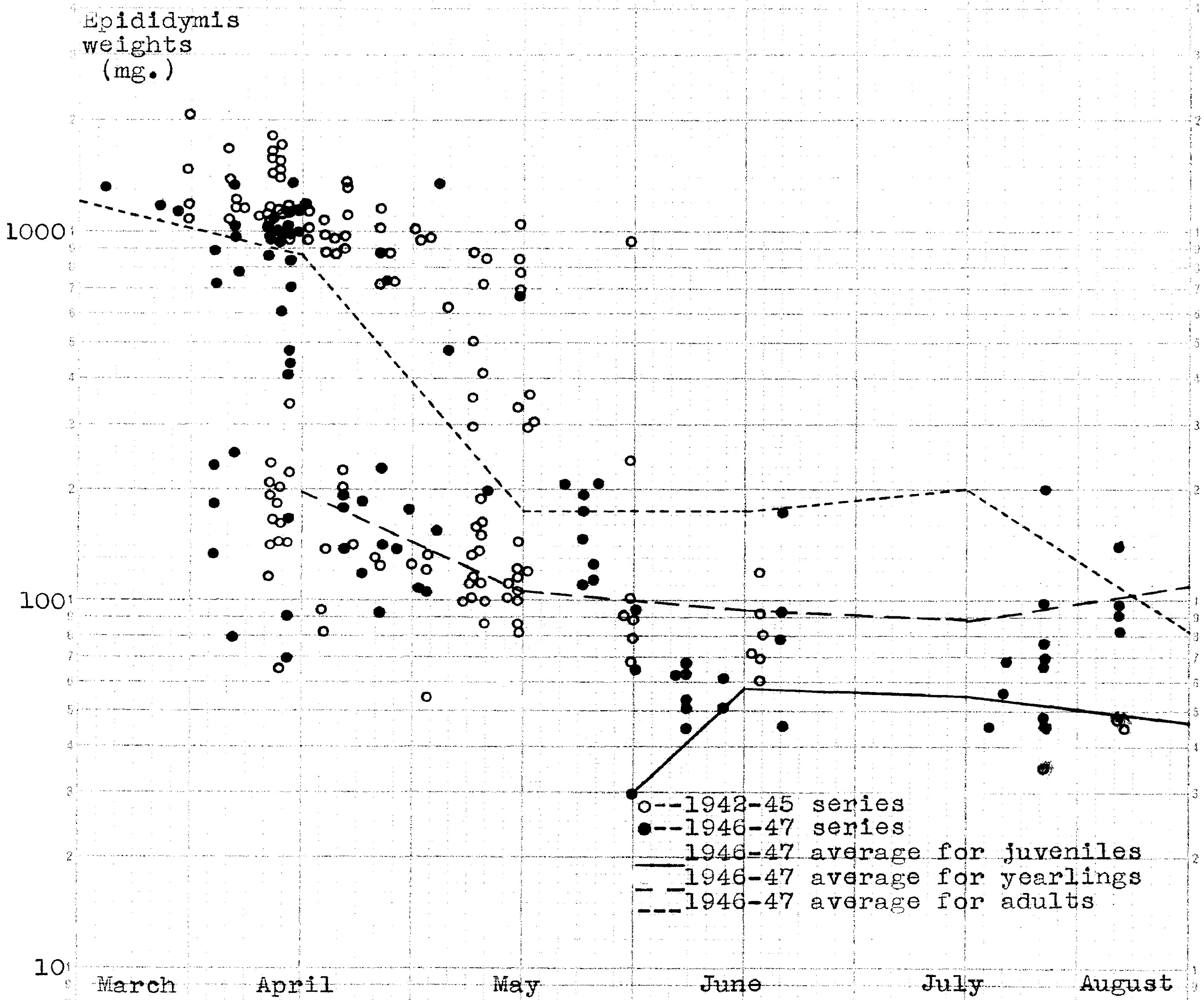


FIGURE 15

DISTRIBUTION OF EPIDIDYMIS WEIGHTS

30 mg. At this time the tubules in the caput portion have an average diameter of 43 u. Epithelial cells of the tubules are cuboidal (about 11 u in height). Epithelial nuclei are rounded and fill the distal two thirds of the cells. Surrounding the tubules are muscle layers which are approximately 9 u thick. The thickness of the capsule of the epididymis is about 33 u at the thinnest part. Intertubular tissue is prominent (Plate V, fig. 19).

About the first of July the epididymides of juveniles weigh their maximum (average 58 mg.) and, for the remainder of the summer, show no observable change except for a possible decrease in weight about the time of estivation (Fig. 15). No spermatozoa are present. Plate V, fig. 20 shows a section of an epididymis from a juvenile taken July 7, 1946. This section is typical of those of juveniles collected throughout the remainder of the summer. During July the average diameter of the tubules is 34 u. The epithelial cells have lengthened slightly (Table V). The nuclei of the epithelial cells have elongated and a clear area about 3 u in width can be seen between their base and the basement membrane. The nuclei nearly fill the proximal portion of the epithelial cells. A decrease is indicated in the thickness of the muscle layer around the tubules and in that of the epididymis capsule, as compared with similar measurements from animals collected the first part of June. During July these measurements average 5.5 and 17 u for the tubule muscle layer and capsule, respectively. Interstitial tissue does not appear to form such a prominent part as it did in fig. 19 (Plate V).

As can be seen from Fig. 15, and, as has been indicated for the testes, the weights of the epididymides of certain of the squirrels collected by Dr. Wright are grouped closely to the median of previously designated juveniles. It is possible that, after the middle of June, the overlap between the weights of the epididymides of juveniles and of yearlings may make it difficult to distinguish between them. Available data, however, indicate that this overlap is probably so small as to be relatively unimportant.

Vasa deferentia. Late in May the diameter of the vasa deferentia of juveniles is 154 u. Fig 21, Plate VI, shows the epithelial cells which line the tube as columnar. They average 40 u in height. The lumen of the tube is small. Numerous darkly stained granules are present in the nuclei of the epithelial cells. The nuclei vary in form from oval to long ovoid. They are close to, or at, the base of the cells. No cilia can be observed on the epithelium. The tubule formed by the epithelium is 88 u in diameter and the muscular coat is 37 u thick. The lamina-propria, (described for humans by Maximow and Bloom, 1944:530) is from 3 to 5 cell layers and nearly 11 u thick. Blood vessels are small in the vas deferens of juveniles.

It is indicated in Table V that, in June, the average diameter of the vasa deferentia of juveniles is 185 u. The condition of the vasa deferentia in these animals during the latter part of June, a time when the tube has attained maximum size, is shown by fig. 22 (Plate VI). There apparently has been little change in the height of the epithelial cells from that of early juveniles and the typical pseudostratified condition of the epithelium with two layers of nuclei is present. Darkly stained granules are evident in the nuclei. The tubule is 83 u in diameter and a definite lumen is present. Apparently there has been an increase in the thickness of the muscular coat (to 39 u) with a slight decrease in the height of the epithelium and the diameter of the tubule as compared with younger animals. Although no change in the number of cell layers can be observed, there is an increase of 3.3 u in thickness of the lamina-propria over that of late May or early June animals. This indicates an increase in size of cells rather than in number.

The vas deferens reaches its maximum diameter by the latter part of June. During July and August there is a decided decrease in its diameter with an accompanying decrease in height of the epithelial cells. Although the longitudinal muscle layer shows no apparent change, the lamina-propria has decreased from 11 to 8 u in thickness, and the epithelial tubule from 88 to 66 u in diameter. The lumen at this time varies in extent from well defined to practically nonexistent. The presence of cilia is questionable. The pseudostratified condition of the nuclei in the epithelium is present (Plate VI, fig. 23).

The above data suggest a possible variation in the rate of physiological maturation of the various organs. While the testes and epididymides show little or no tendency toward a cycle, the vasa deferentia reach a maximum size in June and then decline rapidly during July. This suggests that the vasa deferentia may possibly mature physiologically and somatically at a more rapid rate than do the testes or epididymides. Additional data are necessary, however, before definite conclusions may be reached.

Scrotal skin pigmentation. The amount of pigmentation of the scrotal skin is correlated closely with the breeding activity of male ground squirrels. Wells (1935) states that the pigmentation of the scrotal skin in *C. tridecemlineatus* shows a seasonal rhythm, similar to that of other secondary sexual characteristics. He says that breeding males in April and May have a deep black scrotum from which the pigment diminishes and completely disappears by August. Finkel (1945), who also worked with C. tridecemlineatus, indicates that the pigmentation is due to stimulation by testis hormone of melanophores lying at the dermal-epidermal junction.

A gross and histological examination of the scrotal skin of juveniles of C. columbianus reveal little or no pigmentation beyond that which normally appears in the skin of other areas. Melanophores are small and rounded.

Summary. Juveniles first appear about May 1. At this time their reproductive organs are small and in an immature condition. A rapid increase in size, probably normal somatic growth, is evident until the latter part of June. After this the testes continue to slowly enlarge while the epididymides maintain approximately the peak attained in June. The vasa deferentia, however, decrease in diameter during July and August, a fact which suggests a variation in somatic and physiological maturation between the organs. Little or no pigmentation of the scrotal skin is evident. It is indicated by the above data, that these animals are immature and incapable of breeding.

