THE TURNING OF THE SPERM IN THE ORTHOPTERAN FOLLICLE.

by

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INTRODUCTION

A vast amount of work has been done upon the spermatogenesis of practically all animal forms with varied and diverse results. However, the progress on the whole has been remarkable. For the study of germ cells among all life forms, no more favorable material than that of the Orthoptera has been obtained. Specimens are collected from almost any vicinity. The size and arrangement of the cysts, together with the size of the germcells, make the Orthopteran material one of the most favorable for cytological research.

Possibly, a few words of history along this line may not be out of order. It was in the study of an Orthopteran in which Carnoy in '85 first figured and described the maturation divisions of male germ cells, while in '83 Van Beneden set forth the idea of the haploid number of chromosomes. St. George in '86 described the spermatocyte divisions in Blatta, Wilcox in '95 and in '96 gave a detailed account of metamorphosis of the spermatid in Melanoplus. McClung in '99 discovered the sex chromosome which he named the "accessory chromosome". This was later confirmed by Wilson '05. McClung in a later work '00 described maturation division of Hypiscus, noting the type shapes of the chromosomes.

Sinety, 1901, found that the sex chromosome did not divide in the first spermatocyte division, Sutton found that the chromosomes were paired as to size, later deciding with Montgomery that one of a pair was paternal while the other was maternal. Baumgartner, '02, gave a full account of metamorphosis of spermatids in Gryllus and in '04 showed that the sex chromosome in the spermatogonia is V shaped. In the same account he set forth the fact that the individual chromosomes have individual morphological chracteristics, a fact later confirmed by Wilson and others. Montgomery, '05, set forth the fact that the first maturation division is the reduction division. In 1908 McClung found that the accessory or sex chromosome becomes attached to a bivalent chromosome in the spermatogonial and also in the prophase of the first spermatocyte divisions. Also, there are many other works along this line, but this is enough to give some idea of the great amount of research in the field of spermatogenesis. During the same period of time, as much work has been carried on in the field of fertilization and embryology with as satisfactory results.

It seems that throughout all the researches and investigations upon both spermatogenesis and fertilization, that no mention is made of the movement of the sperm, nor of the spermatid in the follicles except in one or two instances as a matter of comment, and not as a matter of

definite research. Davis, '08, in his work on the spermatogenesis of Dissosteira shows in a text figure the position of the spermatid heads. The sperm heads all point toward the closed end of the follicle, but he does not take up the movement of either the spermatids or the sperm. This figure of Davis has been used by Wilson, '25, Depdola, '28, and others in their works but in the author's study and research, he was unable to find that such an arrangement of sperm indicates all the facts even in Dissosteira, but Davis was placing emphasis upon spermatocyte divisions rather than sperm movements. Bowen, '22, in his studies of Hemiptera, made mention of the spermatids with the heads arranged toward the open end of the follicle but he gives no detailed account of their movements. Baumgartner, '30, in his work on Nemobius fasciatus, calls attention to the fact that he found the sperm "turned early" toward the open end of the follicle and offers a comparative study of his figures with the figures of Davis along this line. He does not agree with Davis.

It was at the suggestion of Dr. Baumgartner that the author, when selecting a problem for research, took up the present work in a study of both fixed and living tissue.

The author has the pleasure of expressing his thanks to Dr.H.H.Lane, head of the department of zoology, for

advise and opportunities in the laboratory, and to Dr. W.J.Baumgartner, professor of zoology, for suggesting the problem, for his continued assistance and much-helpful criticism, also to Sr. Anthony Payne, O.S.B. an associate graduate student of this department, for her assistance and co-operation especially in the making of the photo-micrographs.

MATERIAL AND METHODS

The following investigations are based upon material mostly from two species of Orthoptera, Arphia and Melanoplus. Mostly, adult individuals of different ages were used, although comparative studies were made on extremely young individuals to note the development of the germ cells and cysts within the follicles.

Collections of both male and female specimens were made on the campus of the University of Kansas at different times throughout the fall and winter whenever possible. The male specimens were used for the study of the follicles, the female specimens for the study of the sperm behavior within the sperm receptacles. This material was used either in the fixed form, or as living tissue and proved to be very satisfactory in either case.

In each instance the testes were dissected out in .75% saline or Ringer's solution, and in the study of fixed material, placed immediately in strong Flemming for

twenty-four hours, stained in Heidenhains haematoxylin and mounted in balsam.

In the study of living tissue the intra vitam methods of Baumgartner and Anthony, '30, were followed. The etherized specimen was fastened to the slide with melted paraffin, a ring of the paraffin was run around the specimen to fasten it to the slide, and a ring 10 mm. in diameter was run on the slide to hold the Ringer's solution, being careful that the breathing tubes were not closed. It was necessary to cut off the wings and the larger appendages so that the specimen should have no means of moving its body. After makeing a slit either dorso-median or latero-median, the testes were partially drawn and the testicular membrane removed, thus leaving the two clusters of follicles and the two vasa deferentia uncovered but connected with the specimen. It is well to disturb the specimen as little as possible in the operation. The specimen if properly cared for may live for forty-eight hours. To do the work under the water immersion lens, it is necessary to keep the follicles submerged in Ringer's and in some instances, where the tissues were continually moving, it was necessary to fasten the follicles across the glass with a fine silk thread in order to quiet the motion caused by the heart beats and respiratory movements of the specimen. Especially was this true in some

instances when photomicrographs were taken of the moving clusters. The observations were made either with low power or with the water immersion lens in Ringer's solution and photos were taken in several instances as may be noted in the plates. The observations were made with a Leitz binocular of from 100 to 1200 diameters magnifications. The photomicrographs were made with a Zeis microscope with water immersion of 1200 magnifications in the labratory of Dr. W.J. Baumgartner.

For the sake of clearness it is well to explain the terms used in this work. The word "zone" refers to a portion of the follicle, while "dark belt" refers to a dark belt in the membrane around the follicle separating the two zones indicated below. The term "sperm cluster" is used to denote all the sperm of a single cyst which have gathered into a cluster.

The photomicrographs were made by electric light as were also some of the observations. Some of the observations were made by daylight with very satisfactory results.

OBSERVATIONS

1. Structure of the follicles.

This description of the testis, while it is based primarily upon Arphia and Melanoplus, will apply equally to all Acrididae since the structure is essentially the same throughout the entire family.

The testes lie in the dorsal part of the abdomen from the fourth to the sixth segments and are so closely apposed as to present, upon superficial examination, the appearance of a single organ. Each follicle is covered with a delicate connective tissue membrane and each testis is covered with a delicate three-layered orange colored membrane, the middle layer of which is alveolar in structure, making it spongelike and so serves as a pad or protection to the testis against the body wall and the internal organs.

The follicles are approximately the same length and lie nearly parallel to each other. They are cylindrical in shape, sac-like in form, closed at the distal end, with the larger diameter in the vicinity of the spermatocyte region. The walls of the follicles are connective tissue in part and stain dark with Heidenhains iron haematoxylin. A careful examination shows that the cyst walls do not grow out from the follicular wall, though not fused with it, fig. 1-b. This allows for the probable movement of the cyst along the follicular wall from the distal toward the proximal end, as the cysts are pressed downward due to the growth of the germ cells in the distal end.

The follicles open into the vasa efferentia at the proximal end, and these vasa efferentia open into the vas deferens which leads from the anterior end of the testis back-

ward toward the posterior end of the abdomen where it is connected with the copulatory organs.

a. Fixed Material

In a longitudinal section through the center of the follicle in fixed material, the cyst wall arrangement shows a network of partitions extending out from the follicle wall, and separating the germ cells into clusters. Fig. 1. All the cells in one cluster or cyst show the same stage of development. In the distal end of the follicle these partitions project more or less at right angles while the cyst walls farther down the follicle become bulged toward the open end. Later they bag downward more and more and eventually they come to lie almost parallel with the follicular wall. The long narrow ends of the cyst lead toward the center of the follicle and the wall remnants seem to be loose and distorted.

A remnant of connective tissue may be noted as extending from an elongated cyst wall down the center of the follicle and possibly represents a mass of extended cyst wall remnants, and has been seen to extend in some instances almost to the open end of the follicle. The other remnant of the cyst wall may be observed to lie along side of the follicle wall in the loose or sperm region, while in the

spermatid region, c, this cyst wall may be seen extending outward at an angle to the follicle wall, depending upon its position in the follicle. It serves as a partition between the clusters of cells.

In the spermatid region c, the cysts tend to extend downward in the center of the follicle. This extension of the cyst walls continues well down the follicle and finally the cyst walls disintegrate in the lower part of the follicle. The clusters of spermatid heads are pressed toward the periphery of the follicle with the extension of the cyst wall above. The follicle below the spermatid region contains a fluid granular mass as well as loose cells and tail filaments. In this region there is a core of cyst wall tissue down the center of the follicle for some distance.

