1954

Spermatogenesis in the sand crab Emerita Analoga : a thesis ...

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SPERMATOGENESIS IN THE SAND CRAB EMERITA ANALOGA

A Thesis

Presented to

the Faculty of the Department of Zoology

College of the Pacific

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

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by

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June 1954

INTRODUCTION

Crustacean spermatozoa are among the most peculiarly modified germ cells in the animal kingdom. Many of their striking cytological specializations may be observed in the sperm cells of Emerita analoga, the so-called sand crab. This animal has been used by the writer for studies on spermatogenesis and on spermatozoan behavior during the summer of 1951, 1952, and 1953 at the Pacific Marine Station.

To the writer's knowledge, the classic work of Koltzoff (1906) on the spermatozoa of Galathea squamifera (an Anomuran crab) and the cursory treatment of spermatogenesis in the shrimp, Penaeus setiferus (King, 1948) are the only studies on Crustacean spermatogenesis which have as yet been made.

As stated by J. C. Dan (1952),[1] "Our knowledge of spermatozoa and their role in the fertilization reaction has been acquired through studies approaching the subject from two widely different angles---'straight' morphological observation dealing with fixed specimens, such as the classical drawings of Retzius, or the electron microscopic photographs of Bretschneider; and the physiological studies centering around the fertilization theory of F. R. Lillie and currently

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1 J. C. Dan, 1952, "Studies on the Acrosome. I. Reaction to egg-water and other stimuli." The Biological Bulletin, Vol. 103, No. 1, pp. 54-66.

being pushed close to conclusion by the penetrating biochemical research of Tyler, Hartman, Runnstrom, and their co-workers."

The writer has made a study of fixed, stained testis, and though presenting some aspects of the spermatozoan in vitro, has for the major consideration a "straight" morphological presentation. It is hoped, too, that studies on the male reproductive organs of Emerita analoga will facilitate future investigations on Crustacean spermatozoa.

The writer wishes to thank Dr. Alden E. Noble, Director of the Pacific Marine Station for his valuable aid and advice, without which this research would not have been possible.

PROCEDURES

Collections of sand crabs were made in the inter-tidal zones of sandy beaches. Most of the animals were collected at the Pacific Marine Station, Dillon Beach, California. Some specimens were obtained from the Stemple Creek Estuary and from the Pudding Creek Beach one mile north of Fort Bragg, California. Emerita can be readily located by observing the "V" shaped ripple marks made as the receding wave-wash is cut by their protruding antennae. Though subject to changes over a period of time, the gregarious sand crab was found to congregate in beds at definite beach locations. It is possible, however, that this occurrence was due to

favorable beach currents rather than any tendency on the crabs' part to be "neighborly". Though Mead (1917) found no evidence of the sand crab in zones above the beach washed intermittently by the waves, the writer was able to uncover crabs stranded and waiting the incoming tide, well above the water line. The sand crab, well adapted to its environment, both in color, shape and locomotion is lost if capture is not made immediately after it has been turned up with a shovel. Collectors working in pairs are most successful.

Animals were kept in the laboratory for two months and by all indications could have been maintained indefinitely. Best results were obtained by keeping the animals in a 1x2 foot aquarium covered by not more than 10 inches of sea water nor less than 3 inches. Approximately three to six inches of sand were spread over the bottom of the aquarium. Two continuously operating stone breakers in the aquarium gave sufficient aeration. Emerita is extremely sensitive to putrefaction; one dead organism left in the aquarium for a day was usually lethal to a whole culture. A convenient method of cleaning the tank was to take advantage of the photo-tropic characteristic of Emerita by shining a strong light at one corner of the tank. The animals gathering in this section of the tank were not disturbed as the sand and water were changed.

For the purpose of histological studies, rapid vivisectioning was found to be a most practical method for

removal of the testis. To do this, the operator practiced to perfect techniques that would lose a minimum amount of time between the animals' natural state and tissue fixation. Both ventral and dorsal approaches were investigated. Due to the small amounts of musclature found dorsally and the easily removed exo-skeleton, the dorsal dissection was used. All locomotor appendages including the telson were first removed, and then a complete border around the cephalothorax was cut with dissecting scissors. The exo-skeleton was removed and the integument exposed. Because the testis are completely surrounded and imbedded in the digestive glands, a technique was used which enabled the writer to render the reproductive organs almost entirely free from other tissues. A 5cc syringe with a 20 gauge, slightly hooked, needle was used as a dissecting instrument. This syringe filled with sea water enabled the dissector to clear away unwanted debris by flushing it out of the field. Considerable care was taken to obtain the testis in toto for they are of a very soft consistency, tear readily, and are quite easily confused with the surrounding tissues.

