The Seasonal Spermatogenic Cycle and the Influence of Dehydration on Spermatogenesis in the Kangaroo Rat, *Dipodomys spectabilis spectabilis* Merriam

By

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INTRODUCTION

In recent years, work on the testes of mammals has been distributed over a wide field. Some of the literature of only two phases of the general work will be reviewed briefly here. First, the literature on seasonal spermatogenic cycles in the mammals; second, the literature on some physiological factors which have been shown to modify normal spermatogenic cycles. Tandler and Grosz (1911) found in the mole, *Talpa europaea*, that spermatogenic activity decreased during February and attained its height during March. The increase in size was entirely due to the proliferation of germ cells. Marshall (1911) noted in the hedgehog, that no spermatogenic activity was observable during the period of hibernation, but beginning with the latter part of April and extending to the latter part of October, mature spermatozoa were observed in the ductus deferens. With this period which marked the production of mature sperm, an increase in the size of the testis was observed; this increase was partly

due to the enlargement of the seminiferous tubules, but was largely caused by proliferation of the interstitial cells. Rasmussen (1917) found in the woodchuck, Marmota monax, that during hibernation, which was in the fall and winter months, spermatogenic activity increased markedly and reached its height during the latter part of March and the first of April. This active period was followed by a marked decrease, which reached a minimum in June and July. A complete review of the literature on seasonal cycles of vertebrates has been made by Oslund (1928); however, very few studies have been made on mammals.

The literature on conditions affecting the various testicular elements is extensive and has been reviewed fully by Moore (1926). Experimental cypterchidism has been shown by Moore (1922) and Moore and Oslund (1924) to cause a decided decrease in the amount of germinal epithelium of sheep, which they have demonstrated to be due to a higher temperature in the abdomen than in the scrotum. This has been confirmed by Bissonnette (1926) in cattle. Oslund and Bachman (1926) have shown that x-ray treatment of the testes of rats caused a large reduction in the amount of germinal epithelium, but not total destruction. The same effects were also noted by Tsuzuki (1926) in rabbits. Evans, Burr, and Althausen (1927) found that rats fed a diet consisting of all the necessary ingredients except vitamine E, suffered sterility. The testes showed degeneration of the germinal epithelium of the seminiferous tubules, which could be corrected

before maturity, in most cases, by feeding them a diet containing sufficient vitamine E. Bissonnette (1931c) found that lights of different wave lengths had a marked effect on spermatogenesis in the testes of the European starling, Sturnus vulgaris. In these studies, 3 sets of birds were exposed to white, red, and green light, respectively, and it was demonstrated that in the early days of treatment, those which received white light showed the greatest acceleration in testicular activity, while the birds subjected to red light showed less activity, and the spermatogenic activity of those treated with green light was inhibited; but after 33 days the birds that received treatments of red light surpassed in testis activity those exposed to white light and the spermatogenic activity of those treated with green light was definitely inhibited. Kudo (1921) found in experiments on thirst and its effects on the weights of the various organs of the adult albino rat, that as a result of acute dehydration, the average loss in testis weight was less than half the loss in body weight; but during chronic water inanition, extending over a period of 47 to 55 days, the loss in testis weight was greater than the loss in body weight. In chronic dehydration, the loss in weight of the testis was approximately four times greater than the loss during acute dehydration. Carofeanu and Derevici (1924) deprived dogs, which were fed a diet of dry bread, meat, and salty food, of water for periods varying from 4 to 9 days, and killed the animals at various stages of dehydration. Microscopical examination of the organs

showed lesions in lungs, liver and thyroid, and to a lesser degree in the kidneys, adrenals and stomach; it was also observed that there were differences in the vascular dilatation of different organs, as well as differences in the same organ. No mention was made of the effects of dehydration on the testes.

Since no studies have been made on the physiology of the reproductive organs of desert mammals, the present study was undertaken with the kangaroo rat, Dipodomys spectabilis spectabilis Merriam, for two purposes: first, to collect testes of the kangaroo rat at approximately monthly intervals and to study the macroscopic and microscopic changes which occur in the seminiferous tubules in order to determine whether or not there is a definite spermatogenic cycle; second, to study the effect of dehydration on spermatogenesis in an effort to determine whether or not a decrease in water intake might influence the production of mature, male germ cells. Unlike the albino rat, which has been thoroughly studied, the kangaroo rat has a definite breeding season, the exact limitation of which, however, is unknown. Vorhies and Taylor (1922) have estimated its breeding season to extend from January to August, inclusive; this estimate was based solely on field studies. Histological studies have not been made on the kangaroo rat and therefore no data are available on possible seasonal changes in testis or ovary, neither has this rat been used for laboratory experimentation, and therefore no literature is available on physiological factors which might affect the activity of the males in the perpetuation of

the species. The kangaroo rat, according to Vorhies and Taylor (1922) has a range of Southwestern Arizona, Southeastern and Central New Mexico, the extreme western part of Texas, and parts of the Mexican States of Sonora and Chihuahua, and is somehow so adapted physiologically to semi-arid regions that it does not require free water.

The writer wishes to express his thanks to Dr. G. T. Caldwell, under whose direction this study has been carried out, for his helpful suggestions and advice.

MATERIALS AND METHODS

In this study 93 adult male rats have been used, and the testes of 81 have been studied microscopically. A total of 53 animals was used to study the seasonal spermatogenic cycle, and to serve as controls for the dehydrated animals. Forty animals have been used in the dehydration experiments, to determine effects of water deprivation on spermatogenesis and testis weight to body weight ratios. No animals were trapped in June, July, and August. Table I gives the number of rats caught each month, and the numbers used in each division of this study.

TABLE I

Number of Normal Rats used each Month and Number of Rats Dehydrated each Month

Month	Normal rats	Dehydrated rats
September	5	-
October	-	5
November	10	5
December	10	5
January	5	5
February	10	5
March	5	5
April	4	5
May	4	5
	53	40

In the first experiments, the rats were weighed as soon as they were caught, killed with illuminating gas, the testes removed, tunicae albuginea pricked at each end of its long axis and placed in Allen's B-15 killing fluid, which consisted of saturated aqueous solution of picric acid, 75 c.c.; formalin c.p., 25 c.c.; glacial acetic acid, 10 c.c., warmed to 38°C. with 1.5 grams of chromic acid and 2.0 grams of urea were added just before using. The testes were left in the killing fluid approximately 2 hours, then removed and placed in 70 per cent alcohol containing a small amount of lithium carbonate until all the picric acid had been removed. They were then put into 80 per cent, 95 per cent, and 100 per cent alcohols for 1 hour each. Chloroform was used in clearing, and infiltration was accomplished by adding a small amount of paraffin to the solution containing the tissues; this was followed by three baths of pure paraffin for 1/2 hour each, to remove all traces of the clearing fluid, and then the tissues were embedded in paraffin. It was found that this method of handling produced distortion of the tissue, and resulted in poor fixation of parts of the testis. This was probably due to failure of the fluid to penetrate readily the dense tunicae albuginea. Twenty-two pairs of normal testes were killed and fixed by this method.

