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THE SPERMATOGENESIS OF LEPISMA DOMESTICA

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SIX PLATES (NINETY-FIVE FIGURES)

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INTRODUCTION

Cytologists have long found the class Insecta to be a very fertile field for investigation, but the first order and, according to many, the most primitive, namely, the Thysanura, seems to have been neglected. According to Harvey ('16), only three papers dealing in any way with the cytology of any closely

related forms have been published. The writers, Claypole ('98), on *Anurida maritima*, Lecaillon ('01), on *Orchesella villosa*, and Willem ('00), on *Podura aquatica*, simply report isolated observations which are necessarily incomplete and limited to the class Collembola.

The Lepismatoidea have therefore never been made the subject of a cytological study, and it was in the hope that a survey of this primitive form would throw some light on the present-day cytological problems that this investigation was undertaken.

The completion of the study shows that, instead of the expected simplicity, the process actually is a complicated one, differing only here and there from that already described in other forms. These differences, however, are interesting and, together with the fact that it is the first cytological work in a new class of insects, warrant its presentation.

The work was done at the Osborn Zoological Laboratory at the suggestion of Professor Petrunkevitch beginning in the fall of 1916. During 1917-19 it was practically suspended except for an occasional day or so at Columbia University. It gives me pleasure to express my thanks to Prof. E. B. Wilson for his kindness in giving me laboratory privileges at Columbia and to Prof. Frank R. Lillie for facilities accorded at the Marine Biological Laboratory, Woods Hole, Massachusetts, during the summer of 1919.

Most of all, I am indebted to Prof. Alexander Petrunkevitch, first, for suggesting the problem and later for his unfailing help and criticism.

MATERIAL AND METHODS

Lepisma domestica, commonly called the fire-brat, from its frequently observed habit of running apparently unharmed over hot stones in bakeries, belongs to the class Thysanura of the order Lepismatoidea. It is a fairly common insect in New Haven, and can be kept alive in the laboratory for a considerable period. My method has been to keep them in large glass Stender dishes without covers, since the insects cannot climb up a clean glass wall, and to provide them with a cereal such as corn flakes

to eat. For moisture I have kept shallow dishes filled with moist filter-paper in the Stenders. In spite of these precautions, in the course of a month or so the creatures begin to show a shrinkage of the abdominal region and soon die with the posterior region shrunken fully one-half.

The insects were either killed in xylol or decapitated and the testes dissected out immediately in physiological salt solution. As soon as the body cavity was opened, some fixing fluid was introduced by means of a fine pipette. This renders the tissues more opaque and makes it easier to locate the gonads which are loosely surrounded by fat, as well as to cause better fixation.

For counting chromosomes, Bouin's fluid at 38°C. proved the best, but for general fixation of the cytoplasm as well as of chromatic structures nothing equaled Flemming's strong solution. In addition, Hermann's fluid, Benda's Flemming, 10 per cent formalin, Allen's modification of Bouin, Petrunkevitch's fluid, and Kopsch were tried out and had their special uses.

The testes dissected out, fixed, washed, and dehydrated, were imbedded in paraffin and cut into sections from 3 to 12 μ thick. Sections of 7 μ thickness were found to be very satisfactory for study, and in general this was the thickness used.

The stain used generally throughout was Heidenhain's iron haematoxylin without any counterstain. In addition, various counterstains were tried, and also, in an effort to get a selective stain for mitochondria, Benda's alizarin-crystal violet method. A modification of Cajal's silver-impregnation method by Hortega ('16), especially recommended for centrosomes, was given an inadequate trial with but fair results.

I have also examined a number of splendid slides stained by the safranin-gentian-violet-orange G method, for which I am indebted to Dr. P. W. Whiting.

OBSERVATIONS

External sexual characters

During the spring months of March, April, and May, the adult insects are in the best condition for study, but since it takes some time, probably a year or more, to attain sexual maturity, the early stages may be studied at any time of year in young individuals.

It is comparatively easy to recognize the sexes by their external appearance. The female (fig. 2) has a long median ovipositor extending posteriorly which is quite prominent in the living insect. There is nothing comparable to it in the male (fig. 1), for the penis which could not possibly be confused with the ovipositor is more often retracted and not in view.

Male reproductive system

The testes (fig. 3), of which there are three pairs on each side of the middorsal line, occupy, in mature individuals, a considerable portion of the anterior two-thirds of the abdomen. The testes lie parallel to each other, extending in a ventroposterior direction, and each is connected by a short duct with the vas deferens, which passes as a straight duct posteriorly where it enlarges to form the seminal vesicle. From the seminal vesicle a similar duct extends, which soon enlarges considerably and, after bending upon itself a couple of times, opens into the base of the penis.

Spermatogonia

I have made a long and careful search for primary spermatogonia in the youngest material at my disposal, which consisted of insects only 2 or 3 mm. long, but have not been able definitely to identify them. It is therefore very probable that the primary spermatogonia occur only very early in the life-history. Munson ('06) has described an apical cell which produces early spermatogonia, but this, too, if present at all, would be found in exceedingly young individuals.

One does find cells in quite large numbers at the blind end of the testis, which differ from the ordinary spermatogonia in having large homogeneous nuclei with the chromatin condensed into a single dense mass and irregular in shape (fig. 28). I believe these to be immature Sertoli or nurse cells, for later on one finds such cells, only now they are larger, more elongated, and contain two to four chromatic bodies. Those at the region of the mature spermatozoa are much paler in color and may be wrinkled and twisted upon themselves, indicating perhaps degeneration.

The spermatogonia occupy a considerable part of the blind end of a tubule of the mature insect during the winter and early spring, and can be easily recognized by their position and by the arrangement of the chromatin in the form of clumps attached to each other by linin threads and grouped around the periphery of the nucleus. This arrangement (fig. 4 is a surface view) is the most common and probably represents a resting condition. Although I have not been able to count these clumps of chromatin, the number is easily seen to be more than the haploid, and each one probably represents a spermatogonial chromosome.

The nucleus of the early spermatogonium is quite large and almost equal in size to the nucleus of the growth period. The two or three spermatogonial divisions reduce the nuclear and cell size by apparently not allowing time for growth between divisions (figs. 8 to 11). In prophase the chromosomes are long and bent upon themselves and irregularly scattered throughout the nucleus; later they are drawn into the metaphase plate as shown in figure 5.