Histological techniques were used as follows: Except for the Feulgen reaction, 7% neutralized formalin in sea water and Bouin's solution at 38°c. were found to be satisfactory fixatives for the testicular tissue of E. analoga. Zenker's solution was not found to be a good fixative, because grannules appeared in the cytoplasm of the cells

younger than secondary spermatocytes which obscured nuclear changes. Unfortunately, the writer was unable to verify the significance of these particles. Dehydration was accomplished with a series of progressively concentrated alcoholic solutions. The most successful methods used were to place the tissues, after they had been thoroughly washed in two successive changes each of 25%, 50%, 70%, 80%, 95% alcohol for 5 minutes with two changes of absolute alcohol at the end of the series. In one series, dioxane clearing was carried out with some improvement noted in the slides. The tissues were then placed in equal parts xylene and unmelted paraffin and placed in an incubator at 56°C. for two hours. To complete infiltration transfer was made to pure paraffin for from six to eight hours. Imbedding and microtome cutting were done in the customary manner for paraffin sectioning. A rotary type microtome was used to cut sections from three to twelve microns thick; ten microns were found to be best for the routine tissue studies.

The sections were de-paraffinated with xylene, hydrated in alcohol solutions of decreasing concentrations and placed in water.

Iron hemotoxlyn (Heidenhain) was used for general cytological study and was the most satisfactory and useful of the stains. Sections were mordanted in 0.5% iron alum for thirty minutes and, after careful washings, stained for an equal length of time in hematoxylin. Equally good results

were obtained by leaving sections in both the mordant and stain at 60°C. for one minute. Differentiation was carried out with 0.5% iron alum and also in weak dilutions of hydrochloric acid.

The Feulgen nuclear reaction for detections of thymonucleic acid was found valuable in locating nuclear constituents during the complex changes. Vital stains used were: Janus green B, Neutral red, Methylene blue and Methyl green. Vital studies were made on freshly removed testicular tissues which were teased out and pressed under cover glasses.

GROSS ANATOMY OF THE MALE REPRODUCTIVE SYSTEM

The testes of Emerita analoga occupy a dorsal position in the anterior portion of the cephalothoracic cavity. The testes consist of two lateral, paired lobes measuring 8 to 10mm in length and varying from 1½ to 2½mm in width (plate 2, fig. 2). Posterior to the pyloric stomach and just ventral to the heart is a 2½mm commisure connecting the lobes (Plate 2, fig. 4). There are two subsidiary lobules extending anteriorly on each side (Plate 2, fig. 4) and also one extending slightly posterior.

In microscopic section the vasa efferentia are thin-walled vessels of one epithelial cell thickness and the lumen is filled with spermatozoa. They are very slender, loosely coiled tubules extending from all of the lobules into the bases of the main lobes where they interconnect in somewhat

larger tubules which are aggregated in close proximity. At this point, about 3mm within the testes, they join the vas deferens. The vas deferens on each side has a double flexure curving toward the dorsal side and entering a milky-white translucent body which King (1948) calls (in the shrimp) "ductus ejaculatorius" or "terminal ampoule". These large, paired structures (Plate 2, fig. 1, 4 and 5) measure 2mm by 8mm and are the most prominent land marks observed after the integument had been removed. These whitish, elongate masses are found close to the mid-dorsal line, lying between the commisure of the testis lobes and the pericardial sinus. The bodies, which appear to act as a kind of seminal vesicle pass posterior to the juncture of the cephalothorax and abdomen. In microscopic section this structure was portrayed as a vesicular sac containing a tightly coiled, ribbon-like duct filled with spermatozoa. The duct was found to be 190 μ wide and 30 μ thick. When stretched out to its elastic limits, clumps of small white ovoid masses were observed within it. Since no differentiation of these clumps could be made microscopically, they were presumed to be due to mechanical distortion.

The spermatozoa empty into a sperm duct (Plate 2, fig. 5) which passes anteroventrally for a distance of about 0.5 mm. The duct then turns upon itself and follows a straight course lateroposteriorly to the fifth leg. Situated at the base of this leg is a leaf-shaped (2 mm. x 1 mm.)

dactyl. The whole lower portion of the reproductive system can be examined by pulling off the fifth leg and drawing out the sperm duct and seminal vesicle. This procedure is recommended for the rapid procurement of spermatozoa.

SPERMATOGENESIS

The Spermatogonia

The spermatogonia appear to arise from greatly flattened germinal epithelium arranged around the acinus wall as a lining one cell layer thick. As the developing spermatogonium leaves the germinal layer, it becomes ovoid in shape contrasting sharply with its former flattened oblong appearance. At this stage, the nucleus expands from a small oblong body containing minute chromatin condensations joined by very fine strands. As the nuclear volume increases, the nuclear and cell walls fill out so that the cell appears almost cuboidal. This change in shape is accompanied by an increase in cytoplasmic granules and the spreading out of the closely paced chromatin condensations in the nucleus. The spermatogonia round up and the nuclei, which increase in volume more rapidly than the cytoplasm measures 4.2 u as compared to the cell measurement of 5.6 u (Plate 3, fig. 2). The spermatogonia are recognizable as spherical cells varying slightly in size, containing a large nucleus surrounded by a thin layer of uniform cytoplasm. The chromatin appears in iron haematoxylin preparations to be heavily stained and in

Fuelgen slides appear as purple granules. At full growth, the spermatogonium has one large nucleolus located eccentrically on the nuclear wall.