Figure 1, h, represents a diagonal section of what appears to be sperm heads, no doubt these are out of their natural position. Similar observations have no doubt been made by other observers but the author has as yet found no explanation for this in fixed material. Both the double wall arrangement and the diagonal section of sperm may be considered results of faulty technique. It is easily observed that the diagonal sections of sperm are between the follicle wall and the cyst wall remnants.

In the same figure may be noted the spermatid heads, g, pointing toward the closed end of the follicle and farther down the heads of the sperm clusters are still pointing toward the closed end of the follicle, j. They are in the periphery of the follicle between the follicle wall without, and the cyst wall, m within. It may be observed that the cyst wall, n, extends well down the follicle, where it seems to disappear. In the center of the follicle at k, may be seen a small cluster of cells which seems to be in the remnant of cyst walls. At l, in the same figure may be seen cells, which have every appearance of the "sertoli cells" of Wilson '25 and of Charlton '21. These cells stain darkly and may be seen scattered throughout some follicles. They remain dark when stained with Heidenhains. Just below this mass of cells, in the lower region is a granular fluid mass, o, in which tail filaments may be seen. This substance continues into the vasa efferentia. At m, there may be noted a sperm cluster with the heads pointing toward the open end of the follicle. The follicle wall at g, is seen to be thin and pliable while ar r, it is thicker and less pliable.

Figure 1', which represents a cross section of a follicle of fixed material in the spermatid region, shows a sperm cluster b, in the periphery between the follicle wall a, and the cyst wall c. This cluster certainly is not the position where it originally developed. This position and similar positions of sperm have no doubt been noted by other

observers but no explanation for such positions has as yet been made. These could scarcely be errors of technique, Unless the movements in living tissues have been carefully studied, an interpretation of this figure is rather difficult.

b. Living tissue.

Figure 2, represents a follicle of living tissue in Ringer's solution under the low power. The specimen is rather young as the follicle contains no sperm, but is filled with cysts in different stages of development, with the more mature cysts toward the open end which is as yet unopened. All follicles of the testis show the same relative stage of development, the cysts of each follicle are found to be individual sacs and are independent of each other in development, while all the germ cells in each cyst are in the same stage of development. The general shape of the follicles is cylindrical, tapering toward the distal end which is closed. The largest diameter is in the vicinity of the spermatocyte divisions. The follicle is clear in color, with a slight yellow tinge, and the differences in density of the different intra follicular tissues cause them to be clearly shown. Even the chromosomes of the germ cells are clearly delineated. Every part of the follicle is soft and pliable and responds quickly to any change of pressure

while the entire space within the follicle is filled and the follicle is turgid throughout since no cysts have opened. This figure represents an unopened follicle of about the fourth instar. There are no opened cysts, hence there is no dark belt present, as the dark belt is always noted between the turgid and loose zones.

At a, is the apical cell of Davis '08. This cell could not be seen in all specimens, but when found in one follicle, it could be found in all follicles of that specimen. According to Robertson '30, this cell is somatic tissue as shown by its stain. At b, and at e, are cysts of cells in the first and second spermatocyte divisions respectively. The thick walls of the follicle may be noted at f, They are pliable, true membranes, slightly thicker near the proximal end. Just within the wall, may be seen a cyst of spermatids, ff, the most mature cyst of the entire follicle. The cyst walls are clear and distinct, and conform to such shapes as will occupy the entire space within the follicle.

At g, may be seen a protoplasmic plug closing the opening of the follicle into the vasaefferentia. This is not removed until the copulative or mating stage has been developed.

Figure 3, represents another follicle of living tissue in which the loose zone d, occupies the greater

part of the follicle. C, represents the turgid zone and b, denotes the dark belt in the follicle wall where the cysts have begun to extend down the follicle. Just below this region the clusters have gathered and are in the periphery, all pointing toward the closed end. Farther down the follicle, sperm clusters are moving up and not in a straight line. At g, may be seen two clusters of heads which have turned or oriented farther up the follicle, probably near the dark belt where the cyst walls are turgid. They are about to enter the opening into the vasa efferentia. At f, loose cells are noted, in many observations large masses of these cells were noted throughout the loose zone.

2. Spermatids.

a. Transformation.

The germ cell in passing through the spermatid transformation stage presents several points of extreme interest Bowen '22, separated the transformation stage in to several different phases explaining each phase and following each element of the germ cells carefully in its movements and changes. This suggestion as to the phases has not been generally followed by the other authors and since it is not within the scope of this paper to give a detailed account of the metamorphosis, It is enough that the spermatid in trans-

formation gradually changes from a spherical form with a large pinkish colored nucleus and clear cytoplasm, to a fully elongated sperm. Figure 6 represents a spermatid of living tissue from an opened cyst in saline. It measured seven microns in length at this stage of development. The acrosome a, is a denser darker colored cap at the top of the nucleus. The cytoplasm d, had become elongated surrounding the axial filament ax, which extended about two-thirds the length of the cytoplasmic material, d and well around one side of the nucleus. It was much denser than the surrounding tissues. Along side of the axial filament was a dark granular cone-shaped body, c, extending from the base of the nucleus. It possibly was a remnant of the nebenkern. The axial filament ax- was denser than the cytoplasm. The elongated cytoplasm d, was flattened with concave sides and rounded edges

b. Movement.

The transforming spermatid is quite motile as a living entity. The elongating tail of cytoplasm had a backward-forward rotary movement as shown by the arrows at e, near the end of the tail. The cross section f, gives an idea of an edge view of the tail at this stage. This movement was fairly rapid, about three seconds each and was continous as it rested suspended in the saline solution. The spermatids gradually gather into a cluster

from the scattered position to a point in the uppermost part of the cyst where the points or perforatoria became imbedded in a dense drop of protoplasm, (see photomicrograph figure 29,a.). Cysts of elongating spermatids may be observed in figure 1, c to g. In this instance the cyst extends down the follicle.

3. Sperm Movement.

a. In the follicles.

In figure 4, a follicle of another specimen, is shown in which there are several head clusters moving up the follicle. These clusters have moved spirally around the follicle as noted by the general positions of the tail filaments, and the heads may be seen on the ventral side b while the tail filaments have not spread far from the path of the head cluster.

Figure 5 represents a series of drawings of a follicle of an old specimen since the dark belt a is near to the closed end. At 5:02, by the clock, two clusters of sperm were noted to be moving across the ventral side, below the dark belt a, which marks the division between the turgid and loose zones. At 5:10 one cluster was seen coming into view with the second cluster just behind, while at 5:14 the second one had come well into view. At 5:18 and 5:26 both clusters were crossing the dorsal side of the follicle and at 5:32 the foremost cluster started across the ventral side of the follicles. It may be easily

noted that the clusters were moving just within the follicle wall and between the follicle wall and the cyst walls. Also, that one cluster was a little in advance of the other and that they were at slightly different angles to each other. They moved independently of each other. Z denotes the correction point used to determine whether the follicle was moving rather than the sperm turning. Readings were taken in each instance with regard to these corrections and at the time indicated in the margins. A small nest of cells b, was carefully observed. They were not germ cells and were not in the tissues of the walls.

Plate IV shows a series of figures denoting the several positions of a moving cluster of sperm in a living follicle in Ringer's. The material, except the cluster, is drawn in outline. To be more certain that the cluster was moving, z, a position between two cyst membranes, and y, a gathering cluster of developing sperm, were used as correction points. These points remained fixed throughout the series of observations. The movements were carefully checked as noted in the margins. This cluster no doubt had come from some point farther down the follicle where it had matured into a more motile stage, and from the appearance of the tail filaments it had passed the point y, where it began to turn diagonally across the follicle. The author's first observation was taken at a point marked

5:02. At 5:16 it had crossed approximately one-half the follicle, at 5:25 still farther, and at 5:35, it had reached the opposite wall and had turned. Figure 8 shows its position at 5:47 while figure 9 shows its position at 6:14. It was at points 8 and 9 that the author was able to observe its position with reference to the follicle wall and the intra follicular mass, passing between them. It kept close to the wall and figure 10 shows its position at 6:50 still pointing forward while figure 11 at 6:57 shows the point of the cluster pointing directly across the follicle on the ventral side. The movement of the cluster seemed to slow down, due possibly to the effects of the saline solution or to loss of vitality, or to the fact that the movements are not constant. Figure 12 at 8:15 shows the final position in which the author was able to observe it. The point of the cluster, though not clearly delineated, had not separated and had started backward toward the open end of the follicle. All these observations were made in the loose zone just below the dark belt which marks the limit of the turgid zone of the follicle.

In figure 13, the specimen is no doubt an old one since the dark belt separating the two zones has moved well toward the closed end of the follicle. At d, may be seen the head cluster in the periphery of the follicle. this cluster probably has just begun to move. At b, is another cluster turning against the dark belt, while at

c, are two clusters that have oriented and have started toward the open end. The other clusters which are not moving, have their heads toward the closed end. Throughout the loose zone of the follicle free granular cells may be seen while at the open end may be seen a mass of loose dark granular cells. In other observations clusters were seen in the vas deferens.

