The Thermo-Regulatory Function and Mechanism of the Scrotum

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ABSTRACT

The literature concerned with scrotal function is reviewed, and further studies of this function and the mechanism by which it is accomplished are reported. In the experimental work 18 rats, 12 guinea pigs and 21 rams were used.

Scrotal temperature in the ram was found to be approximately 6.5°C lower than body temperature, while testicular temperature was approximately 4.9°C below body temperature. Scrotal insulation reduced these differences approximately 3.0°C and 2.0°C respectively. Testes removed after 4 days to 16 weeks of insulation indicated progressive degeneration. Partner testes, allowed a 3-weeks recovery period before excision, showed some recovery in cases where insulation period was 2 weeks or more. Scrotal insulation also resulted in a marked increase in the proportion of abnormal spermatozoa ejaculated, followed by an almost aspermatic condition after approximately 3 weeks of insulation. The time required for passage of spermatozoa from testes to the ejaculatory duct ranged from 4 to 13 days (average 8.8 days) in rams in active service.

The effect of abdominal temperatures upon the morphology of fully formed guinea-pig spermatozoa was studied. No increase in rate of degeneration was noted due to abdominal temperature.

Rat testes were subjected to varying degrees of lower than normal temperatures with no notable damage resulting, indicating that testes are less sensitive to lower than to higher than normal temperatures.

Studies of the tunica dartos muscle, both with the scrotum intact and in isolated strips, indicate that this muscle functions very much as a thermostat, relaxing with a temperature increase and contracting with a temperature decrease, being especially sensitive at temperature near the normal testicular temperature, and thus functioning to maintain the testes at a fairly constant temperature. The tunica dartos was found to go into heat rigor at 59.25°C. The effects of various drugs on this muscle are also reported.

Observations on two rams, temporarily of lowered fertility while in high condition (fat) and with a heavy covering of wool, followed by a return to normal fertility after removal of the fleece and some lowering of condition are reported.

The Thermo-Regulatory Function and Mechanism of the Scrotum

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INTRODUCTION

During the past decade the problem of scrotal function has been studied quite extensively by a few workers and their results indicate that the scrotum is a thermo-regulator for the testes, maintaining them at a temperature somewhat below that of the abdominal cavity. The findings which led to this conclusion have been based largely on histological studies of cryptorchid testes and testes that had been subjected to temperatures higher than that in the scrotum. A brief review of the literature pertaining to this function of the scrotum follows.

REVIEW OF LITERATURE

Cryptorchidism is a rather commonly observed phenomenon and the aspermatic condition and lack of normal testis development both micro—and macroscopically has been known for a number of years. Griffiths (1893) found that the testes of the dog, in full spermatogenetic activity, would degenerate in a few months if replaced in the abdominal cavity. Crew (1922) first suggested that the lack of development of cryptorchid testes might be accounted for on the assumption that the abdominal temperature is not that at which the final stages of spermatogenesis occur.

Moore (1922) produced crytorchidism experimentally by re-placing testes of mature rats and guinea-pigs in the abdominal cavity. After three or four months testes treated in this manner were found to be histologically typical of undescended testes, the germinal epithelium being absent, and only a single layer of cells (considered Sertoli cells) persisting around the base of the semi-niferous tubules. Further studies revealed that degeneration of the epithelium was evident seven days after elevation of the testis to the abdominal cavity. In fourteen days the degeneration was well advanced and after twenty or thirty days the germinal epi-thelium was usually absent.

Experimentally cryptorchid rams have given results in line with those described above in the rat, guinea-pig and dog. Moore (1924) found that after a ram's testis had been returned to the abdominal cavity for sixty-five days it had the characteristics of a naturally cryptorchid testis, all cells of the germinal epithelium being absent except a single layer next to the basement membrane.

In addition to histological changes in the experimentally cryptorchid testis there is a decrease in size. Moore (1924) found an appreciable decrease in the size of the guinea-pig testis after having been elevated to the abdomen for thirty days or less, and this diminution in volume was found to continue with longer retention so that after one year of abdominal retention the testis was only about one-fifth its normal size in volume. In the ram Moore (1924) noted a decrease in testicular volume of about one-third after the testis had been returned to the abdominal cavity for sixty-five days.

Obviously the germinal epithelium undergoes degeneration when the testis is elevated to the abdominal cavity. Would such degeneration result if the testis were elevated to the abdominal cavity and yet maintained at a temperature comparable to that of the scrotum? Using rabbits and dogs Fukui (1923), and employing a cooling apparatus on the exterior, proximal to inguinal and abdominal testes experimentally retained, found that by thus keeping the temperature down a testis could be maintained in a nearly normal condition while its mate, also experimentally retained, showed marked degeneration.

Griffiths (1893) found that if the testes of the dog were replaced in the abdominal cavity before completion of sexual differentiation they did not reach the stage of spermatozoon formation, and were but slightly different from purely embryonic testes. Moore (1924) noted a similar condition in the guinea-pig, when he found that degeneration was not so marked in testes when they were elevated to the abdominal cavity before full spermatogenic activity was attained as when elevated after this time. Arrest in development was found to have taken place, and, in general, the earlier after birth elevation of the testis to the abdominal cavity was carried out the less the visible degeneration.

The problem of scrotal function has also been studied by noting the effect of direct application of heat to the scrotum and to the testis upon testis histology. Fukui (1923) exposed rabbit testes to increased temperatures by four methods, namely; (a)

Exposure of the scrotum to sunlight, the exposure being made on clear days in midsummer, (Japan) from 1:00 to 3:00 P. M. (temperature readings not indicated), (b) Warm water bathing of the scrotum at temperatures of 44° to 49° C., (c) Warm air bathing of the "waist part" at temperatures of 40° to 44° C., and (d) Exposure of the scrotum to an arc light. From these experiments the relation between temperature and the minimal time required to produce testicular damage is reported as follows: at 48° C. twenty minutes, at 45° C. half an hour, at 46° C. one hour, at 45° C. two and one-half hours, at 44° C. forty-five hours and at 41° C. about one hundred hours. The body temperature of the rabbit is about 40° C. Fukui also notes having observed cases of what he terms "heat testicles" in dogs, cats, guinea-pigs, goats and one case in a human. Moore (1924) carried on similar experiments with guinea-pigs using hot water, electric light and electric stove as heat sources, and obtained comparable results. Moore (1924) also studied the effect of suspending the testes themselves in warm saline for a time after which they were replaced in the scrotum. Submerging the testes for five minutes in saline at 44° C. resulted in perceptible degeneration and being held in saline at 47° C. for that length of time resulted in marked degeneration of all tubules.

Scrotal insulation provides a favorable method of studying the function of the scrotum since by this means it is possible to increase the scrotal temperature without in any way disturbing the normal connections or coverings of the testes. Moore and Oslund (1924) insulated the scrotum of a ram for eighty days and upon examination of the testes found varying degrees of degeneration in the seminiferous tubules. Some showed a germinal epithelium of normal thickness but with no spermatozoon, in others the variety of the epithelial cells was abnormal, and in others the structure was similar to seminiferous tubules found in cryptorchid testes.

The survival of testicular grafts also sheds some light on the problem of scrotal function. Moore (1923) (1926) made subcutaneous, intramuscular and intraperitoneal grafts of testicular tissue and obtained active mitosis in the germinal epithelium but a normal epithelium with spermatids or spermatozoa was not obtained. However, when such grafts were made on the walls of the scrotum normal seminiferous tubules with characteristic spermatozoa were found after six months.

The recovery of experimentally cryptorchid testes to normal, if the testes were returned to the scrotum after a considerable amount of degeneration had taken place, was noted by Moore (1922). Continuing this study Moore (1926) found that guinea-pig testes regained normal function when replaced in the scrotum after having been held in the abdominal cavity for five months. Spermatozoa were produced within ninety days after scrotal replacement.

The length of time over which degeneration continues in the guinea-pig testes after a brief exposure to high temperatures (46° to 47° C. for 15 to 30 minutes) is about twelve days, according to Young (1927). At the end of this time complete evacuation of the germinal epithelium has taken place except for some spermatogonia and Sertoli cells. The first reconstructive changes become apparent about the time of complete evacuation and reconstruction may be complete and spermatozoa be formed in some tubules by the forty-fifth day following treatment. Regeneration is practically complete about six months after treatment.

The actual difference which exists between the temperature of the abdominal cavity and that of the scrotum has been determined in a few species by Moore and Quick (1924). In rabbits, with an abdominal cavity temperature of about 39.5° C. the scrotal temperature was found to be 1.5° to 2.5° lower. In guinea-pigs a difference of from 2.0° to 3.5° was noted, and in rats the difference found was from 4.0° to 8.0° C. Measurements were made at warm and cool room temperatures and the scrotal temperature seemed to vary directly with the room temperature.

The contributions which are described briefly above indicate clearly that the testes function normally only in the scrotum or when maintained elsewhere at a temperature comparable to that of the scrotum, and that when any marked increase over normal testicular temperature is brought about, whether it be by experimental crytorchidism, scrotal insulation or direct application of heat, spermatogenetic function is impaired or lost. When the testis, so treated, is returned to its normal temperature spermatogenetic function is regained. Thus the scrotum is found to function as a thermo-regulator for the testes.

The heat resistance of cells in various stages of spermatogenesis is of considerable interest. Young (1929) points out that actively dividing primary and secondary spermatocytes and younger spermatids are the first elements to be affected by a

temperature increase, the older spermatids are next to be affected, and subsequently there is degeneration of the spermatozoa, those in the epididymis being more heat-resistant than those in the testes. The spermatogonia are affected less by a temperature increase than the remainder of the germinal epithelium. Thus there seems to be a considerable lowering of heat resistance in the germinal elements during transformation, the heat resistance being regained to some degree as the spermatozoa become completely formed and "ripened" in the epididymis.

The evidence for the increased heat resistance of spermatozoa as they pass from the testis and through the epididymis (mentioned in the preceding paragraph) is based on the duration of motility of spermatozoa from various regions of the male genital tract after having been subjected to higher temperatures. Young (1929).

Heller (1929) gives some further evidence that the thermoregulatory function of the scrotum extends to the fully formed spermatozoa as well as the germinal epithelium. This investigator found that sperm from the isolated epididymis of the guinea-pig were capable of being activated in saline for about twenty-three days if the epididymis remained in the scrotum, but could not be activated after thirteen or fourteen days if the epididymis were held in the abdominal cavity. Similar studies with rats revealed that spermatozoa from the isolated epididymis remaining in the scrotum were capable of being activated for eighteen days, but could not be activated after five days if the epididymis was held in the abdominal cavity.

Since an increased temperature does affect the motility of spermatozoa, the question arises as to whether there is also an effect on the morphology. In semen secured on alternate days from rams with insulated scroti Phillips (1931) found a marked increase in the proportion of abnormal spermatozoa within eight days after insulation was applied. In about three weeks spermatozoa were almost completely absent from the semen. Whether these abnormalities arose from derangement of the spermatogenetic process or from changes in the fully formed spermatozoa was not determined.

No one has described any decrease in sexual desire or change in secondary sex characters in association with testicular degeneration due to increased temperatures, and cryptorchid animals are commonly known to possess normal secondary sex characters and sexual desire.

Knowing that the testes are maintained by the scrotum at a temperature below that of the abdominal cavity and that an increase in temperature results in degeneration of the epithelium and some damage to the fully formed spermatozoa, the problem of the manner in which higher temperatures effect these changes arises. Two possibilities have been presented. Fukui (1923) believes that the degeneration is due to heat lability of testicular proteins at body temperatures or above. On the other hand Moore (1924) suggests that such degeneration may be due to a lack of oxygen and accumulation of carbon dioxide due to vascular stagnation. Heat applied to the scrotum, or directly to the testes produces, as in other parts of the body, a hyperaemic condition associated usually with edema.

Barron (1933) found that Faradization of the external spermatic nerve in rats is followed by a decrease of 0.5° to 1.0° C. in testicular temperature. Section of this nerve in the abdomen is followed by a corresponding increase in testicular temperature. After section of the external spermatic nerve the testes were found to undergo degeneration similar to that produced by heat, inflammation or X-ray. The temperature increase never was observed to persist more than twenty days but the degenerative process continued much longer. Barron believes that this degeneration is due to hyperaemia resulting from vaso-dilation, rather than to the small increase in temperature, that results from section of the external spermatic nerve.

Since hyperaemia results from application of heat to the testicle and is accompanied by degeneration of the germinal epithelium, and in the work by Barron mentioned in the preceding paragraph hyperaemia accompanied by only a slight temperature rise resulted in a similar degeneration, Barron's work would seem to substantiate the view that testicular degeneration at higher temperatures might be due to hyperaemia rather than to lability of testicular proteins at the higher temperatures.

Since the scrotum does maintain the testes at a temperature below that of the abdominal cavity, the mechanism by which this is accomplished becomes of interest. Very little material is available on scrotal physiology from this standpoint. Lieben (1908) reports some findings in his work with the tunica dartos of humans and dogs. The dartos was found to contract when stimulated directly or when many far-removed spots on the body were stimulated. Among the various stimulants studied were applications

of warm or cold water or ether, all of which resulted in contraction of the dartos. The scrotum was found to be relaxed at a uniform room temperature. The dartos was found to be innervated by the sympathetic nervous system in such a manner that each scrotal half receives its nerves from the abdominal cord on the same side. Irritation of the sympathetics and the ramus communicas from the first two sacral segments resulted in contraction of the dartos, but there were no contractions when nerves from other segments were irritated.

Crew (1922) noted that the condition of the scrotum varied with different conditions of atmospheric temperature and suggested that these changes were controlled by the tunica dartos. In considering the problem of scrotal function several questions present themselves. What are the actual differences between scrotal and abdominal cavity temperatures in larger animals? Are the regressive and recovery changes in the testes of larger animals, particularly farm livestock, resulting from heat damage similar to those noted in laboratory animals? How small a temperature increase is necessary to effect such regressive changes in the testis? What is the effect of increased temperature upon the morphology of the spermatozoa? Since higher than normal temperatures result in testicular damage, what would be the effect of lower than normal temperatures? What is the physiology of the thermo-regulating mechanism of the scrotum? What practical application has the study of the problem of scrotal function?

Experiments designed to help answer these questions have been carried out and the findings are reported in this paper.

EXPERIMENTAL WORK

As is indicated in the Introduction results of the studies of several problems are reported in this paper. The data and discussion concerning each problem are reported separately; consequently this section is divided into seven parts.

I. **Comparative Temperatures of the Body, Scrotum and Testes of Rams, and the Effect of Insulation upon Scrotal Temperature.**

Material.—The data herein presented have been collected as an incidental part of the work concerning the effect of insulation upon the testes and upon the ejaculated spermatozoa of rams, and the study of the temperature-regulating mechanism of the scrotum.

Body temperatures were obtained per rectum. Scrotal temperature measurements were obtained by inserting a thermometer through a small incision in the scrotal wall. To obtain testicular temperatures an incision was made through the tunica albuginea and a thermometer inserted directly into the middle of the testis. All temperature measurements were made with a very sensitive thermometer, calibrated to one-tenth of a degree Centigrade, and read with a reading glass.
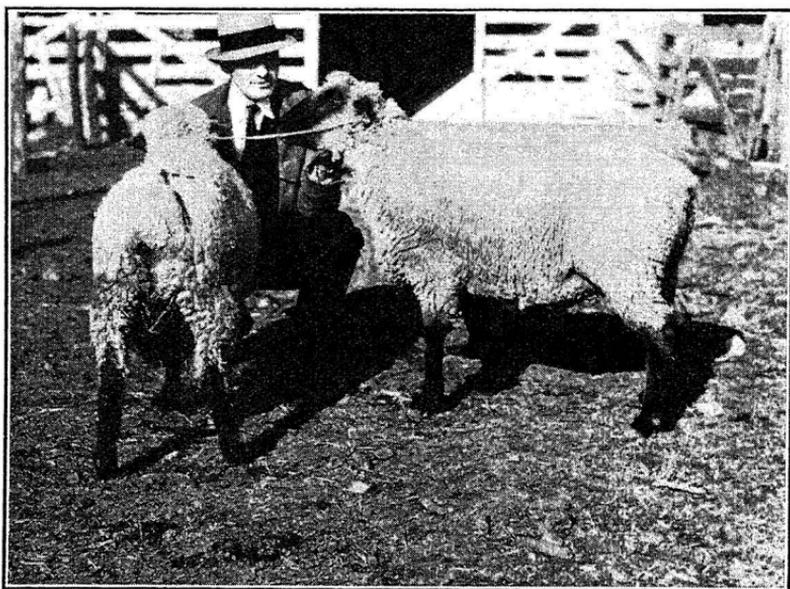
Fig. 1.—Showing the method of scrotal insulation and the type of rams used in the work described in this paper.

The method of scrotal insulation is pictured in Figure 1. The insulation consisted of a sack fashioned to fit the scrotum and made of two layers of blue denim and two layers of cotton batting held in place by straps over the animal's back. The type of rams used may also be noted in Figure 1. They were all purebred Hampshire rams, and were yearlings with one exception, this being a ram approximately four years old (ram K).

Presentation of Results.—Temperature readings of the body, scrotum and testis were obtained from three rams and are shown in Table 1.

TABLE 1.—COMPARATIVE BODY, SCROTAL AND TESTIS TEMPERATURES IN RAMS. (CENTIGRADE)

Ram	Rectal Temp.	Scrotal Temp.	Testicular Temp.	Room Temp.
A	39.0	33.8	34.4	14.0
B	39.8	34.0	34.8	18.8
C	40.1	34.9	35.6	14.4

Scrotal temperature readings from three rams at widely varied room temperatures are shown in Table 2. These readings were made while the rams were under anesthesia, Nembutal (sodium ethyl methyl butyl barbiturate) being the anesthetic used. Room temperatures were changed without disturbing the rams and the length of time after changing the room temperature until the next reading was made is indicated in Table 2.

TABLE 2.—SCROTAL TEMPERATURES OF RAMS AT WIDELY VARIED ROOM TEMPERATURES (CENTIGRADE)

Ram	Room Temp.	Time Since Room Temp. Was Changed	Scrotal Temp.
A	24.2	Several hours	34.0
	−2.0	20 minutes	30.6
	−3.0	30 minutes	27.0
B	21.0	Several hours	33.6
	13.0	45 minutes	33.3
	4.5	45 minutes	31.6
	24.0	60 minutes	33.6
C	24.3	Several hours	32.5
	14.5	20 minutes	31.2

The effect of insulation upon scrotal and testis temperatures is shown in Table 3. Temperature changes in these organs upon removal of the insulation are also presented in Table 3.

TABLE 3.—EFFECT OF INSULATION UPON SCROTAL AND TESTIS TEMPERATURES, AND TEMPERATURE CHANGES AFTER REMOVAL OF INSULATION (CENTIGRADE)

	Ram A	Ram B	Ram C
Body temperature	40.6°	39.5°	40.0°
Time of insulation previous to readings	4 days	1 week	2 weeks
Temp. inside insulation	29.0°	30.0°	32.6°
Room temperature	12.0°	18.0°	20.4°
Scrotal temperatures			
With insulation on	36.4°	36.5°	36.4°
5 minutes after removal	36.0°	35.5°	35.2°
10 minutes after removal	34.8°	33.5°	35.2°
15 minutes after removal	34.6°	33.5°	35.0°
20 minutes after removal	34.2°	32.0°	34.6°
30 minutes after removal	33.8°		32.8°
35 minutes after removal			32.8°
Testis temperatures			
With insulation on	37.0°		37.0°
35 minutes after removal	33.8°		34.0°

The number of animals from which the figures in each of the above tables (Tables 1, 2 and 3) were obtained was necessarily small and is not presented as exactly what would be found in all animals, but they represent the conditions in the rams studied and seem sufficiently clear-cut to indicate what might be found in other rams. The important points in Tables 1, 2 and 3 concerning comparative temperatures of the body, scrotum and testes of the ram are summarized in Table 4.

