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Actividades sexuales del calamar, *Loligo pealii* (Les.)

II. El espermatóforo: su estructura, eyaculación y formación.

Un espermatóforo está compuesto de una masa de espermatozoides, una masa de material de cemento, un grupo de membranas que forman en conjunto un aparato eyaculador y vainas para cubrir la masa de esperma, y tónicas envolventes que suministran fuerza para la eyaculación. Durante esta última la masa de esperma y el cemento son expulsadas por un tubo que se evagina en el extremo oral del espermatóforo. La masa de esperma se rodea de ciertas membranas que forman el reservorio espermático y se fija sobre la hembra por una masa de cemento que se acumula en el extremo del reservorio espermático. El esperma sale por un orificio situado en el extremo libre del reservorio. Los espermatóforos se forman en el órgano espermatofórico, porción especializada del espermoducto. Este órgano presenta un cierto número de divisiones, cada una de las cuales forma una porción del espermatóforo. La cantidad de esperma suficiente para cada uno de estos entra en el órgano de una vez. Por acción ciliar este filamento de esperma se arrolla en una masa espiral cilíndrica y apretada que prosigue girando sobre su eje longitudinal durante su paso por el órgano. A esta estructura se suman materiales producidos en ciertas partes del órgano y de este modo se arrollan membranas y tónicas alrededor del espermatóforo en vías de formación. Después que este se ha formado por completo, la túnica externa se contrae produciendo la turgescencia de dicha estructura. Los espermatóforos una vez formados se acumulan en el saco espermatofórico, en donde permanecen hasta que son utilizados.

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SEXUAL ACTIVITIES OF THE SQUID *LOLIGO PEALII* (LES.)

II. THE SPERMATOPHORE; ITS STRUCTURE, EJACULATION AND FORMATION

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FORTY-ONE FIGURES (SIX PLATES)

The spermatophores of Cephalopods have been mentioned in zoological literature by many writers, but most of the accounts are short, incomplete, and inaccurate, so there seems to be no real need for reviewing the literature as a whole.

By far the best account of the structure and ejaculation of spermatophores of Cephalopods I have seen is given by Émile-G. Racovitza, for *Rossia macrosoma* ('94) in a paper dealing with the habits and reproduction of the species. His descriptions and figures of the spermatophores are much more complete and accurate than those of earlier writers and there seems to be nothing added by later writers of very great importance.

To follow the method by which so complicated a structure is formed by added secretions on the inside of a duct, it is necessary to have rather elaborate figures representing the structure and ejaculation of the spermatophores, so it has seemed best to consider the whole question of structure, ejaculation, and formation together. A previous paper (Drew '11) on the sexual activities of the squid deals with the copulation, egg-laying, and fertilization, and might well follow the observations given in this paper as it deals with the use made of the completed spermatophores.

Part of the laboratory work, which forms the basis of this paper, was done at the University of Maine while I was connected

with the Biological Department of that university, and I am grateful for working space furnished me by the University of Arizona during the winter of 1917. By far the greater part of the work has been done at the Marine Biological Laboratory at Woods Hole, Massachusetts, and all of the material has been obtained at that station.

This species of squid is very abundant in the vicinity of Woods Hole, and any mature male taken from early in the spring until as late as September, and frequently later than that, is sure to have an abundance of spermatophores in the spermatophoric sac.

The spermatophores vary in size according to the size of the animal from which they are taken. Those from very small animals may not be over 8 mm. in length and those from large animals may be as much as 16 mm. in length. They are of course all similar in structure, but the small ones are softer and are not so easily handled in ejaculation observations as the larger ones. As might be expected, there are some slight individual variations in shape and size of parts in the spermatophores of different individuals. The spermatophores of each individual are practically identical in shape and appearance, but may vary slightly in size.