Figure 14 represents a living follicle with an orienting sperm at b, and at c a cluster returning. The orienting sperm is just below the dark belt. The other sperm clusters are not in motion. All these clusters were found to be in the periphery of the follicle just beneath the follicle wall. In this instance no large loops of the tail filaments were noted as were found in several other specimens. A comparative study of Figures, 14 and 14', shows that the spiral movements were either right or left.

Figure 15 represents a follicle in which the movement of the cluster was timed. The time was checked at 7:40. It followed a spiral path in the periphery of the follicle until 8:32 when it stopped moving. In this path the cluster passed one-half way around the follicle. The tail filaments spread out over the intra follicular mass

and did not follow directly the path of the cluster. The waves or loops of the tail filaments became larger as it advanced. (See also figures 19-21). At 8:32 the loops extended entirely across the follicle. At c in the same figure, is another cluster of sperm moving toward the closed end. The filaments are not widely spread. In every observation of sperm approaching the closed end of the follicle, this was found to be the case. The filaments occupied a small path generally when the head was moving toward the closed end, but on returning, the tail filaments tended to spread out in large loops some extending across the entire width of the follicle.

The dark belt a, figure 16 is in the vicinity of the closed end of the follicle, and the region above the dark belt is small when compared to the loose region between the dark belt and the open end. In this condition an old specimen is easily recognized. In this follicle a moving cluster at c was observed at 8:02. It continued moving until 8:26 when it had passed about one-half way round the follicle. The cluster moved between the cyst wall remnants and the follicle wall, as shown at the cluster point at 8:02. The correction point z, did not vary during the observation which was made under low power in saline solution.

Figure 17 shows the position of a returning sperm cluster. The tail filaments have spread in loops aside

from the path of the cluster and denote the general direction only of the sperm. The position of the cluster is such that it shows every evidence that its general movement is spiral. No doubt it has oriented near the dark belt, a.

Another instance of the movement of a cluster is shown in figure 18. In this figure, which is drawn in outline, a cluster was observed at 4:41. It continued moving until 5:07 when it stopped. At 5:00 it was observed passing just within the follicle wall, pressing aside all the intra follicular material including the cyst wall material. The general movement was spiral. Z is the correction point. Two other sperm clusters may be noted in the follicle but they were not in motion. Similar clusters may be noted in the preceding figure.

Figures 19-21 show a sperm cluster moving. The junction of two cyst walls at z was taken as a correction point as it is not probable that there is much rotary movement in the cyst walls. After being convinced that the sperm cluster was in motion, the time was checked with careful attention to the correction point. In figure 20, the different positions are shown at 2:10 and at 2:23 respectively. The cluster crossed diagonally across about one-half the follicle surface. Figure 21 shows the position at 2:44 where it had passed partially around to the

ventral side. In this series of observations the loop of the tail filaments was formed irrespective of the path of the cluster of heads, and spread across the follicle. The small knots or "blebs" of Bowen '22 were easily noted on the filaments. The sperm in the cluster lay in the same plane with the sharp anterior points imbedded in a dense protoplasm, (see also Photo 31-a). The movement of the cluster just within the follicle wall and around the intra follicular mass may be plainly noted in figure 21 at 2:44. The wave motion of the tail filament is shown in figure 22,b. This wave or vibration was whip-like and passed from the cluster down the tail. The individual sperm with a part of the tail filament is shown in figure 23. In several instances, the entire filament was measured and found to be in some cases, twenty-one hundred microns long. This seems to be an extreme length but exceedingly great care was used in floating out the sperm into saline or Ringer's. In some cases the entire cluster of filaments in the follicle could be measured with similar results. The tail filaments were threaded through watery sacs of protoplasm, the blebs of Bowen. The membrane covering these sacs or blebs was continuous along the length of the filament.

The sperm receptacle from a female specimen was removed a short time after copulation, the receptacle was opened and the contents pressed out as shown in figure 28. The sperm a, are easily recognized but not in clust-

ers, yet tail filaments were present. The heads were nearly all in one direction. A solid cluster of sperm was not expected because of the severe methods of technique. The place of the separation of the sperm in the cluster is still a problem.

Photomicrograph 29 shows two sperm clusters taken under the water immersion. These clusters were moving up the follicle toward the dark belt. At a, may be noted the dense protoplasmic drop which was found to persist even through the vas deferens. The body of the cluster b, shows the individual sperm lying parallel to each other. This cluster was moving over a field of tail filaments of other sperm as shown in the back ground at c.

Another and probably the most convincing proof of the turning of the sperm in clusters just below the dark belt, is shown in the photomicrograph, 30. This was taken with intermediate power with a magnification of six hundred diameters. At b, a sperm cluster is turning in the periphery while at f, and at e, other clusters may be seen moving up the follicle. The enlargement of the upper part of the follicle above c, is significant that the cluster b, is turning against the dark belt. The positions of the several cluster heads show that their general movement is spiral and against the follicle wall as may be seen also in photomicrographs taken at the same time of another foll-

icle with the same magnification. The dark belt b, in figure 31, stands out clearly in the spermatid region a, as seen in the background. The follicle wall is clear. One cluster c, is crossing just under the follicle wall and below the dark belt. The movement of this cluster was so rapid that it moved half way across the follicle in the few minutes necessary to arrange the camera. It had come up from the ventral side f, pressing back the cyst wall material g, passing between it and the follicle wall. At d and e may be seen two clusters that have moved spirally toward the dark belt and are about to begin turning. These photomicrographs fail to show any individual sperm outside of the sperm cluster, at this age of the specimen. Several other photomicrographs have been taken, bearing similar proof.

b. In Vas Deferens.

Clusters of sperm leaving the follicle through the vasa efferentia, (figure 24), were observed. At a, may be noted the dark granular protoplasmic mass at the extreme end of the follicle. The walls b, are heavier and thicker. C, shows sperm clusters and loose cells in the lumen. Within the lumen may be seen the sperm clusters and loose cells, specimens of which are shown in figures 25 a, and 26, b.

In the vas deferens, figure 27, a, and b, the sperm clusters were observed to be unbroken and headed toward the copulative organs. The protoplasmic mass, a-2, in which the perforatoria of the sperm are imbedded may be plainly seen. The clusters are not in such a compact body but the sperm are not separated. Associated with the clusters in the lumen are loose cells, smaller than the loose cells observed in the follicle but darker and more granular. These together with a fluid mass filled the lumen.

The movement of the sperm in the vas deferens was clearly observed. The sperm passed through the two vas deferens toward the body surface. After dissecting out the vas deferens in saline the movement was studied.

Two classes of movements were very distinct. One a serpentine or wave-like movement of the entire vas deferens. The waves of vibration traveled in a peristaltic movement, from the follicle end to the body end. These movements were continous and rapid and may be natural movements or due to the stimulii of the operation, see figure 27,b, and c. A second movement of the vas deferens was the moving of a constriction from the follicle end down the tube to the distal end of the vas deferens. It did not fully close the lumen but a partial construction of the tube moved the contents within the lumen more

forward and less backward with each constriction and thus the entire mass moved slowly forward. Within the lumen of the vas deferens sperm clusters were observed in several places along the tube 2-4. It seemed that the body of the cluster of heads had not loosened and the heads were still united at the points in the drop of dense protoplasm.

In several observations, several individual sperm were noted to be free in the granular protoplasmic mass within the vas deferens, no doubt they were torn loose by the constriction of the walls forcing the mass forward. Otherwise there seemed to be little tendancy for the sperm to separate, all remaining in clusters. All sperm heads were turned toward the copulative organs or toward the body opening. Beside the movement of the general mass in the lumen, the individual clusters moved independently in a similar manner as they moved in the follicle. The movement was more rapid.

4. Sertoli Cells.

In several of the figures of this paper, reference has been made to loose cells which are from their appearance not germ cells, figures 1-L, 3-F, 5-B, 27-A- . From these figures one may easily note that these loose cells are found in all parts of the follicle, also in the vas deferens. Their origin is well accounted for by Wilson '25, who considers those cells in the upper part

of the follicle at least, as arising from the early stem cells and performing a nutritive function among the germ cells. In the ovary they seem to be abortive cells. Charlton '21 describes these cells as large and granular. The author has not yet seen these cells in division but Charlton seems to think that he may have seen these cells in amitotic division. From his figures and from a study which the author has made of his material he is unable to confirm Charlton, in his suggestion as to the amitotic division but without question they do arise by division. Baumgartner '30 in his work on Nemobius fasciatus confirmed both Wilson and Charlton in the idea of the nourish-cells at the open end of the follicle, however they may perform other functions than that of nourishment, but as yet the author has found no evidence. In his observations whole lines of cells have been found.

In the spermatogonial region they are evidently nurse cells, and probably they continue that function throughout the follicle and the vas deferens, since they become smaller in size as they continue to pass down the follicle, and the interior mass within the follicle becomes darker and more granular. In the spermatid region they may be found in clusters figure 20-b, in living tissue and floating freely in the intra follicular substance.