TABLE 4.—SUMMARY OF COMPARATIVE BODY, SCROTAL AND TESTIS
TEMPERATURES IN RAMS. (CENTIGRADE)

	Conditions	Number of Readings	Range	Average
Body	(per rectum)	6	39.0-40.6	39.83
Scrotum	Room Temp. 14.0-18.8°	3	33.8-34.8	34.20
Scrotum	Under anesthesia Room Temp. 13-24.3°	6	31.2-34.0	33.03
Scrotum	Under anesthesia Room Temp. −2.0°-4.5°	3	27.0-30.6	29.73
Testes	Room Temp. 14.0-18.8°	3	34.4-35.6	34.93
Scrotum	Insulated	3	36.4-36.5	36.43
Testes	Insulated	2	37.0-37.0	37.00
Scrotum	20-30 mins. after removal of insulation. Room Temp. 12.0-20.4	3	32.0-33.8	32.86
Testes	20-30 mins. after removal of insulation. Room Temp. 12.0-20.4	2	33.8-34.0	33.90

The average rectal temperature noted was 39.83° C. Ritzman and Benedict (1931) report 39.9° C. as the rectal temperature in yearling sheep and 39.2° C. in adult sheep.

It may be noted that the scrotal temperatures observed averaged 34.2° C., or 5.6° C. lower than the rectal temperature. The temperature in the testes was found to be 34.9° C. or 4.9° C. lower than the rectal temperature.

The scrotal temperature readings obtained on rams under anesthesia were slightly lower than temperature readings on normal rams. Two reasons, either or both of which may account for this difference, are (1) that the anesthetic had a slightly depressing effect on body temperature and (2) that the rams were so suspended when the temperatures were taken that the scrotum was more exposed than usual. At low room temperatures (-2.0° C. to 4.5° C.) scrotal temperature was found to be 29.7° C.

The temperature within insulated scrota was found to be 36.4° C. or 2.2° C. above normal. The temperature of testes held in insulated scrota was 37.0° C. or 2.1° above normal. (The scrotal temperature at room temperatures of 14.0-18.0° C. was considered as normal.)

It is interesting to note that upon the removal of scrotal insulation, both testicular and scrotal temperatures dropped below normal. Apparently the scrotum was unable to adjust itself quickly after having been subjected to insulation for a time.

II. The Effect of Scrotal Insulation on the Testes of the Ram.

The object of the experiment herein described was to determine the effect of scrotal insulation upon the testes of the ram, to trace the progress of any degenerative changes, and to note the possibilities of regeneration of testes so damaged.

Material.—Six Hampshire rams were subjected to scrotal insulation. At varying times after applying insulation, same was removed and one testis excised and blocks removed and fixed in Allen's* modification of Bouin's fluid. Three weeks later the second testis was excised, and blocks removed and fixed. Thus blocks of testicular tissue were available for study following the various treatments indicated in Table 5. Two rams died shortly after excision of the first testis.

TABLE 5.—TREATMENT OF TESTES BEFORE BEING EXERCISED

Ram No.	Period of insulation before removal of insulation and excision of first testis	Time from removal of insulation to excision of second testis
7	4 days	
14	1 week	3 weeks
15	2 weeks	3 weeks
13	4 weeks	3 weeks
9	8 weeks	3 weeks
17	16 weeks	

All blocks of tissue were placed in the fixing solution within one minute after excision. The tissues were dehydrated, embedded in paraffin, and sectioned (8-10 micra). Mayer's hemalum and Orange G were used in routine staining.

In discussing the physiology of the male reproductive system Oslund (1928) indicates that all maturing germ cells do not become spermatozoa as some degenerate before reaching definite form. Spermatocytes and spermatids in various stages of degeneration may be seen in the lumina of seminiferous tubules of normal testes.

*Formula for Allen's fluid:

Picric acid (Saturated aqueous solution)	75 parts
Formalin, c.p.	15 parts
Glacial acetic acid	10 parts
Urea crystals	1 part

Presentation of Results.—A description of the condition of testes removed from rams at the end of various insulation periods follows:

Ram 7. At the end of four days of scrotal insulation the testis presented an orderly appearance, the cells of the germinal epithelium being in their normal position and the tubules showing little shrinkage. There were evidences of degeneration in most tubules however. The lumina of some tubules were empty but most of them contained material consisting of spermatids and secondary spermatocytes, a few primary spermatocytes and some spermatozoa. Most tubules had many spermatozoa attached to the epithelium but in a few spermatozoa were neither attached to the epithelium nor in the lumina. The spermatids were apparently normal in some tubules but in many others these cells showed evidences of degeneration and many of them were breaking away from the epithelium. The secondary spermatocytes showed areas of degeneration in some tubules, with several of these cells breaking away from the epithelium. The primary spermatocytes and spermatogonia appeared normal except for a few areas of degeneration. (See Figure 2.)

Ram 14. After one week of scrotal insulation the tubules of the testis had shrunken considerably and the epithelium was tending to become disorganized. Many dark-staining nuclei appeared in all regions of the epithelium. The lumina contained debris and some cells, mostly spermatozoa, spermatids and primary spermatocytes. Spermatozoa were absent in some tubules, many were still attached to the epithelium in others and quite a few were found in the lumina. Of the spermatozoa found in the lumina 150 were observed and of this number 39 appeared normal, 96 were tailless, 3 had coiled tails and 12 still had the middle piece bead attached. The spermatids were showing considerable degeneration and breaking away from the epithelium singly and in groups in many tubules while in others the cell outlines were not clear. Many secondary spermatocytes were also breaking away from the epithelium and much degeneration was evident. The primary spermatocytes and spermatogonia appeared normal in some tubules but there were many areas of degeneration in this region in other tubules. (See Figure 3.)

Ram 15. After two weeks of scrotal insulation, the germinal epithelium presented a generally disorganized appearance and the
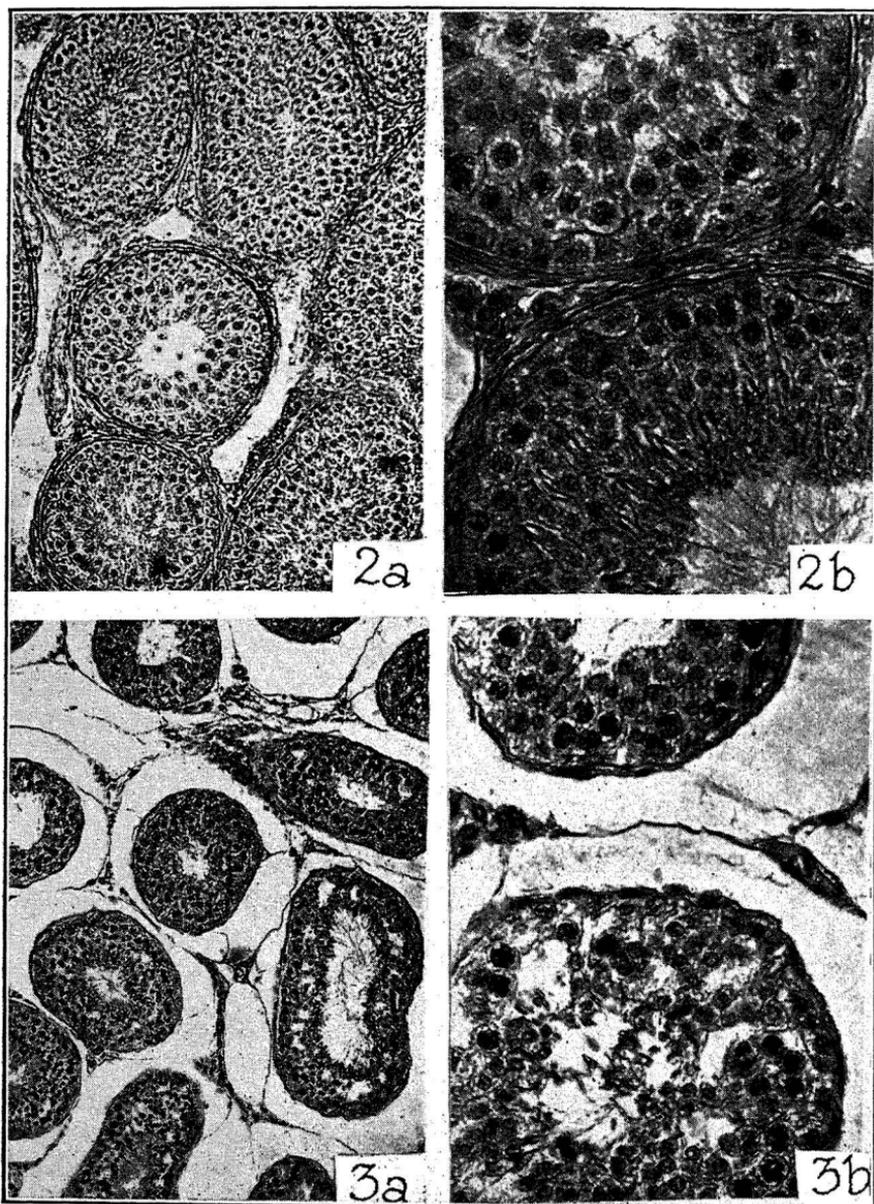
Fig. 2.—Section of testis from Ram 7 after four days of scrotal insulation. (a) 120X, (b) 360X. Note the absence of spermatozoa in some tubules, also sloughing of cells, clumping and disintegration of these cells, and cytolysis of some cells in the germinal epithelium.

Fig. 3.—Section of testis from Ram 14 after one week of scrotal insulation. (a) 120X, (b) 360 X. Note the marked shrinkage of tubules, edematous condition, sloughing and clumping of cells, cytolysis in all regions of the germinal epithelium, pycnosis in many cells, and the chromatoid condition of many nuclei.

tubules were shrunken. The lumina contained debris and degenerating spermatids, secondary and primary spermatocytes and an occasional spermatozoon. No spermatozoon was attached to the epithelium. In some tubules the region normally occupied by the spermatids and secondary spermatocytes was filled with a mass of degenerating material while in others this region was filled with unorganized cells. Some primary spermatocytes appeared in their normal location but others were scattered throughout the tubules. Some degeneration was evident among the spermatogonia. (See Figure 4.)

Ram 13. At the end of four weeks of scrotal insulation, the testis presented a very degenerate appearance. A single row of cells, probably spermatogonia, remained around the basement membrane of the tubules. The remainder of the tubal epithelium was filled with a mass of degenerate material. Spermatozoa were not present. (See Figure 5.)

Ram 9. At the end of eight weeks of scrotal insulation the general appearance of the testis was disorganized and areas of degeneration were evident in all regions of the tubules. Spermatogonia were arranged in a somewhat irregular row on the tubal basement membrane. Most of the tubules contained a considerable number of cells that resembled primary and secondary spermatocytes. These cells were not arranged in definite order, many contained dark-staining nuclei and giant cells resulting from syncytium were common. There was considerable variation in the size of these giant cells. The lumina were either almost completely filled with these cells or with a mass of degenerating material. Some lumina were empty. (See Figure 6.)

Ram 17. At the end of sixteen weeks of scrotal insulation, the testis presented a very disorganized appearance. The tubules were not clearly outlined. Spermatogonia formed a somewhat irregular row around the base of the tubules and a few similar cells were to be found scattered about in the tubules. Besides this, the tubules contained only degenerate material. (See Figure 7.)

The description of the testes are summarized in Table 6. The diameter of seminiferous tubules given is the average of measurements on fifty tubules from each testis studied.

Several points of interest are noted in the reaction of these testes of rams to scrotal insulation.
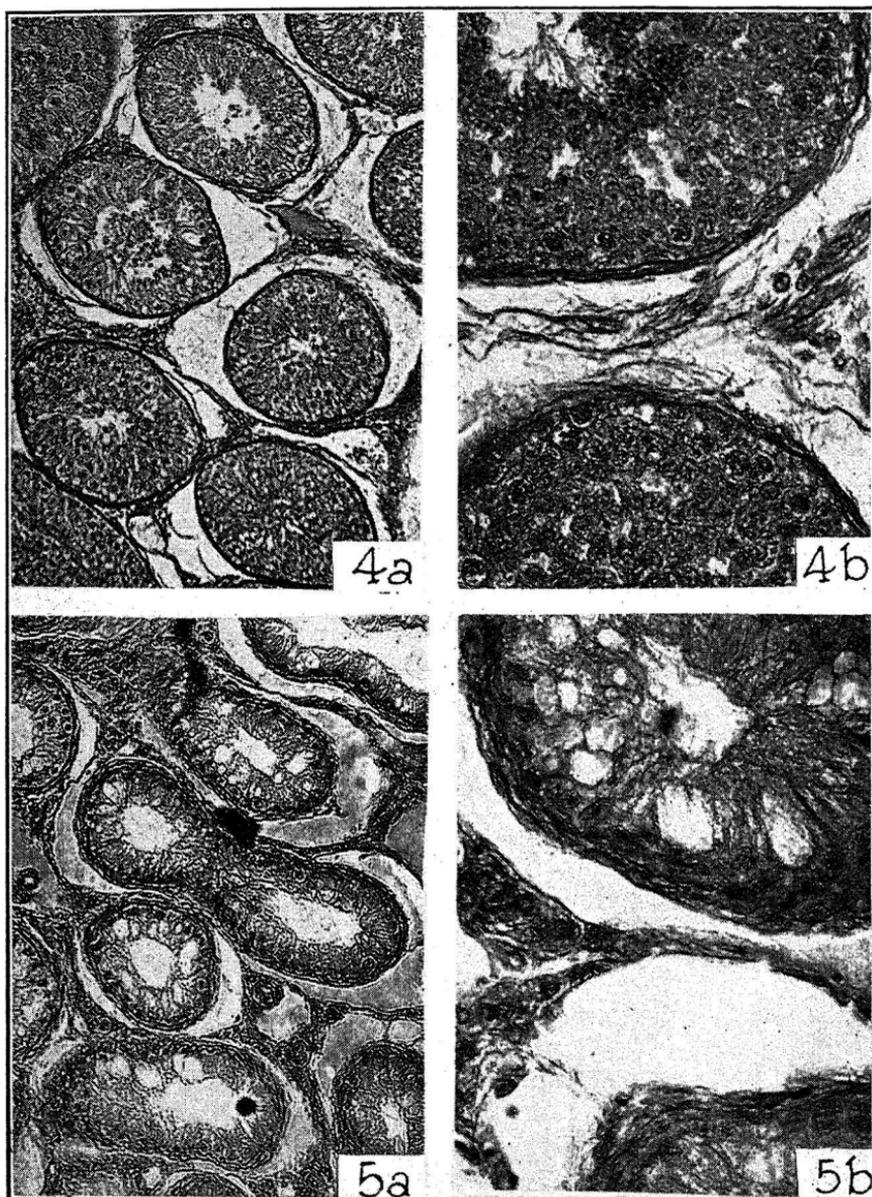
Fig. 4.—Section of testis from Ram 15 after two weeks of scrotal insulation. (a) 120X, (b) 360X. Note the edematous condition, marked pycnosis, sloughing of epithelial cells, cytolysis in all regions of the germinal epithelium, and the absence of spermatozoa except for an occasional one free in the lumen of a tubule.

Fig. 5.—Section of testis from Ram 13 after four weeks of scrotal insulation. (a) 120 X, (b) 360X. Note the marked shrinkage of tubules, edematous condition, absence of epithelial cells except for a single layer on the basement membrane, and the colloidal-like mass of degenerating material in each tubule.
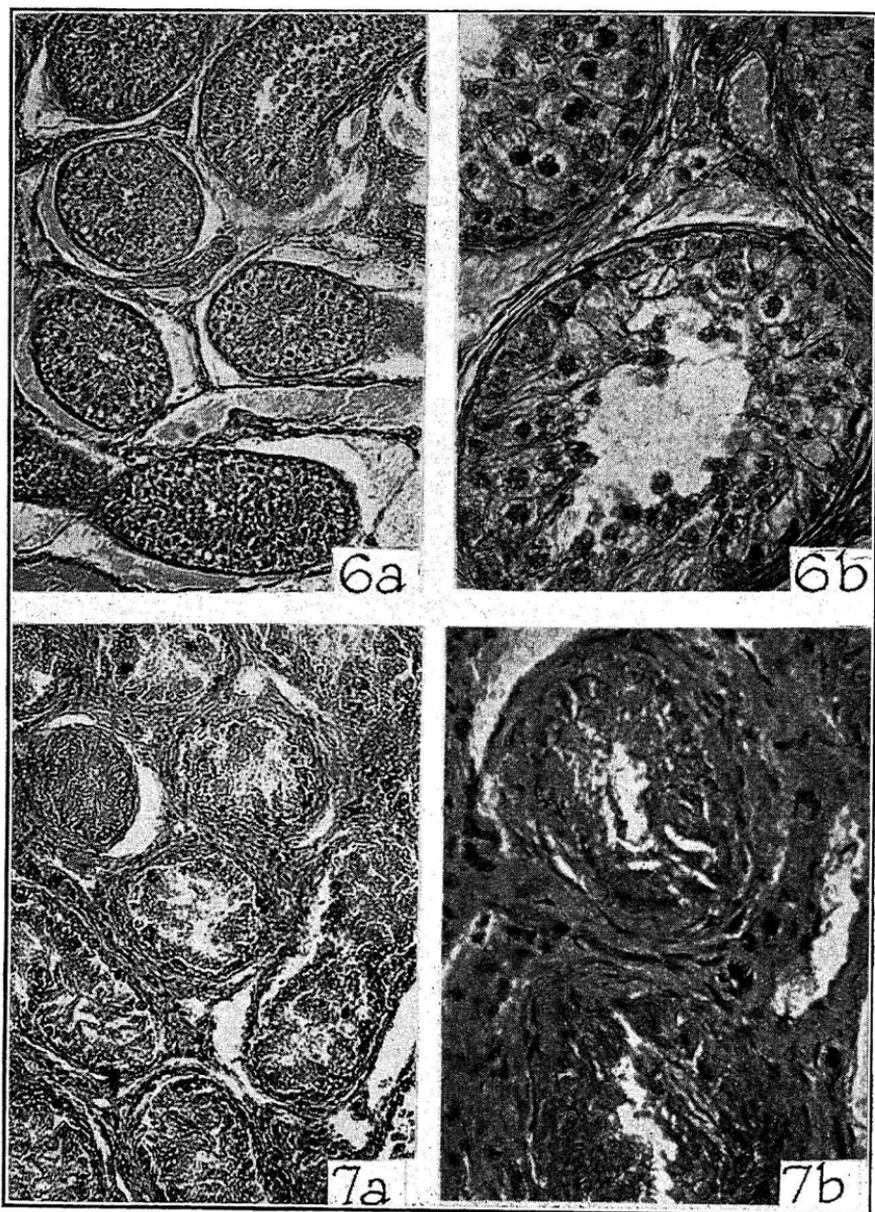
Fig. 6. —Section of testis from Ram 9 after eight weeks of scrotal insulation. (a) 120X, (b) 360X. Note the edematous condition, cytolysis, chromatoid condition of many nuclei, giant cells, and disintegration of cells free in the lumina of tubules.

Fig. 7.—Section of testis from Ram 17 after sixteen weeks of scrotal insulation. (a) 120X, (b) 360X. Note the shrunken condition of the tubules, and the disorganized condition within the tubules where only a few cells around the basement membrane and some disintegrating cells in the lumina remain.