The newly formed spermatogonium goes through a period of growth which is alternately punctuated by mitoses. Interestingly, the onset of mitosis is simultaneous in all the cells of a particular acinus. It was also observed that other cells followed a pattern of uniformity so that cells only in one mitotic phase are observed in the lumen of a particular acinus.

The writer observed numerous prophases, but the later stages of division, while not rare, were less often observed. During telophase and anaphase, the chromosomes appear to be irregular in shape while on metaphase plates they were uniformly rounded and regular in arrangement.

Primary Spermatocytes (Plate 3, figures 2, 3, 4, and 5)

A sharp contrast may be observed between the spermatogonia and the primary spermatocyte since they differ in size, nucleo-cytoplasmic ratio, and shape. The resting primary spermatocyte is about 11.2 μ in diameter while the nucleus measures 7 μ so that there is a ratio of 1:1.75 (cytoplasm to nucleus). The rounded compact spermatogonia differ from the larger irregular and elongated primary spermatocytes in that the spermatocyte's chromatin material is not as dense. In the resting stage (Plate 3, fig. 3) a

delicate network of spireme threads connect with deeply staining chromain substance. The nucleoplasm itself presents a homogeneous ground work which is punctuated by irregularly spaced vacuoles containing less deeply staining substances. The Feulgen reaction reveals a nucleolus in the prophase cells which are invariably seen close to the nuclear membrane. At the initiation of diakinesis the nucleolus disappears and is not seen throughout any of the other stages of spermatogenesis unless an occasional and problematical body appearing in a few spermatids could be interpreted as nucleoli.

Cytoplasmic patterns were not clearly different, yet in some phases of the primary spermatocyte, particularly early prophase and late telophase, a misty condensation was observed adjacent to the nucleus and thought to be mitochondrial material. Verification of this observvation, using vital materials stained with Janus Green B, was not entirely conclusive. Associated with this cytoplasmic condensation was a group of chromophilic materials that took the shapes of closed rings or crescents. Seen particularly well in slides stained with Flemming's tri-color stain and in those mordanted with osmic acid these configurations marked the only clear-cut cytoplasmic structures observed and were presumed to be golgi bodies. These bodies were never more than eight in number and more often four or less were observed. No consistant data could be accumulated regarding their behavior

during diakinesis.

As the primary spermatocyte prepares to divide and the chromosomes condense, a rather pronounced bouquet stage is observed wherein the chronosomal threads are oriented toward one side of the nucleus. Repeated and careful focusing was never rewarded by the sight of centrioles on this side of the nucleus where in many other animals the centrioles are usually observed during the bouquet phenomena. There were many instances however, where a small nongranular spot suggested a cenitriole focal point.

Mieosis appears to occur rapidly because acini containing meiotic cells are not readily located. Spermatogonia, and primary spermatocytes are the most numerous cells found and are therefore probably the longest periods in which cells remain during the developmental process.

The leptotene spireme (Plate 3, fig. 4) and the zygotene bouquet are frequently observed. The writer noted relatively few cells in the diplotene stage but found diakinesis stages more abundant. Even in diakinesis, however, when the compact tetrads are well marked the definition of individual chromosomes is quite difficult. A count of approximately 26 (2 n) chromosomes in tetrads and metaphase plates was averaged from several observations.

At the first metaphase (Plate 3, fig. 5) the chromosomes are arranged around a cleared central area. The cell

is irregular in outline and the cytoplasm is abundant. These cells, measuring 11.8 y, are the largest observed in the spermatogenesis series.

Secondary Spermatocytes

In the interphase the secondary spermatocytes resulting from the first maturation division differ from the primary spermatocytes by their smaller size and the more even appearance of their general cell shape. The nucleus appears to be more vacuolated and the chromatin material less abundant. There actually seems to be no definite resting stages between the first and second maturation divisions as nuclear activity is evident in most all of the secondary spermatocytes. Typical cells (Plate 3, fig. 6 and 7) are found to be 10.5 u in size with a nuclear measurement of 4.9u. The metaphase plate of the second maturation division (Plate 3, fig. 8) is characteristic of the very close, heavy grouping of chromosomes at the metaphase. Many cells were observed in this stage and in anaphase stages. Some secondary spermatocytes, with spindle fibers and other mitotic evidences still discernable, were observed already organizing in the prophase preparatory for another division. Though a study of sperm abnormality was not contemplated in this study speculative conjecture leads the writer to believe that some abnormalities in the mature spermatozooa might arise from a too rapid passing through this secondary spermatocyte stage.