A second method of killing, fixing, dehydrating and clearing was used, based on a modification of Allen's (1919) method. The animals were weighed as before, the testes removed, tunicae pricked in the center of the

testes, placed in the same killing fluid that was used in the previous method, weighed in order to determine whether or not there were seasonal changes in the weights of the tissues, and allowed to remain 30 minutes. The tissues were then removed from the fluid, and cut into sections 4 mm. thick, at right angles to the long axis of the testes, replaced in the fixing fluid, and allowed to remain approximately 3 hours. The dehydration and clearing was accomplished by the apparatus described by Allen (1919), using the same solutions. All fluids were added by the drop method and agitation was maintained by bubbling air through the solutions. For dehydration, the tissues were first placed in 5 per cent alcohol to which several drops of saturated aqueous solution of lithium carbonate had been added. At the end of 2 hours 50 per cent alcohol was added by the drop method, until the mixture had reached a concentration of 30 per cent, when the tissues were allowed to stand in this mixture for 1 hour. Equal parts of 50 per cent alcohol and aniline oil were slowly added until all of the lower grade alcohols had been removed; equal parts of 70 per cent alcohol and aniline oil were added very slowly, then the tissues were changed to fresh 70 per cent and aniline oil and pure aniline was slowly added by the drop method. Allen (1919) recommended that the tissues be placed in fresh fluid of the strength to which they had arrived, before a different solution was added; this was done in all cases. Synthetic oil of wintergreen was used for clearing the tissues, which was also added by the drop method, at a very slow rate. Infil-

tration was accomplished by using graded strengths of paraffin and wintergreen oil, in the following mixtures:

Per cent Paraffin	and	Per cent Wintergreen Oil
10		90
20		80
30		70
40		60
50		50
60		40
80		20
90		10

The tissues were then placed in pure paraffin, where approximately 2-1/2 hours were consumed in infiltration. Following this, the tissues were placed in four changes of pure paraffin, which required 2 to 3 hours and then immediately embedded. Eight pairs of normal testes were killed, fixed, dehydrated, cleared and embedded by this method. Subsequent microscopical examination of these testes revealed considerable distortion of the interstitial materials, and poor fixation in certain areas of the tubules.

A third method of killing, fixing, dehydrating and clearing was used that produced very good results; the animals were killed as before, the testes removed, and a cut made in the tunicae albuginea, at right angles to the long axis and about one-third of the greatest circumference. The testes were placed in Bouin's fixative (saturated aqueous solution of picric acid, 75 c.c.; formalin, 25 c.c.; glacial acetic acid, 10 c.c.; used at room temperature), weighed, and allowed to stay in this fluid approximately 20 minutes. The testes were removed, cut with a sharp razor

blade through their greatest diameter, replaced in the fluid for approximately 10 minutes, then removed, halved again and placed in fresh Bouin's fixative where they remained 18 to 24 hours. The testes were placed in several changes of 70 per cent alcohol, containing 5 drops of saturated aqueous solution of lithium carbonate, until all the picric acid had been removed, and then run up the graded alcohols through 100 per cent. The tissues were cleared in cedar oil, without any intermediate steps between the 100 per cent alcohol and the oil. It was found that tissues could be left indefinitely in cedar oil, without any injurious effects,--Geyer (1917), and Allen (1919). Infiltration was accomplished by saturating the oil with paraffin at room temperature, then placing it in the oven where paraffin was gradually added and the oil poured off until the mixture was almost pure paraffin. The testes were run through three changes of paraffin and embedded. All testes were sectioned at 5 micra, at right angles to the long axis of the organ and approximately 2 mm. from the cut surface of the tissue. This procedure gave representative sections of the different testes. The sections were stained with Harris' haematoxylin counterstained with alcoholic erythrosin, mounted in balsam, and studied at magnifications of 100, 440, and 950 diameters. Five animals were trapped and immediately dehydrated during the following months: January, February, March, April, May, October, November, and December.

The method of dehydration is that of Caldwell (1931). The dehydration apparatus consisted of a large glass bell jar, with a ground glass flange which rested on plate glass and served as the animal chamber. A wire cage divided into five compartments with individual feed boxes, served as the animal container. The cage rested in a shallow pan, which caught the waste food, feces and urine. Much light was eliminated by black paper placed around the bell jar on the outside. Air was drawn into the animal chamber through two William's gas-washing bottles, containing H_2SO_4, through an empty washing bottle, several feet of large-bore glass tubing filled with desiechlora, through a flow-meter and into the animal chamber. From the animal chamber the air was bubbled through a bottle containing concentrated H_2SO_4, through a small bottle that acted as a trap, and finally through a U-tube containing P_2O_5, to a T-tube, one end of which was connected to an electric motor pulp, the other to a water pump. Air was passed through the animal chamber at a rate of 1,000 c.c. per minute. Five rats were dehydrated at one time; they were weighed, tagged, dusted with insect powder, one rat placed in each compartment of the cage and the animal chamber sealed. The animals were fed on Sherman rat diet and were carefully weighed every third day, in order to note the progressive loss of water. However, in this report, only the initial and final weights are included. The average experiment lasted 13 days. When the animals appeared to be in such condition that one or more might not live another 24 hours, they were weighed, killed,

the testes removed and prepared by the same procedure as that for the controls of that month.

EXPERIMENTAL RESULTS

Gross Seasonal Changes in Testes

It was found in the mature male kangaroo rat that the testes were most frequently in the scrotum, but the inguinal canals remained open so that in many animals one testis was in the scrotal position and the other could be changed from scrotal to abdominal or the opposite by the application of slight manual pressure. From the lack of specific information, several immature males were used in the first experiments. These immature males were characterized by having very small testes located in the abdomen with little development of the scrotum. The immature animals have not been included in the study of the seasonal spermatogenic cycle, nor in computing the average monthly testes weight, nor were they used in the comparisons with the dehydrated animals.

The testes varied markedly in gross size, even between the two testes of the same animal; this difference may have been due in some cases to one testis remaining too long in the abdomen and assuming a somewhat cryptorchid condition--Moore (1922). Other factors probably entered into these differences. Rasmussen (1917) found in the woodchuck,

in which the testes occupied two pouches beside the root of the tail during the breeding season, that the testes of the same animal were nearly the same weight.

The testis color was cream, and no changes were observed at different periods of the year, as has been reported by Bissonnette (1932) in the testes of the starling.

Table II shows that a wide variation occurs in the body and testes weights of normal rats trapped the same month, which is also noticeable in the differences in the ratios of testicular weight to body weight. Similar variations in body weight and testes weight were noticeable in the experimental animals (Table III). The average body weights of the animals trapped each month, the average testicular weights and the ratios between these two are given in Table III. It can be observed that the average body weights fluctuate while the testes show a gradual increase in weight beginning with September and reaching a maximum in January and February which is followed by a marked decline in weight in May (Fig. 1). Since no animals were trapped during the summer months, no figures are available for this period. The average weight of testes for May was somewhat higher than that for September (Fig. 1). Because of the small number of rats secured each month, Tables I and II are merely indicative of the average body and testes weights of the rats during the various months. The greatest average testis weight, which was found to be in January, correlates closely with the beginning of the breeding season. Vorhies and Taylor (1922)

caught their first suckling female the latter part of January and the young were approximately one week old. The writer trapped eight females on February 17th, and found that five of these were pregnant, the largest embryos were 6 mm. long; the other three females were neither pregnant nor suckling, which indicated that it was near the beginning of the breeding season.