Yearlings

Yearlings, i.e., Spring Stages II and III, are first found among samples on April 3, 1946. At this date it can be observed (Table V and fig. 14 and 15) that considerable enlargement of the reproductive organs occurs during the period of dormancy. These organs will now be discussed individually.

<u>Testes</u>. At the time squirrels emerge from their first period of hibernation, or soon after, their testes are maximal in size for the age group (Fig.14). The extremely high average for April is due to the few animals whose testis weight is over 1000 mg. The majority (15 of nineteen) of animals of this group have testis weights of less than this. The fact that a large number of the animals, collected during April by Dr. Wright, have testis weights which are less than 1000 mg. seems to support the above data. Testes are turgid, with blood vessels more apparent than in juveniles, and vary from abdominal to scrotal in position. The largest testes are scrotal. Spermatogenic activity apparently seldom reaches that of mature animals as, in only 2 of fourteen animals of Spring Stages II and III, could mature spermatozoa definitely be found. The testis weight of each of these is over 1000 mg. One of them, collected on April 13, 1946,

has testes weighing 2742 mg. and spermatozoa are abundant, i.e., can be found in nearly every tubule. The other, collected April 6, has testes weighing 1310 mg. and only a few spermatozoa are evident. The testes of a third animal, taken April 13, weigh 1221 mg. and the presence of spermatozoa is questionable. Fig. 14 and Table V suggest a possible correlation between size of testes and spermatozoa production as no spermatozoa can be observed in testes of animals collected in April, of which the weight of each pair is less than 1000 mg., whereas, in those having a weight above this, spermatozoa are present in 2, questionable in 1, and absent in the fourth animal.

In testes of ground squirrels of this age group, taken early in April, the seminiferous tubules average 104 u in diameter (Table V). A considerable amount of cell division is evident (Plate VI, fig. 24). Spermatogonia and primary and secondary spermatocytes are abundant, as are Sertoli cells. Spermatids and a definite lumen can be observed in the tubules of the larger testes. No lumen and no spermatids are found in testes weighing less than 1000 mg. Spermatogenesis apparently does not progress beyond the spermatid stage in the majority of yearlings. A comparison of fig. 24 (Plate VI) and fig. 18 (Plate V), indicates a decrease in the amount of interstitial tissue in the testes of yearlings as compared with juveniles. A slight increase, (from 6.3 to 6.9 μ) in the average diameter of nuclei is apparent. With other increases in size there is an increase from 22 μ to 35 μ in the thickness of the tunica albuginea.

The testes start decreasing in size the latter part of April and, by the middle of May, reach the minimum (Fig. 14). The decrease in testis weight is from an average of 721 mg. in April to 176 mg. in May (Table V), a decrease of over 70 percent. Fig. 14 and Table V indicate a slight increase in weight of the testes from this date until August, at which time an extremely rapid increase in weight is shown. This rapid increase is due to the large testes of the three squirrels taken on August 6, 1946, in the Gold Creek area. Insufficient data prevents the drawing of any conclusions concerning the seemingly extreme weight increase mentioned above.

During the latter part of April and throughout the remainder of the summer, nuclei, in which the chromatin threads are clumped together in one portion, leaving the remainder of the nucleus vacuolated (Plate VII, fig. 25), become numerous in the germinal epithelium. Painter (1923) describes similar nuclei in man. He also observed them in the testes of the bat and of the striped skunk. He says of this condition:

> "It is possible that it is a product of poor preservation or a stem in the degeneration of sections of a tubule, but whether either of these explanations is the correct one, such cells probably do not form a step in the normal production of spermatozoa."

Since these nuclei are seldom observed in seminiferous tubules of <u>C. columbianus</u>, in which considerable spermatogenic activity is occurring, and, since the method of fixation was standard for all testes, this phenomenon is probably indicative of regressing testes. Another type of cell, which, although found at all times, is more numerous in the germinal epithelium after regression of the testes has started, is one in which the cytoplasm takes on a grayish stain (Plate VII, fig. 25). These are probably atretic cells. Sertoli cells and spermatogonia are abundant. Primary spermatocytes are relatively scarce unless the cells containing the nuclei with the clumped chromatin can be considered as regressing spermatocytes. No secondary spermatocytes, spermatids, or spermatozoa can be seen and no lumen is present in seminiferous tubules after the first of May. The tubules average 67 and 73 u in diameter during May and July respectively. Interstitial tissue appears more abundant and the rounded nuclei have decreased in diameter from an average of 6.9 u in the testes of spring yearlings to 6.2 u in testes of late summer yearlings (Gold Creek squirrels taken in August are not included). The thickness of the tunica albuginea of a single animal is 33 u. Additional measurements are not available.

No lumen is present in seminiferous tubules of the three animals collected on Gold Creek, August 6, 1946. Numerous Sertoli cells, spermatogonia, and primary spermatocytes and nuclei with clumped chromosomes are interspersed throughout the syncytium to such an extent that, in most tubules, little of the syncytium can be seen. The rounded nuclei in the interstitial tissue average 7.7 u and the seminiferous tubules 116 u in diameter.

The testes of yearling animals are at the maximal size attained by them when they emerge from hibernation in early April. Spermatozoa are produced by at least two of nineteen yearlings. The indications that the testes start to regress almost immediately tend to preclude the possibility of growth and increased spermatogenic activity of the testes after emergence of the animals in the spring. Regression is practically complete in all squirrels by the middle of May. Nuclei with clumped chromatin increase during the period of regression and are present for the remainder of the summer. These are probably indicative of regression.

An examination of Fig. 14 shows that, except for

April, certain testis weights from animals which were collected by Dr. Wright, are grouped around the average a found for the yearlings. It is also shown that the majority of April yearlings, as well as a considerable number of those collected by Dr. Wright, have testis weights between 300 and 700 mg. Histological examination of the testes from the latter animals indicate no essential variation from those described for the yearling age group. The animals therefore can be considered as yearlings. It is possible that non-breeding adults in April would have testes in a similar condition to that as described for yearlings. As only 2 of the animals which were classed as adults (both in Stage IV) in April, have testes in the yearling condition and, as it is possible that some yearlings might attain Stage IV by this time, it appears that non-breeding adults are rare.

<u>Epididymides</u>. The average weight of paired epididymides during April is 196 mg., the minimum being 70 and the maximum 475 mg. Tubules vary from 41 to 134 u with an average of 77 u (Table V). Epithelial cells which line the tubules, as do other tissues in the epididymis, show considerable variation. They vary from tall columnar to cuboidal. An average of 20 u (min. 14, max. 29) in height is indicated. The presence of cilia is questionable. The lumen of the tubules is open in all but two individuals.

Interstitial tissue varies from a small amount to a condition similar to that of juveniles. In the majority of instances, the juvenile condition is shown (Plate VII, fig. 26).

Little variation may be observed in the thickness of the smooth muscle layer around the tubules. The variation is from 5.5 µ for juveniles in July to 5.9 µ for yearlings in April. The maximum and minimum for yearlings is 6.6 and 4.4 µ respectively. The number of cell layers varies from 2 to 4.

With the apparent enlargement of the epididymides of yearlings in April over that of juveniles in July, the capsule has decreased in thickness from 17 µ to 14 µ. This suggests a stretching of the capsule with the enlargement of the epididymis.

The epididymides decrease in size rapidly from the latter part of April until the middle of May, at which time they have almost reached the minimum. A slight decrease in weight is indicated from the middle of May until in July. The epididymides from the three Gold Creek squirrels cause an increase in the average weight for August. The indicated decrease into July is not applied to weight alone as all measurements of the epididymis are smaller in July than in

May (Table V). The epididymides of yearlings in July, taken in the Missoula area, resemble those of juveniles in in July and August (Plate VII, fig. 27).

Among the data collected by Dr. Wright, are numerous epididymides of which the weight and histological condition are similar to what has been described for yearling animals. Because of this it appears that they belong to the same age group.

Epididymides of yearling ground squirrels have attained their maximal weight upon completion of the hibernation period. Regression in size commences soon after the animals emerge and continues until the juvenile condition is reached in mid-May. This condition is maintained throughout the remainder of the summer. Apparently none of the yearling animals in the Missoula area reach breeding condition as spermatozoa are not to be found in the epididymides of any of those collected.

Vasa deferentia. The average diameter of fourteen vasa deferentia from yearlings collected during April is 238 u. The range is from 110 to 374 u. Spermatozoa are present in the testes of the animal which had the maximal sized vas deferens. The epithelial shape varies from cuboidal to columnar and cilia are present in all but one vas deferens. The average length of cilia is 5.8 u. In four individuals the pseudostratified condition of the nuclei of the epithelium is not present. In these four animals the nuclei fill one-half to three-fourths of the space in the cell and they vary in shape. The tube formed by the epithelium has an average diameter of 137 u, and the size of the lumen varies in diameter from zero to approximately 55 u. The lamina-propria is from 2 to 3 cell layers and averages 9.2 u in thickness. The longitudinal muscle layer is 55 u thick. Plate VII, fig. 28, shows a section which is similar to the average condition of the vasa deferentia of yearlings during April.