Spermatids

The oval-shaped, young spermatids, 6.3 u. x 3.5 u. had a spherical, acentric, nucleus, which was 2.8 u in diameter compared to the cytoplasmic diameter of 6.8 u. The cytoplasmic nuclear ratio (np), in Feulgen preparations, demonstrated an interesting phenomenon in which a polar separation of Feulgen positive materials occured. These materials concentrated against the nuclear membrane at the potential posterior and anterior ends. It is entirely within the realm of possibility that these materials eventually surround or envelope the bag like nucleus to form a kind of inner linning of nucleoplasmic materials. This metamorphosis was partially confirmed by tracing the distribution in older cells but could not be wholly confirmed.

There appears to be a lengthening out of the cells and, in areas where mechanical restrictions on space were not existent, the cyloptasmic nuclear ratio appeared to be slightly increased. The elongation of the cell is accompanied by cytoplasmic condensations, percursors of the great convolutions to come. The nucleus in the older spermatids (fig. 11) are surrounded by a space (anloge of the barrel or casule) and gravitate more obviously to the potential anterior end of the cell.

Metamorphosis of the Spermatozoa

It is probably difficult to find a more weird and striking cell metamorphosis, outside the Decapoda, than that witnessed in the spermatogenesis of Emerita. For sake of basic understanding reference is made to structures commonly recognized in most spermatozoam morphology even though in this Crustocean's germ cells the names may not always be properly analogous to the particular function of the part. (Plate 4, fig. 3).

Along with the nuclear migrations and the formation of an adjacent nuclear space the spermatids undergo extensive vacuolation. (Plate 3, fig. 11). The number of cytoplasmic vacuoles varied from two to eight, (fig. 13) becoming progressively more numerous. Appearing as if it were an outgrowth of the nuclear wall itself the space adjacent to the nucleus (Plate 3, fig. 11) extends down through the elongate cell body. Observations of fixed preparations at this stage gave an impression of fragil scoop-shaped cells with thin whisps of cytoplasm and irregularly shaped pycnotic nuclei. However careful focussing revealed a hollowed out central lumen formed within the cytoplasm which apparently contained no cytoplasmic granules nor absorbed any stains. Around the periphery of this space was the frothy vacuolated thined-out cytoplasm. An outgrowth of bristle-like structures was seen to project from the posterior side of the nucleus to ring the top of the newly formed cytoplasmic lumen.

A progressive coalescing of the cytoplasm continues (fig. 14) so that it takes on a less frothy and less vacuolated appearance. The space within seemed to break through the posterior end of the cell forming a completed cylinder blunted at one end by a dark, homogenous staining nucleus (Plate 3, fig. 19). The hair, or cilia-like, ring lengthened somewhat and slanted uniformly in one direction. The large seemingly more heavily stained projections noted on either side of the nucleus were merely views of three or four of the afore mentioned structures (Plate 3, fig. 13, 14, 15 and 18) seen at the edge of the ring. The projections were the anlage of the very elongate processes of the mature spermatozoan.

Living Spermatozoa

Some interesting studies on living spermatozoa were made. As previously explained, methods were devised for rapid procurement of the male sex cells so that valid bases could be established for the recognition of abnormal, disintegrating, and otherwise distorted spermatozoa.

When first expressed from the male Emerita the spermatozoon appeared to have a long, tapering, spine-like tail. This however, proved to be a coalescence of the pseudapodial processes prior to unraveling (Plate 4, fig. 1, 2 and 3). It took a relatively short time, from one to one and a half minutes for the spermatozoon to assume a bristling appearance

as eight to twelve long (40μ to 50μ) processes unfolded. These processes appeared to emanate from a ring of dots located on the collar just below the bag-like nucleus. This ring is a part of the collar that corresponds to what Holtzoff (date) called the proximal centrosome body or centriole I. This neck-like structure, the collar, has another structure in it that takes the vital stain, Janus Green B, quite readily and also correlates quite well with what Holtzoff called mitochondrial bodies in Galathea. The structure is a pair of rounded bodies found just above the inner tube of the capsule. It is interesting to note that this inner cylinder corresponds to the distal centrosome (centriole II) which Holtzoff, in his studies of Galathea squamifera, regarded as a spring-like, trigger mechanism, which was responsible for "shooting" the sperm head into the ovum. The lumen-like inner cylinder stained very lightly with all vital stains and were it not for follow-up experiments with hypotonic salts ($\frac{1}{2}$M $CaCl_2$) and concentrated sea water it might have been merely regarded as a lumen. The writer was able to expand the capsule and observe a slender filament-like structure extending down from the collar. Eventually the cell was "exploded" or lysed away so that only the collar, its processes, fragments of the capsule and the proposed filament-like distal centriole remained attached together. At the distal end of the capsule is a lip-like circular structure which appears to be an apeture into the capsule. With vital stains, in particular neutral red, an

plug structure was observed immediately below this supposed apeture. In fixed slides this plug showed up best in slides stained with Flemming's tricolor preparation.