Table II

Normal rats, months caught, weight of animals, weight of testes, and ratio of testes weight to body weight.

Rat No.	Date	Body Weight in grams	Weight of Both Testes in grams	Ratio of Testes Weight to Body Weight	
58	9/20/31	100.8	0.388	1:259.53	
59	9/20/	119.8	0.676	1:177.2	
60	9/31/	120.9	0.696	1:173.7	
61	9/21/	111.7	0.453	1:244.3	
62	9/21/	108.8	0.4494	1:242.1	
	Average	112.4	0.532	1:219.36	
68	11/8/	111.7	0.7275	1:153.26	
69	11/8/	120.1	0.4705	1:255.25	*
70	11/8/	111.9	0.609	1:183.74	
71	11/3/	113.3	0.6005	1:196.53	
72	11/4/	115.6	0.703	1:164.43	
	Average	114.52	0.622	1:190.64	
79	12/8/	112.8	0.692	1:163.00	
80	12/8/	111.1	0.700	1:158.7	
81	12/9/	114.5	0.647	1:176.83	
82	12/16/	102.9	0.624	1:164.90	
	Average	110.42	0.665	1:166.35	
83	2/8/32	126.5	0.832	1:152.04	
84	2/8/	124.5	0.812	1:153.32	
85	2/24/	101.9	0.633	1:160.83	
86	2/29/	112.9	0.629	1:175.4	
87	2/29/	133.9	0.686	1:195.2	
	Average	119.94	0.7185	1:168.15	

* = Left testis cryptorchid

Table II Continued

Rat No.	Date	Body Weight in grams	Weight of Both Testes in grams	Ratio of Testes Weight to Body Weight
88	3/6/32	111.9	0.597	1:187.43
89	3/6/	109.8	0.713	1:153.9
90	3/1/	113.7	0.465	1:165.37
91	3/16/	107.9	0.596	1:181.04
92	3/16/	121.0	0.628	1:192.67
	Average	112.86	0.644	1:176.06
45	4/28/31	108.1	0.805	1:134.28
46	4/28/	109.6	0.533	1:205.62
47	4/20/	110.6	0.595	1:185.88
48	4/20/	106.5	0.512	1:188.14
	Average	108.7	0.611	1:183.45
54	5/26/	110.1	0.603	1:182.6
55	5/26/	101.7	0.743	1:136.8
56	5/28/	103.3	0.641	1:160.9
57	5/28/	119.7	0.287	1:416.7*
	Average	108.7	0.568	1:160.1

*= Very slightly immature.

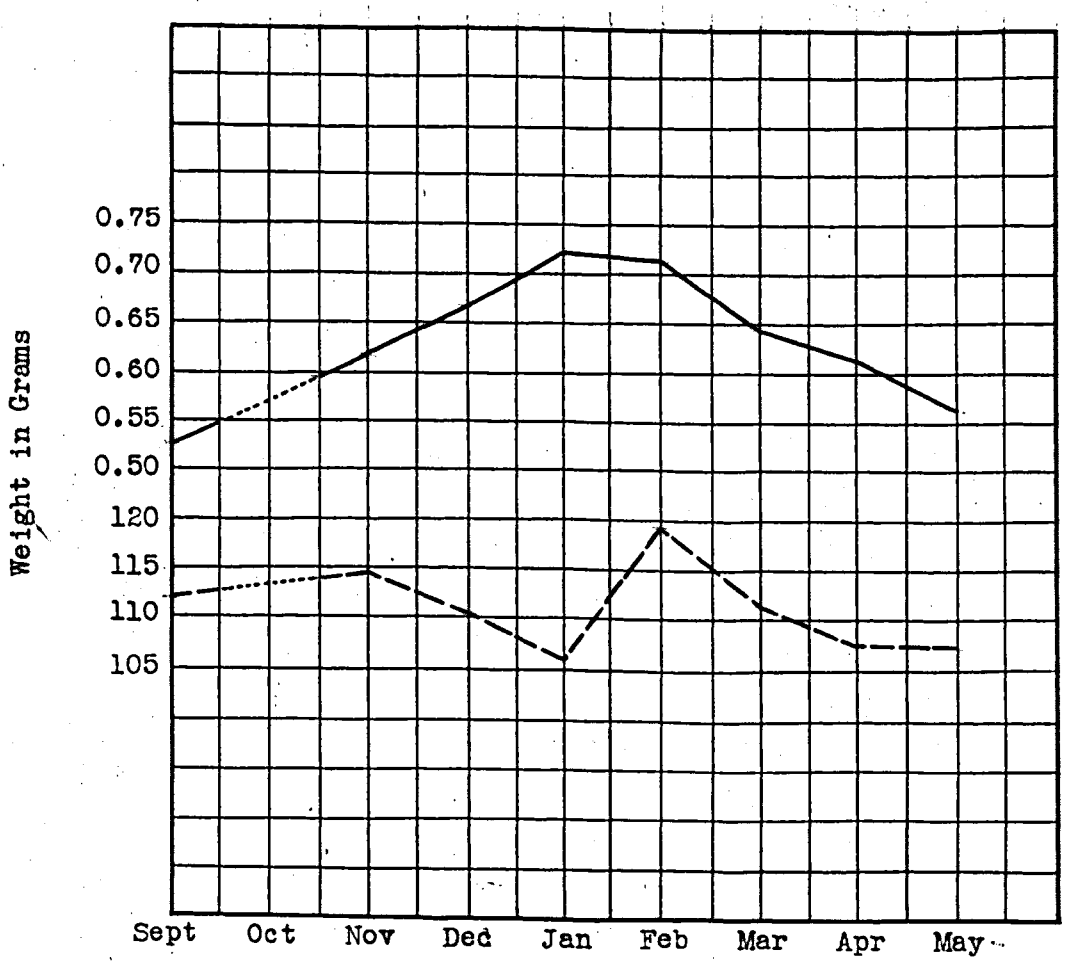


Fig. 1. Graph of testes weight and body weight of normal rats as determined for the months indicated on the ordinate. Solid line represents testes weights. Broken line represents body weights. Dotted line indicates period of one month when no rats were trapped for seasonal cycle studies.