Loose dark staining cells are to be found throughout the loose region of the follicle, and at the proximal end they are often found in clusters until the copulation stage of the specimen, when they may be seen as smaller cell clusters or they may be scattered through the entire lumen of the follicle and vas deferens. The fact that these cells become smaller in size farther down the follicle might be considered as evidence that they continue to perform a nourishing function. They appear yellowish in living tissue and stain very dark in Heidenhains.

DISCUSSION

In as much as the investigations set forth in this work have been carried on in practically a new field and there were practically no sources of information other than the microscope and the suggestions of the author's instructor to which he might refer, the work must of necessity be almost wholly original. Hence, it is needless to state that the progress has been slow and the work has been tedious yet very instructive and interesting, for as observations were made from time to time, new ideas were created and they gave impetus and interest to the work. In the study of fixed material, movements must be assumed and in many instances guessed at in order to formulate an idea or an interpretation.

Living tissue when placed under the microscope, presents a f*a*r different picture from that of fixed

material. Instead of stains and fixations with which one must contend, and which often mislead because of errors in technique, in which the interpretations are uncertain, the living tissues are much more accurate, more clear, and certain positive life movements may be observed. Robertson '17, saw the centrioles gather at the base of the nucleus, Baumgartner and Payne '30, saw the germ cells in division and timed their movements. The mass of protoplasm within the follicle is seen to be highly plastic and pliable, subject to any change of pressure within or from without, ever changing form or position to meet these changes in pressure. To the trained eye, the germ cells under the microscope are living motile entities changing form in metamorphosis preparatory to the performance of a life function. The difference between the soma cell and the germ cell has been defined as the power of the germ cell to reproduce the entire organism. Yet every germ cell in the spermatid and the sperm cycle passes through the same metamorphosis with accuracy and precision. These cells have the power of locomotion and are provided with organelles for that purpose. Every part of the sperm is motile, with the possible exception of the perforatorium or acrosome, and this motility is carried on to the next generation

which in turn goes through the same behavior.

There is an extra follicular source, such as the movement of the internal organs of the body, the contaction of the muscles, the regular and periodic throbs of the heart which are superimposed upon the movements of respiration, all of which cause a continual change of pressure within the body. To this change of pressure there is a response in every part of the follicle which may cause some movement of the germ cells, it is not important when compared to the movements caused by the germ cells themselves.

The open end of the follicle remains closed until the specimen has developed into the copulative age. During this time there is a continuous growth and development of the germ cells . As a result the walls of the entire follicle are turgid and remain so until the closed end of the follicle opens for the exit of the sperm. There is no dark belt as there is no constriction of the follicle wall but when the more mature cyst of spermatids at the open end of the follicle opens, the tension of the follicle wall in that region, is released and a slight constriction of the walls is the result. At this point of constriction the dark belt appears as a ring around the follicle. Under the higher magnification, it appears granular and alveolar with the development of the spermatids and the elongating of the cysts. This belt which

marks the place of division between the loose zone, which has been found by the release of pressure due to the elongating cysts, and the turgid zone, where the cysts have not opened or elongated, moves, with the development of the metamorphosing spermatids and advancing age of the specimen, toward the closed end of the follicle.

Thus there are two zones within the follicle soon after the fourth instar or copulative age is attained. The spermatid movement is within the cyst and of course close in the region of the dark belt with the elongation of the cyst.

It is not the purpose of this paper to discuss in detail the transformation as the work has been thoroughly covered by others. However, a few general statements may not be out of order.

The spermatid stage begins with the telophase of the second spermatocyte division, according to Wilson '25. The spermatids of one cyst are all in the same stage of development at the same time. The nucleii collect near the cyst wall while the cytoplasm collects in the central region of the cyst. The acrosome moves to the head and the nebenkern to the base of the nucleus where it divides and moves down the axial filament. Bowen found "blebs" moving down the filament. Robertson '17 found that the

centrioles move to the base while Baumgartner '30, found that the centrioles in Nemobius pass to the head of the nucleus. During this stage of metamorphosis of the spermatids the cysts elongate extending down the center of the follicle in a funnel form. The tails of the spermatids occupy the extended portion while the heads remain within the cyst proper. The elongating of the cyst above presses the heads of the cyst below toward the periphery of the follicle where the developing spermatids collect into a cluster. As they develop they are gradually pressed downward and backward in the loose zone, toward the open end of the follicle. It is during this period that the spermatids become more threadlike in form and more motile.

The sperm cluster consists of the heads of the sperm from one cyst. The points or perforatoria of the sperm are imbedded in a dense mass of protoplasm. This mass is constant throughout the entire passage of the sperm clusters even through the vas deferens. This mass may correspond very closely to the nourishing cell according to Baumgartner '30. No individual sperm apart from the cluster has been noted in the follicle after the dark belt had appeared.

The cluster, when down the loose zone, starts first toward the closed end of the follicle. The path of the sperm cluster is between the follicle wall and the cyst

wall remnants that have become extended down the follicle. The movements of the clusters are generally spiral. The principal place of turning is just below the dark belt in the loose zone , since the sperm come up against the turgid zone which is a rigion of high pressure, due to the rapid growth of the germ cells within the cysts. Thus they turn against the region of higher pressure following the line of least resistance, orienting around the intra follicular mass and just inside the follicle wall. The author is unwilling to state that they penetrate the intra follicular mass for he has not as yet observed such. And they leave the follicle without having penetrated the intra follicular mass.

The probable cause of the movement of the cluster is the vibrating of both head and the tail filament. The vibration of the sperm head is wave like together with a boring movement. Each sperm head in the cluster has its own individual movement. The vibrations of the tail filaments are larger and whip-like. They travel from the head backward. Each filament is independent of the other filaments, however, the vibrations of several or all filaments may be co-ordinated. When the sperm is moving all filaments of that cluster are in vibration. When one filament begins vibrating very soon all begin vibrating. The clusters do not always move with these co-ordinated vibrations but when it is moving they are

in vibration. The tail filaments may spread out in large loops as when orienting and returning but when approaching the dark belt they are generally more compact. In some instances they appear as a small cylindrical body. The vibrations are not continuous but are spasmodic-a period of vibration and a much longer period of rest. This period of movement and period of rest is characteristic of all animal life in its varied forms. In some follicles no moving sperm clusters could be observed, while in other follicles as many as five clusters were observed to be moving at the same time. The movements of the clusters were independent of each other and occurred at different times. Some were approaching each other, some moving side by side while others were moving in opposite directions. It must be remembered that the sperm clusters and the tail filaments are suspended in the intra follicular fluid.

The behavior of the sperm in moving through the vas deferens is parallel to that in the follicle. They remain in clusters and move with even more vigorous vibrations and at a more rapid rate. This movement is accelerated by the contraction of the walls of the vas deferens in forcing the fluid mass through the lumen. The partial constriction of the walls of the vas deferens is one of the provisions of nature for the protection and preservation of the sperm. This together with the cluster move-

ment of the sperm show some of the economies of nature in providing against loss or destruction in the next generation. The presence of sperm in the female sperm redeptacle is ample proof that nature's provision has been justified.

SUMMARY

1. The Orthopteran follicle of immature males is turgid throughout and remains closed until the copulative age and the first cyst to mature is at the open end of the follicle.

2. After the copulative age or fourth instar there are two zones or regions of the follicle, a turgid zone of unopened cysts, at the closed end and a loose zone at the open end.

3. As the specimen matures in age the turgid zone decreases and the loose zone increases

4. The dark belt separating the two zones appears at the opening of the first cysts of the follicle and gradually moves toward the closed end its rate of movement appreaches almost to zero in very old specimens. The time of appearance and the rate of movement of the dark belt is the same for each follicle of the same specimen.

5. The first locomotor movement of germ cells occurs in the spermatid stage and spermatid heads gather into cyst unit clusters in the periphery of the follicle. Each spermatid and sperm has its own individual vibratory movement.

6. The sperm remain in clusters until leaving the vas deferens.

7. The sperm clusters together with all intra follicular material move gradually down the follicle, during which movement the sperm are maturing and developing stronger motile power.

8. In the lower part of the follicle the more mature sperm clusters move forward toward the closed end, the movements generally spiral,

and orienting or turning against the turgid zone, near the dark belt and return toward the open end.

9. The movement of sperm clusters through the vas deferens is aided by constriction of the walls of the vas deferens.

10. All movements of sperm clusters are in the periphery of the follicle, between cyst and follicle wall.

11. Loose cells possibly nourishing cells are found throughout follicle and vas deferens.

12. The points of the sperm imbedded in the mass of protoplasm which persists through the vas deferens.

13. The movement of the sperm cluster is not continuous but spasmodic a period of movement, and a period of rest.

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EXPLANATION OF THE PLATES

The plates in this work consist of two types, diagrammatic drawings and microphotographs.

Since it was impossible to produce photographs of all the different positions of the sperm, several diagrammatic drawings were made from the low power at 120 magnifications, but were enlarged on the paper.

Drawing 1 and 1' are sections through fixed material. All other drawings and microphotographs are from living tissue.

Figures 2-18, inclusive except Fig. 6, were made under low power.