A study of Table V shows that the vas deferens decreases in size until August, at which time an increase is indicated. This increase is caused by the data from the three Gold Creek squirrels. The vasa deferentia of these three squirrels are similar in appearance to those of May yearlings from the Missoula area.

By July, the diameter of vasa deferentia of yearling ground squirrels has decreased 80 u, the epithelial height from 45 to 22 u, and the other tissues have regressed in proportion (Table V). The regression is such that the vasa deferentia of late summer yearlings are definitely juvenile in appearance (Plate VIII, fig. 29).

Scrotal skin pigmentation. Early in the spring the scrotal skin of the majority of yearlings has a dark grayish color. Melanophores are small but more numerous than in juveniles. In the yearlings which produce spermatozoa, the scrotal skin is black and melanophores form an almost unbroken layer in the basal part of the epidermis.

Melanophores start to disappear and the skin to become lighter in color by the first of May. After the last of May the color of the scrotal skin closely resembles that of juveniles.

Summary. At the time of emergence from hibernation of yearling ground squirrels the reproductive organs and accessories of these animals are at, or close to, the maximal size reached during that year. A possible correlation between the size of testes and the presence of spermatozoa is indicated during early spring, since no pairs of testes of which the weight is less than 1000 mg. produced spermatozoa. Above this weight, spermatozoa are found in 2 of four yearlings and the presence of spermatozoa is possible in a third. Yearling males probably do not attain breeding condition in the Missoula area.

Rapid regression of the reproductive tract commences soon after the yearlings emerge from hibernation and is complete by the middle of May. After regression, except for greater size, the entire tract is juvenile in appearance and remains so until in July. Although it is suggested that the reproductive organs commence to increase in size shortly before the animals estivate in late July, the evidence is inconclusive.

Adults

Adult squirrels (Spring Stages IV to X) emerge from hibernation a week to two weeks before yearlings (Table V and fig. 14 and 15). When the adult males emerge from hibernation their reproductive organs are larger than at any previous time.

Testes. Testes of adults during March are turgid, scrotal, and maximal in size. All stages of spermatogenesis are present (Plate VIII, fig. 30) although mature spermatozoa are more abundant in the testes of animals collected in early April. Interstitial tissue is relatively scarce. Nuclei of interstitial cells average 6.6 μ in diameter. The tunica albuginea is thinner than at any time during the current year (Table V).

Table V and Fig. 14 show that the testes commence to decrease in size soon after the animals emerge from hibernation.

The indicated decrease is probably exaggerated because of the small (199 and 395 mg.) testis weights of two Stage IV animals taken in early April. Histological study of these small testes revealed that their appearance was essentially like that of April yearling testes. Probably these were from yearling animals since the body weights of 256 and 395 gm. are indicative of yearlings in April. By the latter part of April most of the testes have decreased in size and are soft, although still scrotal. A few spermatozoa and considerable detritus may be found in the lumen of the tubules. Large vacuoles are present in the germinal epithelium. Cells with clumped chromosomes have become evident and few spermatocytes or spermatids can be observed. Sertoli cells and spermatogonia are regularly alined in the distal portion of the epithelium. A decrease in the size of the interstitial cell nuclei is indicated as they are 5.5 u in diameter as compared with the 6.5 average for the entire month. Interstitial tissue appears relatively more abundant. The tunica albuginea apparently increases in thickness as the testes decrease in size since it measures about 44 u in thickness the latter part of April and about 20 u the first part. The average diameter of the seminiferous tubules is nearly 107 u.

Figure 14 and Table V show that the testes have completely regressed in size by the middle of May. In appearance of sections the testes of adults closely resemble those of juvenile animals except for the presence of more numerous cells with clumped chromosomes. By the last of May they are juvenile in appearance (Plate VIII, fig. 31).

Since numerous cells with clumped chromosomes are present in the seminiferous tubules of Gold Creek adults taken during the latter part of July and the first part of August, it is possible that regression of the testes occurs neither as rapidly nor as early in the Gold Creek area as in the Missoula area and the squirrels apparently estivate before their testes are fully regressed.

Since, when adult male ground squirrels emerge from hibernation, their testes are maximal in size and numerous spermatozoa are present, they are probably able to breed. Indications that breeding begins soon after emergence are apparent in the decrease in size which takes place at this time. Complete regression is evident by the latter part of May.

Testes of numerous animals collected by Dr. Wright have weights approximating those of the 1946-47 series. Histological examination of a number of these testes shows both groups to be essentially similar. It is probable they are from adult animals. Fig. 14 shows several animals with testis weights which are above average for May. These animals were collected on Gold Creek and the majority of testes contain spermatozoa. Histologically, they are similar to the testes of animals which were collected in the Missoula area the last of April. This suggests that the breeding season in the Gold Creek area is either later than in the Missoula area or is prolonged over a longer period. If a similar pattern is followed by both the Gold Creek and the Missoula area squirrels, probably the first is the true condition.

Epididymides. At the time of emergence of adult ground squirrels in March, their epididymides are at maximal size (Table V and Figure 15). Spermatozoa are numerous and the lumen large (Plate VIII, fig. 32). Further examination of fig. 32 shows the nuclei to be at the base of the high columnar epithelial cells. Table V indicates that the cilia are longer during March than at any other time. The basement membrane averages 1 to 2 cell layers and 2.6 μ in thickness. Interstitial tissue is fibrous and scanty. Measurements of the capsule at its thinnest part indicate that, in March, it is thinner than at any other time during the spring and summer.

A study of Fig. 14 and 15 shows that the epididymides decrease in size at a slower rate than do the testes. Fig. 15 indicates that rapid regression of the epididymides does not set in until near the middle of April. A large part of the decrease in weight of the epididymides which is indicated by the average of the 1946-47 series is caused by the small epididymides of the two Stage IV animals which were discussed in connection with the testes.

By the last of April the epididymides have commenced to decrease rapidly in size. Many tubules contain no spermatozoa. Cilia appear longer but this is probably due to the decreased pressure from the smaller number of spermatozoa. Comparatively more intertubular tissue is evident.

That regression is complete before the end of May is shown by Fig. 15. Table V shows that the decrease in size is general except for an increase in the thickness of the capsule. An additional increase from 1-2 to 3-4 cell layers and from 3.3 to 11 u in thickness of the basement membrane was found. Plate IX, fig. 33 shows a typical section of an epididymis from an adult collected May 30, 1946. Except for a difference in size, as shown by Table V and Plate IX, fig. 33, the condition of the completely regresses adult epididymis closely approaches that of juveniles (Plate V, fig. 20). The clear area between the epithelial cell nuclei and the basement membrane can be plainly seen. No spermatozoa are to be found.

Rapid regression of the epididymides of adult ground squirrels commences during the latter part of April and is complete by the latter part of May. No spermatozoa can be observed in epididymides of animals collected after the first of June. The capsule and basement membrane are thinnest when the epididymis is largest and full of spermatozoa. Stretching of these parts due to internal pressure (possibly caused by spermatozoa) is indicated. No evidence of enlargement of the epididymides during July or August can be seen.

Vasa deferentia. The vasa deferentia, like the testes and epididymides, of adult squirrels are largest when the animals emerge from hibernation. No spermatozoa can be found in the vasa deferentia of 2 of three males collected in March. The animals, in which no spermatozoa can be found in the vas deferens, were collected on March 18 and 26 respectively, and the one with spermatozoa on March 28. During the first half of April spermatozoa can be found in the vasa deferentia of all adult males except the two Stage IV animals previously mentioned.

Plate IX, fig. 34, shows a section of a vas deferens taken from an adult male on March 18. No spermatozoa are present. Epithelium is high columnar (55 u) and involuted.

Cilia are long. The pseudostratified condition of the epithelial nuclei is evident.

By the time spermatozoa reach the vas deferens a decided decrease in the height of the epithelium and length of cilia is noted (Table V). The decrease in cilia length is probably due, not to an actual decrease in length, but to bending caused by the pressure of the spermatozoa. Involutions in the epithelium have largely disappeared. The tube formed by the epithelium has increased from an average diameter of 170 u in March to 190 u in April.

Regression of the duct and relief from spermatozoon distension appears to be simultaneous and, by the last of April, comparatively few spermatozoa are to be found in the majority of the vasa deferentia. Complete regression of the duct, however, does not come until the last of May. Plate IX, fig. 35 shows a section of a completely regressed vas deferens. The diameter of the duct is reduced, the epithelium cuboidal, epithelial cilia height less, and the muscular layer relatively thinner than in animals collected earlier in the year (Table V). The duct apparently remains in this condition until estivation.

<u>Seminal vesicles</u>. The seminal vesicles of adults show greatest size and function during March and April, i.e.,

during the breeding season. At this time the tubules are filled with transparent secretion and separated from others by a comparatively small amount of interstitial tissue. The epithelium is low columnar (Plate IX, fig. 36).

Seminal vesicles of yearling animals during April show little or no secretion, much intertubular connective tissue, and cuboidal epithelium. The tubular lumen is very small or lacking (Plate X, fig. 37).