It is only in the larger and therefore the earlier spermatogonia that good counts of the chromosomes can be made. Figure 6 shows thirty-four chromosomes in a very clear metaphase plate. The chromosomes are of the curved-rod type, differing considerably in size, but close observation fails to show any chromosome or group of chromosomes behaving in any way differently from its neighbors.

In the telophase of the spermatogonial division the chromatin becomes granular and forms a more or less eccentric ring around

the nuclear wall (figs. 11 and 16). At a little later stage one commonly finds an irregular clump of chromatic material representing apparently two spermatogonial chromosomes lying against the nuclear membrane and retaining the haematoxylin stain (figs. 13, 14, and 15). For these chromosomes I shall use the term idiochromosomes, the name given by Wilson ('05) and meaning 'peculiar or distinctive chromosomes.'

In some presumably young cysts the spermatogonial cells are arranged in the form of a rosette, their median ends tapering toward a faintly marked open center and showing an archoplasmic mass or sphere (figs. 4 and 18). Hegner ('14), Meves ('97), and Shaffer ('17), as well as others, have described similar structures and considered them spindle remains. In some cases they figure them as extending from cell to cell. While this is true immediately after division when the spindle remains are very definite (fig. 7), it is not possible later to see any continuation or connection with similar bodies in adjacent cells.

In the spermatogonial region isolated cells are occasionally seen in division, but, strangely enough, the chromosomes are paired and look somewhat like tetrads (fig. 32). The cells themselves are much larger than the spermatogonia and contain but little cytoplasmic staining material in the form of a flaky mass at either end of the cell in which a dark-staining granule may be seen. If these represent division in the Sertoli cells, they are a very rare occurrence. In the older Sertoli cells I have occasionally seen evidence indicating division by amitosis.

The growth period

The stages of the growth period correspond fairly closely with the stages described by Wilson ('12). After the telophase, chromosomes of the last spermatogonial division break up and form a granular ring just inside the nuclear wall, the chromatin arranges itself as previously described in the form of clumps located on the nuclear membrane (Wilson's stage b, similar to fig. 13). In heavily destained material two of these are closely related, one of them being flattened against the nuclear mem-

brane and retaining the dark stain of the haematoxylin (fig. 15). In addition to the idiochromosomes, a similarly staining, small, spherical granule appears (fig. 13). The chromatin clumps now become granular and form an eccentric circle against the nuclear membrane, leaving an open center very much like the condition following the last division. The homogeneous granular border is at first deeply stained, but later loses its affinity for the haematoxylin and appears pale in color (figs. 11 and 16).

The idiochromosomes also seem to break up into unequal spherical bodies, three to eight in number, six being the more common number (fig. 16). In the clear central region the remains of the preceding spindle are quite apparent. Following this stage we have the reappearance of the idiochromosomes (fig. 17), and after that the entire nucleus appears granular, the central clear area disappearing and the two idiochromosomes stand out clearly (fig. 21).

It has not been possible to see anything like an unraveling stage as described by Wilson ('12) for stage c; the granular condition being directly followed by delicate threads (Wilson, stage d, fig. 22) which seem to push out and distort or break the nuclear wall. This is soon followed by the synzesis or contraction stage. Here the threads are drawn closely together and are located more to one side of the nucleus, the plasmosome and idiochromosome thread often remain outside of the contracted mass, as shown in figure 23.

Popoff ('08), Gates ('08), and Whiting ('17) look upon this as due to a rapid absorption of water by the nucleus; in other words, an osmotic effect; however, it has often been considered an artifact. Although at this stage of the growth period the spireme threads stain very intensely, making it difficult to trace the individual threads, it would look as though the filaments became arranged in the form of loops polarized with their free ends near the plasmosome and idiochromosome threads. Later on when the threads have thickened, this bouquet stage is much more clearly seen (fig. 25).

It has not been possible to see a side-by-side union of the spireme threads, the synapsis of Moore ('95), but the number

of filaments certainly is reduced and each one becomes much thicker. The threads now loosen up and occupy practically all the cell, the space between the nuclear membrane and the cell wall being quite small (fig. 24, Wilson ('12), stage f). I have not been able to find the longitudinal splitting of the thread—a process which Wilson ('12) describes as taking place.

There follows a period when it is hard to distinguish the threads as such (fig. 29, Wilson ('12), stage g). Wilson calls it a net-like arrangement. The actual breaking of the threads or pachytene stage is not well exemplified in *Lepisma*, but stage g is soon followed by the clumping of the chromatin into masses irregular in shape and joined together by linin threads (fig. 30). By a further condensation of these masses we get the prochromosomes. The formation of tetrads showing the quadrivalent condition of the autochromosomes is never apparent, neither is there any split indicating a parasynapsis.

The idiochromosomes retain their form and staining reaction until the formation of the delicate filaments (stage d), when they break up and form threads which are darker in color than the other threads, and one may be seen in close relation to a small plasmosome (fig. 35 a). The idiochromosome threads are at first very long and may extend across the entire width of the cell. They appear somewhat beaded, just as is the case with the threads of the autochromosomes.

During the later periods the threads show an end-to-end apposition, being joined by very fine linin fibers (fig. 35 m). The threads now become shorter and thicker assuming the U shape followed by the definite formation of loops with the plasmosome between them (figs. 20 and 35 j). Figures 35 i and h would seem to indicate that the limbs of the loop come together and become still more compact to form clumps lying against the nuclear membrane with the plasmosome still lying between them. For a considerable time the idiochromosome threads show a very clear inequality in that the thread nearest to the plasmosome is the longer (fig. 35 k).

A second small plasmosome may be formed and lies to one side with no attachment to either idiochromosome thread (fig.

35 e and f). Later I believe it fuses with the first, for the latter is seen to increase considerably in size and to show at times a double nature (fig. 35 g and k).

A third body similar in shape and staining reaction to that seen in the spermatogonia becomes quite prominent at this time (figs. 29 and 30), due to a slight increase in size and to the appearance of a clear transparent area encircling it. Painter ('14) describes in spiders similar small dark-staining spherical bodies, which he calls planosomes and which first make their appearance in the late spireme stage and which he was able to trace through the succeeding divisions. The planosomes, according to him, have spindle fibers, and would therefore be comparable to chromosomes, although as a rule they do not divide, but linger near the middle of the spindle and later go to one side.

From his description and figures, this body is the same as the one found in *Lepisma domestica*, only I find it first in the resting stages of the spermatogonia, and have not been able to follow it beyond the prophase stage of the first maturation division.