Figure 6, was made under water immersion at 1200 magnifications.

Figures 19-23, were made under water immersion. The correction point was taken within the field.

Figures 24-28, were made under low power.

Figure 29, was taken under the water immersion.

Figures 30 & 31, were taken at 600 magnifications.

Plate I

Orthopteran follicle, Saggital section. (Low power).

Figure 1. Saggital section.

a. Apical cell.

b. Cyst wall.

c. Cyst of developing spermatids.

d. Cyst wall apart from follicle wall.

e. Lower point of cyst extending downward.

f. Follicle wall.

g. Cyst of developing spermatids, lance shape.

h. Diagonal section of sperm cluster.

i. Tail filaments.

j. Sperm cluster in periphery of follicle.

k. Tissue cells in center of follicle.

l. Loose dark staining cells, (probably Sertoli).

m. Sperm cluster approaching the open end of the follicle.

n. Granular substance near the open end of the follicle.

r. Heavy follicle wall near vasa efferentia.

Figure 1'. Cross section of follicle showing position of the turning of sperm.

a. Follicle wall.

b. Sperm cluster.

c. Cyst wall.

Plate I

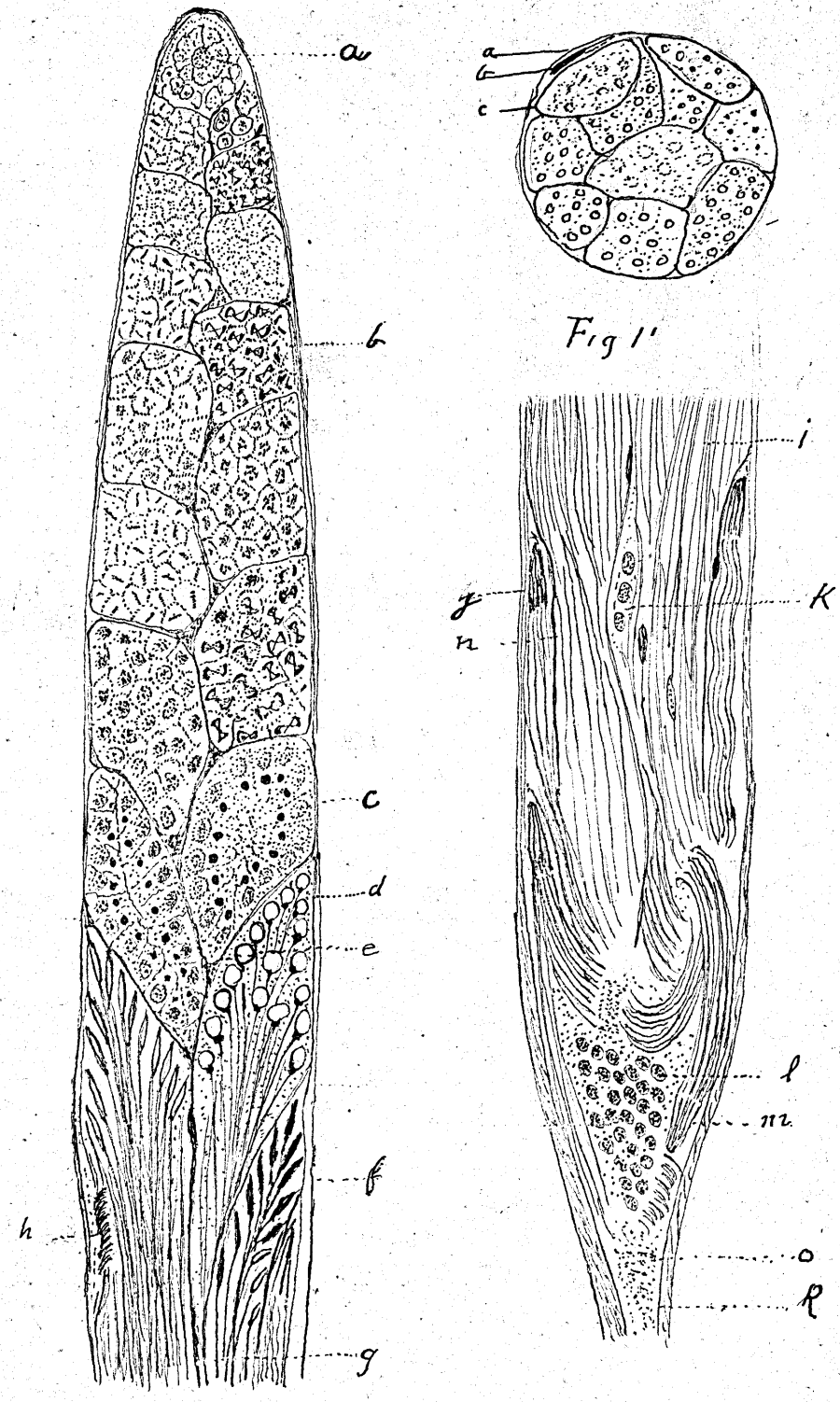


Fig 1.

Plate II

Figure 2. Follicle of young specimen where cysts have not opened. (Living tissue, low power).

a. Apical cell.

b. First spermatogonial division.

c. Second spermatogonial division.

d. First spermatocyte division.

e. Second spermatocyte division.

f. Spermatids beginning to elongate showing the nebenkern.

g. Granular plug of unopened follicle.

Figure 3. Follicle (living tissue) showing different regions. (Low power).

a. Early spermatid region.

b. Dark belt dividing the turgid zone, c, above from the loose zone d, below.

e. Ascending cluster approaching the dark belt.

f. Loose cells.

g. Sperm cluster approaching vasa efferentia.

Figure 4. Follicle (living tissue). Showing the paths. (Low power).

a. Dark belt.

b. Sperm clusters on ventral side moving toward dark belt.

c. Spiral paths taken by sperm clusters.

Plate II

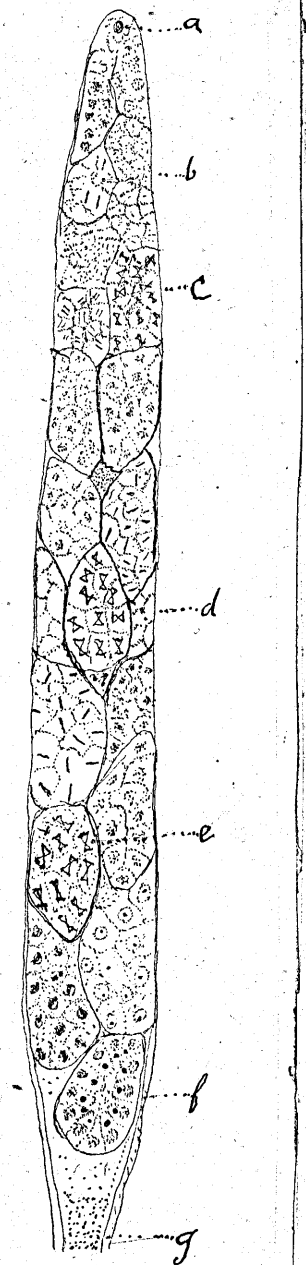


Fig. 2

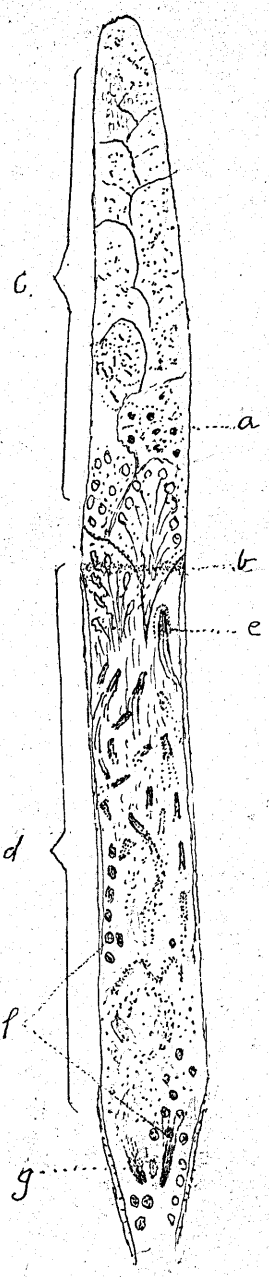


Fig 3.

Fig 4

Plate III

Figure 5. Several dwawings of a follicle of living tissue showing the movement of two sperm clusters.

a. Dark belt.

b. Cluster of loose cells

z. Correction point.

Figure 6. Spermatid in metamorphosis, lining tissue.

a. Acrosome

b. Nucleus.

ax. Axial filament.

c. Funnel shaped body just below the nucleus.

d. Cytoplasm.

e. Cross section of elongating tail.