Since seminal vesicles for the remainder of the summer are not available, it is impossible to discuss or form any conclusions concerning them. Wells (1935) shows, however, that in _C. tridecemlineatus_, the seminal vesicles pass through a seasonal cycle closely correlated with the cycle of the reproductive organs. Probably this is so in _C. columbianus_.

Scrotal skin pigmentation. Adult males during March and April and the first part of May have deep black scrota. About the middle of May the pigmentation commences to disappear and, by the middle of June, the scrotal skin of all squirrls collected was light in color.

Summary. The reproductive tract of adult male ground squirrels is maximal in size at the time the animals emerge from hibernation. Testes are scrotal, and all stages of spermatogenesis can be observed.

Rapid regression of the tract commences about the middle of April and is complete by the last of May.

Possibly, the testes increase in size just before estivation commences but the remainder of the tract shows no such increase.

Discussion and conclusions

Juveniles appear above ground around May 30. At this time their reproductive tracts are extremely small and immature. Growth of the organs continues through June and the maximal size is attained the latter part of June or the first part of July. After this date the testes and epididymides do not gain or lose an appreciable amount, whereas, the vasa deferentia undergo regression in size during July and August. No scrotal skin pigmentation can be observed. Juveniles enter estivation during the latter part of July and the first part of August.

Early in April yearlings emerge from hibernation. Their reproductive organs show considerable enlargement in comparison to those of juveniles. The testes are mostly abdominal but some are scrotal. It is probable that testes which weigh less than 1000 mg. during April never produce spermatozoa. Of nineteen animals, 4 had testes which weighed over 1000 mg. Probably none of these 4 would have bred during the current season since no spermatozoa were observed in their epididymides, and since regression apparently commences soon after emergence from hibernation. By the middle of May, or soon after, regression is complete and the organs appear like those of juveniles.

Adult males probably emerge from hibernation a week or two weeks before the yearlings. Their reproductive organs are maximal in size and activity. Of thirty-one adults collected during March and April, spermatozoa were not present in 2 of them. Both of these animals were in Stage IV and their body weights and reproductive tracts indicated they probably were yearlings.

Regression of the tract is rapid during the latter part of April and the first part of May and is complete by the last of May.

In Fig. 38 testis weights are plotted against epididymis weights. A good correlation is indicated. When the coefficient of correlation was calculated it was found to be high ($r_{xy} = .855$). This extremely high coefficient is probably due to the close correlation shown by the smaller testes and epididymides.

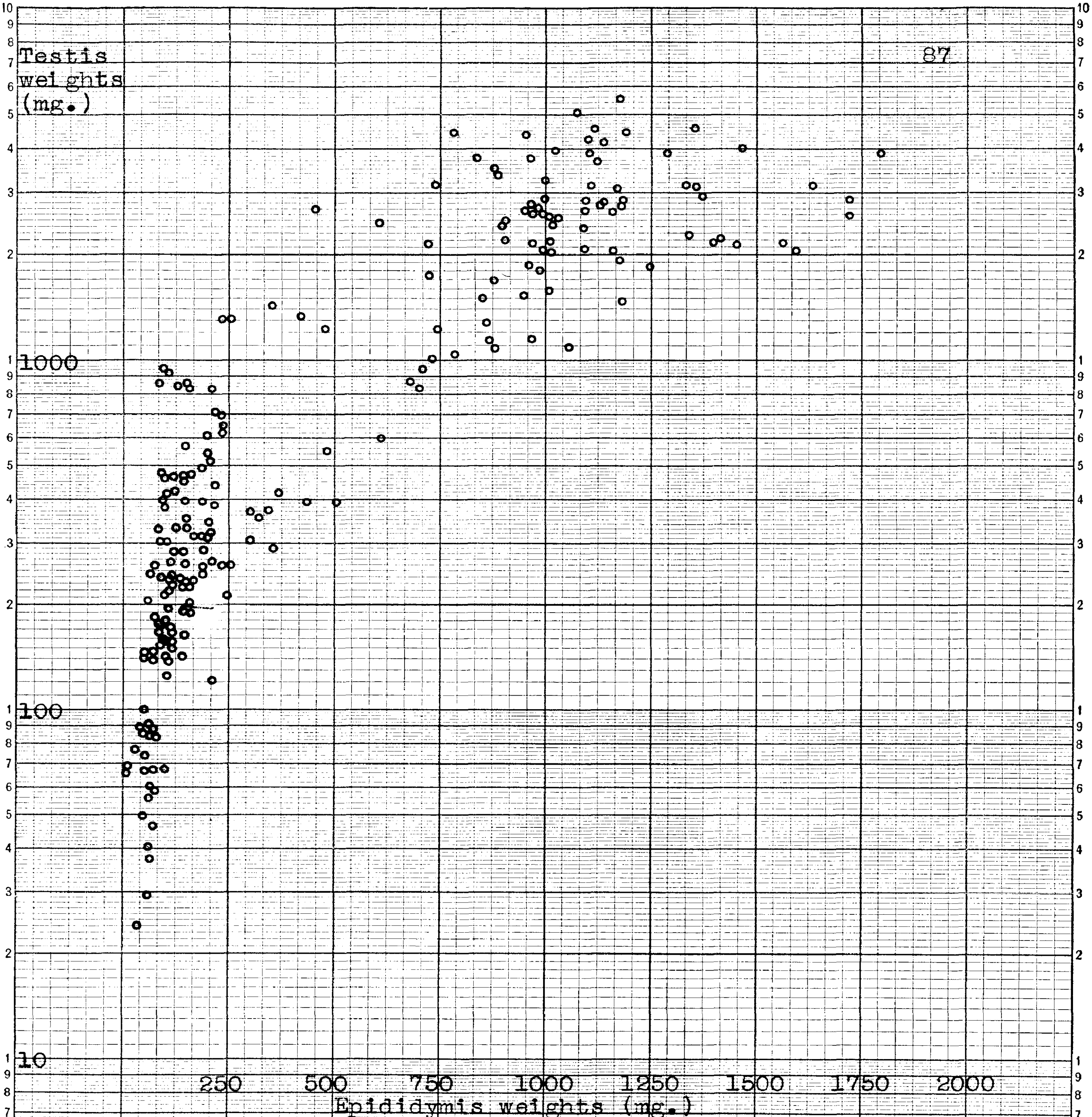


FIGURE 36

TESTIS WEIGHTS PLOTTED AGAINST EPIDIDYMIS WEIGHTS

FEMALES

Since any discussion of the male reproductive cycle would be incomplete without mention of the females, a short, superficial, description will be included. Lack of time prevented a more intensive study. The female cycle will be studied later.

Table VI is a summary, by month, of the gross condition of the vaginal opening and the uterus of the previously determined age groups. Several terms used in the table require explanation.

Johnson et al (1933) describe the opening of the vagina in C. tridecemlineatus and I have found it to be similar in C. columbianus.

Swollen uteri are extremely large in comparison with thread like of short ones. When swollen, they are 35 to 50 mm. in length and 3 to 5 mm. in diameter. When thread-like, the length is 30 to 45 mm. and the diameter less than 1 mm. The uterine horns are short when not more than 25 mm. in length.

Juveniles. Juvenile females were first found in collections on May 30, 1946. One female, weighing 75 gm., was collected. The vaginal opening was closed and the uterine horns small and short in this animal. Little

TABLE VI

SUMMARY BY AGE GROUPS AS DETERMINED BY TOOTH WEAR, CLOSURE OF PARIETAL-FRONTAL SUTURE, AND FUSION OF THE EPIPHYSIS TO THE DIAPHYSIS OF THE FEMUR OF FEMALES

Age	Mo.	No. of anim.	Ave. body wt.	Vaginal aperture Open	Vaginal aperture Closed	Uterus Swol-len	Uterus Thread like	Uterus Sht.	Uterus Preg.	Uterus Post part.
Juv.	M	1	75		1			1		
	J	7	243		7			7		
	J	24	321		24		12	12		
	A	2	351		2			2		
Year.	M	3	289		3	3				
	A	16	302	7	9	10		1	5	
	M	4	342	1	3		3			1
	J	3	455	1	2		2			1
	J	2	562		2		2			
Adult	A	13	422	12	1	4			9	
	M	18	488	18		1			1	16
	J	4	469	3	1					4
	J	5	540	1	4					5
	A	1	532		1					1

change was observed until in July, at which time, the uteri of half the juveniles had lengthened and become thread-like. The uteri of the remaining half were short (Table VI). No evidence that juveniles approach estrus could be observed.

Yearlings. Yearling females (Spring Stage III) were first found in collections in March. Adult females were not collected until April.

Table VI shows that, although the vagina was closed in yearlings which were taken in March, the uterus was swollen.

During April the vagina was open in 7 and closed in 9 animals. Of those having a closed vagina the uterus was swollen in 6, short in 1, and in between short and swollen in 2 instances. The latter are included among the individuals with swollen uteri although, possibly, they would not have attained the larger size of the swollen ones. All but one of the animals with closed vaginas were collected before the 15th of the month. The remaining animal was taken April 20. Of seven animals having an open vagina, 5 (including one with a copulation plug) were pregnant and the uterus was swollen in the other 2. All pregnant animals, except the one with the copulation plug which was taken April 12, were collected after April 22. One of the 2 animals with swollen uteri was taken on April 13 and the other on April 25.