The first spermatocyte

With the condensation of the chromatin segments into the prochromosomes, the nuclear membrane breaks down and two chromosomes located near the periphery are seen joined together by a more or less ribbon-like connection, forming a V-shaped structure. Within or near the arms of the V the plasmosome may be found (figs. 36 and 37). With the exception of the prophase figures in which the idiochromosomes stain more deeply, there is no essential difference in the staining reaction of the idiochromosomes and the autochromosomes; but to make the behavior of the idiochromosomes plain throughout the different stages of the first maturation division, they have been drawn in black, while only the outlines of the autochromosomes are shown (figs. 36, 37, 41, 42, 43, 44, and 45).

The chromosomes arrange themselves on the spindle and in the metaphase plate (figs. 38, 39, and 40), the sex or idiochromosomes are still connected and one pair of the chromosomes is a

little further beyond the metaphase plate, so that in plate view one pair of chromosomes can be seen to be at a different level (figs. 39 and 40). The side view shows how one limb of the V extends farther than the other.

The metaphase plate (fig. 38), in which the idiochromosomes are located in the center and surrounded by a ring of chromosomes, reminds one of the arrangement in some Hemiptera.

There is little change in the position of these joined chromosomes in the anaphase (fig. 41), except for a shortening of the connecting thread and possibly a slight movement of the whole toward the distal pole. Figures 42, 43, and 46 picture the telophase arrangement, the idiochromosomes going undivided to one pole.

There are sixteen chromosomes plus the two idiochromosomes, or eighteen in all, in the first spermatocyte division. Side views have not been counted, owing to the great overlapping of the chromosomes. The plasmosome may be identified during the late prophase (fig. 37), but not definitely after the actual spindle formation. Bodies which are plainly not chromosomes are often seen in relation to the spindle, as the two equal bodies in figure 40, but whether these represent the divided plasmosome or are mitochondrial is not conclusive.

Resting stage of second spermatocyte

In the telophase of the first or early prophase of the second spermatocyte (fig. 46), the chromosomes are breaking up. Some appear unchanged, while others have swollen to a spherical shape and stain more diffusely. It is not possible to identify the idiochromosomes at this time, but a little later, when the resting nuclear stage is reached, the double nature of the idiochromosomes is quite apparent as the nucleolus in one of the now divided cells (figs. 47, 48, 50, and 51). It is not possible to confuse these resting second spermatocytes with the early spermatids, because both nuclear and cell size is much larger. The relative sizes of first and second spermatocytes and spermatids are shown in figure 33, which was diagrammed from measurements of the length

and breadth of ten representative cells of each kind, and the average diameter of each cell and of the ten cells taken.

In the second place, the chromatic nucleolus is distinctly double (fig. 47), while in the spermatid it is single and smaller (figs. 64, 66, and 68).

During the growth and division period, spindle remains stand out quite clearly as one, more usually as two vesicles, formed probably from the central fibers and showing a granular condensation in their interior (figs. 25 and 46).

The formation of the resting stage and the subsequent prophase is a rapid one, as I have observed resting nuclei, prophase, and dividing second spermatocytes in the same cyst. Figures 50 and 51, resting and prophase stages, respectively, are from a slide not particularly well fixed, as the cells are somewhat swollen, but figure 51 is interesting in that it shows the formation of spindle fibers before the nuclear wall has broken down and in figure 50 the idiochromosomes still show their double structure. The resting nucleus, at first granular, breaks up into faintly staining irregular or fantastically shaped entities without any visible unraveling stage and condense quickly into the prochromosomes (figs. 49 and 50).

The second maturation division

With the formation of the spindle for the second maturation division, two types of metaphase plates are seen: one (fig. 53) with eighteen chromosomes and another (fig. 56) with sixteen. In the latter case I have one perfect anaphase (fig. 59), in which both plates can be counted and both show sixteen chromosomes.

It appears that the idiochromosomes are now equal in size and no longer show a connecting thread. In the first maturation division the idiochromosomes were distinctly unequal, but each tapered into a thread connecting it with the other. This thread often seemed ribbon-like, granular, and taking the iron haematoxylin stain like the chromosomes.

It seems to attain its maximum length at the metaphase of the first maturation division and to shorten a great deal by the

time the telophase is reached, and it would appear as though this thread were fused with the smaller idiochromosome so that they both appear equal in the metaphase of the second maturation division. Another factor in favor of this hypothesis is that the chromatic nucleolus of the resting stage shows a double structure with hardly any inequality.

In the early anaphase (fig. 55) all the chromosomes show a longitudinal split near their centers, except two which represent, I believe, the divided idiochromosomes. The anaphase often shows the chromosomes arranged in the form of a ring (fig. 62). In figure 63 the chromosomes are at the poles and are beginning to form a nuclear membrane, but no change has taken place in the centrosomes. Figure 60, a late telophase, shows that one chromosome differs from the rest in being elliptical, while the others are V- or U-shaped and slender. A still later telophase is figured in figure 61, the chromatin now being massed at the poles. Two types of spermatids are formed, those with sixteen and eighteen chromosomes, respectively.

The centrosome in the spermatogonial and maturation divisions

In the archoplasmic mass or sphere representing the remains of the previous spindle one may occasionally see two dark granules (fig. 18), which I take to be the divided centrosomes. In the division figures of the spermatogonia centrosomes are difficult of demonstration, but in a few slides I can make them out as definite single granules at the poles of the spindle (fig. 12). I have never seen astral rays or anything comparable to a centrosphere at the time of division, but during the resting stages the centrosome is found in a granular sphere.

From the division figure of the last spermatogonial mitosis until shortly before the synaptic or contraction stage, the centrosome has not been traced, and when it does appear a considerable metamorphosis has taken place. At about the time when the fine spireme threads are being changed into loops, a granular mass can be made out at one end of the cell, and in this mass appear two short, stubby rods lying parallel to each other.

Later (figs. 20 and 26) the rods lengthen and show small granules at their ends. At first the two rods form an angle of 180° , but this angle is later decreased to 90° or less. Each rod now divides, but the halves remain attached by their granule ends, forming a pair of V-shaped centrosomes, each V representing a divided centrosome. This whole process is a rapid one, for all stages as well as the separation of the V's for some distance may be seen in cells which show little change otherwise. The migration is about completed and the V's nearly at the poles by the time the prophase condition is reached (fig. 30). During the succeeding division the apex of the V is directed toward the chromosomes, while its limbs touch the surface of the cell. The V may open considerably, nearly to a straight angle, so that a large part of the outer surface of the rods is in contact with the cell wall. The cells may also show a slight depression at the poles (fig. 39).