Ram	Insulation period	Tubule dia. (Micra)	Material in Lumina of Tubules	Spermatozoa	Spermatids	Secondary Spermatocytes	Primary Spermatocytes	Spermatogonia
6	4 days	177.1	Nothing in a few. Cells, mostly spermatids and secondary spermatocytes, and some spermatozoa.	None in a few tubules. Most tubules had many attached to epithelium, and a few in lumina.	Normal in some tubules. Areas of degeneration in many.		Normal in most tubules. Areas of degeneration in some.	
14	1 week	146.8	Debris and some cells, mostly spermatozoa, spermatids, and secondary spermatocytes.	Many in lumina and many attached to epithelium. None in some tubules.	Cell outlines not clear. Much degeneration and shedding into lumina.	Much degeneration and shedding into lumina.	Areas of degeneration and dark-staining nuclei in many tubules. Appear normal in some.	
15	2 wks.	152.7	Debris and some cells, mostly spermatids, secondary and primary spermatocytes. An occasional sperm.	None in epithelium. An occasional one in lumen.	Disorganized. In some tubules mostly mass of degenerating material. In others made up of unorganized group of cells.		Some normal, others scattered thruout tubules.	Some degeneration and tending to become disorganized.
13	4 wks.	114.3	Mass of degenerate material.	None	An occasional cell in this region. Mostly degenerate material.			Single row around base of tubules.
9	8 wks.	131.8	Some lumina empty. Others contained degenerating material and some were filled with cells.	None	None	Most tubules contained cells similar to these but disorganized. Dark staining nuclei common. Much syncitium, so much variation in size.		Irregular row around base of tubules.
17	16 wks.	102.6	Degenerate material, with a few cells, probably spermatogonia.	None	None			Irregular row around base of tubules.

As in laboratory animals the spermatids and secondary spermatocytes were the first to be affected by the increased temperature, followed by the primary spermatocytes and then some disturbance of the spermatogonia. A notable amount of degeneration was present after four days of scrotal insulation. In a subsequent section the studies on spermatozoa ejaculated by these rams during insulation are reported. The great proportion of spermatozoa with tailless heads observed in the tubules of the testis from Ram 14 (one week of insulation) indicates the source of this type of spermatozoa which appeared in the semen in such large numbers following insulation.

The fact that spermatozoa were not found in the epithelium and only an occasional one in the lumina of tubules of a testis after two weeks of scrotal insulation indicates the quickness with which an animal is rendered sterile by a small increase in scrotal temperature.

A marked decrease in the diameter of the seminiferous tubules is noted as the length of the insulation period increases. There was also a decrease in the gross dimensions of the testes as may be seen in Table 7. The "volume" given is the product of multiplying the three dimensions given and is of course not the true

TABLE 7.—EFFECT OF SCROTAL INSULATION UPON THE SIZE OF RAM TESTES

Ram No.	Length of insulation	Testis dimensions (cm.)	Testis "Volume"
7	4 days	5.6 x 5.7 x 8.0	255.3
14	1 week	4.6 x 4.9 x 7.5	169.0
15	2 weeks	5.4 x 5.9 x 10.2	324.9
	(3 weeks recovery)	5.3 x 5.9 x 10.1	315.8
13	4 weeks	4.3 x 5.0 x 7.0	150.5
	(3 weeks recovery)	4.5 x 4.7 x 8.2	173.4
9	8 weeks	4.6 x 5.0 x 7.8	179.4
17	16 weeks	2.7 x 3.7 x 6.2	61.9

volume. The size of two partner testes, after three weeks' recovery, is also included. Obviously the testis from Ram 17 is greatly reduced in size as compared to the others. Apparently there was considerable variation in the size of testes at the beginning of insulation, since a comparison of the two testes from Ram 15 indicate that after two weeks of insulation the testis was no smaller than its partner that was allowed three weeks of recovery. After four weeks of insulation the testes of Ram 13 had apparently decreased in size somewhat since the testis removed at the end of insulation was smaller than one removed after a recovery period of three weeks.

The condition of testes removed from Rams 14, 15, 13 and 9 after a three weeks' recovery period following insulation is of

interest when compared with partner testes removed at the end of the insulation period. A description of these testes follows.

Ram 14. After the testis had been subjected to scrotal insulation for one week, followed by a three-week period without insulation the germinal epithelium was more disorganized and degenerate than in the partner testis removed at the end of one week of insulation. More cells and debris from degenerating cells were present in the lumina, and most of the epithelium, except the spermatogonia, was gone in some tubules. Spermatozoa were absent in many tubules, while almost all tubules in the testis removed at the end of one week of insulation contained spermatozoa. (See Figures 3 and 8.)

Ram 15. In comparison with a testis removed at the end of two weeks of scrotal insulation the testes subjected to two weeks of insulation and then allowed a three weeks' recovery period showed much reconstruction and the cells in the epithelium were organized in a nearly normal manner. Comparatively few cells and debris were to be found in the lumina. Spermatozoa were absent in a few tubules but were present in considerable numbers in the epithelium in most tubules, while at the end of two weeks of insulation no spermatozoon was present in the epithelium and only an occasional one in the lumina. (See Figures 4 and 9.)

Ram 13. In the testis removed at the end of four weeks of scrotal insulation only a single layer of cells remained around the base of the tubules and the lumina were filled with degenerate material. After being allowed a three weeks' recovery period the partner testis still presented a disorganized appearance, some tubule lumina were empty, while others contained masses of degenerating material. However, instead of a single layer of spermatogonia around the base of the tubules, primary spermatocytes were present in most tubules and a few contained secondary spermatocytes. Spermatids or spermatozoa were not observed. (See Figures 5 and 10.)

Ram 9. Instead of the disorganized appearance presented at the end of eight weeks of scrotal insulation this testis removed after being allowed a recovery period of three weeks showed marked reorganization. Most tubule lumina were filled with debris and degenerating cells, but spermatogonia and primary spermatocytes were arranged in their normal positions in most tubules, secondary spermatocytes and spermatids were present in many tubules and some spermatozoa were also present. (See Figures 6 and 11.)
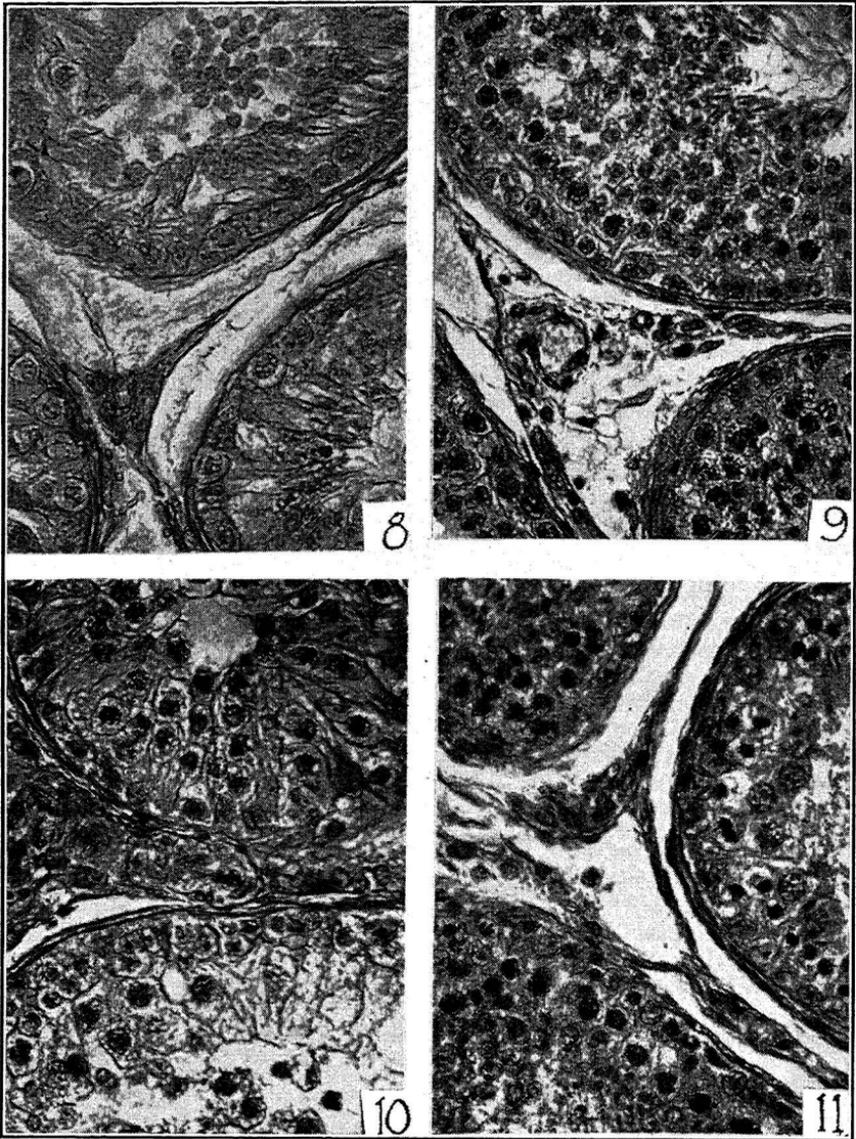
Fig. 8.—Section of testis from Ram 14 after one week of scrotal insulation followed by a three weeks' recovery period. 360X. Note the sloughing of epithelial cells, and marked cytolysis in all regions of the germinal epithelium. Degeneration is more marked here than in the partner testis removed at the end of insulation three weeks earlier indicating that degeneration, once started, goes to completion before regeneration begins. (cf. Fig. 3).

Fig. 9.—Section of testis from Ram 15 after two weeks of scrotal insulation followed by a three weeks' recovery period. 360X. Note that the epithelium has regained a nearly normal organization and spermatozoa are being formed. (cf. Fig. 4).

Fig. 10.—Section of testis from Ram 13 after four weeks of scrotal insulation followed by a three weeks' recovery period. 360X. In the partner testis removed three weeks earlier only a single layer of cells was found around the basement membrane. The presence of many primary spermatocytes in this testis gives evidence of recovery. (Cf. Fig. 5).

Fig. 11.—Section of testis from Ram 9 after eight weeks of scrotal insulation followed by a three weeks' recovery period. 360X. Instead of the disorganized condition found in the partner testis removed three weeks earlier this testis has spermatogonia and primary spermatocytes arranged in a nearly normal order, indicating marked regenerative changes during the recovery period. (Cf. Fig. 6).

Young (1927) observed in guinea-pig testes subjected to high temperatures for brief periods that degeneration continued for about twelve days, at which time the tubules were largely evacuated of germinal epithelium and regeneration begun. Insulation is not comparable to high temperature applications for brief periods but from the conditions observed in Ram 14, it is obvious that degeneration was not complete at the end of one week of insulation but continued after removal of the insulation. The condition of the testis from Ram 14 after being subjected to one week of insulation and then allowed a three weeks' recovery period is not greatly different from the testis of Ram 13 removed at the end of four weeks of insulation. In both testes just mentioned the time from the beginning of insulation was four weeks.

Testes subjected to longer periods of insulation than one week (two, four, and eight weeks) and then allowed a recovery period of three weeks all showed obvious signs of regeneration. Evidently the germinal epithelium of these testes had more nearly undergone complete degeneration at the end of the respective periods of insulation than had the testes subjected to only one week of insulation and consequently were able to begin regeneration more quickly after removal of the insulation.

Photomicrographs of sections of the testes obtained at the end of various periods of scrotal insulation, and of testes after being subjected to scrotal insulation for various periods and then allowed a three weeks' recovery period are shown in Figures 2 to 11.

III. The Effect of Scrotal Insulation Upon Ejaculated Spermatozoa of the Ram.

Material.—The materials for this work consisted of semen recovered from Hampshire rams which were subjected to scrotal insulation for varying lengths of time, namely: four days, one week, two weeks, four weeks, eight weeks, and sixteen weeks.

Before applying the insulation a few semen samples were obtained from each ram. During and after the insulation period the rams were allowed three services every third day, a sample of semen being obtained at the first service on each of these days. At the ends of the insulation periods one testis was removed from each ram, and three weeks later the remaining testis was removed, for histological study.

After recovering the semen, smears were made by placing a drop of the semen on a slide and lightly drawing it out with another slide. The smears were allowed to air dry, then were fixed with heat (in oven at 100° C. for twenty minutes), cleared in "chlorozene"*, washed, dried, stained for twenty seconds in Ziehl-Neelson's carbol fuchsin†, washed and dried.

In an earlier section of this paper it was noted that the insulation used increased the temperature of the scrotum about 2.2° C. above normal. Observations include the types and numbers of abnormal spermatozoa that were produced by this amount of insulation. Five hundred spermatozoa were observed in each smear, except in cases where spermatozoa were so scarce as to make the counting of so large a number impracticable. In such cases only three hundred spermatozoa were observed.

Presentation of Results.—The types of abnormal spermatozoa, the number of each type, and the total number of abnormal spermatozoa observed in the semen of each ram before, during and after insulation are presented in Tables 8 to 13, inclusive. The numbers represent the number of each type of abnormality per thousand spermatozoa counted.

TABLE 8.—NUMBERS AND TYPES OF ABNORMAL SPERMATOZOA OBSERVED IN
SEMEN OF RAM 7

Time of obtaining semen	Tailless heads	Coiled tails	Tapering heads	Enlarged middle pieces	Broken at neck	Total no. abnormal spermotozoa per M
8 days before insulation	12	60			4	76
5 days before insulation	14	38			2	54
2 days before insulation	14	62	2		8	86
First day of insulation	18	22			2	42
Fourth day of insulation	12	32		2	2	48

Note: Insulation removed and one testis removed at end of fourth day. (See Fig. 2.) Ram died soon after removal of testis.

The chief types of abnormal spermatozoa observed are illustrated in Figure 12. Other types that were observed include dark staining heads, head of normal size but with a short middle piece and tail, and in one sample of semen several objects that appeared to be tails, with no middle pieces, but attached directly to small fragments of heads were noted.

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*"Chlorozene" is a commercial product containing 5 % chlorozene (paratoluyene-sodium-sulpho-chloramide) in combination with sodium bicarbonate, sodium chloride, saccharin and eucalyptol. It is produced by the Abbott Laboratories of North Chicago, Illinois.

†The formula for Ziehl-Neelson's carbol fuchsin is as follows:
Saturated alcoholic solution of basic fuchsin ---1 part
5 % solution of carbolic acid water--9 parts
Use while fresh.

TABLE 9.—NUMBERS AND TYPES OF ABNORMAL SPERMATOZOA OBSERVED IN SEMEN OF RAM 14

Time of obtaining semen	Tailless heads	Coiled tails	Tapering heads	Enlarged middle pieces	Broken at neck	Dark staining heads	Small heads	Filiform middle pieces	Middle piece beads	Short middle piece and tail	Total No. abnormal spermatozoa per M.
Before insulation_____	216	8	8	--	--	--	--	--	--	--	232
Before insulation_____	200	26	4	--	10	--	--	--	--	--	240
1st day of insulation_____	152	70	6	2	4	2	--	--	--	--	236
4th day of insulation_____	176	26	8	2	8	--	12	2	6	--	240
7th day of insulation_____	28	38	--	--	8	--	--	--	--	--	74
Insulation taken off and one testis removed at end of seventh day											
10th day after beginning insulation	26	42	--	--	--	--	--	--	--	--	68
13th day after beginning insulation	134	122	--	2	8	--	--	--	--	--	266
16th day after beginning insulation	78	192	6	--	6	--	--	--	--	--	282
19th day after beginning insulation	No spermatozoa in semen										
22nd day after beginning insulation_	128	90	66	--	2	--	4	--	32	--	322
*25th day after beginning insulation	164	92	56	--	16	--	--	--	20	--	352
*28th day after beginning insulation	300	116	52	12	48	--	--	--	2	2	532

*Few spermatozoa.

Note: Second testis removed at end of 28th day after beginning of insulation. Ram died soon after castration.

TABLE 10.—NUMBERS AND TYPES OF ABNORMAL SPERMATOZOA OBSERVED IN SEMEN OF RAM 15

Time of Obtaining Semen	Tailless heads	Coiled tails	Tapering heads	Enlarged middle pieces	Broken at neck	Filiform middle pieces	Middle piece beads	Large heads	Total No. abnormal spermatozoa per M.
Before insulation	12	6	--	--	--	--	--	--	18
Before insulation	6	6	--	--	2	--	--	--	14
Before insulation	12	12	--	--	2	--	--	--	26
1st day of insulation	24	12	--	--	2	--	--	--	38
4th day of insulation	20	28	--	--	4	--	--	--	52
7th day of insulation	6	26	--	2	--	--	--	--	34
10th day of insulation	50	44	--	--	8	--	--	--	102
13th day of insulation	112	80	2	14	22	10	--	--	240
Insulation taken off and one testis removed at end of 14th day									
16th day after beginning insulation	52	64	30	--	10	--	2	--	158
19th day after beginning insulation	38	100	14	--	4	2	--	--	158
22nd day after beginning insulation	28	470	42	4	6	--	--	--	550
25th day after beginning insulation	154	198	54	2	--	--	--	2	410
28th day after beginning insulation	50	184	68	4	26	2	--	----	334
31st day after beginning insulation	70	204	62	18	22	--	4	--	380
34th day after beginning insulation	250	44	--	--	24	--	--	--	324
Second testis removed at end of 35th day after beginning insulation									
37th day after beginning insulation	186	58	42	2	18	2	--	--	308
40th day after beginning insulation	*142	122	44	4	16	--	--	--	328
43rd day after beginning insulation	*								
46th day after beginning insulation	*								
49th day after beginning insulation	*								
52nd day after beginning insulation	**								
55th day after beginning insulation	**								
58th day after beginning insulation	**								
61st day after beginning insulation	**								
64th day after beginning insulation	**								
67th day after beginning insulation	**								
73rd day after beginning insulation	**								

*Few spermatozoa. **Very few spermatozoa.

TABLE 11.—NUMBERS AND TYPES OF ABNORMAL SPERMATOZOA OBSERVED IN SEMEN OF RAM 13

Time of Obtaining Semen	Tailless heads	Coiled tails	Tapering heads	Enlarged middle pieces	Broken at neck	Filiform middle pieces	Short middle piece and tail	Total No. abnormal spermatozoa per M.
Before insulation_____	80	66	2	---	6	--	--	154
Before insulation_____	62	46	--	---	4	--	--	112
Before insulation_____	20	14	--	---	--	--	--	34
Before insulation _____	12	14	--	---	2	--	--	28
1st day of insulation_____	26	32	--	---	2	--	--	60
4th day of insulation_____	16	342	--	---	4	--	--	362
7th day of insulation_____	38	520	--	---	6	---	--	564
10th day of insulation_____	612	162	--	---	6	--	--	780
13th day of insulation_____	**							
16th day of insulation_____	**		-					
19th day of insulation_____	465	275	5	105	5	15	--	870
22nd day of insulation_____	505	240	10	55	30	10	25	875
25th day of insulation_____	**							
28th day of insulation_____ _ _	**							
	Insulation taken off and one testis removed on 28th day							
31st day after beginning insulation	**							
34th day after beginning insulation	**							
37th day after beginning insulation	**							
40th day after beginning insulation	**							
43rd day after beginning insulation	**							
46th day after beginning insulation	**							
49th day after beginning insulation	**							

Note: Second testis removed on 49th day. Ram died soon after castration.

*Few spermatozoa.   **Very few spermatozoa.

TABLE 12.—NUMBERS AND TYPES OF ABNORMAL SPERMATOZOA OBSERVED IN SEMEN OF RAM 9

Time of Obtaining Semen	Tailless heads	Coiled tails	Tapering heads	Enlarged middle pieces	Broken at neck	Filiform middle pieces	Middle piece beads	Short middle piece and tail	Large heads	Total No. abnormal spermatozoa per M.
Before insulation	26	26	--	--	12	--	--	--	--	64
Before insulation	30	24	--	--	--	--	--	--	--	54
1st day of insulation	20	18	--	--	2	--	--	--	--	40
4th day of insulation	10	14	--	--	2	--	--	--	--	26
7th day of insulation	52	40	--	--	20	--	--	--	--	112
10th day of insulation	746	128	2	6	6	--	--	--	--	890
13th day of insulation	244	134	--	4	12	2	--	--	--	400
16th day of insulation	*56	166	20	--	4	2	2	2	--	250
19th day of insulation	*162	114	27	3	3	--	--	--	2	309
22nd day of insulation	**									
25th day of insulation	**									
28th day of insulation	**									
31st day of insulation	**									
34th day of insulation	**									
37th day of insulation	**									
43rd day of insulation	**									
46th day of insulation	**									
49th day of insulation	**									
52nd day of insulation	**									
55th day of insulation	**									
Insulation taken off and one testis removed on 56th day										
58th day after beginning insulation	**									
61st day after beginning insulation	**									
64th day after beginning insulation	**									

Note: Became sick and died following testis removal on 56th day.