Plate III

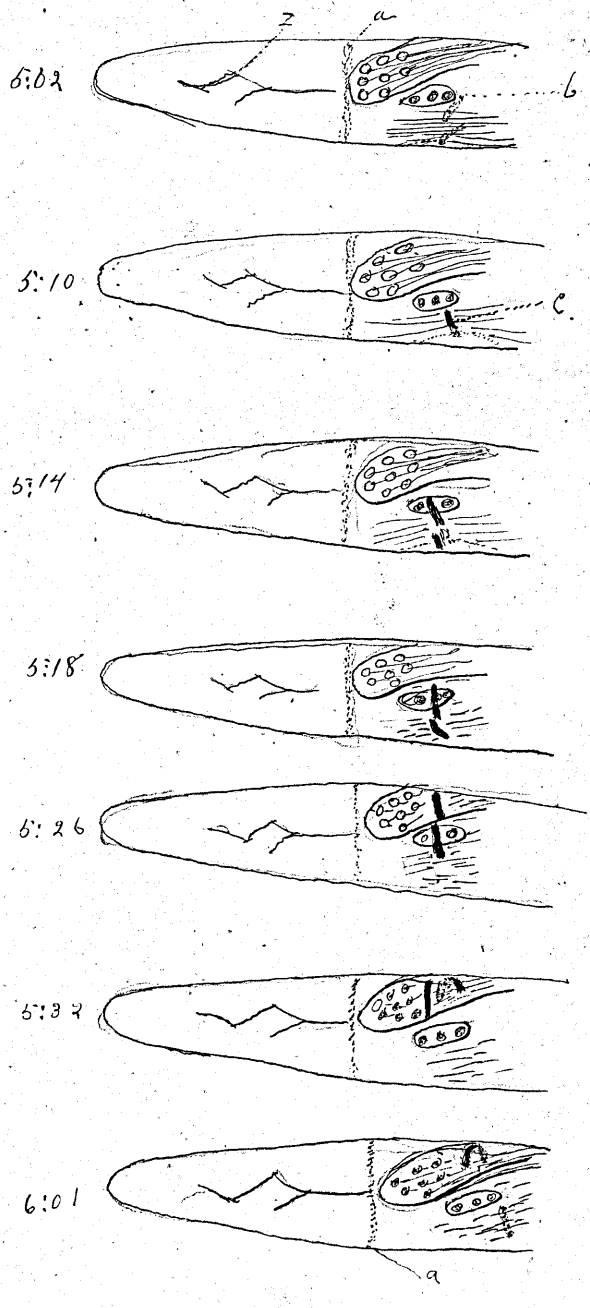


Fig 5

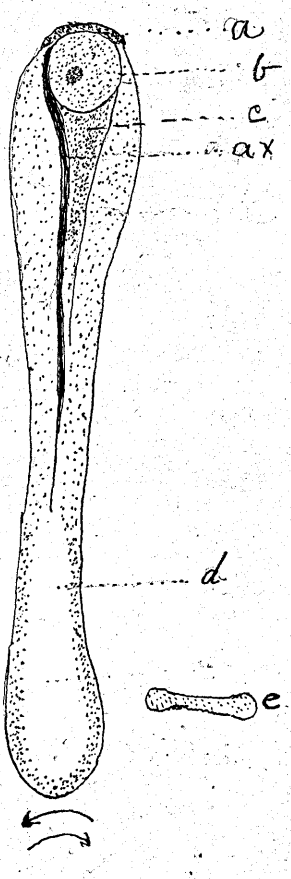


Fig. 6

Plate IV

Figure 7. Part of loose region of follicle just below dark belt a.

b. Turning sperm cluster.

c. Tail filaments showing blebs and general path of sperm.

d. Resting cluster.

z & y. Correction points.

Figures 8-12. Different positions of the cluster at different readings as shown in margin of each. (Much enlarged).

Plate IV

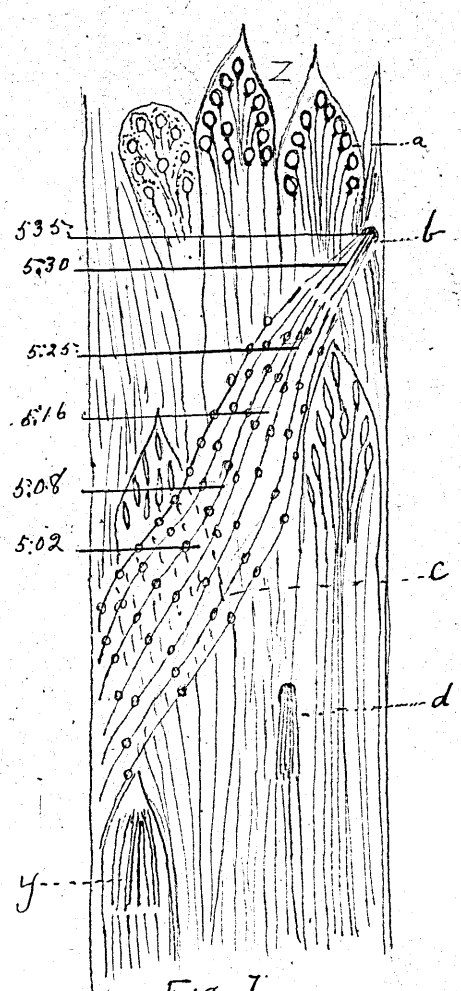


Fig 7

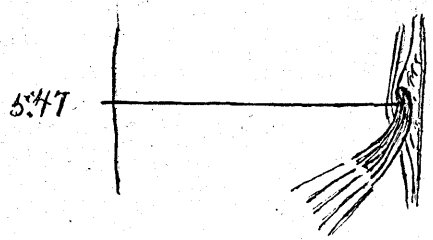


Fig 8

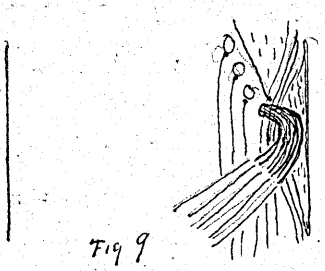


Fig 9

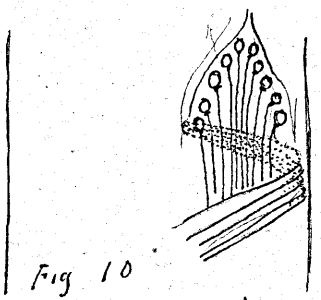


Fig 10

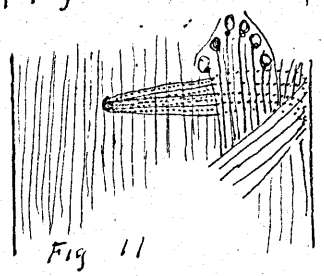


Fig 11

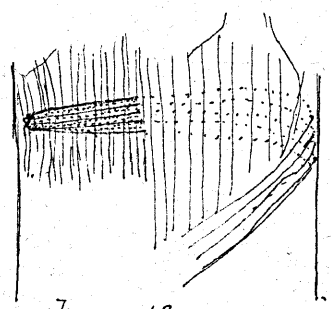


Fig. 12

Plate V

Figure 13. Follicle showing loose zone.

a. Dark belt dividing the zones.

b. Cluster of sperm in act of turning.

c. Two sperm clusters about to leave the follicle.

d. An approaching cluster.

Figure 14. Follicle showing sperm cluster approaching the turgid zone.

a. Dark belt dividing the zones.

b. Approaching sperm cluster.

c. Returning sperm cluster.

Figure 14'. Follicle showing positions of several sperm clusters.

a. Dark belt, dividing the zones.

b. Path of a moving cluster between cyst wall material and follicle wall.

c. Returning cluster.

Figure 15. Follicle showing movement of a cluster with time checked in margin.

a. Dark belt.

b. Tail filaments of a returning cluster.

c. Sperm cluster about to leave the follicle.

x. Correction point.