Of the four yearling females examined in May, 3 had closed vaginas and thread-like uteri. The 4th was parous and the vagina was open.

In June, conditions similar to those found in May were observed except that the uterus of the single parous female in June was much smaller (almost thread-like) than that of the one in May.

By July the vagina was closed and the uterus thread-like in all yearlings examined.

Table VI shows that 7 of twenty-eight (one in four) yearling females breed. It is possible that more attain estrus but do so after the testes of the males have regressed and consequently, they are not bred. Whether this is so may be shown by a more intensive study of the reproductive organs of the female.

Adults. Of thirteen adults examined in April, all except one (Stage IV) had open vaginas. Nine of these were pregnant. The uterus was swollen in the remaining 4 (including the animal with the closed vagina). The latter animals were taken before April 10. The first adult in which implantation sites could be seen was collected on April 10. Since this was true, copulation must have occurred several days previous to this date.

By May, parturition had occurred in all adult females except 2. Of these 2, one was pregnant with large embryos, and the vagina was open and the uterus swollen in the other. The pregnant animal was examined on May 3 and probably would have given birth within a few days. The other probably would not have produced young during the present year.

The vaginal opening was closed in 1 of four parous females taken in June. The uteri of all four females showed placental scars.

By July, the vaginal opening was closed in 4 of five parous adult females (Table VI).

Table VI shows that 36 of forty-one adult females bred during 1946 and 1947. Of the remaining animals 4, and possibly all 5, might have bred.

<u>Summary</u>. Juveniles first appear in collections the last of May. At this time their vaginal openings are closed and their uteri short and small. Little change could be observed in the size of their uteri during the remainder of the summer.

Yearlings emerged from hibernation in March. Their vaginal openings were closed and the utrri swollen. In April, the vagina was open in approximately one-half of the animals. The uterus was swollen in 10 of sixteen animals. Of five animals collected between April 23 and 29, 4 were pregnant. The vagina was open and the uterus swollen in the fifth. Apparently, about 1 of four yearling females bred during 1946 and 1947.

Table VI indicates that adult females were in breeding condition at the time they emerged from hibernation, whereas, yearlings were delayed. The major part of copulation probably took place between April 1 and 15.

The first pregnant female was taken on April 10 and the first post-partum female on May 3. In one yearling female, trapped in the field and kept in the laboratory, parturition occurred 23 days and 4 hours after she had been captured. An examination at the time of capture revealed no evidence of recent copulation.

Thirty-six of 41 adult females were pregnant or parous and 4 of the remaining five might have become pregnant had they not been killed so early in the season.

SUMMARY

A study of 493 Columbian ground squirrels (239 males and 254 females) taken in the vicinity of Missoula, Montana, between 1942 and 1947 leads to the following conclusions:

1. There was a sex ratio of 51.5 % females to 48.5 % males.

2. Three age groups, i.e., juveniles, yearlings, and adults, based upon tooth wear, closure of the parietal-frontal suture, body weight, and fusion of the epiphysis to the diaphysis of the femur, can be distinguished.

3. When considered by month and within the various age groups, males consistently weigh more than females.

4. The size and condition of the male reproductive tract is closely correlated with the indicated age of the animals, especially during March, April, and May.

5. Juveniles leave their nest and appear above ground when about 30 days of age. Their reproductive tracts are small and, except for the vas deferens, show little change during the summer. Spermatogenic activity in the testes does not progress beyond the spermatogonial stage during the first summer of the juvenile life.

6. Yearling males emerge from hibernation early in April. Their reproductive tracts have attained maximal size for the current year. No lumen and no spermatids were found in paired testes weighing less than 1000 mg.

Spermatogenesis did not progress beyond the spermatid stage in the majority of yearlings. Regression of the reproductive tract commences in April and, by the latter part of May, is so complete that the tract is indistinguishable from those of the juveniles. Possibly no yearling males reach breeding condition.

7. Adult males emerge from hibernation 1 or 2 weeks ahead of yearling males. The reproductive tracts of the adults are maximal in size and activity. The testes are turgid and scrotal. All stages of spermatogenesis are present. Regression of the tract commences soon after the animals have emerged from hibernation and is complete by the end of May.

8. A high coefficient of correlation was found to exist between testes and epididymis weights ($r_{xy} = .855$).

9. Yearling females were first collected in March. About 1 in four yearling females bred in 1946 and 1947. Yearling females probably do not reach a condition of estrus until after the majority of adult females have copulated.

10. Adult females were first collected in April. The majority are probably in estrus upon emerging from hibernation. To a great extent, copulation occurred between April 1 and 15. Of forty-one adult females collected during 1946 and 1947, 36 were pregnant or parous.

LITERATURE CITED

Allanson, M.
1932. The reproductive processes of certain mammals. Part III--The reproductive cycle of the male ferret. Proc. Roy. Soc. London. Ser. B, 110

1933. The reproductive processes of certain mammals. Part V--Changes in the reproductive organs of the male grey squirrel (Sciurus carolinensis). Phil. Trans. Roy. Soc. London. Ser. B, 222:47-96.

Bond, A.
1942. Early spring studies upon comparative body, testes, and bacculum weights of male Columbian ground squirrels, (Citellus columbianus columbianus). (Unpublished manuscript in office of P. L. Wright).

Coco, R. M.
1942. Notes on vaginal smears in the ground squirrel, (Citellus tridecemlineatus). Proc. La. Acad. Sci., 6:83-84.

Deanesly, R. and Parks, A. S.
1933. The reproductive processes of certain mammals. Part IV--The oestrous cycle of the grey squirrel, (Sciurus carolinensis). Phil. Trans. Roy. Soc. London. Ser. B, 222:47-96.

Erickson, T. S.
1940. The post-natal development of the young striped gopher or ground squirrel, (Citellus tridecemlineatus). Proc. So. Dak. Acad. Sci., 20:49-52.

Evans, F. C., and Holdenried, R.
1943. A population study of the Beechey ground squirrel in central California. Jour. Mammal., 24:231-260.

Finkel, M. P.
1945. The relation of sex hormones to pigmentation and to testis descent in the opossum and ground squirrel. Amer. Jour. Anat., 76:93-148.

Foster,
1911. (1911, p. 2 Bulletin of Washington Experiment Station). (Not seen. Quoted by Shaw, 1924).

Hall, E. R.
1926. Changes during growth in the skull of the rodent, (Otospermophilus grammurus beecheyi). Univ. Calif. Pub. Zool., 21:355-404.

Hamilton, W. J. Jr.
1934. The life history of the rufescent woodchuck, (Marmota monax rufescens). Ann. Carnegie Mus., 23:85-178.

Howell, A. H.
1938. Revision of North American ground squirrels. North Amer. Fauna. No. 56.

Johnson, G. E., Foster, M. A., and Coco, R.
1933. The sexual cycle of the thirteen-lined ground squirrel in the laboratory. Trans. Kans. Acad. Sci., 36:250-269.

Linduska, J. P.
1947. Longevity of some Michigan farm game mammals. Jour. Mammal., 28:126-129.

Longhurst, W.
1944. Observations on the ecology of the Gunnison Prairie dog in Colorado. Jour. Mammal., 25:24-36.

Maximow, A. A., and Bloom, W.
1944. A textbook of histology. (Philadelphia and London, W. B. Saunders Co.) 4th ed., vii + 695 pp.

Moore, A. W.
1937. Some effects of altitude and latitude on the Columbian ground squirrel. Jour. Mammal., 18:368-369.

Moore, C. R., Simmons, G. F., Wells, L. J., Zalesky, M., and Nelson, W. O.
1934. On the control of reproductive activity in an annual breeding mammal, (Citellus tridecemlineatus). Anat. Rec., 60:279-289.

Mossman, H. W., Lawlah, J. W., and Bradley, J. A.
1932. The male reproductive tract of Sciuridae. Amer. Jour. Anat., 51:89-155.

Painter, T. S.
1923. Studies in mammalian spermatogenesis. Jour. Exp. Zool., 37:309.

Sarnat, B. G., and Hook, W. E.
1942. Effects of hibernation on tooth development. Anat. Rec., 83:471-492.

Shaw, W. T.
1918. The Columbian ground squirrel, (Citellus columbianus columbianus). The Monthly Bull. Calif. State Comm. Hort., 8:118-128.

1924. The home life of the Columbian ground squirrel. Canad. Field Nat., 38:128-130, 151-153.

1925a. Duration of the aestivation and hibernation of the Columbian ground squirrel, (Citellus columbianus) and sex relation to the same. Ecology, 6:75-81.

1925b. The hibernation of the Columbian ground squirrel. Canad. Field Nat., 39:56-61.

Shaw, W. T.

1925c. The seasonal difference of north and south slopes in controlling activities of the Columbian ground squirrel. Ecology, 6:157-162.

1925d. Breeding and development of Columbian ground squirrels. Jour. Mammal., 6:106-113.

1925e. Observations on the hibernation of ground squirrels. Jour. Agr. Res., 31:761-769.