The spermatozoa were observed as they floated in egg water, solutions of sea water and in close approximation with ova. At no time, however, was seen the phenomenal activity of the "exploding" spermatozoam as reported in Holtzoff's study: "Die Schwanzkapsel spielt die Rolle eines eine bedentende Energie anfweisenden Fortbewegungsorganes, welches bei der, den Sprung des Spermiums nach sich Ziehenden Explosion frei wird. Nach der explosion hat die Kapsel fur den Befruchtungsprozess keine Bedentung nehr und kann nun ausserhalb der Ei hulle bleiben und ganz abfallen.

"Der vordere Ring des distalen Central-korpers dient wo dersebe zur Ansbildung gelangt (Galathea), als Grenze zwischen Hals und Kapsel und an dieser Stelle lost sich letztere ab.

"Der hintere Teil des distalen Centralkorpers, welcher die Fahingkeit besitzt aufzuschnellen, und in welchem ein gewisses Quantum Elastizitatsenergie aufgespeichert ist, spielt eine orientierende Rolle bei der Kapselexplosion indem er fur den normalen Verlanf der betzteren von Bedentung ist. Dieser Teil des Centralkorpers wird nach beendigter Explosion ebenfalls unnotig und fallt zusammen mit der Kapsel ab."

"Zun Schluss des gegenwartigen Kapitels und auf grund der oben berichteten Facten und Erwagungen will ich versuchen, naher auf die Zweckmassigkeit der einzelmen Teil des Decapoden sperm einzergehen. Heir will ich die die Richigkeit meiner Erwagungen betreffenden Ruckbehalte welche seinerzeit in Text notig waren, nicht meher vorausschicken. Der Kopf des spermiums, ebenso wie die Koffortsatze der S. cephalacantha enthalt den kern und in erster linie das chromatin, welches nach der allgemein auerkannten Auschanung der Trager der erblichen Eigensch aften ist; in Berfruchtung sprozesse wird der kopf ganz in das Ei hineingefuhrt.

"Die formativen Faden des Kopfes bestimmen dessen schrauben formige, mit scharfen Kanten versehene Form, welche dem kopfe den Weg im Ei hahnen.

"Der Hals enthalt den proximalen Central korpen, welcher nach Boveri fur den Berfruchtungsprozess unentbehrlich ist, und von welchem samtliche Centralkorper des Embryos abstammen Der Hals dringt zusammen mit dem kopf in das Ei ein.

"Die Function von Halsfortsatze der S. dercantha und der Kopffortsatze der S. cephalacantha bestecht in der orientation of the sperm auf der Eioberflache vor der Kapselexplosion. Bei der Befruchtung mussen die Kopf

fortsatze mit in das Ei eindrigen, wogegen die Halsfortsatze."[2]

Fortunately several observations were made in which the spermatozoon did orient itself on the egg membrane (Plate 4, fig. 5). This was done in a seemingly timid manner. The spermatozoon settled uncertainly, sticking none too securely by first one process and then orienting itself as other spines hooked onto the vitelline membrane so that the bag-like head pointed downward toward the egg and the capsule extended upwards. It was at this point that the writer expected to see the phenomenon of the nuclei being forced into the egg by a capsular reaction, but repeated efforts to observe the phenomenon noted by Holtzoff were not rewarded. Attempts to simulate natural conditions, including temperature controls and a variety of salt concentration experiments, always gave negative results. It was concluded that some factor, unknown to the writer, found in the turbulent natural habitat of Emerita analoga must be the responsible agent for setting off the fertilization mechanisms of this highly specialized Anomuran crab.

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2 N. K. Holtzoff, 1906, "Studien uber die Gestalt der Zelle Untersuchungen uber die Spermien der Decapoden, als Einleitung in das Problem der Zellen Gestalt" Archiv fur mikr, Anatomie Bd 67 S 364-571 Taf XXV-XXIX.

SUMMARY

Studies on spermatogenesis in the sand crab, Emerita analoga, have been made and are herein reported and figured. The anatomy of the male crab, with special emphasis on the reproductive system is described and illustrated. The techniques found most productive are outlined. Collections and observations were made at the Pacific Marine Station, Dillon Beach, California during the summers of 1949-1953.

EXPLANATION OF PLATES

Plate 1

Photograph of male Emerita analoga, ventral and dorsal views X2.

Plate 2

Anatomy of the male reproductive organs of Emerita analoga.

Fig. 1 Dorsal view of male reproductive system X4.

Fig. 2 Cross section of male through protocephalon X3½.

Fig. 3 Cross section of male 0.5 mm posterior to protocephalon X

Fig. 4 Cross section through gnathothorax X3½.

Fig. 5 Cross section 0.5 mm posterior to gnathothorax X3½.

Plate 3

Figures of spermatogenesis in Emerita analoga X 2420.