The spindle fibers all lead to the centrosome region, but an actual attachment of the fibers to the centrosomes, while taken for granted, does not show clearly in sections.

This V arrangement can be identified up to a late telophase of the primary spermatocyte, but I have not traced it through the resting stage of the second spermatocyte. Each second spermatocyte would receive one V, but when the rods reappear in the division figure they are divided, a single rod at either pole lying against the inner surface of the cell wall and oriented parallel to each other, but at a slight angle with the cell axis. The division or separation of the V's as well as their migration to opposite poles must take place during the resting period.

The centrosome rod can be traced through every succeeding stage to the early spermatid, where it may be seen lying free in the cytoplasm (fig. 65). In exceptional cases, as in figure 54, the rods have granules at their ends, or we may find a number of granules or fragments and no rod, as in figure 58.

The spermatid

The young spermatid cell is considerably smaller than the resting stage of the second spermatocyte. The chromosomes clump together, form a nuclear membrane, and quickly break

up. The nucleus appears round in polar view, but oval if looked at from the side. Later the nucleus becomes spherical, the chromatin appearing finely granular and congregated at the boundaries of the nucleus leaving an open center (fig. 66). One-half the cells show an idiochromosome nucleolus which usually presents a spherical part extending into the nuclear cavity and a flattened area against the inner surface of the nuclear membrane, while the other cells do not possess an idiochromosome nucleolus.

The methods of fixation and staining have a great deal to do with the structures observed in the spermatid. When strong Flemming is used for fixing followed by Heidenhain's iron haematoxylin, the cytoplasm of the early spermatid contains such a mass of intensely staining material that the nuclear membrane is made out only with difficulty. The same stain after Bouin's fluid brings out the nucleus and centrosomes, but not the cell inclusions.

At the very first, the cytoplasmic structures are somewhat loosely aggregated around the nucleus, but particularly between the nucleus and the last division plane. The centrosome can easily be followed from the telophase; located at first on the cell wall of the dividing second spermatocyte, it later moves inward, occupying the space between the cell wall and the nucleus (fig. 65).

As it moves around to get between the nebenkern and the nucleus, it turns 90° and comes to lie with one end on the nuclear membrane and the other against or near the cell wall (figs. 66 and 67). The rod-shaped centrosome now frequently shows a granule or enlargement at the nuclear end.

The nebenkern has meanwhile formed a broad ring of densely staining granular material in the center of which spindle remains of the last division appear and on either side two spherical bodies become visible (fig. 69), exactly like those seen in the two maturation divisions, and undoubtedly represent old spindles. In cross-section they appear as rings with their boundaries staining in varying degrees, often looking like crescents, and may possess a darker staining center. Looked at from the side, they take the form of rods with faintly stained material between them.

When the centrosome is in contact with the nuclear wall, one usually sees a granule at its inner end (fig. 66), and later a granule similar in size located near it on the membrane (fig. 67). This suggests the breaking away or division of the granule at the base of the centrosome.

The centrosome may now change its position, being found in the region of the nebenkern or even at the opposite side, and shortly the delicate axial filament is seen pushing from the cell and occasionally carrying a small clump of cytoplasm with it, very much as has been described by Buder ('15) in the Lepidoptera and called by him 'plasmaklumpchen.'

The single granule arising from the centrosome increases considerably in size and divides, giving rise to two granules which move apart and come to lie against the nuclear membrane and closely applied to it (figs. 65, 70, 71, 72, and 73). About this time or a little later a somewhat larger, round body condenses out of the nebenkern ring, as shown in figure 72.

Outside of the breaking up of the idiochromosomes and a slight tendency to become pale and homogeneous, the nucleus remains the same during the above changes in the cytoplasmic inclusions. In the stage which follows, the delicate axial filament is quite obvious and its outgrowth from the distal end of the rod centrosome is very clear. The rod has swung so that now it is in contact with the nucleus throughout its entire length and the thread is seen traversing the space between nucleus and cell wall (fig. 74). The nucleus contains numerous dark-staining granules. The spindle remains are prominent, their borders have increased in thickness, and now appear as irregular-shaped thick-walled vesicles. The nebenkern ring, cleared of the spindle remains and of the various granules as well as of several aggregations of granular mitochondria, now rounds itself up into an oval-shaped dense mass which later becomes round (fig. 76). No structure is at first apparent except a heavily stained granular body, but one soon sees a vacuolization of its border and we get the rosette nebenkern of many writers (fig. 74).

Of the three granules already mentioned, those arising from the centrosome have either disappeared or have become so

closely adherent to the nuclear membrane as to seem a part of it, while on the other hand the other body appears slightly larger (figs. 77 and 78).

The nucleus continues to stain darker, due to the enlargement of the chromatin granules, and these may become joined to each other and give the appearance of short threads (fig. 75). The vacuolization of the nebenkern continues at the expense of the central body which becomes smaller. The walls of the peripheral vacuoles break down, the spaces becoming larger and larger, until there is but one vacuole, which may exceed even the nucleus in size, with a small heavily staining central part (figs. 80 and 81).

From this period on, the axial filament is in close relation to the central body of the nebenkern, which in well-fixed material is now seen to be made up of a spireme-like thread. I have been able to follow it throughout the greater part of its course and I feel almost certain that it is a single continuous thread (fig. 80). The cell now begins to lengthen somewhat and the central part of the nucleus to stain heavily, the chromatin moving toward the nuclear center, leaving a clear transparent border (figs. 78 to 83).

Unless one is fortunate with his fixation and staining, the central part of the nebenkern shows no structure, but appears as a glassy elliptical body suspended in the single large vacuole by means of the axial thread, but it can be seen very clearly that the tail filament never enters the central body, but comes to lie against it. The vacuole membrane lengthens out as it increases in size, while the central thread-like structure breaks up into several large and many small vesicles (figs. 81 and 82). There are also mitochondria-like structures located between the nebenkern and the nucleus as well as some distal to the nebenkern.

The nebenkern membrane forms apparently the sheath of the axial thread, some cytoplasm forming clumps around the distal part of the thread, but the vesicles in large numbers fill the spaces between the spermatozoa as they increase in length. The middle-piece anlage enlarges, and by a turning of the nucleus, the axial filament comes to lie against it (figs. 85, 86, 88, and 90).

The body then flattens out against the nucleus and later elongates slightly (figs. 85 and 90). The nucleus lengthens, and as it does, the axial filament between the middlepiece and the centrosome does likewise. The centrosome, however, is now at the apex of the nucleus and will hereafter be considered as the acrosome.