*Few spermatozoa.   **Very few spermatozoa.

Time of Obtaining Semen	Tailless heads	Coiled tails	Tapering heads	Enlarged middle pieces	Broken at neck	Middle piece beads	No. of abnormal spermatoza per M.
Before insulation	130	24	---	2	22	---	178
Before insulation	60	40	---	---	4	---	104
1st day of insulation	110	54	---	---	8	---	172
4th day of insulation	50	68	---	---	12	---	130
7th day of insulation	22	26	---	---	4	---	52
10th day of insulation	24	398	---	---	---	---	422
13th day of insulation	248	248	6	---	6	4	512
16th day of insulation	No spermatozoa ejaculated						
19th day of insulation	934	8	14	---	4	---	960
22nd day of insulation	876	44	20	---	8	---	948
25th day of insulation	**						
28th day of insulation	**						
31st day of insulation	**						
34th day of insulation	**						
37th day of insulation	**						
40th day of insulation	**						
43rd day of insulation	**						
46th day of insulation	**						
49th day of insulation	**						
52nd day of insulation	**						
55th day of insulation	**						
58th day of insulation	**						
1st day of insulation	**						
64th day of insulation	**						
67th day of insulation	**						
70th day of insulation	**						
af73rd day of insulation	**						
7 th day of insulation	**						
79th day of insulation	**						

**Very few spermatozoa.

Tailless   Coiled Tail   Coiled Tail

Tapering Head   Broken at Neck   Small & Large Heads

Enlarged Middle   Middle Piece   Filiform Middle
    Piece   Bead   Piece

Double Head   Double Tail   Cytoplasmic Extrusion
                                      at Base of Head
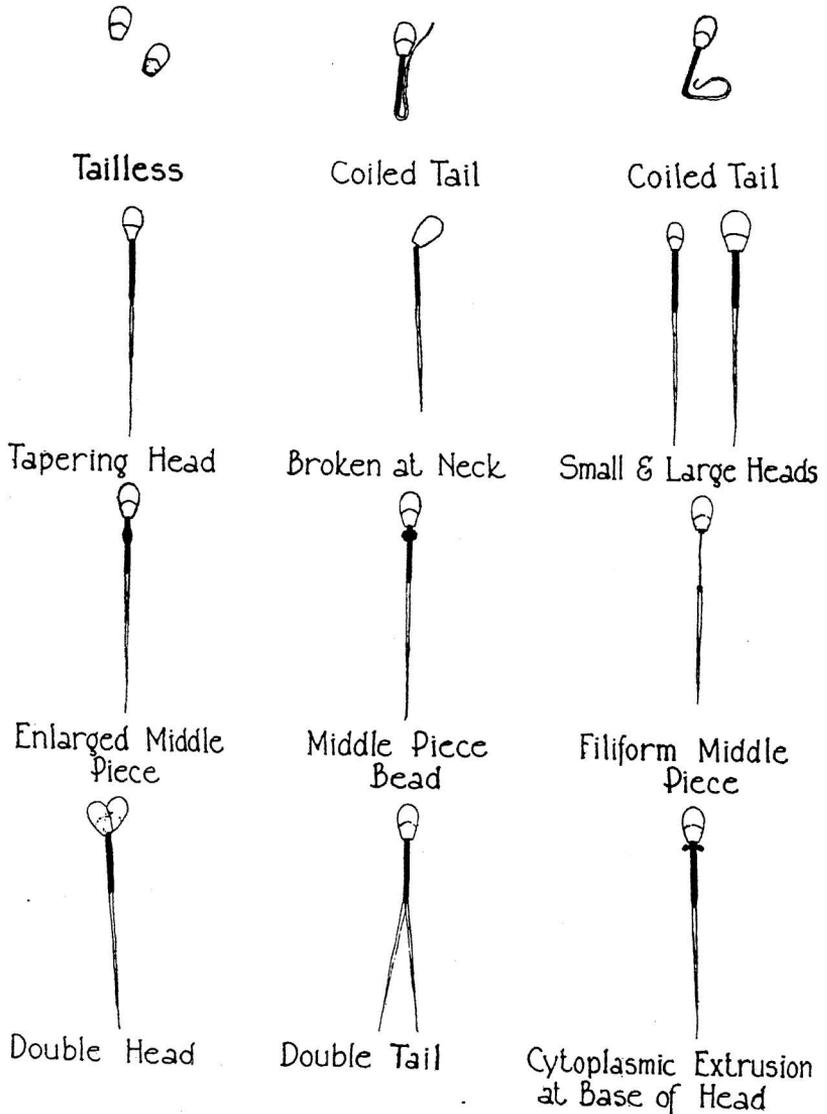
Fig. 12.—Types of abnormal spermatozoa.

Table 14 gives a summary of the total number of abnormal spermatozoa per thousand noted in the semen before, during, and after insulation of each of the six rams of which detailed information is given in Tables 8 to 13 inclusive.

The preceding tables (Tables 8 to 14 inclusive) present several interesting points. Rams 14, 13, and 17 show high abnormal

TABLE 14.—EFFECT OF SCROTAL INSULATION AS NOTED ON EJACULATED
SPERMATOZOA. FIGURES GIVEN DENOTE TOTAL NUMBER OF
ABNORMAL SPERMATOZOA PER THOUSAND SPERMATOZOA

Time of obtaining semen	Ram 7 4 day insul.	Ram 14 1 week insul.	Ram 15 2 weeks insul.	Ram 13 4 weeks insul.	Ram 9 8 weeks insul.	Ram 17 16 weeks insul.
Before insulation	76	232	18	154	64	178
Before insulation	54	240	14	112	54	104
Before insulation	86		26	34		
Before insulation				28		
1st day after beginning insulation	42	236	38	60	40	172
4th day after beginning insulation	48	240	52	362	26	130
7th day after beginning insulation	(Ram died)					
10th day after beginning insulation		74	34	564	112	52
13th day after beginning insulation		68	102	780	890	422
16th day after beginning insulation		266	240	**	400	512
19th day after beginning insulation		282	158	870*	250*	960
22nd day after beginning insulation		322	550	875*	**	948
25th day after beginning insulation		352*	410	**	**	**
27th day after beginning insulation		532*	334	**	**	**
31st day after beginning insulation		(Ram died)	380	**	**	**
34th day after beginning insulation			324	**	**	**
37th day after beginning insulation			308	**	**	**
40th day after beginning insulation			328*	**	---	**
43, 46, 49 days after beginning insulation			**	**		**
52, 55, 58, 61, 64 days after beginning insulation				(Ram died)	**	**
67, 70, 73, 76, 79 days after beginning insulation			**		(Ram died)	**
Time of removal of insulation and one testis	End of 4th day of insul.	End of 7th day of insul.	End of 14th day of insul.	End of 28th day of insul.	End of 56th day of insul.	End of 108th day of insul.
Time of removal of second testis		End of 28th day begin. insul.	End of 35th day begin. insul.	End of 49th day begin. insul.		

*Few spermatozoa   **Very few spermatozoa.

spermatozoon counts during the first few days, the count dropping down considerably in a short time. This condition was noted in several rams by McKenzie and Phillips (1933) and is believed to be the result of physiological degeneration of spermatozoa in the vas deferens. The rams had been sexually inactive for some months preceding their use in this work. After a few services these degenerating spermatozoa were cleared out and normal spermatozoa were ejaculated. A comparable condition was noted in guinea-pigs by Simeone and Young (1931) in their study of the fate of non-ejaculated spermatozoa.

In Rams 14, 15, 13, 9 and 17 a marked increase in the proportion of abnormal spermatozoa ejaculated was noted within a short time after applying insulation. The time of appearance of the first increase of abnormalities varied somewhat and will be discussed in a later paragraph. Ram 7 died before the effect of insulation upon the ejaculated spermatozoa could be noted.

The effect of insulation upon the number of the various types of abnormal spermatozoa is of interest. The number of tailless heads and spermatozoa with coiled tails increased very markedly as a result of insulation. It seems possible that the coiled tail abnormality might be a predecessor of the tailless head since, especially in Rams 13 and 17, it may be noted that a marked increase in coiled tails appeared first, followed shortly by a marked decrease in the proportion of coiled tails. Assuming that most of the increase in abnormalities noted resulted from interference with spermatogenesis, a more probable explanation would seem to be that the increased temperature resulted in coiled tails on the spermatozoa that were practically mature at the time of insulation, while the tailless variety resulted from interference at an earlier stage in spermatogenesis.

In all cases an increase in the proportion of tapering heads was noted after the insulation had been applied for some time. An increase in the number of enlarged middle pieces was noted in Rams 14, 15, 13 and 9, and this was especially marked in Ram 13 where 105 enlarged middle pieces per thousand appeared on the nineteenth day of insulation. A notable increase in the proportion of spermatozoa broken at the neck occurred in Rams 14, 15 and 13.

Other types of abnormalities that appeared in the various rams at various times after the beginning of insulation include small heads, filiform middle pieces, middle piece beads, large heads, and short middle pieces and tails.

In Rams 15 and 19 it will be noted that the number of abnormal spermatozoa ejaculated increased to a peak, then lessened before the supply of spermatozoa was exhausted. The explanation offered here is that the main bulk of abnormal spermatozoa produced by the increase in temperature was given off at the time of the highest abnormality count just mentioned and after this time, since spermatozoa were not being formed, the most of the spermatozoa given off were those held along the sides of the vas deferens and epididymis, and not passing through with the main group of spermatozoa.

As was indicated above, the time at which the first increase in abnormal spermatozoa ejaculated was noted varied in the different rams. This is given, along with other data, in Table 15 and ranges from four to thirteen days.

Table 15.—Showing Relation of Number of Days and Services Follow-
ing Insulation to Time of Appearance of Abnormalities in
Semen and Exhaustion of Sperm Supply

Ram	No. of days after beginning of insulation till first increase in no. of abnormal spermatozoa	No. of services from insulation until first increase in abnormal spermatozoa was noted	Days from beginning of insulation until sperm supply was exhausted	No. of services from beginning of insulation until sperm supply was exhausted
14	13	12		
15	10	11		
13	4	4	13	9
9	7	5	22	18
17	10	9	25	15
Average	8.8	8.2	20	14

The data in Table 15 are of especial interest in connection with the time required by spermatozoa to pass from the testes to the distal end of the vas deferens. Assuming that all or most of the sperm abnormality increases resulted from changes in spermatozoa during spermatogenesis, and thus having their origin in the testes, the time from the beginning of insulation until the appearance of the abnormalities, produced by this insulation, in the ejaculated semen would be an approximate measure of the maximum time required by those spermatozoa in traversing the epididymis and vas deferens. There are obviously individual differences in the time required for this passage, and this is further substantiated by the figures given in Table 15 showing the number of services from the time of insulation until an increase in the proportion of abnormal spermatozoa was noted. Where fewer days were required for the increase in abnormal spermatozoa to appear, also fewer services had been had by the ram from the time of beginning of the insulation until the time of the first increase in abnormalities.

It is also interesting to note in Table 15 that those rams in which abnormal spermatozoa appeared most quickly in the ejaculated semen after insulation were also the ones in which a scarcity of spermatozoa was first noted.

The average time required for the appearance of the first increase in abnormal spermatozoa was 8.8 days, and up to this time (from the time of insulation) an average of 8.2 services had been allowed. Abnormalities were probably not produced immediately so the time required for spermatozoon passage from the testes to the distal end of the vas deferens was, at the rate of service indicated, probably somewhat less than 8.8 days. In the guinea-pig Toothill and Young (1931) found the time required

for the passage of spermatozoa from the head of the epididymis to the anterior end of the vas deferens to be between 14 and 18 days. When the animal had had two electrically produced ejaculations the time was about two days less.

In Rams 13, 9 and 17 (insulated for 4, 8 and 16 weeks respectively) the supply of spermatozoa was almost exhausted at 13, 22 and 25 days after the beginning of insulation. A few spermatozoa were to be found in the semen after this time, but, as was noted in a preceding section of this paper, spermatozoa were not being produced and these few remaining spermatozoa were probably some that had remained in the vas deferens and epididymis. Ram 15 was insulated for two weeks, and although spermatogenesis had practically ceased, as was noted in a preceding section, a sufficient number of spermatozoa were held in the epididymis to keep a notable number in the ejaculated semen until the remaining testis recovered sufficiently to produce more. Eight days after removal of the second testis the supply of spermatozoa was practically exhausted. Ram 15 continued to copulate at regular intervals for six weeks after complete castration, but his interest diminished considerably during this time.

It may be noted that four of the six rams died after removal of the first or second testis. The rams were in good health up to this time and the only reasons suggested for so many deaths are (1) that the group of rams, all closely related, may have had poor resistance to infection, or (2) some particularly virulent type of bacteria might have been present in unusual numbers in the shed where the rams were kept.

IV. The Effect of Abdominal Temperature on the Morphology of Guinea-Pig Spermatozoa.

In a previous section of this paper the effect of scrotal insulation upon the ejaculated spermatozoa of the ram was described. Within a short time after the beginning of insulation a marked increase in the proportion of abnormal spermatozoa was noted. The question arises as to whether these abnormalities resulted from changes in fully formed spermatozoa or whether they resulted from faulty spermatogenesis after the testicular temperature was increased by insulation.

Material.—To determine the effect of abdominal temperature upon fully formed spermatozoa guinea-pigs were used. The technique is described briefly below.

Guinea-pigs were anesthetized, a region on the abdomen shaved, an incision made along the median line of the abdomen, and the testes withdrawn through this opening. The epididymi were then ligated at a point approximately at the mid-point from either end of the testes. One testis was then returned to the scrotum and the other sutured to the abdominal wall.

This operation left the proximal epididymis connected with the testes normally, but the distal epididymis and the vas deferens were isolated from any sperm supply. At various periods after operating, animals were killed and spermatozoon smears made from the following sections of the genital tract, slides being made from both the scrotal and abdominally retained tract:

(a) Testes, (b) Proximal epididymis, (c) Distal epididymis, (d) Proximal vas deferens, (e) Distal vas deferens.

The times of obtaining smears were as follows: 4 days, 1 week, 2, 4, 6 and 8 weeks after operation. No slide was obtained from the scrotal testis and tubes at six weeks after operating. By comparing the condition of spermatozoa from the distal epididymis and the vas deferens when held in the body cavity with spermatozoa from these tubes when in their normal position any effect of the abdominal temperature on the fully formed spermatozoa could be noted.

The slides were prepared as follows: after removal of the genital tract sections were removed from each of the divisions noted above, macerated in Ringer's solution, and smears made from this solution. The smears were stained with Ziehl-Neelson's carbol fuchsin. This technique is described in detail in an earlier section of this paper.

Presentation of Results.—The various types of abnormal spermatozoa, the proportion of each type, and the total number of abnormal spermatozoa per thousand observed in the slides from each division of the genital tract (both the normally located and the abdominally retained divisions of the tract) at the various periods after operation are given in Tables 16-20. Observations on spermatozoa from each division of the genital tract are given in a separate table.

The names of the various types of abnormalities which are listed are largely self-explanatory, and in general are the same as those previously described in ram spermatozoa in the section concerning the effect of scrotal insulation upon the ejaculated spermatozoa of the ram. One type of abnormality, listed in the tables as "Triple or more tails" appeared to be groups of three or more degenerating spermatozoa, the heads of which had become fused so as to present a single, but somewhat irregular outline.

In each of Tables 16-22, the last column indicates the total number of abnormal spermatozoa observed per thousand exclusive of those having coiled tails. This column is included in addition to the one headed "Total No. Abnormal Spermatozoa per M" since it seems probable that the coiled tail abnormality in this case might.result from treatment in preparing the slides, or that this might be a characteristic of the younger spermatozoa.

In Table 16, it will be noted that spermatozoa were not found in testes held in the abdomen two weeks or longer. After being held in the abdomen one week, spermatozoa were very scarce. In testes returned to the scrotum after epididymis ligation spermatozoa were plentiful at all the times after operating that slides were made and, though there was some variation in the number of abnormal spermatozoa observed (136 to 237 per M), there seemed to be no tendency for the number to increase as the length of time after epididymis ligation increased.

Normally very few spermatozoa could be found in the proximal epididymis, so they apparently passed rather quickly from the testes to the distal portion of the epididymis. The spermatozoa found in the proximal epididymis in these animals then must have collected there after ligation.

In Table 17, it will be noted that no spermatozoon was found in the proximal epididymis at 4 and 8 weeks after ligation of the epididymis, the testis being held in the scrotum during these times. In Table 16, it may be noted that in the corresponding testes spermatozoa were present. Apparently the spermatozoa in this portion of the epididymis at the time of, or shortly after ligation had degenerated and none had come in from the testes although spermatozoa were present there.

In epididymi held in the abdomen spermatozoa were found only at four days and one week after ligation and elevation to the abdomen. At one week after ligation and abdominal retention,

the number of abnormal spermatozoa was considerably higher than in any of the other smears from this section of the tract. These abnormalities probably resulted from faulty spermatogenesis due to the increased temperature and came into the proximal epididymis soon after ligation.

To obtain the numbers presented in Table 21, the numbers from Tables 18, 19 and 20 were averaged, so the numbers represent averages of the abnormal spermatozoon numbers observed in the distal epididymis and in the proximal and distal vas deferens. The figures have in turn been combined to obtain averages for comparing the condition of spermatozoa held in the epididymis in its normal location with those held in the abdomen. The six weeks abdominal stage was not included in Table 21 since there was no corresponding scrotal stage for comparison.

The numbers in Table 22 were taken, with one exception, from Table 21. These figures illustrate the gradual course of degeneration of the spermatozoa in these sections of the male genital tract after the supply of spermatozoa was shut off by ligation. The rate of degeneration appeared to be no faster in tubes retained in the abdomen than when in their normal position.

Young (1929) and (1931) Simeone and Young (1931) found that spermatozoa were normally removed from the vas deferens of sexually inactive guinea-pigs without passing on to the urethra, the removal process being the degeneration and resorption of the aged spermatozoa. Spermatozoa were not found in the distal epididymis nor in the vas deferens at eight weeks after ligation of the epididymis so all spermatozoa must have degenerated and been resorbed by this time.

In Table 21 the average total number of abnormal spermatozoa in tubes in their normal location was 147 and 120 in those abdominally retained (Table 21). Thus there is no indication from this work that abdominal temperatures increase the rate of degeneration from a morphological standpoint. By analogy these results would indicate that the increase in abnormal spermatozoa ejaculated by rams following subjection to scrotal insulation which was described in a previous section of this paper probably resulted from faulty spermatogenesis.