Histological Seasonal Changes in Testes

The testis of the kangaroo rat was found to be typical of the mammals and is composed of seminiferous tubules, with interstitial material and blood vessels between them. The tunica albuginea composed the outer covering of the testis proper, and the basement membrane surrounded the individual tubules. The tubule consisted of a germinal epithelium, which extended from the basement membrane to a lumen, which was in the center of the tubule; this epithelium contained two types of cells: first, the Sertoli cells; second, the sexual cells. The nuclei of the Sertoli cells were located close to the basement membrane, and the cytoplasm extended in columns toward the lumen. In the rat these cells appeared somewhat flattened and the cytoplasm took a deeper stain with erythrosin than did the cytoplasm of the sexual cells. In many sections studied, the Sertoli cytoplasm contained spermatids, extending from the nuclei to the edge of the lumen. These nuclei of the spermatids could be readily observed since they stained with haematoxylin. The germinal cells constituted the remainder of the tissue of the tubule, and in the kangaroo rat it was found that multiplication, division and reduction of these cells occurred in "waves" along the tubule (Fig. II), which resulted in differences in the appearance of the various tubules in a given section (Fig. 5). Allen (1918) described this phenomenon in the albino rat, but Bissonnette (1932) did

not find it present in the starling.

In September the testes of the kangaroo rat showed some activity in the germinal epithelium; this was based upon comparisons with testes of other months. All stages of spermatogenesis were found, and the lumina of many tubules were partially filled with the tails of spermatids and mature sperm, but relatively few of the latter were found in comparison with other months (Figs. 2, 3, and 4). The germinal epithelium of some tubules was crowded with dividing spermatogonia and others with primary spermatocytes in the different stages of mitosis. The average section of this month proved that the animals were sexually active, as far as the production of mature, male germ cells was concerned. The interstitial material was scanty in proportion to the amounts observed in the testes of animals trapped during the winter months (Fig. 2). The tunica albuginea and the basement membranes were very thin, which indicated that there was considerable tension on them; some disintegration was present in the germinal epithelium, but it was very unevenly distributed in a single slide, and a wide variation was found between different sections.

The testes collected in November showed a higher degree of activity in the average section when compared to the normal testes of the previous month (Fig. 5). Upon examination it was found that the cells were active, which was true for all stages of multiplication, division, and reduction. Many tubules were crowded with spermatids,

PLATE II

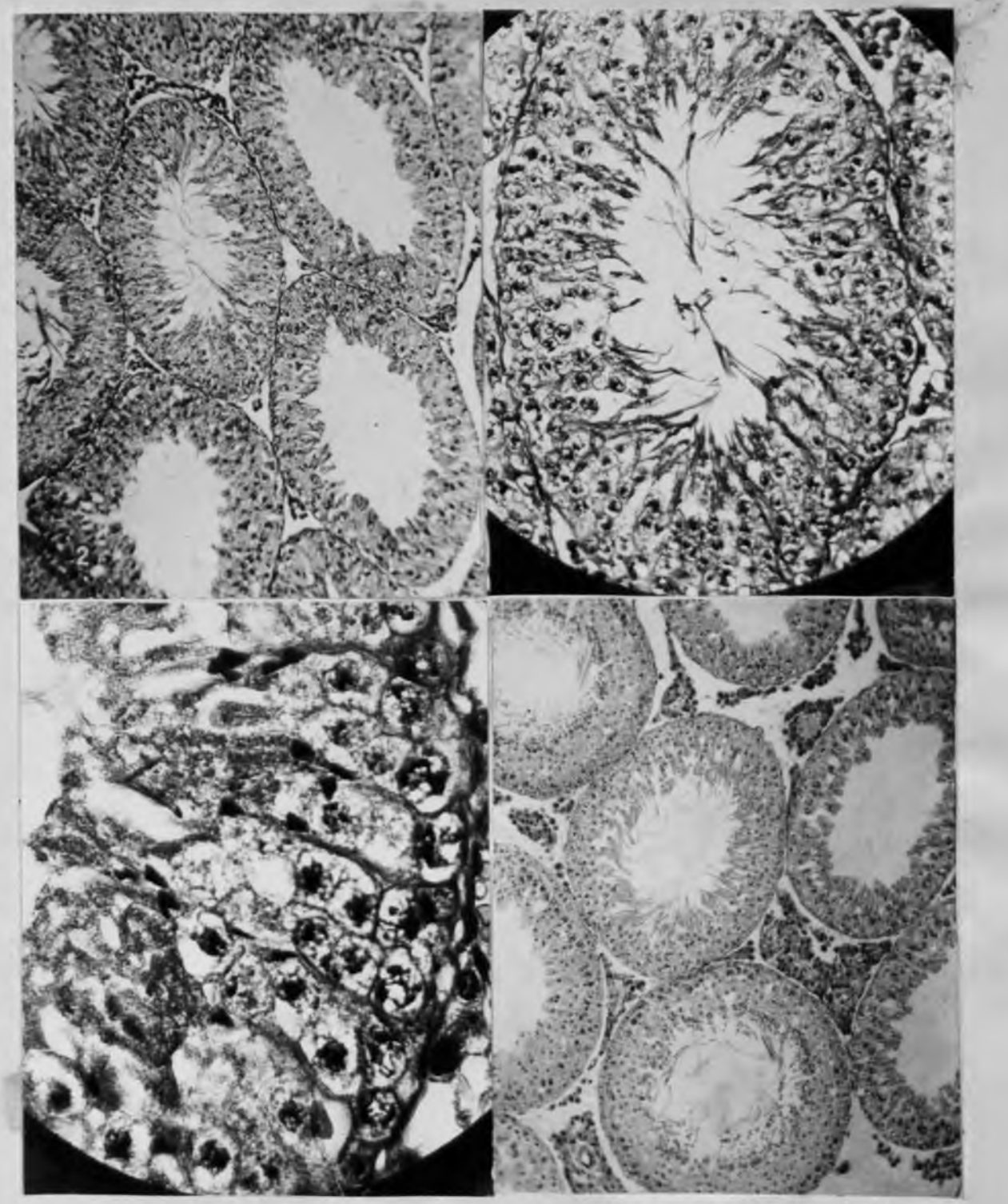
Fig. 2. Microphotograph of section through the testis of normal rat number 58, trapped in September. x 100.

Fig. 3. Microphotograph of a cross-section of one of the tubules in Fig. 2. x 300.

Fig. 4. Same tubule as shown in Fig. 3. x 800.

Fig. 5. Microphotograph of section through the testis of normal rat number 68, trapped in November. x 100.

PLATE II



some were found without tails, close to the basement membrane in the Sertoli tissue, while others with tails were nearer, or on the edge of the inner margins, with the tails extended into the lumina. Both the tunica albuginea and the basement membranes were thin in the testes of this month. Disintegration of the germinal epithelium was found to be present in varying degrees, both in the same section and in different sections. The interstitial material was slightly more prevalent in comparison with the amount observed in November; this material was not measured quantitatively so the exact differences are not known.

The normal testes secured in December were found to be more active than any secured in the fall. The individual sections showed active spermatogenesis, and all the stages of maturation were observed. It was found that a very large number of tubules were so crowded with spermatids and nearly mature sperm around the lumen, that it gave the appearance of a "ring" of cells. In some tubules the lumina were almost obliterated by the tails of the spermatids and in others they were almost filled with a syncitial-like mass; it is not known what caused this "filling in", but since germinal disintegration was present in varying degrees, this may have been, in part, the cause. The interstitial material appeared relatively greater than in the previous months.