1926. A short season and its effects upon the preparation for reproduction by the Columbian ground squirrel. Ecology, 7:136-139.

Simmons, G. F.

1946. The problem of delayed puberty in a monestrous wild animal, (*Citellus tridecemlineatus*). (Abstract) Anat. Rec., 94:368.

Snedecor, G. W.

1940. Statistical methods applied to experiments in agriculture and biology. (Ames, Iowa, Collegiate Press, Inc.). 3rd ed., xiii + 422 pp.

Stockard, A. H.

1929. Observations on reproduction in the white-tailed prairie-dog, (*Cynomys leucurus*). Jour. Mammal., 10:209-212.

Wade, O.

1927. Breeding habits and early life of the thirteen-lined ground squirrel, (*Citellus tridecemlineatus, Mitchell*). Jour. Mammal., 8:269-276.

Wells, L. J.

1935. Seasonal sexual rhythm and its experimental modifications in the males of the thirteen-lined ground squirrel, (*Citellus tridecemlineatus*). Anat. Rec., 62:409-447.

1936. Effects of synthetic androsterone on accessory reproductive organs of the male ground squirrel. Proc. Soc. Exp. Biol. and Med., 34:525-528.

Wells, L. J., and Gomez, E. T.

1937. Hypophysectomy and its effects on male reproductive organs in a wild mammal with annual rut, (*Citellus*). Anat. Rec., 69:213-227.

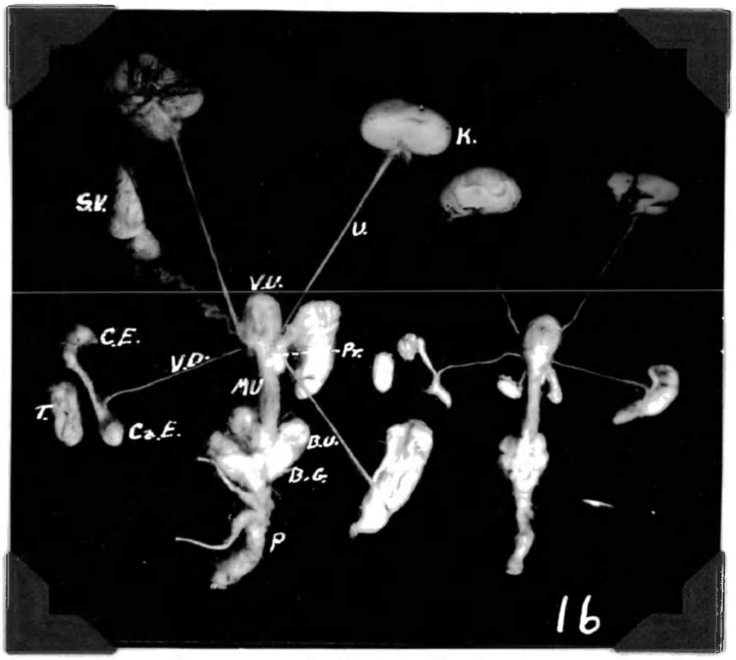
Wells, L. J., and Moore, C. R.

1936. Hormonal stimulation of spermatogenesis in the testis of the ground squirrel. Anat. Rec., 66:181-200.

Wells, L. J., and Overholser, M. D.
1940. Effects of estrogen on the genital tract and urethra of anestrous female ground squirrels. Anat. Rec., 78:43-57.

Wells, L. J., and Zalesky, M.
1940. Effects of low environmental temperatures on the reproductive organs of male mammals with annual aspermia. Amer. Jour. Anat., 66:429-446.

PLATE IV



Adult   Yearling

REPRODUCTIVE TRACTS FROM ADULT AND YEARLING MALE <u>C. COLUMBIANUS</u> WHICH WERE TAKEN DURING THE PERIOD THE TRACTS WERE REGRESSING. (mag. x $\frac{1}{2}$)

The organs are in their normal relationships except for the penis which has been stretched out, and one seminal vesicle which has been partially dissected to show the coarse tubular nature.

Key to figure 16

B.G., bulbo gland
B.U., bulbo urethra
Ca.E., caudal epididymis
C.E., caput epididymis
K., kidney
M.U., muscular urethra
P., penis
Pr., prostate
S.V., seminal vesicle
T., testis
U., ureter
V.D., vas deferens
V.U., vesica urinaria

PLATE V

Explanation of figures

All figures on Plates V through X magified x 266.

17 Portion of testis of juvenile, No. 387 taken May 30, 1946. This animal weighed 80 gm. and was the smallest male collected. A Stage I animal.

18 Portion of testis of a juvenile, No. 396 taken June 20, 1946. The condition is typical of juvenile testes from this time until estivation occurs. A Stage I animal.

19 Portion of caput epididymis of No. 387. Note that intertubular tissue forms a considerable portion of the picture.

20 Portion of caput epididymis of a juvenile, No. 425 taken July 21, 1946. Compare with fig. 19. Note lightly stained area between epithelial nuclei and the basement membrane. A Stage II animal.

PLATE V

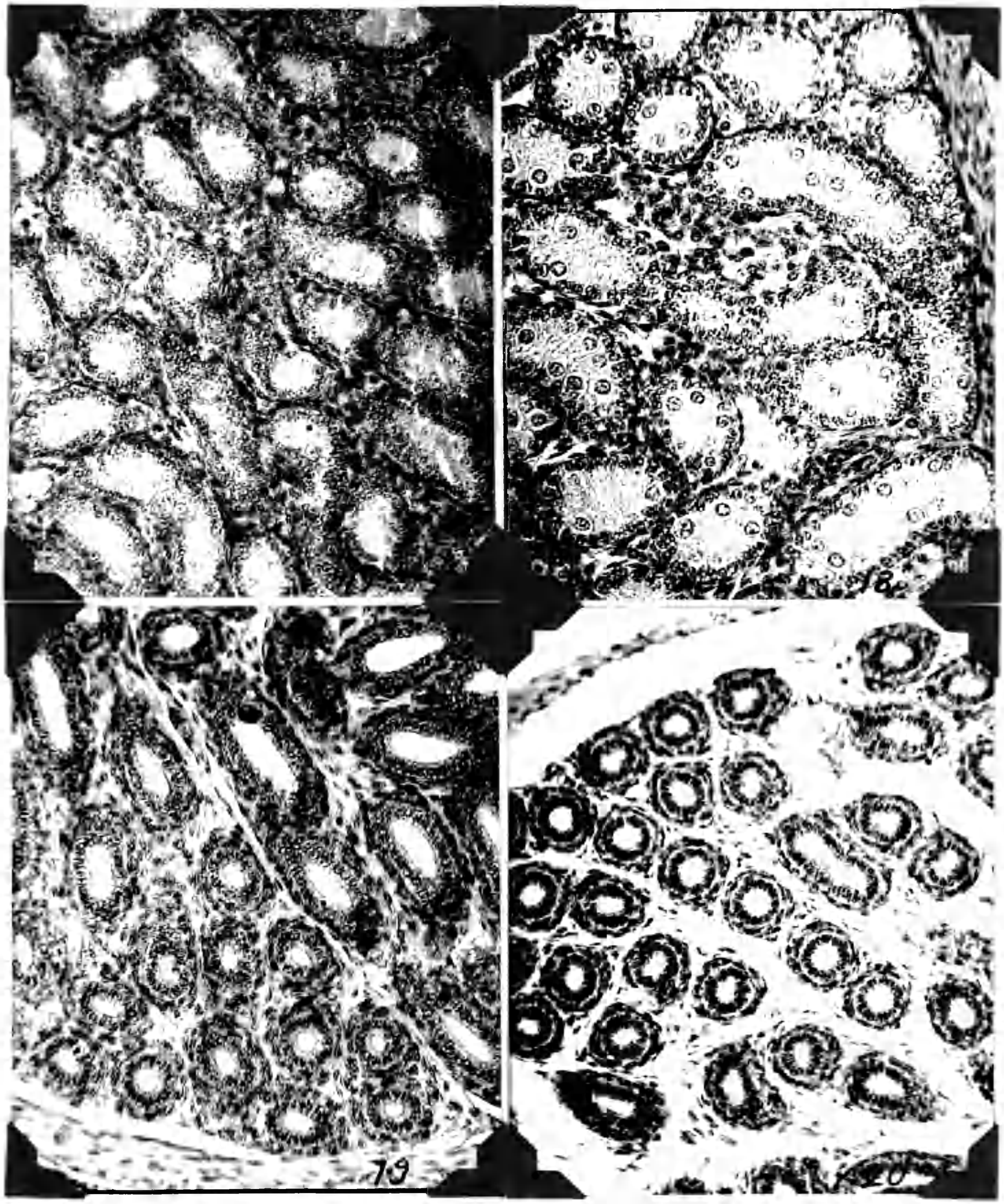


PLATE VI

Explanation of figures

21 Vas deferens of animal No. 387. The lumen is small and the nuclei of the epithelium fail to show the pseudostratified condition which is evident in the vas deferens of more mature animals.

22 Vas deferens of juvenile No. 399, taken June 20, 1946. Note the general increase in size over fig. 21. A Stage I animal.