The number of spermatophores carried by each individual varies with the size of the animals (the smaller having fewer than the larger), with the season of the year, and with the frequency of copulation. May and June are probably the months when sexual activity is at its greatest. Usually at this time the spermatophoric sacs are gorged with spermatophores. A large individual may have as many as four hundred fully formed spermatophores stored at one time.

The formation of the spermatophores is evidently rather rapid. Several, perhaps several dozen, may be formed in a day. It is difficult to determine with anything like accuracy what the rate may be, but after a male has used large numbers of spermatophores in repeated copulations, a day or two is sufficient to bring the supply almost if not quite to normal.

STRUCTURE

Each spermatophore consists of a white opaque mass of spermatozoa surrounded by almost transparent liquids and membranes, a mass of material, rather opaque but not so opaque as the mass of spermatozoa, which lies against one end of it, and a brownish spiral filament together with a number of membranes of varying degrees of transparency on the other side of the body just mentioned. For convenience we may speak of these main divisions as the sperm mass (fig. 1, *SM*), the cement body (*CB*) which lies at one end of the sperm mass and was called by Racovitza the 'faux boyaux,' and the ejaculatory apparatus (*EA*), composed of the complicated group of membranes and the spiral filament which joins the cement body and occupies the smaller end of the spermatophore. It is also convenient to speak of the portion of the spermatophore occupied by the ejaculatory apparatus as the oral end, since from this end ejaculation takes place.

Figure 1, a complete spermatophore, shows that the aboral is considerably larger than the oral end. The sperm mass is little more than half the diameter of the spermatophore and does not extend to the extreme aboral end of the spermatophore. There is some difference in the amount of space posterior to the sperm mass, and likewise in the diameter of the portion of the spermatophore in which the sperm mass lies in different spermatophores. This is due largely to the fact that the spermatophores, when liberated take up water rapidly, and consequently swell and change in shape. This condition is easily controlled by passing the spermatophores into solutions of formaldehyde. Full strength formaldehyde will do no harm, but 10 per cent formalin is sufficient for ordinary purposes.

In studying the structure I have found that staining the formalin-treated spermatophores in dilute solutions of Ehrlich's triple stain or in Ehrlich's triacid stain and mounting in glycerin jelly has been very helpful. The stains are selective and stain different parts different colors or shades. The glycerin jelly will clear and preserve without shrinking the specimens badly.

The stains are not permanent, but when kept in the dark will last for some months. Formalin will harden gelatin, so mounts of formalin specimens become quite permanent, unless they are kept in a very damp place without being sealed.

The spermatophore is very turgid and elastic. This is due to the outer covering, the outer tunic (fig. 2, *OT*), which is a tough and elastic membrane. It is transparent, rather thin, and of about even thickness except at the extreme anterior end, where it becomes thickened and sculptured for the attachment of membranes of the ejaculatory apparatus, and where it is modified to form the cap (*C*) covering the oral end. The cap ultimately loosens and allows the spermatophore to ejaculate. The name cap which is applied to the portion that covers the oral end is somewhat confusing. The covering is formed by winding and cementing down around this end a thin leaf of outer tunic material which is continued as a long thin thread, the cap thread (*CT*), from the oral end of the spermatophore. This thread serves to loosen the winding of the cap when it is pulled, and ejaculation processes are immediately started.

The outer tunic gives the strength and elasticity to the spermatophore. The very great turgidity is due to the strain to which this tunic is subjected. When this tunic is punctured or cut, the contents escape rapidly and the tunic shrinks because of its elasticity.