Fig. 1 nurse cell

Fig. 2 spermatogonium

Fig. 3 spireme stage of primary spermatocyte

Fig. 4 bouquet stage of primary spermatocyte

Fig. 5 metaphase of primary spermatocyte

Fig. 6 and 7 secondary spermatocyte

Fig. 8 metaphase plate of secondary spermatocyte

Fig. 9 anaphase of secondary spermatocyte

Fig. 10 daughter cells separating into spermatids

Fig. 11 and 12 spermatids. Figure 11 shows more prominently the anloge of the barrel.

Fig. 15 metamorphosis of the spermatozoon showing incipient barrel or capsule.

Fig. 16 optical section through nuclear end of metamorphosing spermatozoon.

Fig. 18, 19, and 20 Later metamorphosis of spermatozoon.

Fig. 21 mature spermatozoa expressed from sperm duct.

Plate 4

Fig. 1 and 2 typical spermatozoa X 847

Fig. 3 diagramatic representation of a typical expanded spermatozoon.

Fig. 4 section of a portion of the testis X 138.

Fig. 5 diagramatic representation of the spermatozoan position preparatory to fertilization approximately X 200.

ABBREVIATIONS

Bstg - branchia stegite, Bstgf - branchia stegite fold, Cbt - cross bridge of testis, Coe - coelom, Dig - digestive gland, Epst - epistome, Exo - exoskeleton, Gi - gills, Gm - gastric mill, Gn - gnathothorax, Hrt - heart, Int - intestine, Mrpd - meropodite of third maxilliped, Mth - mouth, Mus - muscle, Pr - protocephalon, Sbrc - subbranchial canal, Sep - septa between gastric mill and esophagus, Spd -

ABBREVIATIONS (continued)

sperm duct, St - sternum, Sv - seminal vesicle, Tes - testis, Vd - vas deferens.

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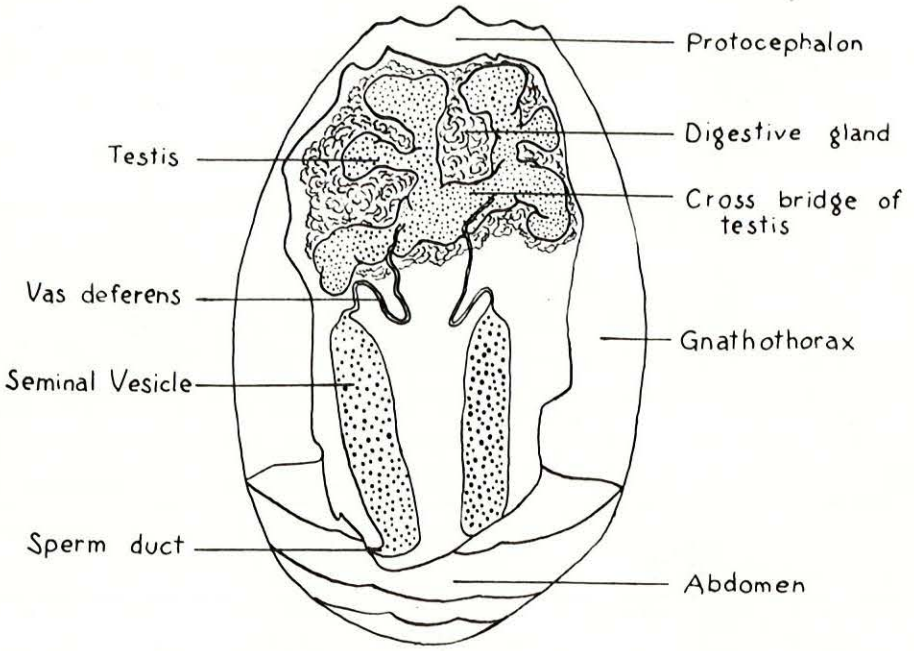
PLATE 1

PLATE 2



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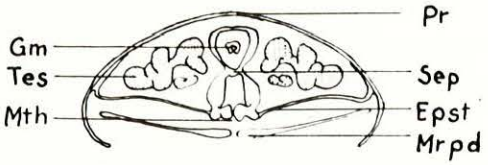

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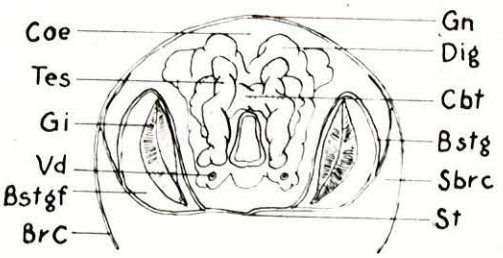

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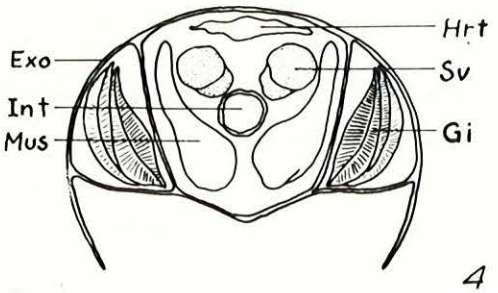