Plate V

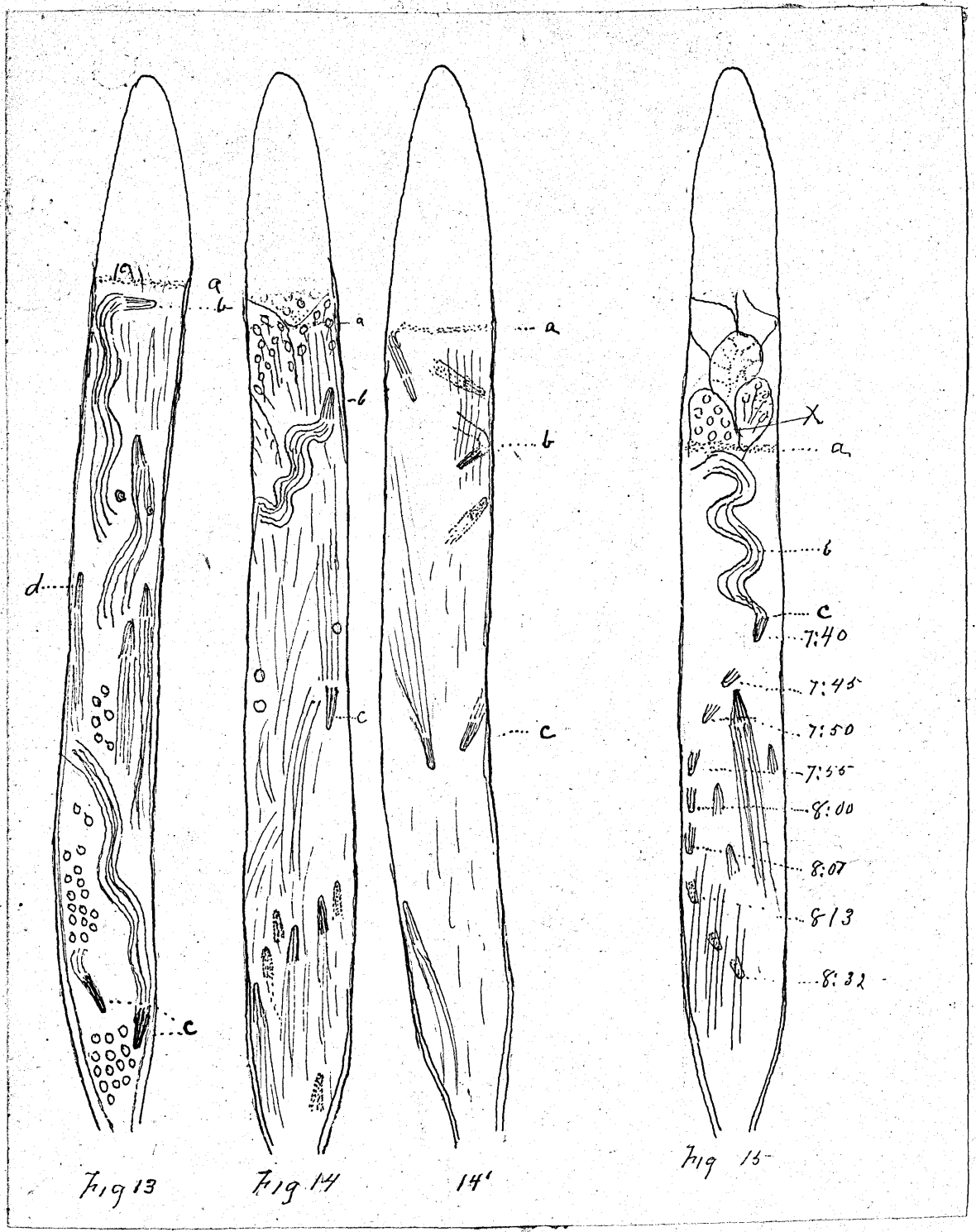


Fig 13 Fig 14 14' Fig 15

Plate VI

Figure 16. Section of follicle showing an approaching sperm.cluster. (Time checked in margin).

a. Dark belt.

b. Sperm cluster turning on ventral side.

c. The same cluster at 8:02.

d. Cyst wall remnant pressed back.

z. Correction point.

Figure 17. An outline of follicle showing showing position or a returning cluster.

a. Dark belt region.

Figure 18. A returning cluster in movement (time checked in margin).

a. Dark belt.

z. Correction point.

Plate VI

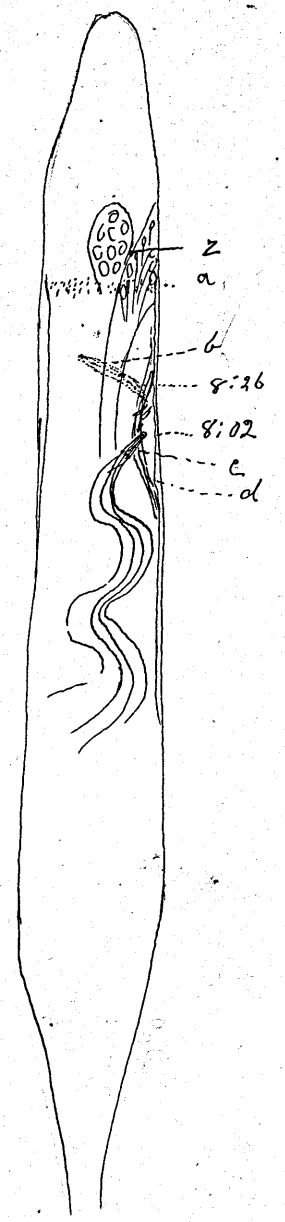


Fig. 16.

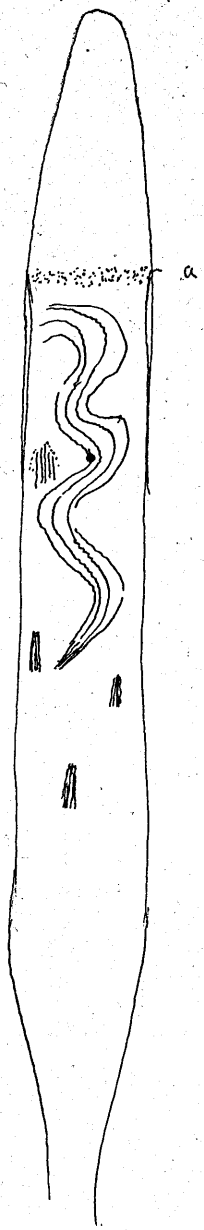


Fig 17

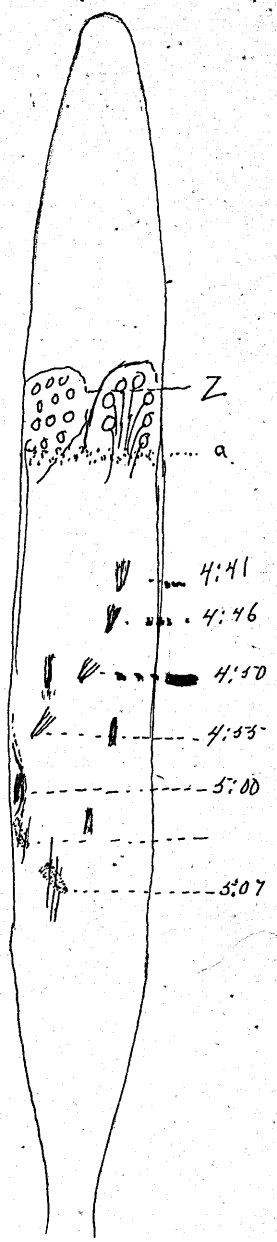


Fig 18

Plate VII

Figure 19. Section of follicle-living tissue showing position of sperm cluster before it started moving.

a. Dark belt.

z. Correction point.

Figure 20, & 21. Same sections as figure 20. The cluster moving. (Time checked in margin).

a. Dark belt.

b. Blebs on the tail filaments.

Figure 22, a, sperm cluster and filaments.

b. Single filament showing waves.

Figure 23. Single sperm head and tail filaments.

a. Head.

b. Tail filament.

Plate VII

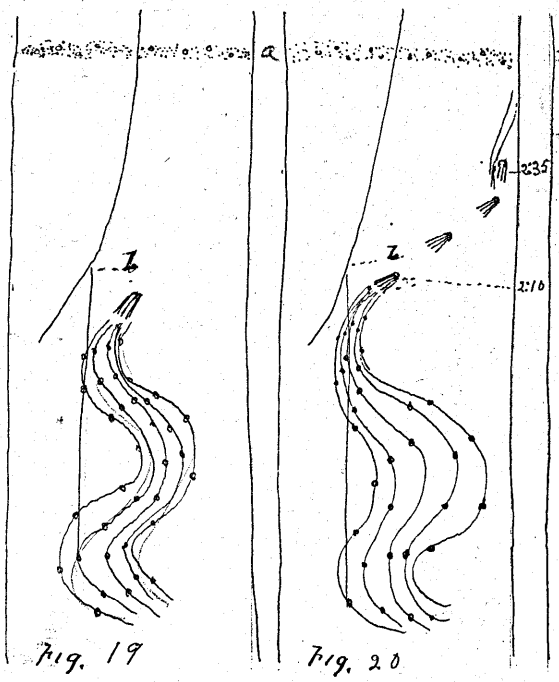


Fig. 19

Fig. 20

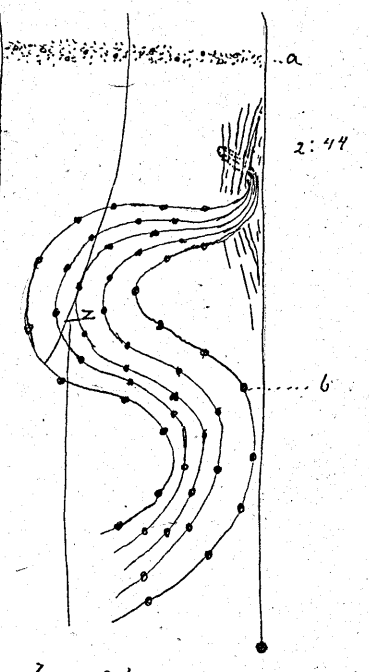


Fig. 21

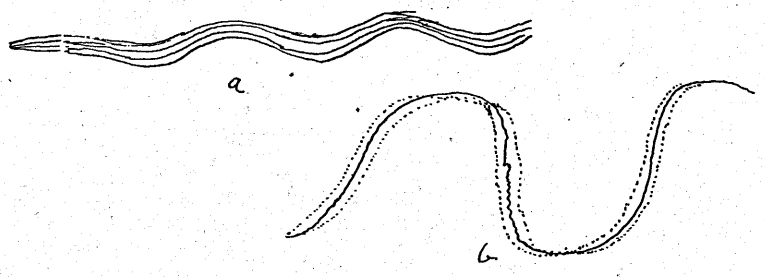


Fig 22

Fig 23

Plate VIII

Figure 24. Part of open end of a follicle and the vasa efferentia.

a. Granular substance in lumen of follicle.

b. Heavy walls.

c. Sperm clusters within lumen of vasa efferentia.