23 Vas deferens of juvenile No. 447, taken August 6, 1946. Note general decrease in size as compared with fig. 21 and 22. Although smaller than fig. 21, more specialization is shown in the more fully developed lumen and in the arrangement of the epithelial nuclei. A Stage II animal.

24 Portion of testis from a yearling No. 292, taken April 3, 1946. Numerous primary and secondary spermatocytes are evident but no spermatozoa. Compare with fig. 18. A Stage III animal.

PLATE VI

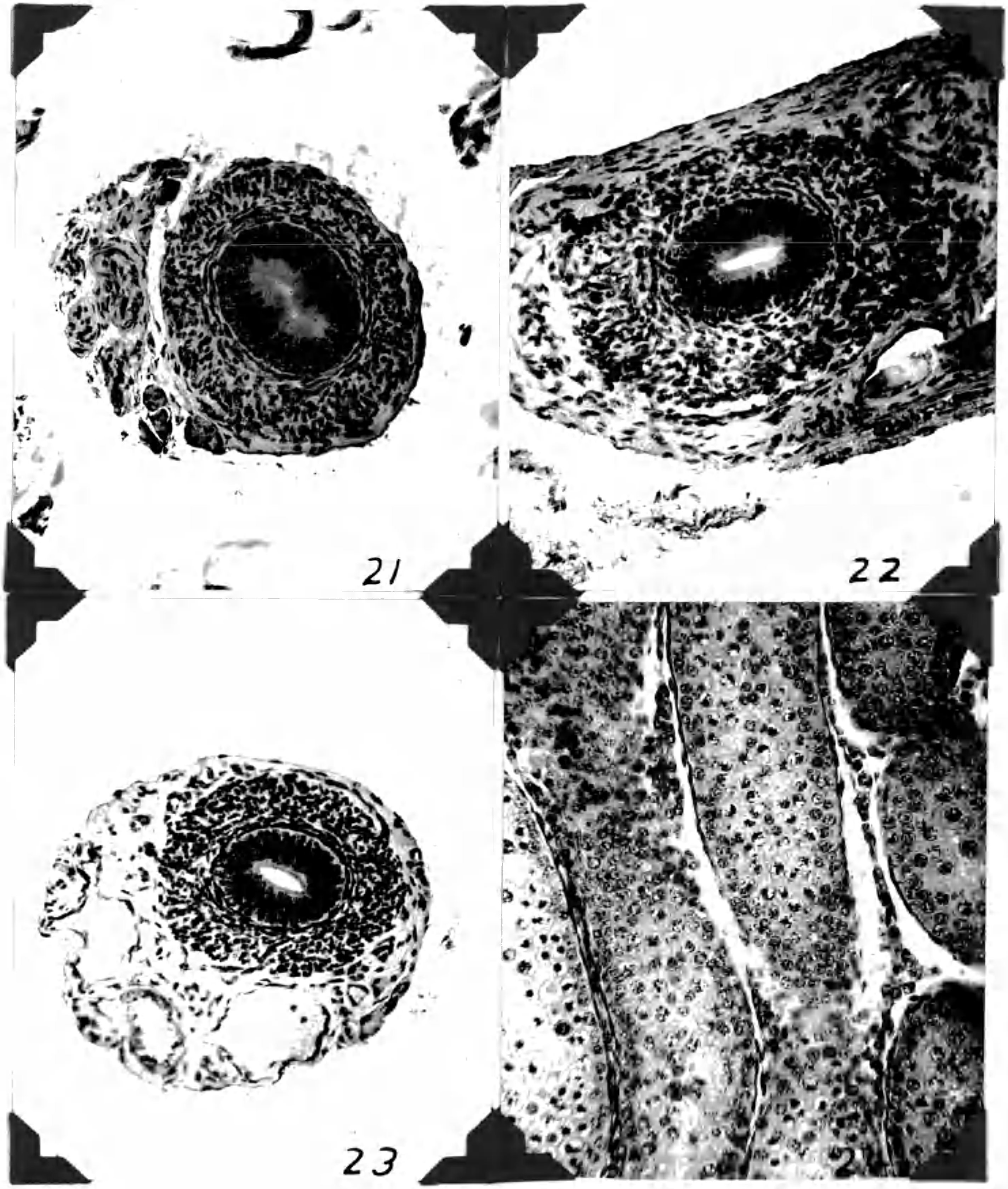


PLATE VII

Explanation of figures

25 Portion of regressing testis of yearling No. 275, taken May 24, 1946. Nuclei with clumped chromatin (C.C.) are evident as well as cells with darkly staining cytoplasm (D.S.). Note the juvenile appearance of the majority of seminiferous tubules. A Stage IV animal.

26 Portion of caput epididymis of yearling No. 293, taken April 3, 1946. The tubules are much larger than they were at the time the juveniles estivated. Compare with fig. 20. A Stage III animal.

27 Portion of regressed caput epididymis of a yearling No. 384, taken May 30, 1946. Note similarity between this figure and fig. 20. A Stage III animal.

28 Enlarged vas deferens of yearling No. 293. Specialization is shown by the presence of cilia and in the pseudostratification of the epithelial nuclei. The pseudostratification is more evident than in previously discussed groups.

PLATE VII

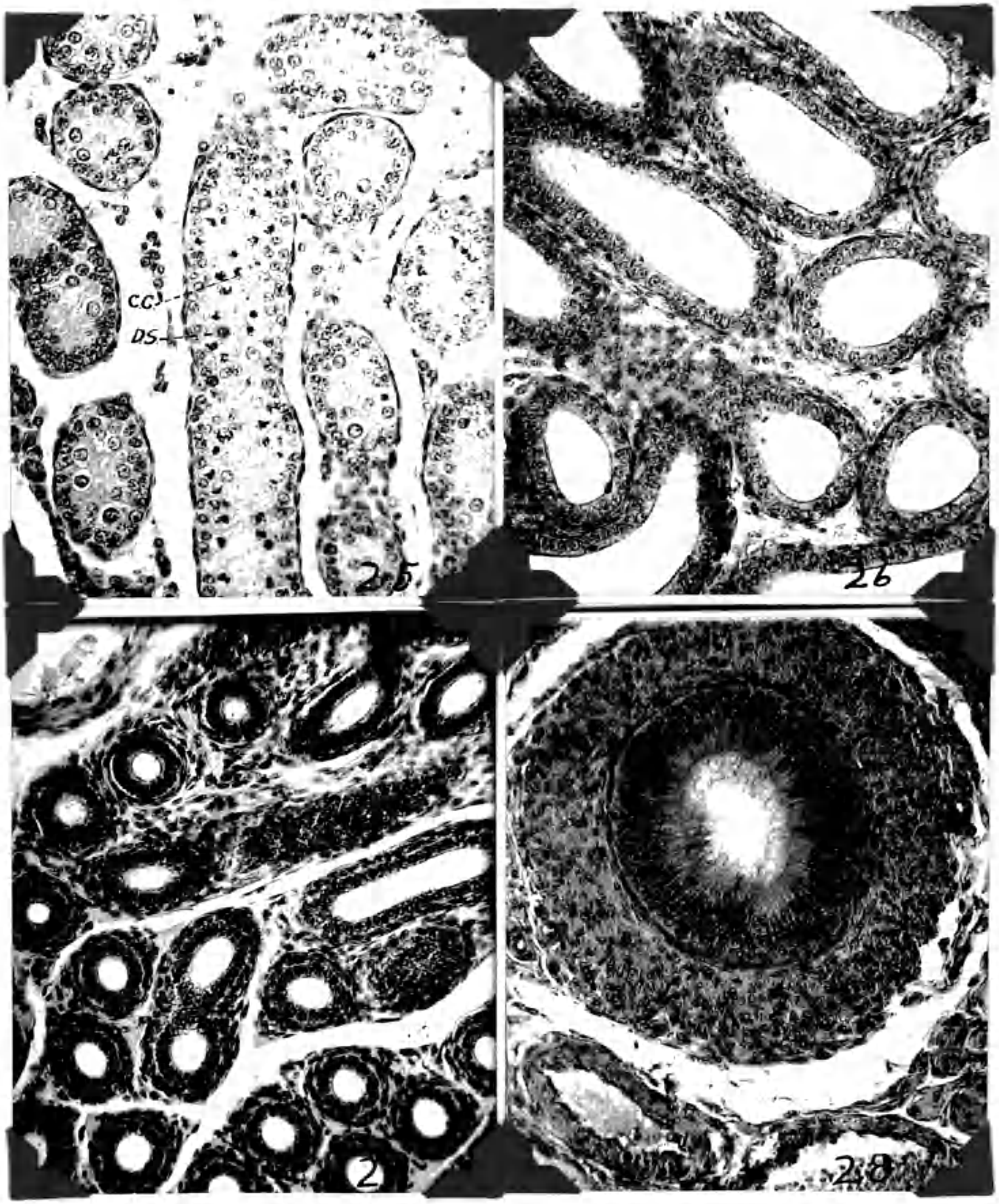


PLATE VIII

Explanation of figures

29 Regressed vas deferens of yearling No. 454, taken August 6, 1946. Compare with fig. 28 and note the extreme reduction in size. Also note the juvenile appearance. A Stage V animal.