The condition in the normal testes in January showed the greatest activity of any month studied; this could be observed in the number of "rings" of spermatids and mature sperm seen in the average section of this period. Almost all lumina contained some tails; the nuclei of these spermatids and the heads of the sperms were embedded at various depths in the Sertoli cytoplasm. Some tubules showed uneven disintegration in the germinal epithelium. The interstitial material was so distorted that adequate comparisons with testes of other months could not be made. The blood vessels were prominent in the intra-tubular spaces and were apparently larger than in the previous months.

The testes collected in February were found to have approximately the same activity as those studied in January; the sections showed marked variation in spermatogenesis in different tubules (Fig. 6), as they did in January. Many "rings" of spermatids and spermatazoa were observed (Fig. 8); also the amount of interstitial material was large and the blood vessels were prominent in the intertubular spaces (Fig. 7). Disintegration of the germinal epithelium in some tubules was marked, which may have caused the "filling in" of the lumina (Fig. 8).

The degree of sexual activity in the testes secured in March appeared slightly less than in January and February and because the production of germ cells occurred in waves, it was difficult to compare sections as to the

PLATE III

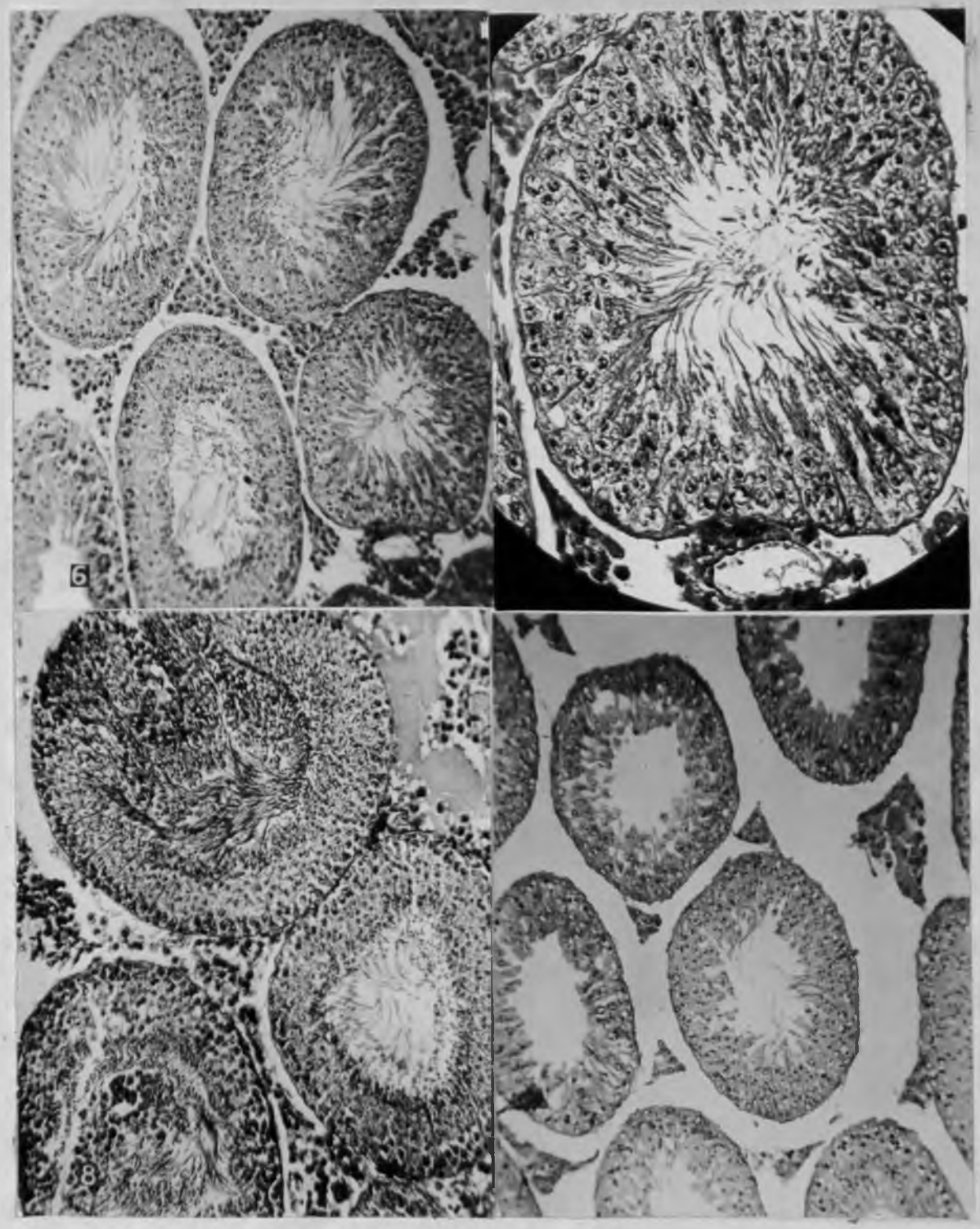
Fig. 6. Microphotograph of section through the testis of normal rat number 84, trapped in February. x 100.

Fig. 7. Microphotograph of a cross-section of one of the tubules in Fig. 6. x 300.

Fig. 8. Microphotograph of section through the testis of normal rat number 86, trapped in February. x 100.

Fig. 9. Microphotograph of section through the testis of normal rat number 56, trapped in May. x 100.

PLATE III



relative amounts of activity. Some tubules were crowded with spermatids, which formed "rings". The interstitial material was approximately equal that in January and February (Fig. 11).

The activity of the testes collected in April was slightly less than in March. The individual cells were observed in the various stages of mitosis; also cells were found in the different stages of maturation, which showed that the gonads were in an active condition. Some tubules were found to contain mature sperm, but the relative number of these cells was much less than in January and February. A "filling in" of the lumina of some tubules was noted, as has been mentioned before, but was present to a less degree than in February. The interstitial material was fairly prominent, but the average amount in each section appeared to be much less than was found in March. This difference was entirely too great to be the result of fixation.