At the time when the nebenkern membrane and its vesicles have completely broken up and are only apparent as end products ensheathing the elongated tails or located between the filaments, the nucleus is still spherical, compact, and does not take the haematoxylin stain very well (fig. 82). It still has a clear area about it and some mitochondrial material about the middle-piece anlage, which is a quite prominent body located usually on the opposite side of the nucleus from the acrosome. The axial filament arising from the acrosome at the apex of the nucleus is bent backward and passes near the middle-piece anlage.

The further changes are the loss of the clear ring about the nuclear chromatin by the spreading out of the chromatic material. The nucleus shows better staining qualities. The axial filament comes to lie nearer to the middle-piece and the latter may sometimes show one or more bubbles or vesicles, which are possibly mitochondrial, in relation to it (figs. 84, 85, and 86).

The nucleus now begins slowly to elongate and seems sometimes to pull away from the acrosome, so that part of the latter body may project beyond the nucleus. The nucleus continues to lengthen, the chromatin to appear paler in color. The middle-piece enlarges and elongates (figs. 88, 89, 90, and 91). The axial filament strand between the acrosome and the middle-piece remains applied against the nucleus and sometimes may show one or several splits in the thread, leaving an elliptical opening. In material stained for mitochondria, a cloud of granules seems to gather about the thread (fig. 87). Although it cannot be traced directly, I am of the opinion that this axial filament, which is loosely applied to the outer nuclear surface, becomes the undulating membrane of the mature spermatozoon.

Thompson ('17, p. 267) suggests the formation of the undulating membrane from a free flagellum in the Trypanosomes, as follows: "It is a plausible assumption to suppose that, as the flagellum waves about it comes to lie near and parallel to the body of the cell, and that the frill or undulating membrane is formed by the clear fluid protoplasm of the surface layer springing up in a film to run up and along the flagellum, just as a soap-film would be formed in similar circumstances." Of course the axial filament in this case is located between the nucleus and the outer cell wall, but it seems a reasonable hypothesis to think of the axial filament as having become loose from the nucleus and as able to draw out the thin layer of cytoplasm some little distance from the nucleus forming the undulating membrane.

The nucleus and middle-piece now become drawn out to considerable length, the acrosome decreases in size and we see a slight projection of the nucleus extending beyond the acrosome. The elongated nucleus stains darker and darker until no structure can be made out. From this point until the mature spermatozoa are reached I have not been able to make observations (figs. 93 and 94).

The spermatozoa

A study of the mature spermatozoa has been made by teasing the contents of the seminal vesicle in a minute quantity of physiological salt solution and either studying them alive in the solution or fixing the teased material in osmic acid fumes, hot corrosive sublimate, Bouin or strong Flemming, and staining.

The unstained living contents make an interesting study when examined by means of the dark-field microscope. In addition to the spermatozoa with their waves of movement extending from anterior to posterior end of the undulation membrane, there are a large number of small elliptical bodies performing active brownian movement. I thought at first that these bodies were the true spermatozoa and the others giant spermatozoa, but further study convinced me that this was not the case, for no tails could be found upon the small bodies, they stained only by plasma stains, and furthermore no stages in their development could be made out.

Occasionally the lumen of the vas deferens is partially filled with granules differing in size, and as the cells lining the vas deferens may show similar granules in their cytoplasm, I have considered the granular material of the lumen to be secretion products of the cells. Although the bodies present in the seminal vesicle are a little longer than broad and show a little difference in their size relations, yet I think they represent the secretion found in the vas deferens. Munson ('06) considers the epithelial cells of the vas deferens of the butterfly *Papilio* to have a secretory function, but unfortunately he does not figure or describe the process.

An added fact of interest is that the bodies are transmitted during copulation and are found in the seminal receptaculum of the female, which suggests that they have some function yet unknown to us.

To ascertain their nature, smears of the seminal vesicle were made, fixed in hot corrosive sublimate, and stained in orcein, safranin and orange G, Delafield's haematoxylin and orange G, safranin and malachite green, safranin and bleu de Lyon, Delafield's haematoxylin and erythrosin. In every case it was found that the granules took only the cytoplasmic stains. In order to test the possibility of the granules' being of a fatty nature, fresh smears were stained in sudan III, but the result was negative. Smears treated with ether showed also no effect of the latter on the granules.

When the spermatozoa are examined in the fixed and stained condition (text fig. A and fig. 95), one finds a long chromatin staining thread ending in a transparent fine point, the acrosome having disappeared, and extending from near the apex a conspicuous undulating membrane. The free edge seems formed of a little denser material and represents in all probability the proximal part of the axial filament, i.e., that part between the acrosome and the middle-piece.

It is almost impossible to see just where this membrane leaves off distally as it gets narrower gradually, but I should say that about the anterior two-thirds of the spermatozoon is provided with the membrane. It is not possible to find any trace of the

middle-piece or to see where the nucleus leaves off and the tail filament begins, as the latter structure becomes finer and more transparent until it is almost impossible to see where it ends.

Text fig. A Mature spermatozoon of *Lepisma domestica* arranged from successive camera-lucida drawings. $\times 2800$.

I have noted a tendency for the tails of the living spermatozoa to stick together. The spermatozoa are very long, measuring from 400 to 660 μ .

Mitochondria

Mitochondrial structures are present in the spermatogonia, but in very small numbers, and it is difficult to make them out. At the beginning of the growth period they appear clearly as a dark-staining crescent-shaped mass usually located at one end of the cell. This mass soon breaks up and forms some six to eight bodies differing from each other in shape and size (fig. 21), but retaining an almost constant number. These bodies take the stain intensely and appear as the most prominent structures during the entire growth period. As the cell increases in size, the mitochondria becomes so conspicuous, even by ordinary iron-haematoxylin staining, that the cytoplasm seems like a dark border about the nucleus, and in this dark-staining mitochondrial matrix the clumps stand out clearly.

During the first maturation division the ring of granular mitochondria encircles the entire spindle, while the larger bodies are scattered about the cytoplasm and are located near but not on the spindle. The mitochondrial material seems to divide equally, half the granular material as well as four clumps going to each cell (fig. 39). In the resting stage of the second spermatocyte the mitochondria forms a narrow dark-staining ring about the nucleus, but the clumps are no longer apparent (figs. 49, 50, and 51). In figure 54 the granular mass is seen arranged about the spindle of the second division, while figure 61 indicates how they gather about the chromatin at the poles of the young spermatids. In the description of the spermatids the further history of the mitochondria has already been given.