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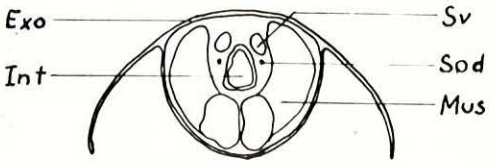

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PLATE 3

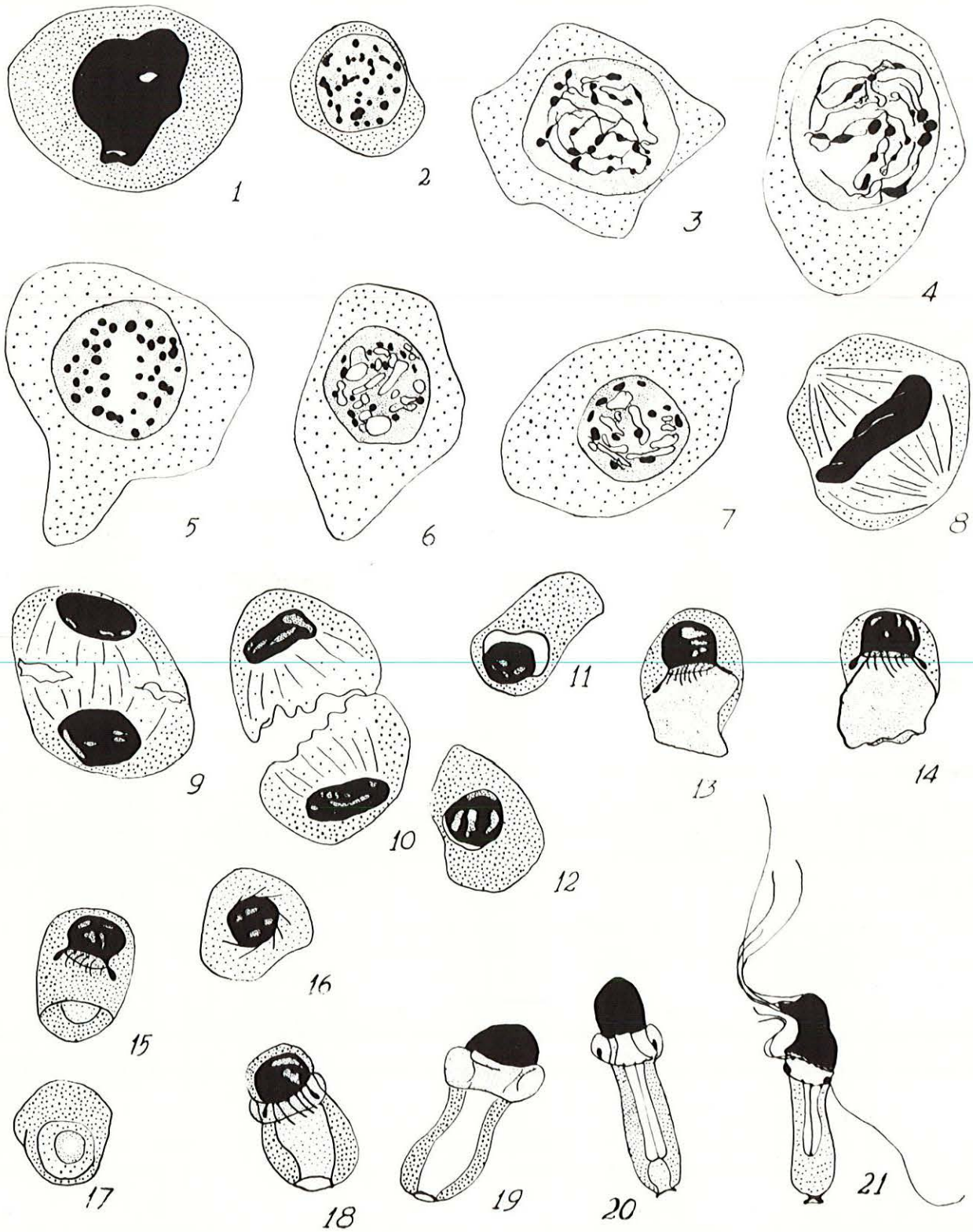
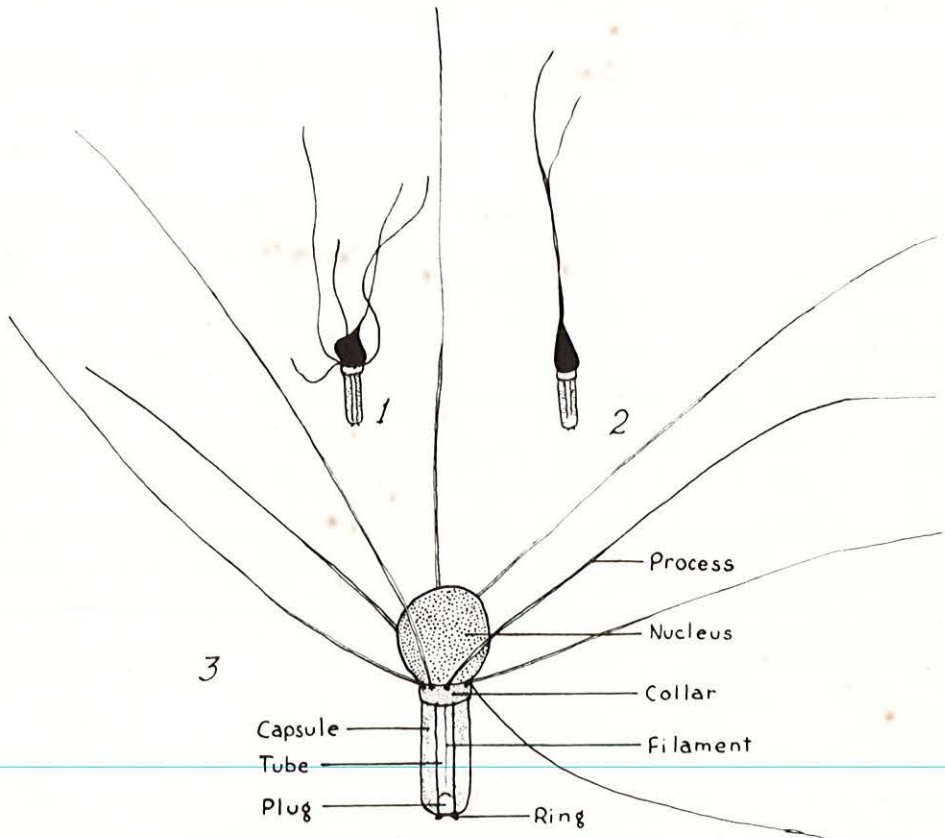