The minimum sexual activity observed occurred in the testes of the animals secured in May and the typical section in this month was quite different from those collected in December, January and February (Figs. 9 and 10). Upon examination the various stages of spermatogenesis were found, which proved that cell activity was present, as in the previous months, but the relative number of tubules that showed "rings" of spermatids and nearly mature spermatozoa, with tails in the lumina was greatly reduced. The obliteration of the lumina, which was probably caused by disintegration of the epithelium, was greatly reduced when compared with that

PLATE IV

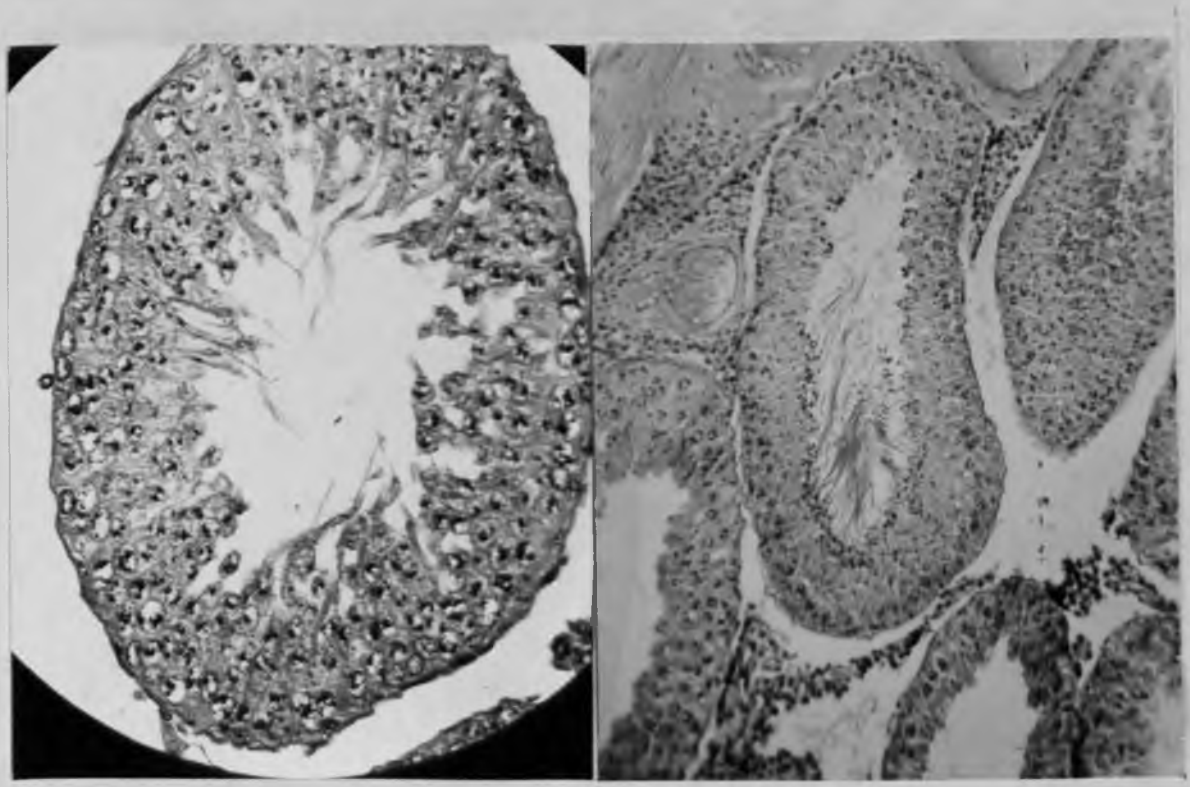


PLATE IV

Fig. 10. Microphotograph of cross-section of one of the tubules in Fig. 9. x 300.

Fig. 11. Microphotograph of section through the testis of dehydrated rat number 94, dehydrated in March. x 300.

of December, January, and February. Here again it was found that disintegration was very irregular in the different tubules. The interstitial material was markedly reduced in volume when compared to the testes studied in December, January, February and March.

Histological Changes in Testes After Dehydration

The testes of the dehydrated rats were studied microscopically in an attempt to determine whether or not water deprivation produced histological changes in the structure of the testis when compared with the testes of the controls. The tissues of the dehydrated animals were compared with the control animals in the following points: the size of the tubules, the thickness of the germinal epithelium, the degree of vacuolization and disintegration of the elements of the testis, the activity of the individual cells of the tubules and the appearance of the interstitial material. It was decided without using quantitative methods that no marked difference in size of the individual tubules occurred in the experimental animals, when compared with the controls, since there were noticeable variations within the testes of a single control. The basement membranes appeared to show the same degree of folding, which was probably due to the method of preparation. No differences could be found in the degree of vacuolization and disintegration between the two groups, since there were varying amounts in the same preparation.

In comparison of the normal and dehydrated rats in respect to the degree of cell activity, no conclusive differences could be observed; this was partly due to the fact that spermatogenesis occurred in "waves," which caused the individual tubules to be at different stage of development. Because of the lack of time and also due to the poor preparation of some of the tissues, it has been impossible to make microscopic quantitative measurements, which may have revealed testicular changes due to dehydration.

Gross Effects of Dehydration on Testes

The average length of dehydration of the experimental rats was 13 days, during which time they were kept in dry air, but were given food, which contained only 6 to 8 per cent available moisture. It was found that approximately 80 per cent of the animals survived for this period and consumed food throughout the experiment. Many appeared at the end of 13 days as though they were able to exist for long periods.

Kudo (1921) observed that one albino rat, which was subjected to total inanition ate about half its tail. This was observed in only one rat of the 40 animals dehydrated in these experiments. Kudo (1921) also reported that some dehydrated rats were observed in which the conjunctivae were congested, which has been observed in approximately three animals in these experiments.

Table III gives in detail the history of each rat dessicated, with the dates of the experiment, the normal weight of the animal, the weight after dehydration, the loss in body weight in grams, the loss in body weight in per cent and the ratio of the dehydrated testes weight to the total dehydrated body weight. It was found that marked variations occurred in the per cent of body weight loss between animals dehydrated at the same time. This is shown in the group dehydrated in April in which animal No. 41 lost 36.6 per cent of its body weight and No. 44 of the same group lost 25.3 per cent of the body weight; both of these animals were dehydrated under identical conditions.

TABLE III

Dehydrated rats, dates of experiment, initial weight of experimental animal in grams, weight after dehydration in grams, loss in weight grams, loss in weight percent, weight of dehydrated testes in grams and the ratio of testes weight to body weight.

Rat No.	Duration of Exp.	Initial Wt. of Exp. Animals grams	Wt. after Dehy. grams	Loss in wt. gms.	Loss in wt. percent	Wt. of Dehy. Testes grams	Ratio of Testis to Body Wt.
63	10/12/31	110.3	85.7	24.6	22.3	0.732	1:117.1
64	to	115.2	86.3	28.9	25.8	0.478	1:180.5
65	10/24/	121.1	92.5	28.6	23.6	0.455	1:203.2
66*		.....	....	....	....	.....	.....
67*		.....	....	....	....	.....	.....
	Av.	115.52	88.16	27.36	27.2	0.555	1:166.96
74	11/11	125.4	93.6	31.8	25.3	0.597	1:156.55
75	to	123.5	93.6	29.9	24.2	0.501	1:186.82
76	11/24	117.6	91.1	26.5	22.5	0.580	1:157.16
77		115.7	92.8	22.9	19.8	0.4855	1:191.23
78		123.9	93.3	30.6	24.7	0.576	1:161.84
	Av.	121.2	92.8	28.3	23.3	0.548	1:170.72
8	12/4/30	110.2	80.3	29.9	27.13	.....	.....
9	to	112.6	82.5	30.1	26.73	.....	.....
10	12/18/	112.2	75.0	27.2	33.1	.....	.....
11		115.3	84.1	31.2	27.32	.....	.....
12*		.....	....	....	.....	.....	.....
	Av.	112.5	80.4	32.1	28.57	.....	.....