DISCUSSION

The resting stage

While in many animals no resting nucleus is formed following the first maturation division, the chromosomes of the telophase being quickly transformed into the prophase of the second division, there are quite a number of exceptions reported in the literature.

Murray ('98) finds a well-marked resting nucleus in the Pulmonates, *Helix* and *Arion*. McGill ('04) found it to happen occasionally in the dragon-flies, and Painter ('14) describes it as occurring in the spiders with the accessory chromosome persisting as a nucleolus. Kingsbury ('01) finds in the salamander *Desmognathus fusca*, that a nuclear membrane is formed following the first maturation division, but that the chromosomes never lose their individuality.

In *Lepisma domestica* the chromosomes, with the exception of the idiochromosomes, entirely break up and a nuclear membrane is formed. While it is undoubtedly of short duration, still the outward individuality of the autochromosomes is lost and the second division is preceded by their reformation.

The idiochromosomes

Wilson ('09) divides the sexual differences of the chromosomes into five and possibly seven types. *Lepisma domestica* falls in line with his type IV in which "the male has a pair of idiochromosomes, half the spermatozoa receiving both and hence two more than the other half."

Only one form has been found which has this arrangement, the coreid species *Syromastes marginalis* L. This form was first described by Gross ('04) and again by Wilson ('09). The accessory chromosome arises by a synapsis of two spermatogonial chromosomes which divide equationally in the first spermatocyte, but fail to divide in the second.

Lepisma domestica differs in that the two spermatogonial chromosomes do not fuse, but remain separate and joined by a stout thread. They pass undivided to one pole in the first spermatocyte division, but separate in the second. Wilson's prediction that the female of *Syromastes* would have two more chromosomes than the male, he afterward found to be the case. Reasoning in a similar manner, *Lepisma domestica* females should have thirty-six chromosomes, but unfortunately I have been unable to make any chromosome counts so far in the female.

Synapsis and reduction

It has not been possible in the ordinary chromosomes or auto-chromosomes to see whether there is either a side-by-side union of the spireme threads, a parasynapsis, or an end-to-end conjugation, a telosynapsis. It is clear, however, that the spireme threads in postsynaptic stages are much thicker and are present in fewer numbers. Whether they are half the leptotene number or not could not be made out.

In the case of the idiochromosomes the conclusions are clearer. Each idiochromosome breaks up into a spireme thread and the two threads eventually unite end to end, one of them being attached to a large plasmosome. From these threads two chromosomes are formed by the condensation of the chromatin, but they still remain united by a thread which is probably linin in nature and along which, when the thread lengthens, the chromatin is drawn out.

Synapsis, or a side-by-side conjugation, if it takes place at all, does so following the telophase of the first maturation division. That the idiochromosomes do come into a very close relation is shown by the longitudinal split apparent in the idichromosome nucleolus of the resting nuclei of the second spermatocyte (figs. 47 and 50).

If, as is generally conceded, the spermatogonial chromosomes represent two groups, one of maternal and the other of paternal chromosomes, and the homologous pairs conjugate at synapsis then each of the idiochromosomes represents one spermatogonial chromosome. A further proof of this is that the thirty-four spermatogonial chromosomes, judged from their size, are all of the same valence, i.e., bivalent. After synapsis the autochromosomes are quadrivalent, but definite four-part tetrads are not apparent during the prophase, and at the metaphase the chromosomes are dumb-bell-shaped, the longitudinal pairing leaving no trace. However, in one cell I have found the idiochromosomes at the time of anaphase showing a bivalent construction (fig. 45).

The first maturation division separates the dumb-bell-shaped chromosomes transversely (fig. 39), and probably represents a reduction division, as the idiochromosomes go to one pole undivided. The second division of the autochromosomes is clearly a longitudinal one (fig. 55), while the idiochromosomes separate transversely, so that it would seem that this represents an equational division of the autochromosomes and the separation of the idiochromosomes one from the other.

The centrosome

The single- or double-rod type of centrosome has been described in a variety of forms. Meves ('98) and Buder ('15) have described them in the Lepidoptera, and Sewertzoff (Meves, '00) in Orthoptera, and Korff ('01) in the Coleoptera. In plants Von Mottier ('98) found them in the tetraspore mother cell of *Dictyota dichotoma*. Korff also found them in the sperm cells of the domestic hen and duck, while Hortegea ('16) figures them for the ganglion cells and brain of man.

Von Mottier does not consider them homogeneous, but to arise from small granules. In the beetles, Korff shows them to be very like those found in *Lepisma domestica*, but he has not reported any granule in relation to the rods at any time. He finds the limbs of the V separating in the late telophase and appearing parallel to the polar axis in the spindle. In *Lepisma domestica* the centrosomes are always oblique to the axis, but parallel to each other.

We have seen the centrosome first as one or two small granules, then as double rods with or without end granules, and still later as single rods which only occasionally show a granule. In very rare cases, instead of single rods, the centrosome consists of several granules in the polar position. From these observations the form of the centrosome would seem to be a variable quantity. It is interesting in this regard that Korff ('01) was only able to see the V-shaped centrosome in the sperm cells of the drake and rooster, while all the other cells of the body showed centrosomes consisting of single granules.

The acrosome

Although a number of the older writers, notably Platner ('89), Niessing ('96), Field ('95), and Moore (94'), have described the acrosome as arising from the centrosome, Wilson ('06) comes to the conclusion that the work of Henking ('91), Wilcox ('96), and Paulmier ('99) show conclusively that in the insects the acrosome is derived from the nebenkern.

That this is not the case in *Lepisma domestica* can be easily proved, for in every stage from the telophase of the second division until the oldest transformation stage in which it was possible to identify structures, the centrosome rod and its change into the acrosome can be followed.

It might perhaps be argued that the granule is really the centrosome and the rod only a product of the centrosome, formed in somewhat the same manner as the 'battonet' or rodlet in the spermatid of the Pribilof fur seal. As described by Oliver ('13), it arises as a prolongation from the anterior centrosome. In any event, the acrosome owes its origin either directly or indirectly to the centrosome.

Goldsmith ('19) describes in the tiger-beetle a condition, which in view of my own work on the acrosome and middle-piece, is very suggestive. He figures an extra nuclear plate or middle-piece which is formed at the point of junction of the axial filament and the nucleus. It contains several chromatin-staining bodies to which the axial filament is attached. These chromatin bodies move to one side and then toward the anterior end of the nucleus, the filament coming at last to lie against the elongated mitochondrial body (nebenkern) and the bodies to assume a bivalent appearance at the anterior end of the nucleus. The middle-piece becomes drawn out into a granular thread continuous with the axial filament, while the acrosome appears later and fuses with the other two bodies.