Figure 25 & 26. Sertoli cells.

a. Well up in Spermatocyte region.

b. In Vas deferens.

Figure 27. Vas Deferens.

a. Vas deferens not in movement.

1. Heavy contractile walls.

2. Sperm clusters moving toward the sperm duct.

3. Thin membrane outside the heavy walls.

b. Showing serpentine movement of vas deferens.

4. Point of sperm cluster.

c. Partial constriction of vas deferens.

Plate VIII

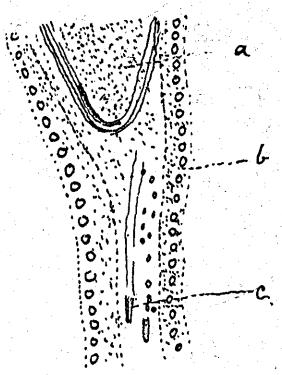
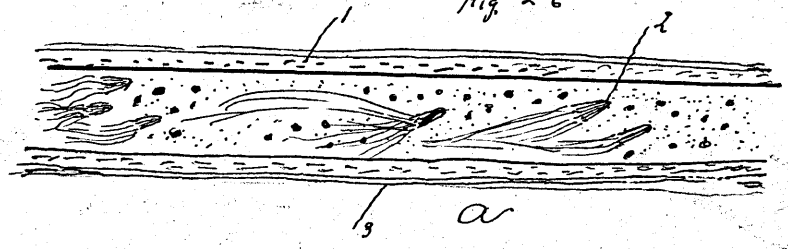


Fig 24

Fig. 25

Fig. 26



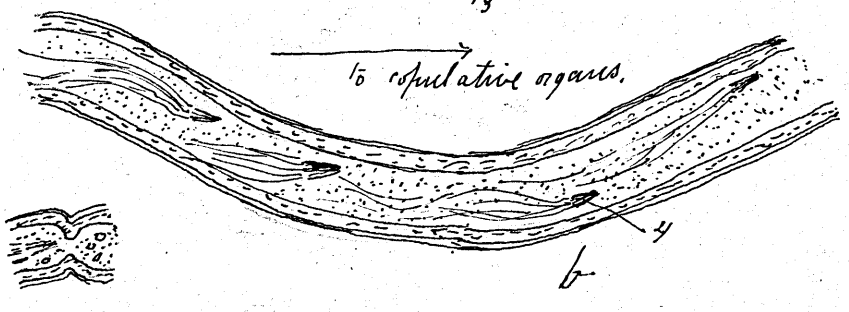


Fig 27

Plate IX

Figure 28. Female sperm receptacle pressed open.

a. Active sperm found in receptacle.

b. Wall of receptacle.

Figure 29. Cluster of sperm (water immersion).

a. Protoplasmic drop in which may be seen perforation of sperm.

b. Sperm heads.

c. Intra follicular mass of filaments.

Plate IX

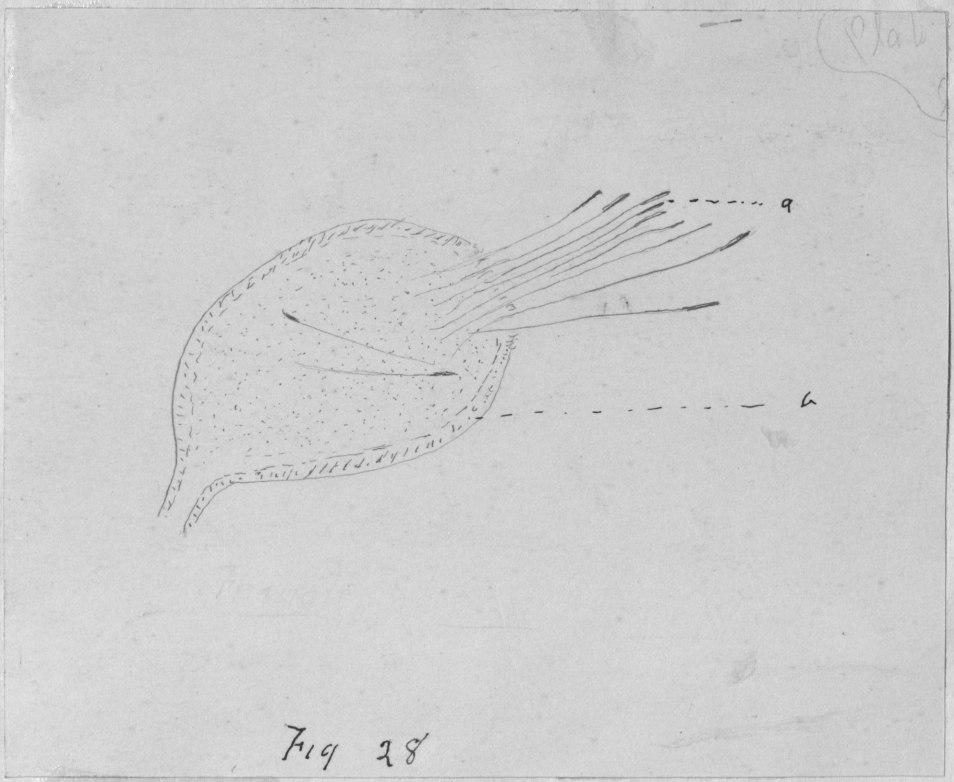


Fig 28

Fig 29

Plate XX

Figure 30. Microphotograph (intermediate) showing the orienting or turning of the sperm clusters.

a. Tail filaments and blebs, near dark belt.

b. Approaching sperm cluster turning.

c. Cyst wall remnants pressed aside.

d. Follicle wall.

e. Approaching cluster.

f. Sperm cluster arising from below.

g. Interior protoplasmic mass and filaments.

Figure 31. Microphotograph in dark belt region.

a. Spermatids.

b. Dark belt.

c. Orienting sperm cluster.

d-e. Approaching sperm.

f. Follicle wall.

g. Cyst remnant pressed aside.

Plate X

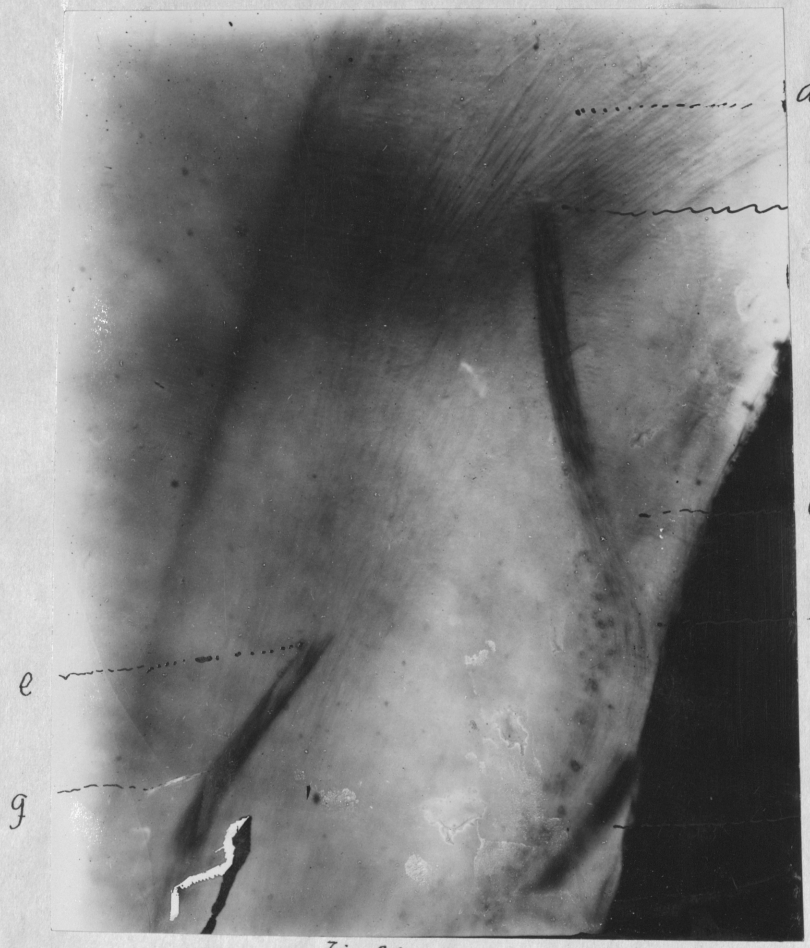


Fig 30

Fig 31