* Died during experiment

TABLE III Continued

Rat No.	Duration of Exp.	Initial Wt. of Exp. Animals grams	Wt. after Dehy. grams	Loss in Wt. gms.	Loss in Wt. per-cent	Wt. of Dehy. Testes grams	Ratio of Testes Body Wt.
24	1/31/31	105.3	81.7	23.6	22.41	.....	.....
25	to	107.6	88.4	19.2	19.70	.....	.....
26	2/12/	106.6	80.3	26.3	24.67	.....	.....
27		104.6	73.4	31.2	29.9	.....	.....
28		107.0	71.8	35.2	32.9	.....	.....
	Av.	106.2	79.1	27.1	25.91	.....	.....
29*	2/25/	.....	....	....	.....	.....	.....
30	to	108.5	71.4	37.1	34.1	.....	.....
31	3/10/	116.2	92.9	23.3	20.0	.....	.....
32		93.2	64.4	28.8	30.9	.....	.....
33		119.2	98.8	20.4	17.1	.....	.....
	Av.	109.27	81.8	27.4	25.52	.....	.....
93	3/21/32	131.2	97.0	34.2	26.0	0.737	1:131.6
94	to	122.1	92.1	30.0	24.57	0.502**	1:183.4
95	4/5/	113.1	76.9	36.2	32.01	0.465	1:165.3
96		135.2	92.9	42.3	31.21	0.618	1:150.32
97		114.1	97.9	26.2	22.09	0.569	1:149.2
	Av.	123.1	89.3	33.7	27.1	0.644	1:155.9
40*	4/28/31	.....	....	....	....	.....	.....
41	to	136.4	99.8	36.6	26.8	0.6012	1:166.0
42	5/11	110.7	81.8	28.9	26.1	0.3650±	1:224.1
43		125.8	96.0	29.8	23.7	0.7016	1:136.8
44		102.4	77.1	25.3	24.7	0.4098	1:188.1
	Av.	118.9	88.6	30.1	25.3	0.5194	1:163.6

* Died during experiment
** Left testis cryptorchid
± Right testis cryptorchid

TABLE III Continued

Rat No.	Duration of Exp.	Initial Wt. of Exp. Animals grams	Wt. after Dehy. grams	Loss in Wt. gms.	Loss in wt. per-cent	Wt. of Dehy. Testes grams	Ratio of Testes to Body Wt.
49	5/12/31	114.6	84.8	30.0	26.1	0.600	1:141.0
50	to	108.1	81.9	26.2	24.2	0.569	1:143.9
51	5/25/	126.3	101.6	24.7	19.5	0.432	1:234.7
52*		.....	.....	....	....	.....	.....
53		100.3	77.7	22.6	22.5	0.336	1:230.7
	Av.	112.3	86.45	25.87	23.1	0.568	1:187.5

* Died during experiment

Table IV is a comparison of the data on the dehydrated and control rats. Five groups of dehydrated rats are included with the respective control rats for each group; three groups of dehydrated animals were not included, because in the early experiments the testes were not weighed and therefore the loss in testicular tissue due to acute dehydration could not be determined. The average individual weight of the control animals was 110.7 grams, while the average initial weight of the dehydrated animals was 118.21 grams. Therefore the average rat which was subjected to dehydration was 7.51 grams heavier at the beginning of the experiment than the average control animal. This was not the result of selection, but purely the result of chance in trapping. The average weight of the individual rats comprising the five groups was 89.09 grams at the end of dehydration, which was a loss of 26.26 per cent of the body weight. Kudo (1921) observed that albino rats in normal air which were given food ad libitum and 5 grams of milk per day weakened rapidly and survived only approximately 10 days, during which time some difficulty was encountered in the refusal of the animal to eat. It was found in these experiments on the kangaroo rat, in which the animals were constantly subjected to dry air and were only given the water which occurred in the air-dried food, 6 to 8 per cent, that approximately 80 per cent were able to withstand dehydration for 13 days without reaching the vital limit. Very little trouble was encountered with the dehydrated animals due to refusal of food.

This marked difference between the albino rat and the kangaroo rat clearly indicates that the kangaroo rat is so physiologically adapted that it is able to conserve the water in its tissues during periods of extreme water deprivation.

The average weight of the testes of the controls was 0.6177 grams and the average weight of the testes of the dehydrated animals was 0.5379 grams, which is a difference of 12.92 per cent. Because of the number of animals used in these experiments the error due to individual variations in testes weight is materially reduced, and the difference in weights of the testes of the dehydrated and control animals is undoubtedly significant. Kudo (1921) found in the albino rats which were subjected to varying degrees of acute thirst for periods from 6 to 16 days, that the body loss was 36.1 per cent and the testes loss was 15.1 per cent. The results of the present experiments show that in the kangaroo rat, acute dehydration also resulted in a loss in testis weight which is approximately half the loss in body weight. Kudo (1921) also reported that two albino rats fed on a diet of maize, without water or milk, lost approximately 36.7 per cent of the body weight in 6-1/2 days, during which time the animals were kept in normal air. These experiments clearly show that the kangaroo rat is able to withstand dehydration approximately twice as long as the laboratory white rat, with a percentage body weight loss that is noticeably less, regardless of the fact that the kangaroo rats were subjected to much more severe dehydration than the albino rats.

TABLE IV

TABLE IV

Comparison of averages of control weights with the weights of the dehydrated rats showing gross seasonal changes resulting from dehydration

Rat No.	Date	Normal Wt.* of Control Rats	Normal Wt. of Exper. Rats	Wt. of Rats after Dehy.	Loss in Weight from Dehy.	Per cent Loss in Body Wt. Dehy. Rats	Testes Wt. of Normal Rats	Testes Wt. of Dehy. Rats	Per cent Diff. in Wt. of Control and Dehy. Testes	Ratio of Testes Wt. to Body Wt. Controls	Ratio of Testes Wt. to Body Wt. Exper. Animals
40-44 Exper.	April May		118.9	88.6	30.1	25.03		0.5194			1:178.77
45-48 Controls	April May	108.70					0.6112		6.61	1:183.45	
49-53 Exper.	May		112.3	96.45	25.87	23.14		0.4848			1:187.57
54-57 Controls	May	105.03					0.6627		24.87	1:160.1	
63-67 Exper.	Oct.		115.52	88.16	27.36	27.2		0.555			1:166.96
58-62 Controls	Sept.*	112.4					0.5482		-1.02	1:219.36	
74-78 Exper.	Nov.		121.2	92.88	28.34	28.77		0.5482			1:170.72
68-72 Controls	Nov.	114.52					0.6221		17.65	1:190.64	
93-97 Exper.	March April		123.14	89.36	33.79	27.18		0.5822			1:155.98
88-92 Control	March April	112.86					0.6444		9.65	1:176.06	
AVERAGE		110.70	118.21	89.09	29.09	26.26	0.6177	0.5379	12.92	1:185.82	1:172.0

* All weights in grams
‡ Last part of the month

DISCUSSION

Marshall (1911) noted that most wild animals had a definite breeding season, whereas related domestic animals had a season that was much longer and in many cases no periods of sexual inactivity were present. This difference in the breeding seasons is found in the difference between the kangaroo and the domestic albino rat. The kangaroo rat has not been used for a laboratory animal, but Vorhies and Taylor (1922) estimated that the breeding season extended from January to August, inclusive. The albino rat has been thoroughly studied in the laboratory and it is well known that no periods of sexual inactivity occur in the males.