It would appear that the chromatin-staining bodies, to which the axial filament is attached, must be the centrosomes, and that their change in position, due to the rotation of the nucleus, is exactly parallel with what occurs in *Lepisma domestica*.

While no opinion as to the origin of the acrosome is advanced, the fact that it arises in relation to the granules from which the axial filament originally developed would point to the probability of a centrosomal origin. The granular thread which he considers to be the drawn-out middle-piece is, in *Lepisma domestica*, simply the proximal end of the axial filament carried to the apex of the cell along with the centrosome, and it would seem as though such an interpretation could be made of Goldsmith's results.

The middle-piece

Wilson ('06, p. 337), in speaking of the essential structures in a spermatozoon, lists the middle-piece as a body which "either contains a formed centrosome or a pair of centrosomes, or is itself a metamorphosed centrosome."

It is altogether possible that the middle-piece in *Lepisma domestica* arises from one or both of the granules which we found to have their origin from the centrosome and later traced them to where they were closely applied to the nuclear wall. In fact, we would naturally expect something of the kind, but unfortunately the later history of the granules could not be followed. The question is further confused by the presence of a body which condenses from the nebenkern ring and which, from its size, staining power, and position, would point to its becoming the middle-piece. Furthermore this body can be found practically in all the stages up to the apposition of the middle-piece to the nucleus, but here again we are stopped, for we have not seen the actual formation of this body into the middle-piece. It is possible that a further study will solve this difficulty.

Comparison with Orthoptera

At the first glance there seems to be a similarity between the spermatogenesis in *Thysanura* as described above and that in *Orthoptera* as previously described and particularly given by Payne ('16) for *Gryllotalpa borealis*, but there are also differences which cause one to question whether the resemblance is not more superficial than real.

In the first place, the general shape and arrangement of the chromosomes in the spermatogonia of *Grylotalpa borealis* are very much like similar stages in *Lepisma domestica*. Payne also has described changes in the mitochondrial mass of the spermatid (his figures E, F, and G, pl. 2), which have almost exact counterparts in *Lepisma domestica*. Then again his figure J on plate 3 shows an axial filament in which the cytoplasm is so arranged in waves as to look like the undulating membrane found in the Thysanura.

A comparison of the group of chromosomes associated probably with sex in the two forms shows, however, several important differences. In *Grylotalpa borealis* Payne finds a single chromosome which does not divide in the first maturation division and therefore could be directly compared with the idiochromosomes of the Thysanura were it not for the fact that the single chromosome is associated with an unequal bivalent chromosome and in division always goes to the same pole as the large 'end' of the unequal chromosome. Therefore, the two resulting secondary spermatocytes differ not only in that one has an extra chromosome, but also in that the same cell possesses the large 'end' of the unequal chromosome, while the smaller part passes to the other cell. Payne favors the view that these chromosomes represent a triad group rather than an unequal pair of idiochromosomes and an accessory chromosome.

Payne has not been able to trace the centrosome of the second maturation division through to the spermatid, and in fact has not been able to demonstrate the presence of the centrosome in the spermatid at all, although he presumes that the body from which the axial filament arises and which later becomes the middle-piece may be a centrosome. Further, he describes the acrosome as arising from an elongated body which suddenly appears *de novo* in the cytoplasm, whereas in *Lepisma domestica* the acrosome is formed from a rod-like centrosome.

SUMMARY

1. The male *Lepisma domestica* has three pairs of testes on each side, each testis connected by a duct with the respective vas deferens.

2. The blind end of the testis contains the youngest stages.

3. Primary spermatogonia are formed very early in life.

4. Thirty-four chromosomes are present in the spermatogonia. A chromatic nucleolus is present in the resting stage of the spermatogonia.

5. The growth stages follow the description given by Wilson ('12).

6. A planosome is seen in the resting stage of the spermatogonia appearing in the growth stages as a much larger body.

7. One or two plasmosomes appear shortly after formation of the spireme threads, and disappear later.

8. There are eighteen chromosomes in the first maturation division. The two idiochromosomes pass undivided to one pole.

9. The autochromosomes divide longitudinally in the second division, while the idiochromosomes do not. Instead, they separate, each spermatid receiving one idiochromosome.

10. The form of the centrosome is changeable, but its almost constant presence either in the shape of a granule or of a rod indicates that it may be a permanent cell structure.

11. A chromatic nucleolus is present in half of the spermatids.

12. The nebenkern is formed from granular mitochondria, the remains of the last and of two previous spindles.

13. The axial filament grows from the end of the rod-shaped centrosome which forms the acrosome.

14. Another body, presumably derived from the nebenkern, forms the middle-piece.

15. The nebenkern, after separating out the spindle remains and several accumulations of mitochondrial material, form a vacuolated body which furnishes a sheath for the axial filament.

16. The axial filament persists and forms the undulating membrane of the mature spermatozoa.

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EXPLANATION OF PLATES

All the figures, with the exception of the first three, were made with a Zeiss 2-mm. apochromat. objective and Zeiss compensating ocular no. 12. In order to get as high a magnification as possible, drawings were made at table level, giving an enlargement of about 1850 diameters.

ABBREVIATIONS

a, acrosome
af, axial filament
c, centrosome
i, idiochromosomes
m, mitochondria
mp, middle-piece
n, nebenkern
o, ovipositor

p, penis
pl, plasmosome
ps, planosome
s, spindle remains
sv, seminal vesicle
t, tubules
vd, vas deferens
x, middle-piece anlage

PLATE 1

EXPLANATION OF FIGURES

- 1 Posterior segments, ventral surface, of male *Lepisma domestica*. $\times 20$.
- 2 Posterior segments, ventral surface. of female *Lepisma domestica*. $\times 20$.
- 3 Testes of one side showing connection with vas deferens and seminal vesicle. $\times 20$.
- 4 Spermatogonium, surface view, showing resting condition of nucleus and the attraction sphere.
- 5 Prophase of spermatogonium.
- ✓ 6 Metaphase plate early spermatogonium. Thirty-four chromosomes joined by linin threads.
- ✓ 7 Telophase spermatogonia showing persistent spindle remains.
- 8, 9, 10, and 11 Nuclei of spermatogonia decreasing in size with each division.
- ✓ 12 Spermatogonial metaphase from the side. Centrosome as single granule at poles.
- 13, 14, and 15 Resting stages spermatogonia to show idiochromosomes.
- 16 Spermatogonium showing the breaking up of the idiochromosomes.
- 17 Beginning of growth period. Idiochromosomes reformed.
- 18 Spindle remains from spermatogonium with two centrosome granules.
- 19 Same as the preceding from early growth period with V-shaped centrosomes.
- 20 Centrosome rods with granules at their ends, before division. Plasmosome and idiochromosome loops.