TABLE 16.—NUMBERS AND TYPES OF ABNORMAL SPERMATOZOA FROM GUINEA-PIG TESTES IN SCROTUM AND IN ABDOMEN. NUMBERS INDICATE NUMBER PER THOUSAND SPERMATOZOA

Guinea-pig number	Time after ligation of epididymis and location of testis	Tailless heads	Coiled tails	Tapering heads	Enlarged middle pieces	Broken at neck	Bent middle pieces	Broken middle pieces	Filiform middle pieces	Double heads	Double tails	Double heads and tails	Dark-staining heads	Total No. abnormal spermatozoa per M.	Total No. abnormal spermatozoa per M. exclusive of coiled tails
16	4 Days S*	159	342	3	6	15	27	6	--	6	15	--	--	579	237
17	1 Week S	96	664	24	8	--	8	--	16	--	--	--	--	816	152
18	2 Weeks S	72	40	32	56	36	4	--	4	--	8	--	--	252	212
20	4 Weeks S	36	196	8	16	8	--	4	32	--	32	--	--	332	136
19	8 Weeks S	92	112	12	8	12	--	--	40	4	--	--	8	288	176
16	4 Days A**	84	136	4	28	16	12	32	--	20	4	16	4	356	220
17	1 Week A	220	80	20	20		(Very few spermatozoa)			20	20	10	--	370	290
18	2 Weeks A	No spermatozoa													
20	4 Weeks A	No spermatozoa													
21	6 Weeks A	No spermatozoa													
19	8 Weeks A	No spermatozoa													

*S—in scrotum   **A—in abdomen

TABLE 17.—NUMBERS AND TYPES OF ABNORMRL SPERMATOZOA FROM PROXIMAL EPIDIDYMI OF GUINEA-PIGS (SCROTAL AND ABDOMINAL). NUMBERS INDICATE NUMBER PER THOUSAND SPERMATOZOA

Guinea-pig number	Time after ligation of epididymis and location of testis	Tailless heads	Coiled tails	Tapering heads	Enlarged middle pieces	Broken at neck	Bent middle pieces	Broken middle pieces	Middle piece beads	Double tails	Total No. abnormal spermatozoa per M.	Total No. abnormal spermatozoa per M. exclusive of coiled tails
16	4 Days S	38	440	8	16	2	2	--	--	4	510	70
17	1 Week S	110	288	6	16	6	2	2	--	--	430	142
18	2 Weeks S	99	12	12	45	6	9	--	--	--	183	171
20	4 Weeks S	no spermatozoa										
19	8 Weeks S	no spermatozoa										
16	4 Days A	10	282	12	18	--	24	42	4	--	392	110
17	1 Week A	252	72	8	40	12	8	4	--	--	396	324
18	2 Weeks A	no spermatozoa										
20	4 Weeks A	no spermatozoa										
21	6 Weeks A	no spermatozoa										
19	8 Weeks A	no spermatozoa										

TABLE 18.—NUMBERS AND TYPES OF ABNORMAL SPERMATOZOA FROM DISTAL EPIDIDYMI OF GUINEA-PIGS (SCROTAL AND ABDOMINAL). NUMBERS INDICATE NUMBER PER THOUSAND SPERMATOZOA

Guinea-pig number	Time after ligation of epididymis and location of testis	Tailless heads	Coiled tails	Tapering heads	Enlarged middle pieces	Broken at neck	Bent middle pieces	Broken middle pieces	Double heads	Double tails	Dark-staining heads	Triple or more tails	Total No. abnormal spermatozoa per M.	Total No. abnormal spermatozoa per M. exclusive of coiled tails
16	4 Days S	56	222	30	16	2	2	4	--	20	--	--	352	130
17	1 Week S	14	370	6	10	8	--	6	--	16	--	--	430	60
18	2 Weeks S	22	86	4	6	--	--	14	--	72	--	110	314	228
20	4 Weeks S	30	166	10	14	6	106	206	4	--	--	--	542	376
19	8 Weeks S	no spermatozoa												
16	4 Days A	22	242	14	16	--	4	8	--	12	--	--	318	76
17	1 Week A	6	234	6	4	--	2	2	2	12	2	4	274	40
18	2 Weeks A	14	72	14	2	4	6	13	--	62	--	108	296	224
20	4 Weeks A	46	12	2	4	2	228	16	--	--	--	--	310	298
21	6 Weeks A	14	20	10	12	--	320	30	--	4	--	--	410	390
19	8 Weeks A	no spermatozoa												

TABLE 19.—NUMBERS AND TYPES OF ABNORMAL SPERMATOZOA FROM THE PROXIMAL VAS DEFERENS OF GUINEA-PIGS (SCROTAL AND ABDOMINAL). NUMBERS INDICATE NUMBER PER THOUSAND SPERMATOZOA

Guinea-pig number	Time after ligation of epididymis and location of testis	Tailless heads	Coiled tails	Tapering heads	Enlarged middle pieces	Broken at neck	Bent middle pieces	Broken middle pieces	Middle piece beads	Double heads	Double tails	Dark-staining heads	Triple or more tails	Total No. abnormal spermatozoa per M.	Total No. abnormal spermatozoa per M. exclusive of coiled tails
16	4 Days S	14	292	32	12	--	--	4	--	--	8	2	--	364	72
17	1 Week S	44	356	12	4	12	--	--	--	--	12	--	--	440	84
18	2 Weeks S	12	72	8	8	4	--	--	56	--	--	--	68	228	156
20	4 Weeks S	60	249	18	27	18	--	87	12	--	--	3	--	474	225
19	8 Weeks S	no spermatozoa													
16	4 Days A	2	266	42	8	2	--	4	--	--	10	--	--	334	68
17	1 Week A	12	296	14	6	--	--	--	--	--	4	--	89	332	36
18	2 Weeks A	75	63	21	3	--	--	12	--	3	53	--	--	319	256
20	4 Weeks A	84	14	10	4	8	10	2	--	--	4	--	--	136	122
21	6 Weeks A	27	15	9	42	--	501	18	--	--	--	--	--	612	597
19	8 Weeks A	no spermatozoa													

TABLE 20.—NUMBERS AND TYPES OF ABNORMAL SPERMATOZOA FROM THE DISTAL VAS DEFERENS OF GUINEA-PIGS (SCROTAL AND ABDOMINAL). NUMBERS INDICATE NUMBER PER THOUSAND SPERMATOZOA

Guinea-pig number	Time after ligation of epididymis and location of testis	Tailless heads	Coiled tails	Tapering heads	Enlarged middle pieces	Broken at neck	Bent middle pieces	Broken middle pieces	Middle piece beads	Filiform middle pieces	Double tails	Triple or more tails	Total No. abnormal spermatozoa per M.	Total No. abnormal spermatozoa per M. exclusive of coiled tails
16	4 Days S	16	168	14	10	8	--	4	10	--	2	--	232	64
17	1 Week S	16	308	6	2	2	--	2	--	40	2	44	338	30
18	2 Weeks S	66	96	6	6	2	--	6	--		--	--	266	170
20	4 Weeks S	36	192	38	18	12	--	70	6	--	--	--	372	180
19	8 Weeks S	no spermatozoa												
16	4 Days A	22	286	26	10	--	--	10	--	--	2	--	356	70
17	1 Week A	18	266	6	8	--	--	4	--	4	4	--	310	44
18	2 Weeks A	15	175	25	--	5	--	--	--	--	20	20	260	85
20	4 Weeks A	60	21	9	30	12	--	9	--	--	--	--	141	120
21	6 Weeks A	15	12	12	24	6	483	3	--	--	3	--	558	546
19	8 Weeks A	no spermatozoa												

TABLE 21.—NUMBERS AND TYPES OF ABNORMAL SPERMATOZOA FROM DISTAL EPIDIDYMIS, PROXIMAL AND DISTAL VAS DEFERENS OF GUINEA-PIGS. FIGURES ARE AVERAGES TAKEN FROM TABLES 18, 19, AND 20

Guinea-pig number	Time after ligation of epididymis and location of testis	Tailless heads	Coiled tails	Tapering heads	Enlarged middle pieces	Broken at neck	Bent middle pieces	Broken middle pieces	Middle piece beads	Filiform middle pieces	Double heads	Double tails	Dark-staining heads	Triple or more tails	Total No. abnormal spermatozoa per M.	Total No. abnormal spermatozoa per M. exclusive of coiled tails
16	4 Days S	29	228	25	13	3	1	4	3	--	--	10	1	--	317	89
17	1 Week S	25	345	8	5	7	--	3	--	--	--	10	--	--	403	58
18	2 Weeks S	33	85	6	7	2	--	7	--	--	--	56	--	74	270	185
20	4 Weeks S	42	202	22	20	10	35	121	6	--	1	--	1	--	460	258
Average		32	215	15	11	5	9	34	2	--	1	19	1	18	362	147
16	4 Days A	15	265	27	11	1	1	7	--	1	1	8	1	1	335	70
17	1 Week A	12	265	9	6	--	1	2	--	--	1	7	1	1	306	41
18	2 Weeks A	35	103	20	2	3	2	9	--	--	1	45	--	72	292	189
20	4 Weeks A	63	16	7	13	7	79	9	--	--	--	1	--	--	195	179
Average		31	162	16	8	3	21	7	--	1	1	15	1	18	282	120

TABLE 22.—TOTAL NUMBER OF ABNORMAL SPERMATOZOA (EXCLUSIVE OF COILED TAILS) OBSERVED IN DISTAL EPIDIDYMIS, PROXIMAL VAS DEFERENS AND DISTAL VAS DEFERENS

Length of time after ligation	Abnormal spermatozoa per M in tubes in normal position	Abnormal spermatozoa per M in tubes in abdominal position
4 days	89	70
1 week	58	41
2 weeks	185	189
4 weeks	258	179
6 weeks	---	511
8 weeks	no spermatozoa	no spermatozoa

V. The Effect of Low Temperatures on the Testes of the Rat.

All the work which has been previously reviewed or reported in this paper has had to do with the effect of higher than normal temperatures on the testes and spermatozoa. Would lower than normal temperatures affect the testes and spermatozoa in any way,—or to state the problem in another way, is there an optimum temperature for normal spermatogenetic function?

In a preliminary trial three rats were used, the treatment being as follows: anesthesia was produced with nembutal, after which an area on the abdomen was shaved and prepared for operation. An incision was then made along the midline of the abdomen and one testis withdrawn and suspended in a bath of Ringer's solution maintained at 17-19° C. for a period of thirty minutes, one hour or two hours. The testes were then returned to the scrota, and after ten days the animals were killed and both treated and normal testes removed and fixed in Allen's fluid*. Blocks of tissue were then dehydrated, cleared, imbedded in paraffin, sectioned and stained.

These testes, after the treatment described above, showed no notable evidences of derangement of the germinal epithelium when compared with the normal or untreated testes.

Testes of another group of 15 rats were exposed to lower than normal temperature by external applications of cold or cool water. This was done upon anesthetized animals, by placing a gauze pack on the scrotum and keeping this pack cooled with a constant stream of water. The temperatures, period of treatments, and time after treatment until removal of the testes are given in Table 23.

*Formula for Allen's fluid:
  Picric acid (Sat. aqueous sol.)   75 parts
  Formalin, c. p.   15 parts
  Glacial acetic acid   10 parts
  Urea crystals   1 part

TABLE 23.—OUTLINE OF LOW TEMPERATURE EXPOSURES OF RAT TESTES

Temperature C.	Length of treatment	Time from treatment until testis removal
6-8°	1 hour	4 days
	1 hour	7 days
	15 minutes	10 days
	30 minutes	10 days
	1 hour	10 days
	2 hours	10 days
	3 hours	10 days
15°	1 hour	10 days
	2 hours	10 days
	3 hours	10 days
20°	1 hour	10 days
	2 hours	10 days
	3 hours	10 days
25°	1 hour	10 days
	3 hours	10 days

These testes, and testes from control animals, were fixed in Allen's fluid, dehydrated, cleared, imbedded, sectioned and stained.

A study of these sections revealed no evidence that the exposure to low temperatures resulted in any notable derangement of the germinal epithelium.

Further work is necessary to determine the amount and degree of exposure to lower than normal temperatures required to cause testicular damage, but it seems obvious from the material presented that testes are much less susceptible to damage from exposure to low temperature, than they have been found to be from exposure to higher than normal temperatures.

VI. The Physiology of the Tunica Dartos of the Ram

In preceding sections of this paper it has been noted that the scrotum maintains the testes of the ram, and the testes of various other species, at a temperature below that of the body cavity. It was also noted that if the scrotal temperature was increased, spermatogenesis was seriously interfered with. The mechanism by which the scrotum maintains the testes at this lower temperature has never been studied to any extent. Among stockmen it has been rather common observation that during hot weather the scrota, especially of bulls and rams, are much more pendulous than in cooler weather. This indicates that the scrotum does react to temperature changes, but little information seems to be available concerning the nature, time relationships, and the importance in testis-temperature control of this reaction. A study of the scrotal physiology of the ram was undertaken in an attempt to supply information on these points.

Material.—Eight Hampshire rams have been used in studying reactions of the intact scrotum. The method of study was to
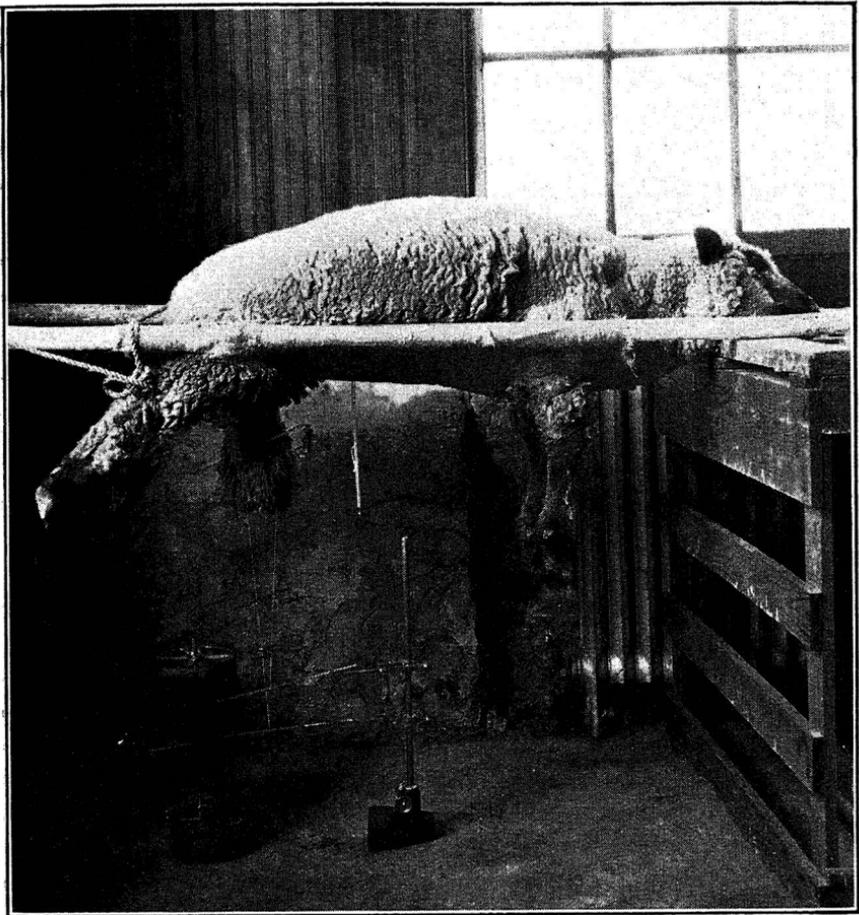
Fig. 13.—Showing the arrangement of ram and apparatus for studying reactions of the scrotum.

anesthetize the rams with nembutal, place them in the manner shown in Figure 13, with apparatus for recording scrotal reactions placed under the ram. The arrangement of the apparatus varied somewhat in different rams. In the set-up pictured in Figure 13, it may be noted that there are two recording levers, one attached to the tip of the scrotum and one attached at a point midway between the tip of the scrotum and the point of attachment to the body. Recording levers were attached to the scrotum by a thread passed lightly through its skin. A thermometer is suspended near the scrotum for the recording of room temperatures, and a second,

finely graduated thermometer, is inserted through an incision in the scrotum to obtain temperatures within the scrotum itself.

Some of the rams were used several times so that a total of thirteen observations were made upon intact scrota, two upon castrate scrota, and one on the scrotum of a ram including observations immediately before and after castration. Thus a total of sixteen observations were made, ranging from one and one-half to six hours in length.

Strips of the tunica dartos were also removed from a number of rams, mounted in Ringer's solution and subjected to various temperature changes. Also. various drugs (adrenalin, ephedrine, pilocarpine and atropine) were used in an attempt to determine the innervation of this tissue. These experiments will be discussed in detail later.

In the various studies of scrotal physiology a total of thirteen rams have been used. They have been labeled in series, Rams A, B, C, etc., for convenience. The experimental results will be described in three sub-sections as follows: reactions of intact and castrate scrota in rams; reactions of isolated strips of the tunica dartos to temperature changes; the innervation of the tunica dartos.

Reactions of Intact and Castrate Scrota in Rams.—In presenting this material it seems best to describe in detail, with illustrations, some of the records obtained. Also a table is given summarizing briefly the results of all observations. All the records were obtained under field conditions. The illustrations shown were copied directly from photographs of the original kymograph records. The time is shown on the base line of all records, in one minute intervals. All movements are magnified 2X.

In Figure 14 two consecutive records are shown that were obtained from Ram B. The observations were begun early in the morning and the room temperature was 16° C. at that time. The temperature gradually increased and was 19° C. at the beginning of the second record. The various contractions and relaxations undergone by the scrotum of this ram at the temperatures noted may be seen in Figure 14.

Not long after the end of the second record shown in Figure 14, the room temperature had increased to 22.5° C. During several records obtained at this and slightly higher temperatures the scrotum was completely relaxed. One of the last of these records is shown in Figure 15. At this time the room temperature had increased to 24.0° C. Increasing the temperature in the immediate
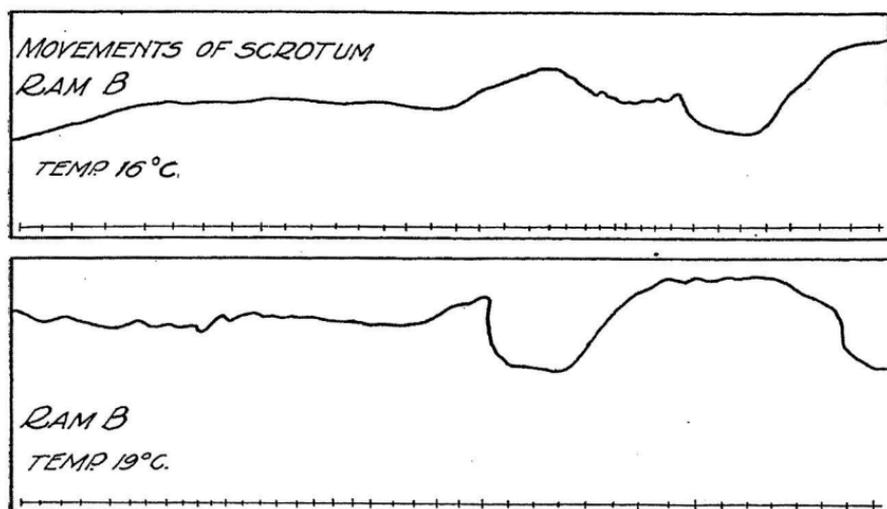
Fig. 14.—Movements of the scrotum in Ram B. Temperature 16° to 19° C. Time in one minute intervals.
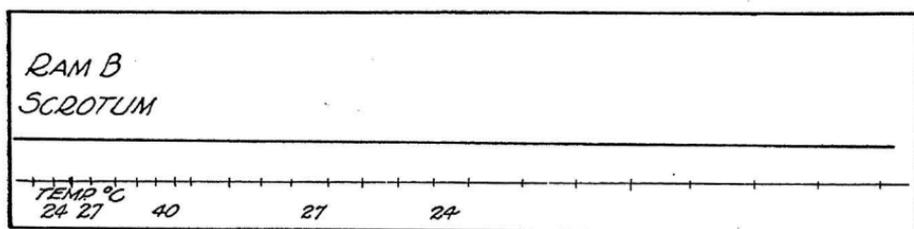
Fig. 15.—Record from Ram B consecutive with those shown in Figure 14. Room temperature 24° C. Time in one minute intervals. Temperature increased with electric heater.

vicinity of the scrotum to 40.0° C. with an electric heater resulted in no relaxation beyond that observed at 24.0° C.