In this study of the kangaroo rat the presence of a definite seasonal spermatogenic cycle has been demonstrated. The height of the seasonal activity was found to occur during the months of December to March, inclusive, and it has been found that the spermatids were so numerous in some tubules that it formed a "ring" around the edge of the lumen. The period of least activity was observed in the testes collected in May. Since no studies were made during the summer months, it is possible that the period of least activity may be between May and September. This appears to be possible since more activity was observed in the tubules in September than in May. Vorhies and Taylor (1922) trapped 45 females in the month of June and 21 of these were "breeding;" this field study shows

that the animals were sexually active during this summer month, but no records are available for the other summer months.

A definite increase in testicular weight occurred in the kangaroo rat from September to February, which was followed by a decrease in April through May. Such seasonal changes have been noted by: Marshall (1911) in the study of the hedgehog, Tandler and Grosz (1911) in the mole, and Rasmussen (1917) in the woodchuck. These studies in the kangaroo rat showed that the changes in testicular weights were closely correlated with the periods of greatest sexual activity. Because the relative volumes of the interstitial material were not measured in this study on the kangaroo rat, the exact monthly differences are not known. Marshall (1911) in the study of the hedgehog, found the same relationship between sexual activity and the volume of interstitial material estimated in this study. Tandler and Grosz (1911) found in the testis of the mole that during the maximum spermatogenic activity, the volume of the interstitial material was at the minimum and immediately after breeding, when the sperms had disappeared from the tubules, the interstitial material increased and reached its maximum during the period of least sexual activity.

No noticeable changes in the size of the tubules were observed in the kangaroo rat during the different seasons of the year. A difference in the size of the tubules of the testis has been reported by Bissonnette (1930) in the work on the European starling. In these studies on the kangaroo

rat it was found that the individual tubules of the same section of the testis varied markedly in area and it would therefore have been difficult to determine any seasonal changes in the size of the tubules without the use of quantitative methods.

Spermatogenesis was found to occur in waves along the tubules in the kangaroo rat which has also been noted by Allen (1918) in the albino rat. Because of this it has been difficult to determine the relative amount of activity in one section as compared to another.

Dehydration of the kangaroo rats produced an average testis weight loss of 12.92 per cent when the dehydrated testes were compared to the controls of that period. During the same period of dehydration the animals lost 26.62 per cent of their body weight. It is possible that the loss of 12.92 per cent in testis weight was not sufficient to produce histological changes which would be observed by the methods of study used in these experiments. Carefeanu and Derevici (1924) have shown that acute water deprivation in dogs produced lesions in some organs, but no mention was made of the effect of dehydration on the testis. In this study no observable lesions were found in the testes of the kangaroo rat, but these may have occurred, if the dehydration had been less severe and had extended over a longer period. Kudo (1921) has shown that chronic dehydration which extended over periods of 47 to 55 days in albino rats resulted in the testes becoming softer and somewhat atrophic in appearance. Kudo (1921)

has also shown that the average loss as a result of acute dehydration in the albino rat was 36 per cent, and the testis loss was 15.1 per cent; also that chronic dehydration caused a loss of 52.4 per cent in body weight and a testis loss of 59.9 per cent. The loss in testicular weight in the kangaroo rats in acute dehydration was approximately half the body weight loss, which is approximately the same ratio that Kudo (1921) found in the rats. This similarity between the results obtained by Kudo (1921) and the results obtained in these experiments is marked, which indicates the possibility that a greater decrease in weight of the testes may have occurred if the water loss had been more gradual and extended over a much longer period. This would approximate the conditions that might occur in the arid and semi-arid regions during periods of unusual drought.

Jackson and Smith (1931) have shown that albino rats put on a restricted water intake from 25 to 47 days old and given food ad libitum, could be kept at a constant weight by regulating the water intake. They also observed that the testes were approximately normal in weight. This distinctly shows that albino rats are able to maintain nearly normal testis weight, even though the body weight is below normal, because of water deficiency. Jackson and Smith did not state whether mature sperms were found in the seminiferous tubules or whether the accessory glands, the prostate and seminal vesicles, were normal. Therefore, if chronic

dehydration as reported by Kudo (1921) causes a greater amount of weight loss in the animal, than does acute dehydration, and results in a marked decrease in the weight of the testes, it is possible that it may affect the prostate and seminal vesicles to the extent that sterility may result, even though the testes are apparently normal, as has been reported by Jackson and Smith (1931). Marshall (1911) reports that Walker (Johns Hopkins Hos. Report XVI, 1911) found in albino rats that removal of the prostate gland together with the seminal vesicles did not inhibit sexual activity in the rat but caused sterility. The removal of either the prostate or seminal vesicles did not produce sterility. In the kangaroo rats which were subjected to acute dehydration in these experiments, the accessory glands may have been so affected by water deprivation that sterility resulted.

SUMMARY AND CONCLUSIONS

1. These studies have demonstrated the presence of a well-defined seasonal cycle in the activity of the testis of the kangaroo rat. The most activity was found in the winter months, from January to March, inclusive. The minimum activity observed occurred in May, but the actual minimum may occur during the summer months, for which period no studies were made.

2. There was a definite increase in the weight of the testis from September to February, which was followed by a decrease from March to May, inclusive. The changes in weight were closely correlated with the histological findings.

3. The interstitial material of the testis apparently reaches its maximum during December, January, and February, which corresponds generally with the height of the spermatogenic activity. No quantitative methods were used to determine the relative volumes of interstitial material for the different months, so that exact differences can not be given.

4. Spermatogenesis was found to occur in "waves" along the seminiferous tubules in the testes of the kangaroo rat. This agrees with the report of others on the albino rat.

5. No histological changes were observed in the testes of acutely dehydrated rats, when they were compared to the normal testes of the same period. However, it has been emphasized that the variations in different tubules of the same testis, as well as variations in the testes of different animals trapped on the same date, make such a study extremely difficult unless quantitative methods are used.

6. Kangaroo rats have been dehydrated in dry air, without acute starvation, on a diet containing 6 to 8 per cent available moisture for approximately 13 days without reaching the average vital limit. Such dehydration resulted in an average body weight loss of 26.26 per cent and an average loss in weight of testes of 12.92 per cent, which indicates a physiological protection of germinal tissues against water losses.

It has been clearly shown that the kangaroo rat is able to withstand dehydration approximately twice as long as the laboratory white rat, with a percentage body weight loss that is noticeably less, regardless of the fact that the kangaroo rats were subjected to much more severe dehydration than the albino rats.

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