PLATE 4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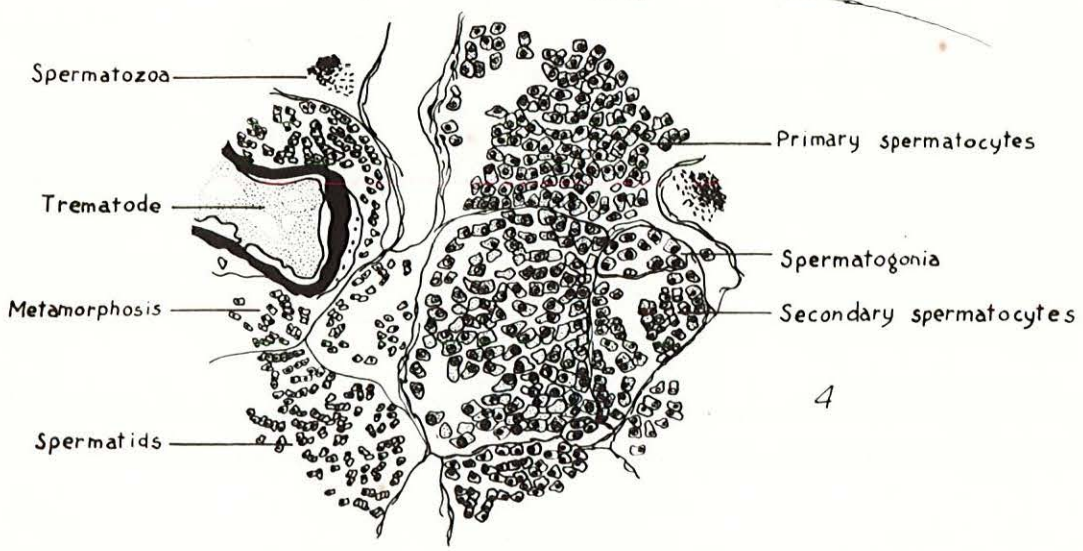
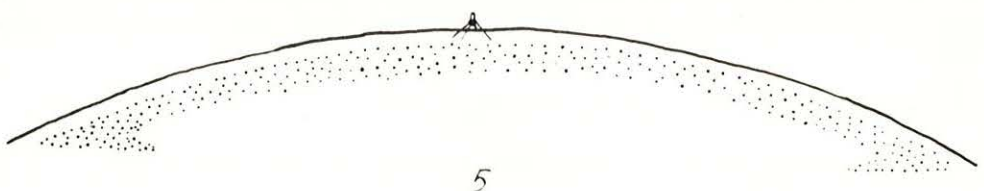
2
3
Process
Nucleus
Collar
Capsule
Tube
Plug
Filament
Ring


Spermatozoa
Trematode
Metamorphosis
Spermatids
Primary spermatocytes
Spermatogonia
Secondary spermatocytes
4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