The reaction of the scrotum of Ram C when the temperature of the air in the immediate vicinity of the scrotum was increased with an electric heater is shown in Figure 16. Here, however, the room temperature was 7.0° C. and when increased to 21.0° C. the scrotum relaxed. The relaxation continued for a few minutes after removing the heat, the reason being that the air surrounding the scrotum cooled more quickly than the scrotum itself. As the air in the immediate vicinity cooled the scrotum again contracted.
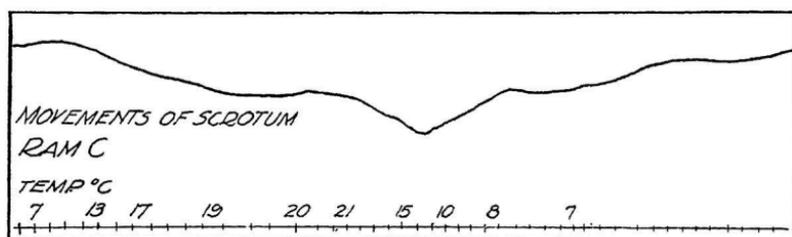
Fig. 16.—Effect of temperature increase on scrotum of Ram C. Temperature increased with electric heater. Time intervals are one minute.
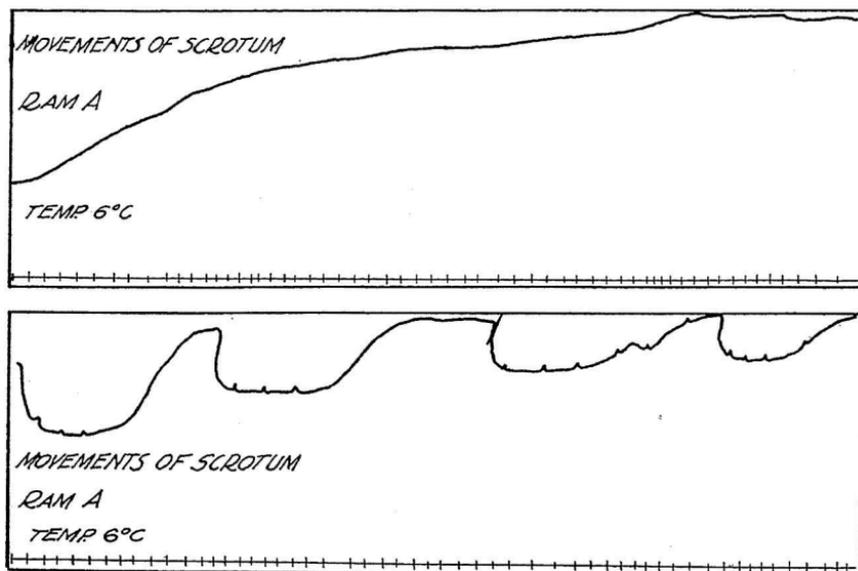
Fig. 17.—Consecutive records showing scrotal activity in Ram A at 6° C. Time in minute intervals.

Two consecutive records obtained from Ram A are shown in Figure 17. The room temperature was 6.° C. It will be noted that the scrotum first underwent a long wave of contraction. then underwent shorter waves of relaxation and contraction. After obtaining these records Ram A was castrated. Immediately after castration further records were obtained but no activity was noted. However, when the temperature in the vicinity of the castrate scrotum was increased to 26.5° C. with an electric heater

the scrotum relaxed, contracting again when the heat was removed. The record is similar to that shown in Figure 16.
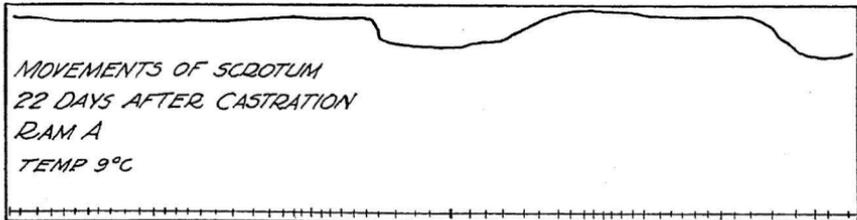
Fig. 18.—Record showing activity of the scrotum of Ram A twenty-two days after castration. Room temperature 9° C. Time in one minute intervals.

Twenty-two days after castration observations were again made on Ram A. The room temperature was 9.0° C. The castrate scrotum was found to be undergoing waves of relaxation and contraction as is shown in Figure 18. When the temperature in the immediate vicinity of the scrotum was increased to 26.5° C. with an electric heater, the scrotum relaxed.
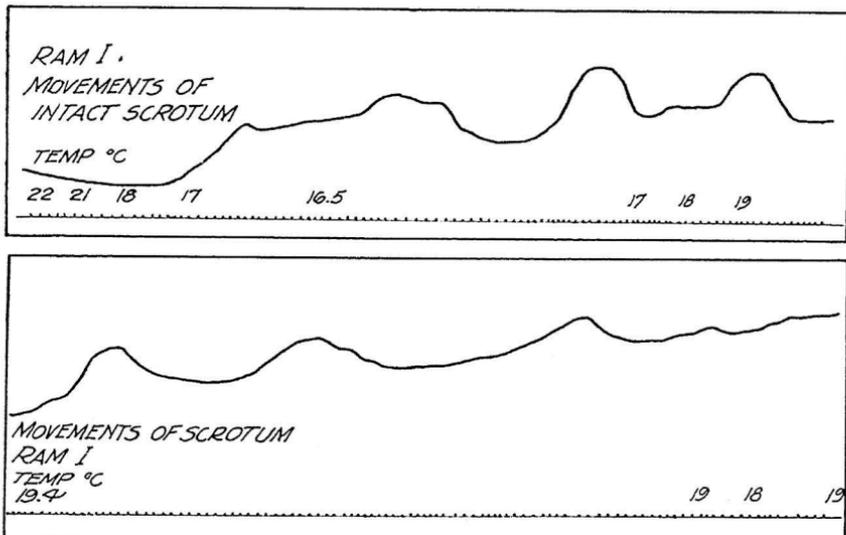
Fig. 19.—Consecutive records showing scrotal movements in Ram I. Room temperature changes are indicated. Time in one minute intervals.

Two consecutive records obtained from Ram I are shown in Figure 19. The room temperature at the beginning of the observation was 22.0° C. It was quickly changed to temperatures ranging from 16.5° to 19.4° C. (Quick temperature changes in records previously discussed were brought about by an electric heater and the change was only in the vicinity of the scrotum. In the present record and those which follow, the temperature of the entire room was controlled.) In Ram I, the scrotum was relaxed at 22.0° C. but when the temperature was lowered as was previously indicated, the scrotum contracted gradually during the two records shown, and at the same time underwent waves of relaxation and contraction. After these records, the room temperature was increased to 26.0-27.0° C. and the scrotum relaxed to some extent but had not relaxed to its original length when the ram began to revive after forty minutes at this room temperature.
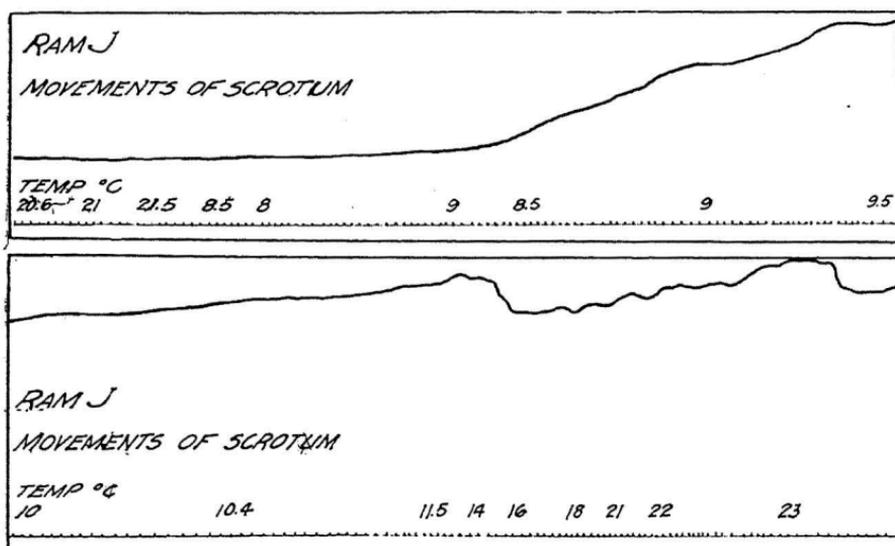
Fig. 20.—Consecutive records showing scrotal movements in Ram J. Room temperature changes are indicated. Time is in one minute intervals.

Two consecutive records obtained from Ram J are shown in Figure 20. The temperature at the beginning of these records was 20.6—21.5° C. and at these temperatures the scrotum was relaxed.

The temperature was lowered to 8.5-10.4° C. and the scrotum underwent a long wave of contraction. When the temperature
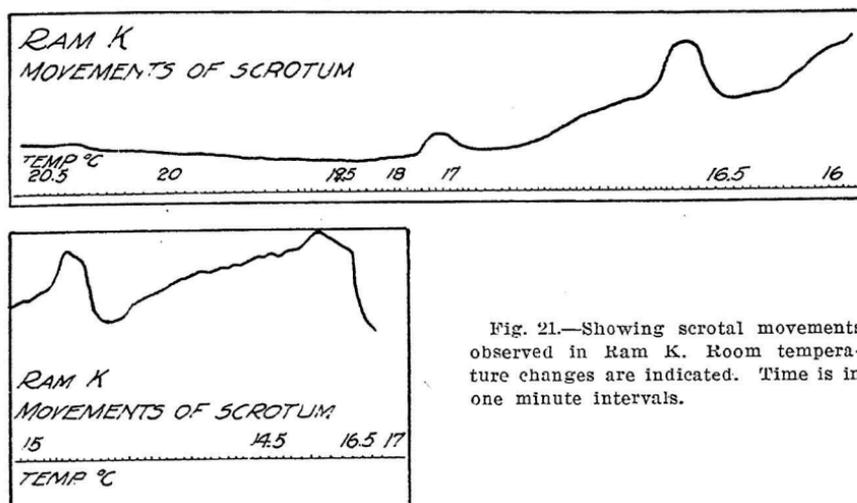
Fig. 21.—Showing scrotal movements observed in Ram K. Room temperature changes are indicated. Time is in one minute intervals.

was raised gradually to 23.0° C., the scrotum began to undergo small waves of relaxation and contraction, but at the end of these records was still in a contracted state.

Records obtained from Ram K are shown in Figure 21. At the beginning of these two records the room temperature was 20.0 to 20.5° C. and the scrotum was relaxed. The temperature was then lowered to 18.0 and gradually to 14.5° as is shown on the record. The scrotum underwent gradual contraction with waves of relaxation and contraction superimposed upon the gradual wave of contraction.

At another time the three consecutive records shown in Figure 22 were obtained on Ram K. Three lines besides the base line appear in these records. The upper line, marked A, was made by a recording lever attached at the point where the scrotum joins the body; the second line, marked B, was made by a recording lever attached midway between the point where the scrotum joins the body and the tip of the scrotum; the third line, marked C, was made by a recording lever attached to the tip of the scrotum. The object of this arrangement was to determine whether the movements observed were caused by general contraction and relaxation of the entire scrotum, or whether these contractions and relaxations were confined to one portion of the scrotum.
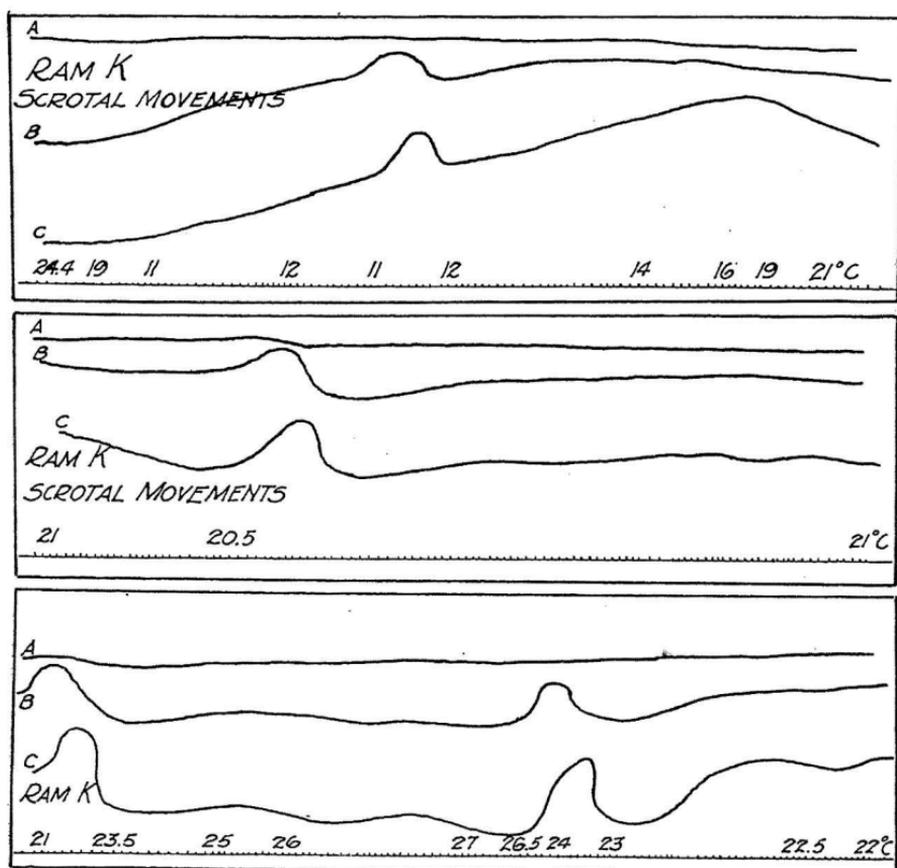
Fig. 22.—Consecutive records showing scrotal movements in Ram K. The upper line, (A), was made by a recording lever attached at point where scrotum attaches to body; lower line, (C), was made by recording lever attached to tip of scrotum; (B) was attached midway between (A) and (C). Room temperature changes are indicated. Time given in one minute intervals

The temperature at the beginning of the record in Figure 22 was 24.4° C. The temperature was lowered as may be seen on the record and a long wave of contraction set in, with one short relaxation wave superimposed. At a point preceding the short wave of relaxation the total altitude of the contraction of the scrotum had been 3.9 cm. and 3.7 cm. of this had been brought about by the upper half of the scrotum. At the peak of the long wave of contraction the total contraction altitude was 8.3 cm. and 5.1 cm. of this brought about by the upper half of the scrotum. The temperature was increased to 20.5-21.0° C. and remained so during the second record. The total relaxation from the highest point in the first record to the lowest point in the second record

was 7.2 cm. and the upper half of the scrotum was responsible for 4.0 cm. of this relaxation. During the third record the temperature was increased to 26.5° C. and the scrotum became relaxed. The amount of relaxation from the highest point at the beginning of the third record to the lowest point just beyond the middle was 5.8 cm. and the upper half of the scrotum was responsible for 3.2 cm. of this relaxation. Immediately following this low point the temperature was lowered to 23.0° C. A wave of contraction 3.8 cm. in altitude followed and the upper half of the scrotum was responsible for 2.3 cm. of this contraction.
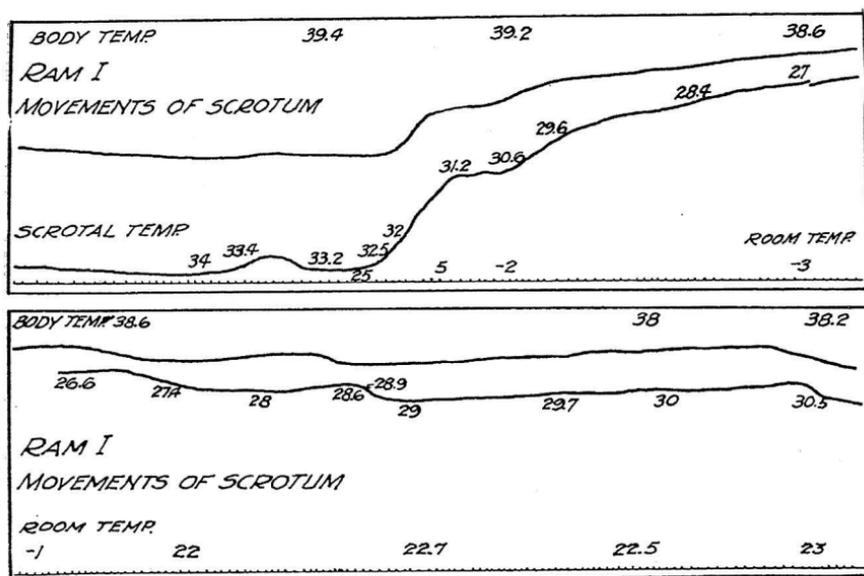
Fig. 23.—Consecutive records showing scrotal movements in Ram I. Lower line made by recording lever attached to tip of scrotum, upper line by lever attached at middle of scrotum. Room temperature changes indicated on base line, body temperatures at top of records, and scrotal temperature along lower line. Time in one minute intervals.

In Figure 23 records on Ram I similar to the ones described in the preceding paragraph for Ram K are shown. In the first record in Figure 23, a long wave of contraction is shown. The altitude of this contraction was 10.5 cm. and the upper half of the scrotum was responsible for 5.4 cm. of this contraction. At a point earlier in the wave of contraction, the total altitude was 5.6 cm. and 3.3 cm. of this was accounted for by the upper half of the scrotum. Records on these two animals are not sufficient to draw conclusions but they do indicate that the entire scrotal

wall is concerned in the movements described and that the upper half may be somewhat more reactive than the lower half.

Two consecutive records from Ram I are shown in Figure 23. In addition to room temperatures, body and scrotal temperatures are also shown. Body temperatures were obtained per rectum. Scrotal temperatures were obtained by inserting a thermometer through an incision in the scrotal wall and placing the bulb just beneath the tunica dartos on the opposite side of the scrotum. The thermometer used was graduated to one-tenth of a degree and was read with the aid of a reading glass to insure greater accuracy.

The slight decrease in body temperature during the observations was probably due to the anesthetic.

The room temperature was 24.2° to 25.0° C. at the beginning of the records shown in Figure 23 and the scrotum was relaxed at these temperatures except for one small contraction-relaxation wave. The scrotal temperature was between 33.2° and 34.0° C. The room temperature was then decreased to and held between −3.0 and −1.0° C. for some time. A long wave of contraction set in and at the same time the scrotal temperature decreased until after about one and one-quarter hours at this low temperature the scrotum was so shortened that the testes were held close up to the body and the scrotal temperature had recreased to 26.6° C. The room temperature was then returned to 22.0° to 23.0° C. After an hour and one-half at this increased temperature, the scrotum had relaxed only a small amount. In some of the records already described a similar condition has been noted. This slow response to increased room temperature is apparently explainable on the basis of the slow increase in scrotal temperature noted in Figure 23. In one and one-half hours after returning the room temperature to 22.0°, the scrotal temperature increased from 27.4 to 30.5° only and was still several degrees below the temperature recorded at the beginning of the first record.