PLATE 2

EXPLANATION OF FIGURES

- 21 First spermatocyte. Nucleus homogeneous except for idiochromosomes.
- 22 First spermatocyte showing fine, loosely arranged leptotene thread. Idiochromosomes very pale in color.
- 23 Early contraction stage of first spermatocyte. Plasmosomes with idiochromosome thread to one side.
- 24 First spermatocyte showing a spreading out of the threads following contraction. Plasmosome and idiochromosome threads shown in surface view.
- 25 Bouquet stage of first spermatocyte. Only a few loops shown in drawing.
- 26 Pachytene stage of first spermatocyte.
- 27 Same as the preceding, only somewhat later.
- 28, 31, and 34 Sertoli cells associated with different stages in the development of the spermatozoa.
- 29 Confused or net-like stage of first spermatocyte. Planosome prominent.
- 30 Early prophase of first spermatocyte. Centrosomes have migrated nearly to poles. Two plasmosomes present.
- 32 Dividing cell from spermatogonial region. Possibly a Sertoli cell. Chromosomes paired.
- 33 Diagram to show the relative sizes of cells of first and second maturation divisions and of the spermatid.

PLATE 3

EXPLANATION OF FIGURES

35 The plasmosomes and idiochromosomes during the growth period of the first spermatocyte showing formation of spireme threads and their contraction into the prochromosomes.

36 and 37 Prophase of first spermatocyte showing connected idiochromosomes.

38 Metaphase plate of first spermatocyte with idiochromosomes in center.

39 Side view metaphase of first spermatocyte to show dumb-bell-shaped chromosomes and their transverse division. Idiochromosomes joined together.

40 Same as the preceding.

41, 42, 43, and 44 Side views of dividing first spermatocyte, showing idiochromosomes during anaphase and telophase.

45 Oblique view of anaphase of dividing first spermatocyte. Thirty-three chromosomes plus the idiochromosome complex which here shows each idiochromosome as bivalent.

PLATE 4

EXPLANATION OF FIGURES

- 46 Early second spermatocyte, showing the breaking up of the telophase chromosomes. Position of spindle remains typical.
- 47 Resting stage of second spermatocyte.
- 48 Same as the preceding, the chromatic nucleolus found in only half the cells of the second spermatocyte.
- 49 Early prophase of second spermatocyte.
- 50 Same as the preceding, chromatic nucleolus double. Ring of mitochondrial granules around the nucleus.
- 51 Resting stage of second spermatocyte, showing formation of spindle fibers before the prophase stage is reached.
- 52 Side view metaphase of second spermatocyte.
- 53 Eighteen chromosome metaphase plate of the second spermatocyte.
- 54 Side view metaphase plate of second spermatocyte. Rod centrosomes with granules at inner ends.
- 55 Early anaphase of second spermatocyte with the autochromosomes dividing longitudinally, while the idiochromosomes have separated transversely.
- 56 Metaphase plate of second spermatocyte. The sixteen chromosome type.
- 57 Later anaphase of second spermatocyte.
- 58 Same as the preceding with granules at poles instead of rods.

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PLATE 5

EXPLANATION OF FIGURES

- 59 Two anaphase drawings of second spermatocyte from same cell. Sixteen chromosomes in each.
- 60 Spermatids from division of eighteen chromosome second spermatocyte. The divided idiochromosomes differ in shape and are darker stained than the autochromosomes.
- 61 Young spermatids before the reorganization of nucleus.
- 62 Anaphase of second spermatocyte, showing ring-like arrangement of chromosomes.
- 63 Early formation of nucleus of spermatid.
- 64 Spermatid with resting nucleus and nucleolus.
- 65 Early spermatid with rod centrosome.
- 66 Same as the preceding, showing centrosome with granule at base lying against nuclear membrane.
- 67 Same as the preceding. The granule broken off.
- 68 Same as the preceding. The granule much enlarged.
- 69 Elements of nebenkern from early spermatid.
- 70 Spermatid showing division of granule.
- 71, 72, and 73 The nebenkern ring from spermatid, showing separation of granules and formation of new body from the nebenkern.
- 74 Central part of nebenkern of spermatid, showing rosette form and spindle remains.
- 75 Spermatid showing vacuolization of nebenkern border further advanced. Nuclear granules larger and stain darker.
- 76 Spermatid showing centrosome applied to nuclear membrane.
- 77 and 78 Spermatid showing clear space forming around chromatin of nucleus. Middle-piece anlage present.

PLATE 6

EXPLANATION OF FIGURES

- 79 Same stage as figure 78. Centrosome rod shown clearly.
- 80 Spermatid with chromatin of nucleus more condensed. Thread-like structure in middle of nebenkern vacuole. Mitochondria between nebenkern and nucleus as well as distal to nebenkern.
- 81 Same as the preceding, but threads of nebenkern have broken and now form vesicles.
- 82 Same as the preceding showing linear arrangement of small vesicles.
- 83 Spermatid of a slightly later state than the preceding.
- 84 Spermatid with nebenkern contents thrown off.
- 85 and 86 Spermatids. Side and surface view adjacent cells to show relation of axial filament to middle-piece anlage. Filament thicker on account of mitochondrial granules in relation to it.
- 87 Spermatid showing mitochondrial granular mass in relation to middle-piece and axial filament.
- 88 Spermatid showing secondary relationship to middle-piece anlage.
- 89 Spermatid. A later stage to show mitochondrial ribbon about the proximal part of the axial filament.
- 90 Spermatid nucleus elongating and staining darker. Middle-piece getting longer.
- 91 Same as the preceding.
- 92 Elongation of nucleus of spermatid. Mitochondria about filament.
- 93 Spermatid. A still later stage, no mitochondria shown.
- 94 Spermatid nucleus very much drawn out. Acrosome at apex and darker part indicates position of middle-piece.
- 95 Part of anterior end of mature spermatozoon. The undulating membrane with its thicker border is well shown.

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