30 Seminiferous tubules from an adult No. 456, taken on March 18, 1947. All stages of spermatogenesis are found although mature sperm appear more abundant around the first of April. Note paucity of interstitial tissue. A Stage V animal.

31 Portion of regressed testis from an adult No. 385, taken May 30, 1946. A few nuclei with clumped chromatin are present. Note juvenile appearance. A Stage VI animal.

32 Tubules of caput epididymis of an adult No. 456. Note spermatozoa packed tubules. Epithelium is high columnar and its nuclei are regularly arranged at the base of the cells. Note comparatively small amount of intertubular tissue. Compare with fig. 19 and 26.

PLATE VIII

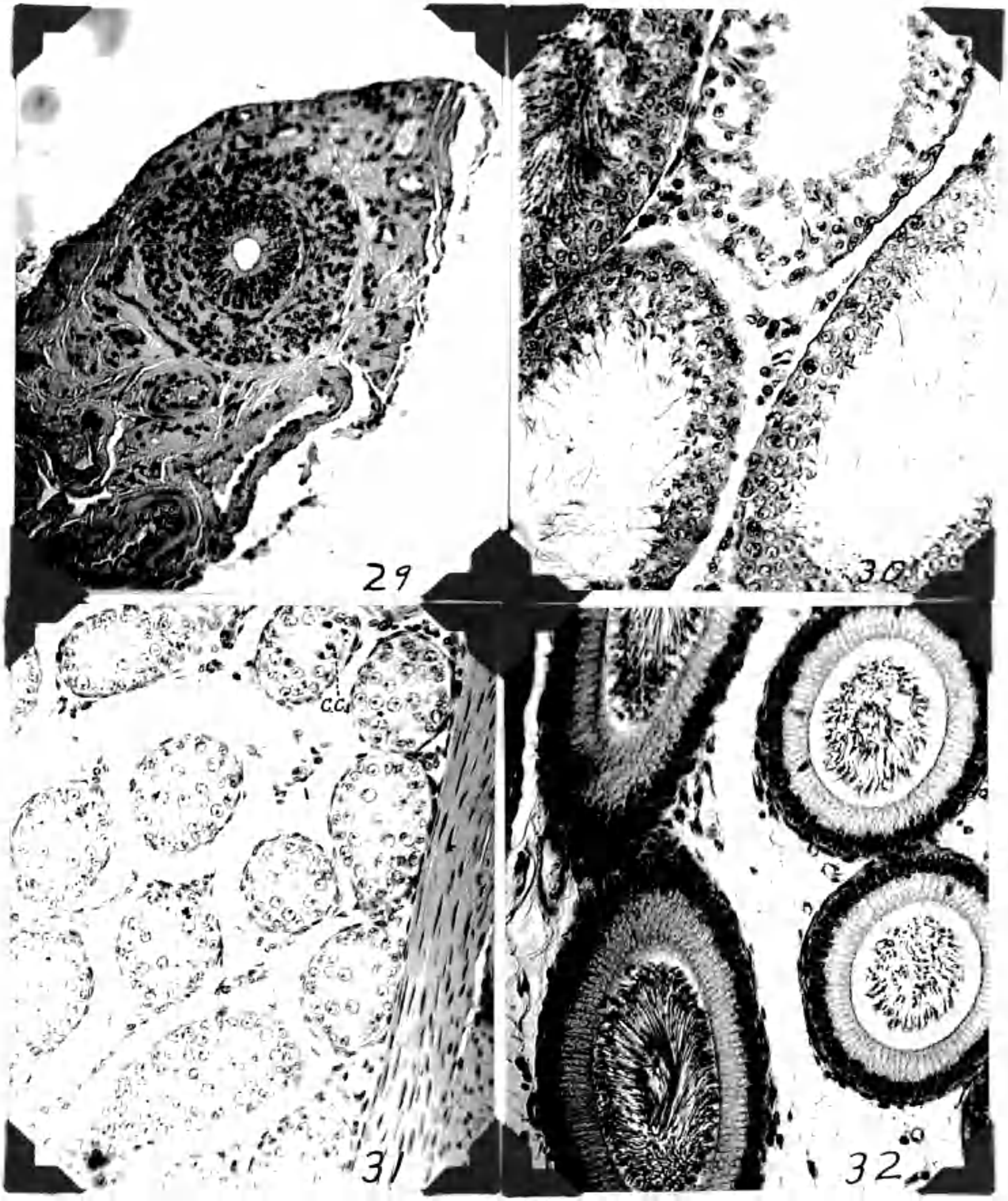


PLATE IX

Explanation of figures

33 Portion of regressed caput epididymis from an adult No. 385. Note the lightly stained area between the epithelial nuclei and the basement membrane. A similar condition was noted in the regressed epididymides of yearlings. Compare with fig. 20 and 27.

34 Vas deferens of an adult No. 456. Infolding of epithelium largely disappears upon arrival of spermatozoa into the tube.

35 Regressed vas deferens of an adult No. 373, taken May 19, 1946. Compare with figure 34. A Stage V animal.

36 Portion of seminal vesicle of adult No. 456. Large tubules are filled with secretion, and intertubular tissue is relatively scanty.

PLATE IX

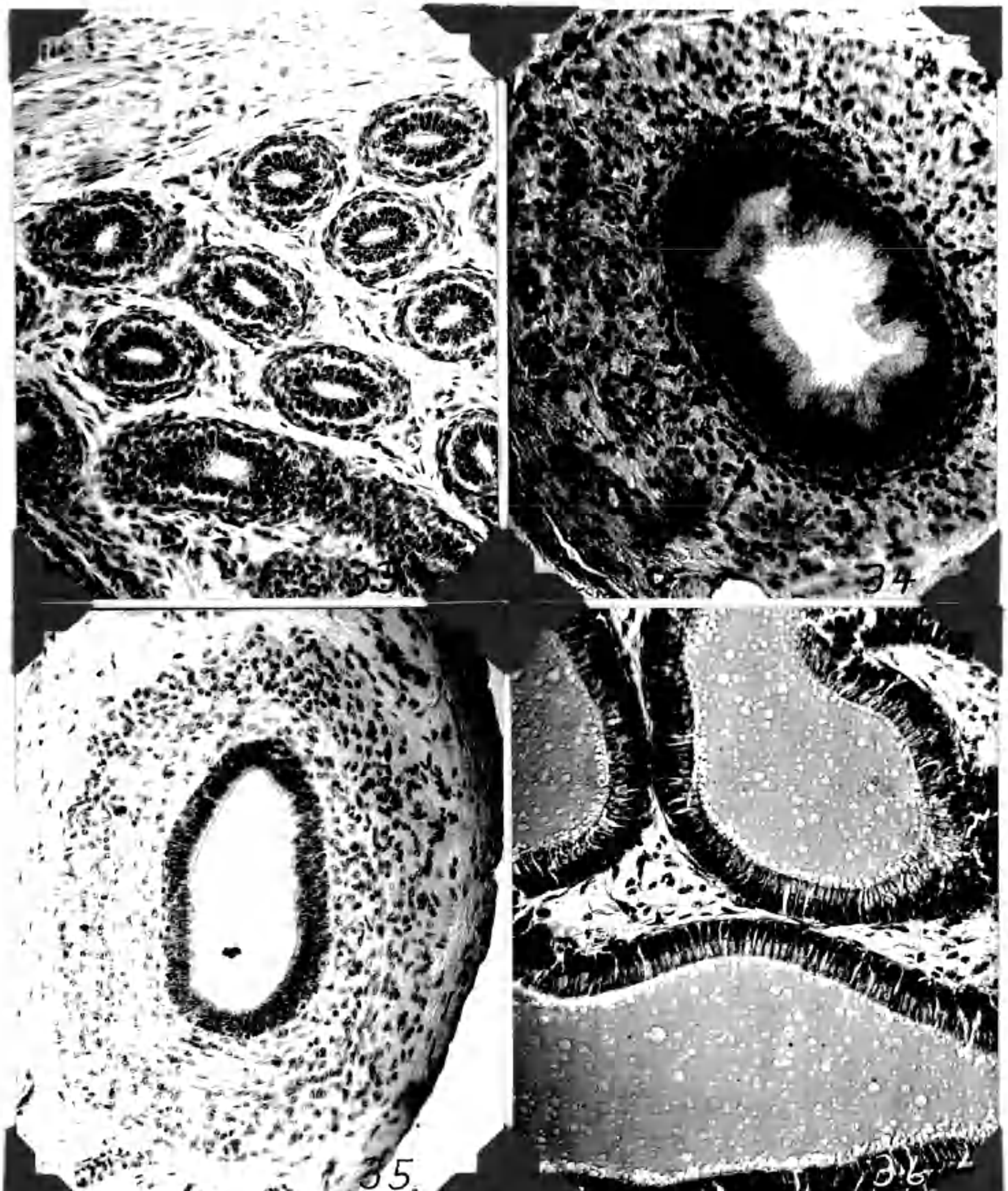


PLATE X

Explanation of figures

37 Portion of seminal vesicle of yearling No. 465, taken April 26, 1947. Compare with figure 36. Note small tubules and comparative large amount of interstitial tissue.

PLATE X