A record from Ram K is shown in Figure 24. Room and scrotal temperatures were recorded. Throughout the first record and for twenty minutes in the second the room temperature was maintained between 21.0° and 24.0° C. It will be noted that during this time the scrotal temperature was maintained between 33.1° and 33.9° C. The relationship between the various scrotal temperatures and the length of the scrotum is shown clearly in this record. It will be noted that a drop in scrotal temperature of a fraction of a degree resulted in contraction. This contraction,
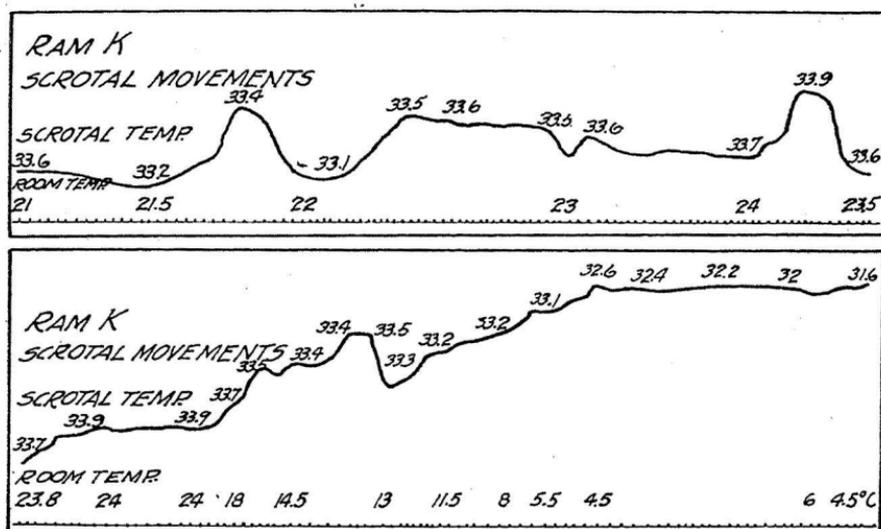
Fig. 24.—Consecutive records showing scrotal movements in Ram K. Room temperature changes are indicated on the base line. Scrotal temperatures are given along the line indicating scrotal movements. Time is in one minute intervals.

by drawing the testes nearer the body, lessening the surface area of the scrotum and thickening the scrotal wall, resulted in an increase in temperature. After an increase of a fraction of a degree in temperature the scrotum again relaxed. In the second record the temperature of the room was lowered gradually to 4.5° C., then maintained near that point during the remainder of the record. As soon as the scrotal temperature began to decrease, the scrotum began to contract and by this contraction maintained the scrotal temperature above 33.1° C. for a considerable time. When the scrotum had contracted until the testes were held against the body and no further contraction seemed possible, the temperature within the scrotum dropped still lower (to 31.6° C.).

A study of the records which have been described and of other records which have been obtained seem to indicate that the scrotum acts very much as a thermostat maintaining the testes at a fairly constant temperature. As has been indicated the scrotum of the ram is not capable of compensating completely for very low temperatures, and it was indicated that the scrotum could only relax to a certain extent so would not be able to compensate entirely for very high room temperatures. It would seem that when rams are maintained at temperatures of 22.0 to 24.0° C. or

above the scrotum remains relaxed, while at temperatures below about 5.0° C. the scrotum contracts and remains so. At temperatures intermediate between these points the scrotum seems to undergo constant adjustment, functioning to maintain a constant temperature. Reactions of the scrotum to room temperature changes are rather slow. This is to be expected for two reasons. First, the tunica dartos which is responsible for these reactions is composed of fibro-elastic tissue and smooth muscle (Sisson—1914) so reacts slowly. Second, the scrotum of the ram is usually covered with some wool and it is necessary for any temperature change to penetrate this wool and the scrotal skin before it can affect the tunica dartos. A study of the reactions of the dartos itself to temperature changes has been made and is described later in this paper.

Another point of interest has been observed in studying the intact scrotum. Two rams died while under observation and in both cases the scrotum contracted sharply almost immediately after respiration stopped. One of these records is shown in Figure 25. After the contraction, which lasted about five minutes, the scrotum relaxed almost as quickly and remained so. No explanation is given for this reaction.
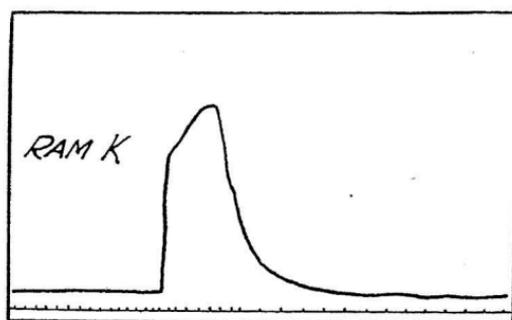

RAM K

Fig. 25.—Records showing reaction of scrotum in Ram K at death. Contraction began almost immediately after respiration stopped. Time in one minute intervals.

The scrotum has shown no reaction to such stimuli as pinching or piercing with a needle or to such severe stimulation as making an incision through the skin and dartos and inserting a thermometer.

The records which have been described in the preceding pages and other similar records that have been obtained are summarized briefly in Table 24. The records which have been previously described are indicated in one column of the table.

TABLE 24.—SUMMARY OF STUDIES OF THE SCROTUM OF THE RAM

Ram	Length of observations	Conditions and treatment	Movements observed in scrotum	Duration of each movement noted (mins.)	Altitude of each movement (cms.)	Figure no. indicates that that record is shown in text
A	3 hrs. & 20 mins.	Room temp. 12°-13° C.	Scrotum undergoing relaxation-contraction waves	15-25	3.6-5.8	
		Temp. increased to 37.5° C. (heater)	Scrotum relaxed	25	10.3	
		Temp. lowered to 12.5° C.	Scrotum contracted	25	9.3	
B	4 hrs. & 30 mins.	Room temp. 16°-20°C.	Many small contraction-relaxation waves	1-2	.3-1.0	Fig. 14
			Several larger relaxation-	10-20	3.6-5.5	
		Room temp. 22.5°-24°C.	Scrotum relaxed and remained so			
		Temp. increased to 40° C. (heater)	No further relaxation			Fig. 15
		Temp. lowered to 24° C.	Scrotum remained relaxed			
C	1 hr. & 30 mins.	Room temp. 6°-8° C.	Long wave of contraction	40	8 8	
		Temp. increased to 21°C. (heater)	Scrotum relaxed	20	5.0	
		Temp. lowered to 7° C.	Scrotum contracted	25	5.0	Fig. 16
A	4 hrs.	Room temp. 6° C.	Long contraction wave	60	9.0	Fig. 17
			Relaxation-contraction waves	10-16	2.5-5.9	
		Ram castrated	No movements observed			
		Temp. increased to 26.5° C. (heater)	Scrotum relaxed	23	7.0	
		Temp. lowered to 6° C.	Scrotum contracted	29	6.2	
D	3 hrs. (Scrotum very short and with little wool)	Room temp. 8°-9° C.	Gradual wave of contraction	110	6.1	
		Temp. increased to 28° C. (heater)	Scrotum relaxed	45	7.9	
		Temp. lowered to 9° C.	Scrotum contracted	20	5.2	
A	1 hr. & 30 mins. (22 days after castration)	Room temp. 9° C.	Scrotum undergoing relaxation-contraction waves	12-20	2.1	Fig. 18
		Temp. raised to 26.5° C. (heater)	Scrotum relaxed	14	5.6	

TABLE 24.—SUMMARY OF STUDIES OF THE SCROTUM OF THE RAM—(CONT'D)

Ram	Length of observations	Conditions and treatment	Movements observed in scrotum	Duration of each movement noted (mins.)	Altitude of each movement (cms.)	Figure no. indicates that that record is shown in text
I	1 hr. & 30 min.	Room temp. 16.8° C.; gradually increased to 21.5° C.	Scrotum relaxed gradually	55	2.8	
		Temp. lowered to 9.5° C.	Scrotum contracted	30	8.8	
J	4 hrs. & 15 mins.	Room temp. 20.6°-21.5° C.	No movements noted			
		Temp. lowered to 8°-11° C.	Wave of contraction	130	10.0	Fig. 20
		Temp. raised to 23° C.	Scrotum relaxed somewhat undergoing small relaxation-contraction waves but not to original level			
K	2 hrs. & 40 mins.	Room temp. 19.5°-20.5° C.	No movements noted			
		Temp. lowered to 16.5° C.	Wave of contraction	50	6.6	Fig. 21
		Temp. maintained at 15°-17° C.	Relaxation-contraction	30	3.4-4.8	
I	5 hrs. & 30 mins.	Room temp. 22° C.	Scrotum in relaxed condition			
		Temp. lowered to 17°-19° C.	Long wave of contraction	215	9.5	Fig. 19
			Small relaxation-contraction waves superimposed	20-30	1.3-2.5	
		Temp. raised to 26° C.	Relaxation wave with small relaxation-contraction waves superimposed	60	3.0	
		Temp. held at 26°-27° C.	Some relaxation but not to original level when ram revived	40		
K	6 hrs.	Room temp. 24.4° C.; lowered to 12°-14°	Long wave of contraction (Short relaxation superimposed)	100	8.3	
		Temp. raised to 20.5-21° C.	Gradual relaxation	145	5.0	
			Contraction-relaxation waves superimposed	10	4.0-4.8	Fig. 22
		Temp. raised to 25°-27° C.	Relaxation to same level as at beginning of observations			
		Temp. lowered to 22-24° C.	Wave of contraction	8	3.9	
			Relaxation-contraction waves	30	3.5	
I	1 hr. & 15 min.	Room temp. 24.5° C.	Contraction-relaxation waves	10-15	2.8-5.4	
		Temp. lowered to 14.5° C.	Wave of contraction (small contraction waves superimposed)	20	6.3	

TABLE 24.—SUMMARY OF STUDIES OF THE SCROTUM OF THE RAM—(CONT'D)

Ram	Length of observations	Conditions and treatment	Movements observed in scrotum	Duration of each movement noted (mins.)	Altitude of each movement (cms.)	Figure no. indicates that that record is shown in text
M	3 hrs. & 30 min.	Room temp. 16.4-18.2° C.	No movements noted			
	(55 days after castration)	Temp. lowered 13.5° C.	Wave of contraction (short relaxation wave superimposed)	30	2.7	
K	4 hrs. & 30 mins.	Room temp. 21°-24°C.	Contraction-relaxation waves	15-30	2.2-4.4	
		Temp. lowered gradually to 4.5° C.	Wave of contraction (short relaxation-contraction waves superimposed)	95	10.2	Fig. 24
		Temp. raised to 24° C.	Slight relaxation	70	2.7	
I	4 hrs.	Room temp. 24.2-25° C.	Scrotum in relaxed condition			
			One small contraction-relaxation wave			Fig. 23
		Room temp. lowered to 1.0° C.	Long wave of contraction	60	10.6	
		Temp. raised to 22°-23° C.	Slight relaxation	90	2.0	
K	1 hr.	Room temp. 23° C.	Scrotum in relaxed condition. Ram died. Sharp wave of contraction	5	9.2	Fig. 25
			Relaxation followed quickly	8	9.2	

Reactions of Isolated Strips of the Tunica Dartos to Temperature Changes.—Lieben (1908) gives the following description, from Eberth, of the tunica dartos: The tunica dartos is a smooth muscular coat consisting of two layers. The outer layer is very delicate, clings closely to the skin of the scrotum and has fibers arranged in various directions. The inner and by far the stronger layer consists of muscular fibers which for the greater part are turned in the direction from the anus to the root of the penis. In the region of the raphe the tunica dartos of each scrotal half turns over on the connective tissue-like dividing wall of the scrotum; thus each testis rests in an individual sac of smooth musculature.

Strips of the tunica dartos for study were removed from along the longitudinal axis of the scrotum. After removal the dartos was dissected from the skin and mounted in Ringer's solution with one end attached to a recording lever so that any movements might

be recorded. These strips were from one-half to three-fourths of an inch in length. Any movements are magnified 8X on the records.

The dartos strips used were obtained as follows: one from Ram A, three from Ram E, one from Ram F, three from Ram G. four from Ram H, one from J, making a total of thirteen strips from six rams. Each strip was subjected to several temperature changes. The reactions in all strips were essentially the same so all the records will not be described in detail.
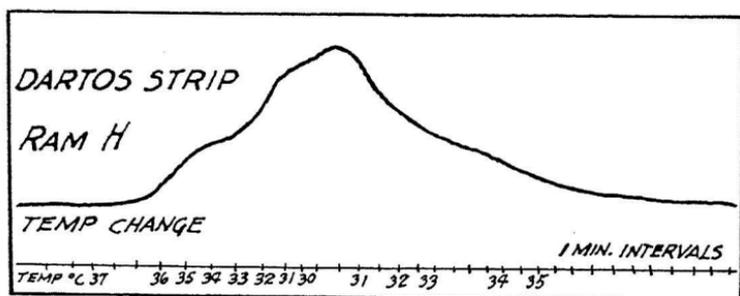

Fig. 26.—Reaction of a dartos strip from Ram H to temperature changes. Time in one minute intervals.

A portion of one record from a dartos strip from Ram H is shown in Figure 26. With a constant temperature of 37.0° C. the strip maintained a constant tone. When the temperature was lowered to 30° the strip contracted. The temperature was then raised to 36.0° C. and the strip relaxed, but not quite to the original level at 37.0° C.
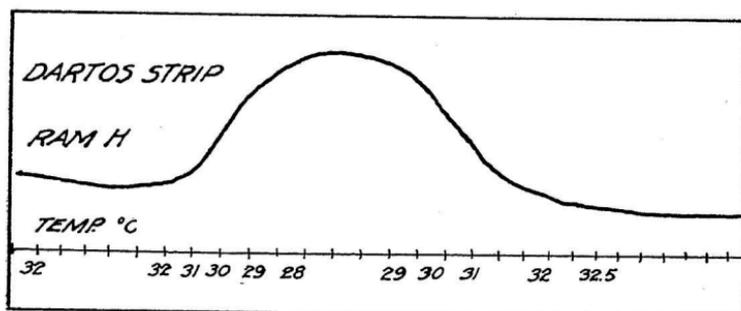
Fig. 27.—Reaction of a dartos strip from Ram H to temperature changes. Time in one minute intervals.

A portion of one record from another dartos strip from Ram H is shown in Figure 27. This is from the first part of the record and the slight relaxation at the beginning of the record was due to adjustment to the weight of the lever. When the temperature was lowered from 32.0° to 28.0°, the strip contracted sharply. When the temperature was increased to 32.5° C., the strip relaxed to a point somewhat below its original level at 32.0° C.
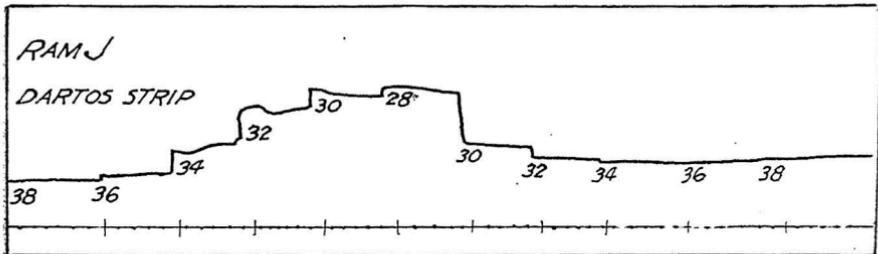

Fig. 28.—Reaction of a dartos strip from Ram J to temperature changes. Time in one minute intervals. Drum stopped for a few minutes at each change of temperature. Stops indicated by heavy marks along base line.

On one record obtained from a dartos strip from Ram J, the drum was stopped while each temperature change was being made thus building up the "stair-step" effect shown in Figure 28. It is interesting to note that the greatest contraction from a two degree temperature drop was between 34.0° and 32.0° C. The next greatest contractions from a two degree drop were the "steps" on either side of the one just mentioned, namely, 36.0 to 34.0° and 32.0 to 30.0° C. It will be remembered that the scrotal temperature of the ram, except at low room temperatures, is approximately 33.0 to 34.5° C. The amount of contraction between 38.0° and 36.0°, and between 30.0 and 28.0° was relatively small.

Another interesting feature of this record is the fact that when the temperature was increased from 28.0 to 30.0° C., the relaxation was much greater than was the contraction when the temperature was lowered from 30.0 to 28.0° C. The relaxations beyond that point were relatively small. It may be noted in several of the records on intact scrota that the scrotum contracted slowly, then relaxed at a comparatively rapid rate. This reaction might be explained on the basis of the record just described.

The number of dartos strips studied is admittedly small, and further study is necessary for a full understanding of the reactivity of this tissue to temperature changes. The studies thus

far, however, indicate clearly that the tunica dartos does not undergo spontaneous movements as many of the smooth muscle tissues of the body do, and that this tissue is very sensitive to temperature changes, contracting as the temperature decreases and relaxing as the temperature increases, within limits, of course. These findings further substantiate the idea, previously discussed, that the scrotum acts very much as a thermostat functioning to keep the testes at a fairly constant temperature.

TABLE 25.—HEAT RIGOR IN THE TUNICA DARTOS

Ram	Temperature °C.	Condition of Muscle	Altitude of movement described (cm.)	Time required for movement described (min.)	Previous treatment of strip
A	40.0	In relaxed condition			Pilocarpine
	52.0 to 58.0	Relaxed further	1.1	3	and
	60.0	Contracted	4.4	6	Atropine
G	40.0	In relaxed condition			Ephedrine,
	50.0 to 55.0	Relaxed further	.8	6	Pilocarpine
	59.0	Contracted	3.7	8	and Atropine
G	38.0	In relaxed condition			Ephedrine,
	50.0 to 57.0	Relaxed further	1.6	8	Pilocarpine
	60.0	Contracted	3.3	9	and Atropine
E	40.0	In relaxed condition			Ephedrine,
	50.0 to 56.0	Relaxed further	1.6	5	Pilocarpine
	58.0	Contracted	4	10	and Atropine
E	38.0	In relaxed condition			
	38.0 to 60.0	No further relaxation			
	60.0	Contracted	7.3	8	
E	40.0	In relaxed condition			
	40.0 to 58.0	No further relaxation			
	58.0 to 59.0	Contracted	9 0	8	

Six tunica dartos strips, obtained from three rams, have been subjected to sufficiently high temperatures to produce heat rigor. The data are presented in Table 25. All of the strips were apparently completely relaxed at 38.0 to 40.0° C. or above, up to 50.0 to 52.0° C. The first four strips listed underwent further relaxation after the temperature reached 50.0 to 52.0° C. and while it was being increased up to 55.0 and 58.0° C. This last relaxation was not noted in the other two strips, and these two strips had not been subjected to any drug treatments as are indicated for the

first four strips. Some effect of the drugs may have been responsible for this relaxation which took place above 50.0 to 52.0° C. In all the strips heat rigor set in at between 58.0 and 60.0° C. the average for the six strips being 59.25° C.

The Innervation of the Tunica Dartos.—Lieben (1908) briefly describes Langley's work in which it is reported that the scrotum contracts subsequent to irritation of the abdominal trunks, of the sympathetic system and upon irritation of the rami communicans from the first and second sacral roots.

Lieben (1908) undertook a further study of the innervation of the tunica dartos using the dog, and some observations on the human, and the results are described briefly. It was found that the scrotum normally exists in a tonus which slowly disappears when both abdominal sympathetic trunks are severed. If the scrotum were halved and subsequently one of sympathetic trunks severed the scrotal half on that side rapidly lost its tone, while no change was noted in the opposite side. In an uninjured scrotum electrical stimulation of either abdominal sympathetic trunk was found to produce rapid and intense contraction of the entire musculature of the tunica dartos, while only a one-sided contraction resulted in scrota which had been halved. Electrical stimulation of the rami communicans from the two first sacral segments resulted in scrotal contractions in all cases while irritation of the higher rami communicans was not so successful. Electrical stimulation of the cut spinal cord was also found to produce contraction of the tunica dartos.

In this study several isolated strips of the tunica dartos were subjected to the action of various drugs (adrenalin or epinephrine, ephedrine, pilocarpine and atropine) in an attempt to obtain some further knowledge of the type of innervation supplied to the tunica dartos. Descriptions of the physiological action of the drugs just mentioned are numerous. The brief account given below concerning their use in the study of the innervation of muscular tissues is taken from Howell (1927).

The effect of epinephrine or adrenalin is the same as stimulation of the sympathetic nerves, and may result in either contraction or relaxation of the muscle depending upon whether the sympathetic innervation is motor or inhibitory. It has been shown to exercise its action on the connections between the postganglionic fibers and the muscle. Adrenalin has no effect on the parasym-

pathetic system. Ephedrine is a compound similar in structure to epinephrine and having a similar physiological action.

The effect of pilocarpine upon the muscle is identical with stimulation of the parasympathetic nerves, while atropine exercises a sedative effect upon muscles innervated by the parasympathetic system. When pilocarpine is used, followed by atropine, the atropine counteracts the effect of the pilocarpine. Atropine apparently poisons out the parasympathetic nerve endings.

The effect of ephedrine was observed on six isolated strips of tunica dartos. The dilutions used varied from 1 part in 10,000 to 1 part in 500,000. In all cases a slow wave of contraction set in immediately after addition of ephedrine to the Ringer's solution in which the strips were held. A record from one of these strips is shown in Figure 29. When placed in fresh Ringer's solution these strips relaxed.

DARTOS STRIP

RAM G

TEMP 31°C
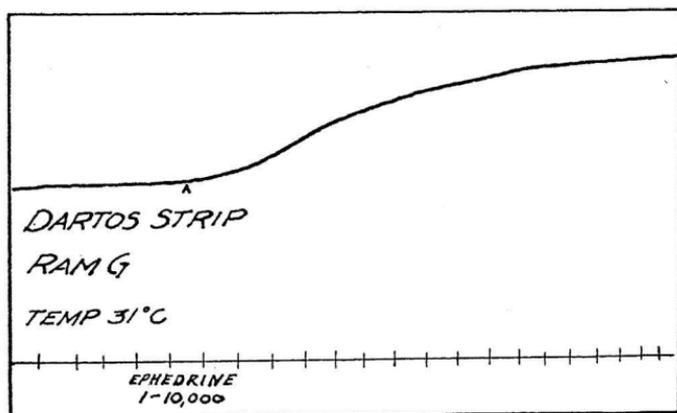
EPHEDRINE
1-10,000

Fig. 29.—Reaction of a dartos strip from Ram G to ephedrine (1 to 10,000) administered at point indicated on record just before wave of contraction set in. Heavy marks on base line indicate one minute intervals.

The effect of epinephrine (or adrenalin) was observed on only two strips of tunica dartos, dilutions of one part in 10,000, and one part in 80,000 being used. As may be noted in Figure 30, the contraction was much more rapid and of greater altitude than that resulting from ephedrine. Also these strips, after having been subjected to epinephrine, could not be returned to their original level by several changes of Ringer's solution.

Eight strips of the tunica dartos were treated with pilocarpine (dilutions of 1 to 10,000 and 1 to 100,000) followed by atro-
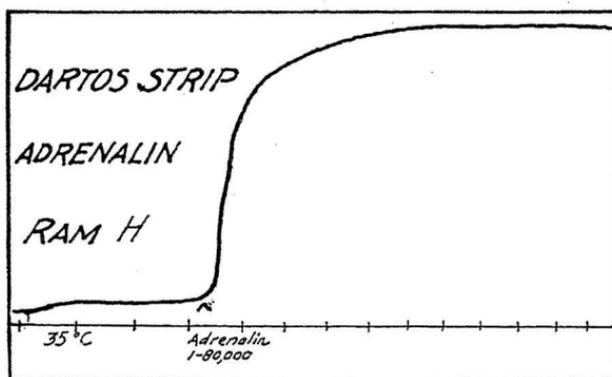
Fig. 30.—Reaction of a dartos strip from Ram H to adrenalin (1 to 80,000). Long marks on base line indicate one minute intervals.

pine (dilutions of 1 to 10,000 and 1 to 100,000). In all but one of the strips a slow wave of contraction set in when pilocarpine was applied. When atropine was applied the contraction stopped and the strips gradually relaxed to their original level. A record from one of these strips is shown in Figure 31.
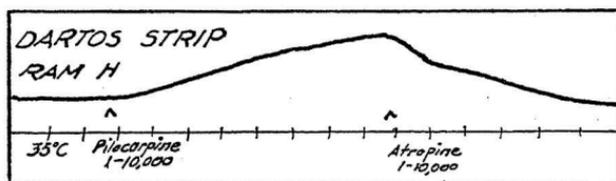

Fig. 31.—Reaction of dartos strip from Ram H to pilocarpine and atropine. Long marks on base line indicate one minute intervals.

The work done with drugs was very limited, but the results seem to indicate both a sympathetic and parasympathetic motor innervation of the tunica dartos of the ram. Further work is necessary, however, before these results could be accepted as conclusive.

VII. Temperature As a Factor in the Fertility of Rams

McKenzie and Phillips (1934) reported that rams ejaculating semen containing spermatozoa of which more than 140 per thousand were abnormal were usually of reduced or low fertility. In an earlier section of this paper it is reported that scrotal insulation was followed by a marked increase in the proportion of abnormal

spermatozoa ejaculated.   When insulation was continued for long periods an azoospermatic condition developed.   While the above work was in progress a few of the rams were allowed to breed ewes at various times before and during insulation.   At services before insulation, and after two, four and seven days of insulation the ewes were settled, but after thirteen or more days of insulation the ewes failed to settle.   The number of breedings was small but results give some indication of the quickness with which insulation affected fertility.

A very interesting case of the practical significance of the effect of temperature on fertility was presented by one ram that was observed.   Ram L, a purebred Shropshire and an excellent individual, was in show condition at the beginning of the 1932 breeding season.   Thirty-four ewes were field bred to this ram. At the end of two months none of the ewes had settled and the ram was removed from the flock.   Shortly after this time a semen sample was obtained (11-2-32) and subsequently several more samples were secured.   At the time of removal from the flock this ram was still quite fat and possessed a heavy fleece.

The types and numbers of abnormal spermatozoa observed in the semen samples secured from Ram L are given in Table 26. It will be noted that up to January 4, 1933 the abnormality count was quite high (648 to 838 per M).   On January 20, 1933 the ram was sheared, a heavy fleece being removed from both the body and scrotum.   Eleven days after shearing (January 31) the abnormality count was still 772 per M, but twenty-five days after shearing (February 14) the abnormality count had dropped to 468 per M, and thirty-four days after shearing (February 23) the

TABLE 26.—SHOWING NUMBERS AND TYPES OF ABNORMAL SPERMATOZOA IN SEMEN FROM RAM L. (IN NUMBERS PER THOUSAND)

Date of obtaining semen	Tailless heads	Coiled tails	Tapering heads	Enlarged middle pieces	Broken at neck	Broken middle pieces	Bent middle pieces	Total no. abnormal spermatozoa per M
11- 2-32	660	95	--	--	4	-	-	759
11- 8-32	576	248	--	--	14	-	-	838
11-17-32	248	390	2	2	6	-	-	648
1- 4-33	380	318	--	4	4	-	-	706
1-20-33	Ram	sheared						
1-31-33	274	482	--	4	12	-	-	772
2-14-33	220	240	2	--	6	-	-	468
2-23-33	74	76	--	--	8	-	-	158
3-16-33	50	104	2	2	4	-	-	162
4-20-33	128	176	2	--	14	-	-	320
5-18-33	302	336	--	--	6	-	r	644
7-20-33	444	156	56	20	16	-	-	692
8-28-33	770	24	8	4	8	2	2	818†

†Spermatozoa scarce.

abnormality count was 158 per M. By April 20, 1933, the fleece had again grown to over an inch in length and the animal was gaining in condition. At this time the abnormality count had risen to 320 per M, and on July 20 and August 28, 1933 the counts were 692 and 818 per M respectively.

Ram L was again sheared about September 1, 1933 and placed with a flock of forty ewes. Most of the ewes lambed and judging from lambing dates most of the ewes were settled during the latter part of October, so it is apparent that after shearing the testes again returned to normal spermatogenesis.

Body temperature readings on this ram are of interest. Three readings taken before shearing averaged 39.8° C. while five readings taken during the month following shearing averaged 39.1° C. A reading taken after the wool had grown out to about one inch in length was 39.7° C.

Ram M, also a purebred Shropshire, was observed during the 1933 breeding season. The ram was brought to the University of Missouri flock direct from the show circuit about September 1, 1933 and was sheared shortly afterward. At the time of arrival a semen sample was obtained which contained very few spermatozoa and showed an abnormality count of 548 per M. This ram was turned in with his flock after being sheared and semen samples obtained on October 6 and November 4 showed abnormality counts of 92 and 68 per M respectively. These abnormality counts are given in detail in Table 27.

TABLE 27.—SHOWING NUMBERS AND TYPES OF ABNORMAL SPERMATOZOA IN SEMEN OF RAM M (IN NUMBERS PER M)

Date	Tailless heads	Coiled tails	Tapering heads	Enlarged middle pieces	Broken at neck	Total abnormal sperm per M
9-1-33	488	50	4	4	2	548*
10-6-33	40	40	4	2	6	92
11-4-33	36	18	6		8	68

*Very few spermatozoa.

The breeding record of Ram M is as follows: He was turned with his flock on September 5, 1933. All ewes were served three times, and the first ewe lambed on February 28, 1934, so that the first service at which this ram settled a ewe was approximately October 4, 1933, one month after he was turned with his flock. Twelve of his fourteen ewes lambed at dates corresponding to breedings between October 4 and October 21, 1933.

It would seem that the scrota of Rams L and M, while under the handicap of a heavy fleece and high condition, were unable to maintain the testes at a temperature low enough to permit normal spermatogenesis. After removal of the fleece, and with some lowering of condition, the testes apparently were returned to a normal temperature and were then able to resume normal spermatogenesis.

SUMMARY

The literature concerned with scrotal function is reviewed and all the evidence clearly indicates that testes which are normally held in scrota do not continue their normal spermatogenetic function if they are placed in a warmer environment even for a short time. Recovery of testes so treated when returned to their normal environment is also reported. The evidence then clearly indicates a thermo-regulatory function of the scrotum whereby the testes are maintained at a temperature lower than that of the body cavity and at which the spermatogenetic tissue is able to function normally.

Further studies of this scrotal function, and a study of the mechanism by which it is accomplished are reported. The ram has served as the chief experimental animal, with some work being carried out on guinea-pigs and rats. The more significant findings are as follows:

(1) In the rams studied the body temperature readings averaged 39.8° C., scrotal temperatures at room temperatures of 13.0° to 24.0° C., averaged 33.3° C., and at these room temperatures the average testicular temperature was 34.9° C.

(2) The amount of insulation used in this work increased the scrotal temperature to 36.4° C. and the testicular temperature to 37.0° C.

(3) Ram scrota were insulated for periods varying from four days to sixteen weeks. In general, the longer the insulation period, the more marked the degeneration of the germinal epithelium found upon histological examination. No spermatozoon was observed in the tubules after periods of insulation longer than two weeks. A marked decrease in testis size was noted after sixteen weeks of scrotal insulation.

(4) Testes were secured from four rams after having been subjected to scrotal insulation for 1, 2, 4 and 8 weeks respectively,

followed by a three weeks recovery period. Partner testes removed at the end of the insulation periods were also studied. In the first ram degeneration was greater at the end of the recovery period than at the end of one week of insulation, while in the last three rams some recovery was noted, the recovery being less as the insulation period increased.

This is in accord with the findings of other workers, namely, that once degeneration is started, even by a brief application of heat, much of the germinal epithelium tends to break down before rebuilding sets in.

(5) Following scrotal insulation a marked change in the morphology of the ejaculated spermatozoa was noted, both the number and types of abnormal spermatozoa increasing considerably. With continued insulation very few spermatozoa were to be found in the semen after the 22nd day of insulation. Abnormal spermatozoa were observed in the tubules of one ram after one week of insulation.

(6) Assuming that the increase in abnormal spermatozoa ejaculated resulted from faulty spermatogenesis rather than from degeneration of fully formed spermatozoa, the appearance of the first increase in abnormal spermatozoa would indicate the time required for those spermatozoa to be transported from the testes to the ejaculatory duct. In five rams this time varied from 4 to 13 days, the average being 8.8 days. Three services were allowed every third day.

(7) A decrease in the length of time fully formed spermatozoa retain the power of becoming motile by subjection to abdominal temperatures has been reported. Would such treatment affect the morphology of the spermatozoa? Guinea-pigs were used in studying this problem, the spermatozoa from isolated epididymi and vasa deferentia, located normally and abdominally, being observed. There was no morphological evidence that the spermatozoa degenerated more quickly in the abdominally located tubes than in those located in the scrotum.

(8) The testes of rats were subjected to lower than normal temperature. No notable effect upon the germinal epithelium was observed. In studying scrotal temperatures in rams one observation was made after the animal had been exposed to a room temperature of −1° to −3° C. for approximately 45 minutes and the scrotal temperature dropped to 26.6° C. Such low temperatures,

or even lower, must be common among breeding males in cold climates, yet do not lead to breeding troubles; so it would seem that the testes are less susceptible to injury at lower than at higher than normal temperatures.

(9) The physiology of the tunica dartos of the ram has been studied and it has been found to function very much as a thermostat would, furnishing the mechanism by which the thermo-regulatory function of the scrotum is accomplished. Significant findings concerning the tunica dartos of the ram are:

(a) At low temperatures the intact scrotum is contracted, holding the testes close to the body, while at high temperatures it is completely relaxed. At intermediate temperatures (6.0° to 24.0° C.) there is constant adjustment, the tunica dartos contracting and relaxing as a result of very small decreases or increases in scrotal temperature.

(b) Isolated tunica dartos strips react quickly to temperature changes, contracting as the temperature decreases and relaxing as it increases. It seems most sensitive to changes at temperatures approximating the normal scrotal temperature.

(c) Reactions of isolated tunica dartos strips to adrenalin, ephedrine, pilocarpine and atropine have indicated motor sympathetic and parasympathetic innervations.

(d) Isolated strips of the tunica dartos have gone into heat rigor at 58.0° to 60.0° C., the average being at 59.25° C.

(e) In two cases when animals died while being observed the intact scrotum contracted sharply immediately after breathing stopped, and became completely relaxed a few minutes later.

(10) Two rams were observed in which the testes, under the handicap of high condition (fat) and a heavy covering of wool, were unable to continue normal spermatogenesis. After removal of the fleece, with some coincident lowering in condition, the testes regained normal spermatogenetic function. The condition in these rams parallels that observed in rams subjected to increased scrotal temperatures by scrotal insulation.

CONCLUSIONS

1. The literature reviewed and material presented clearly indicate a thermo-regulatory function of the scrotum.

2. The immediate cause of degeneration of the germinal epithelium following an increase in scrotal temperature is still uncertain. The evidence to date would seem to indicate an indirect effect, perhaps vasostagnation and carbon dioxide accumulation, due to the increased temperature.

3. Testes are apparently less sensitive to a decrease than to an increase in temperature.

4. Abnormal spermatozoa that appear in the semen of rams following scrotal insulation apparently result from changes in the morphology of spermatozoa developing at the time of insulation rather than from changes in fully formed spermatozoa.

5. The time required for the passage of spermatozoa from the testes to the ejaculatory duct varies in different rams. The average time required in rams observed was 8.8 days, the ram being allowed three services every third day.

6. The scrotum of the ram functions very much as a thermostat maintaining the testes at a fairly constant temperature considerably below that of the body cavity. The tunica dartos muscle provides the mechanism by which this function is accomplished.

7. A heavy fleece and high condition (fat) frequently results in lowered fertility or in sterility in rams fitted for shows and fairs. Two cases observed seemed to parallel the condition in rams subjected to scrotal insulation so it would seem that the scrota of these two rams were unable to maintain the testes at a sufficiently low temperature for spermatogenesis while under the handicap of heavy fleece and high condition.

8. Rams of lowered fertility due to heavy fleece and high condition (fat) apparently recover normal spermatogenetic function quickly if relieved of the fleece and allowed some coincident lowering of condition. This should be of considerable practical value to sheep breeders, especially those in warmer climates.

BIBLIOGRAPHY

Barron, Donald H. *The Trophic Influence of the Spermatic Nerve of the Testicle.* Anat. Record, v. LV (1933), Supplement, p. 6.

Crew, F. A. E. *A Suggestion as to the Cause of the Aspermatic Condition of the Imperfectly Descended Testis.* Jour. of Anat. v. LVI (1922), pp. 98-106.

Fukui, N. *On a Hitherto Unknown Action of Heat Ray on Testicles.* Japan Med. World, v. III (1923), pp. 27-28.

Fukui, N. *Action of Body Temperature on the Testicle.* Japan Med. World, v. III (1923), pp. 160-163.

Griffiths, Joseph. *Structural Changes in the Testicle of the Dog When it is Replaced Within the Abdominal Cavity.* Jour. Anat. and Physiol., v. XXVII (1893), pp. 482-499.

Heller, Richard E. *New Evidence for the Function of the Scrotum.* Physiol. Zool., v. II (1929), pp. 9-17.

Howell, William H. *Textbook of Physiology.* Ed. 10, Philadelphia and London, W. B. Saunders Co., 1933.

Lieben, S. *The Physiology of the Tunica Dartos.* Arch. f. d. ges. Physiologie, v. CXXIV (1908), pp. 336-352.

Moore, Carl R. *Cryptorchidism Experimentally Produced.* Anat. Record, v. XXIV (1922), p. 383.

Moore, Carl R. *On the Relationships of Germinal Epithelium to the Position of the Testis.* Anat. Record, v. XXV (1923), p. 142.

Moore, Carl R. *Testicular Reactions in Experimental Cryptorchidism.* Amer. Jour. Anat., v. XXXIV (1924), pp. 269-316.

Moore, Carl R. *Heat Application and Testicular Degeneration; The Function of the Scrotum.* Amer. Jour. Anat., v. XXXIV (1924), pp. 337-358.

Moore, Carl R. *The Activity of Displaced Testis and Its Bearing on the Problem of the Function of the Scrotum.* Amer. Jour. Physiol., v. LXXVII (1926), pp. 59-68.

Moore, Carl R. *Scrotal Replacement of Experimentally Cryptorchid Testes and the Recovery of Spermatogenetic Function (Guinea-pig).* Biol. Bul., v. LI (1926), pp. 112-128.

Moore, Carl R. and Oslund, Robert. *Experiments on Sheep Testis.* Amer. Jour. Physiol., v. LXVII (1924), pp. 595-607.

Moore, Carl R. and Quick, Wm. J. *The Scrotum as a Temperature Regulator for the Testes.* Amer. Jour. Physiol., v. LXVIII (1924), pp. 70-79.

McKenzie, Fred F. and Phillips, Ralph W. *Measuring Fertility in the Ram.* Jour. Amer. Vet. Med. Ass'n., v. LXXXIV (1934), pp. 189-202.

Oslund, Robert M. *The Physiology of the Male Reproductive System.* Jour. Amer. Med. Ass'n., v. XC (1928), pp. 829-830.

Phillips, Ralph W. *Observations on the Spermatozoa of the Ram and Their Application to the Determination of Fertility.* Master's Thesis, University of Missouri, 1931.

Ritzman, E. G. and Benedict, F. G. *The Heat Production of Sheep under Varying Conditions.* Technical Bul. 45, (1931), New Hampshire Agr. Exp. Sta., Durham, New Hampshire.

Simeone, Fiorindo A. and Young, Wm. C. *The Fate of Non-Ejaculated Spermatozoa in the Genital Tract of the Male Guinea-pig.* Jour. Exp. Biol., v. VIII (1931), pp. 163-175.

Sisson, Septimus. *The Anatomy of the Domestic Animals.* Ed. 2, Philadelphia and London, W. B. Saunders Co., 1914.

Toothill, Martha C. and Young, Wm. C. *The Time Required by Spermatozoa in Passing Through the Ductus Epididymidis of the Guinea-pig as Determined by Means of India Ink Injections.* Anat. Record, v. L (1931), pp. 95-107.

Young, William C. *The Influence of High Temperatures on the Guinea-pig Testis.* Jour. Exp. Zool., v. XLIX (1927), pp. 459-499.

Young, William C. *Is the Attainment of Full Spermatozoon Maturity Attributable to Some Specific Action of the Epididymal Secretion?* Jour. Morph., v. XLVII (1929), pp. 479-495.

Young, William C. *Functional Changes Undergone by Spermatozoa During Their Passage Through the Epididymis and Vas Deferens.* Jour. Exp. Biol., v. VIII (1931), pp. 151-162.