Inside the outer tunic is the middle tunic (figs. 1 and 2, *MT*). This is closely applied to the outer tunic, thickened over the whole aboral end and gradually thins out orally, after the sperm mass is passed. The aboral portion is rather thick and granular, but transparent. After the region of the cement body is reached, the granular character is lost, and it is very difficult to determine whether a membrane is present or whether the space is filled with liquid. However, it can sometimes be easily traced. Under certain conditions of distension or ejaculation of the spermatophore, a line appears that can be accounted for only as the inner border of such a membrane. This portion is quite transparent, differing greatly in appearance from the granular membrane in the aboral end, but under favorable conditions

it may be traced to the oral end of the spermatophore, where it seems to end against a ridge on the inside of the outer tunic. The middle membrane of the ejaculatory apparatus, to be described later, is firmly attached to the other side of this same ridge. The middle tunic is soft, evidently elastic, forming an elastic cushion, and is evidently capable of taking up water rapidly. When a spermatophore is removed from a spermatophoric sac and placed in sea-water, the middle tunic immediately begins to increase in thickness and soon the spermatophore begins to ejaculate, or, if the cap holds, the outer tunic is ruptured by the increased internal pressure.

If the outer tunic be cut in such a spermatophore, the sperm mass is driven through the opening and the middle tunic thickens to occupy the space formerly occupied by the sperm mass. The combined swelling of the middle tunic and the elastic shrinking of the outer tunic nearly obliterate the space formerly occupied by the sperm mass. Evidently these two tunics are concerned in supplying the force that causes the ejaculation of the spermatophore.

Closely applied to the sperm mass is a very thin and not very definite membrane, the inner tunic (fig. 2, *IT*). This is frequently hard to identify, as it has nearly the same appearance as the mucilaginous material with which the sperm are mixed, and it is very closely applied to the mass. In the figures the part of the inner tunic covering the sperm mass is represented by a single line.

During the formation of the spermatophore, when the material of this tunic is wrapped around the sperm mass, it is easily distinguished in sections. In spermatophores which have not been completely formed, before final shrinking takes place, the coiling of the sperm thread of the sperm mass is quite distinct, and here the inner tunic is seen in the spaces between the coils of the sperm thread, separated slightly from the sperm mass.

Connecting the sperm mass and the cement body is a thin cylinder of transparent material, the connecting cylinder (fig. 6, *CC*) which seems to be a continuation of the core of the mucilaginous material with which the spermatozoa are mixed. The

inner tunic is seen here as a thin sheet surrounding this cylinder and extending from the sperm mass to the cement body.

On the cement body the inner tunic becomes applied to the outer membrane (fig. 2, *OM*) which covers this body. From this point on the inner tunic (*IT*) is thicker and very easily seen. It, together with the outer membrane, to which it is closely applied, leaves the cement body where it abruptly narrows and continues toward the oral end of the spermatophore nearly to its extremity, as part of the ejaculatory apparatus. The two structures are very similar in appearance, but the dividing line between them is distinct.

Just before reaching the point where the ejaculatory apparatus is thrown into loops, both the inner tunic and the outer membrane thicken to form a distinct ring. The dividing line between the two structures is easily followed for some distance into the thickening and is then hard to trace. I find, however, in the forming spermatophore, and occasionally in completely formed specimens which have been mounted some months in glycerin jelly, that the line of separation can be indistinctly traced nearly through this thickening, and I am inclined to think that the oral end of this thickening may be taken to be the extremity of the inner tunic. Beyond the thickening there is no indication of a double character and this part is probably the continuation of the outer membrane only. In the thickening the two structures are evidently more closely applied than elsewhere if, indeed, they are not fused.

The free end of the outer membrane is further on among the loops of the ejaculatory apparatus. It is easily seen in a specimen which is cut so that the loops of the ejaculatory apparatus straighten out, (fig. 3). This free ending of the inner tunic and outer membrane on the ejaculatory apparatus is of importance in studying the method of ejaculation.

The middle and inner tunics are entirely separate from one another. Where they touch they do not adhere. Between them is an actual or potential space (figs. 1 and 2, *SL*) filled with clear liquid. This space is always visible behind the sperm mass, in the region of the cement body, and along the ejacu-

latory apparatus. It is most easily seen behind the sperm mass, but there is evidently considerable liquid along the sides of the ejaculatory apparatus. This is especially shown during ejaculation.

The sperm mass (figs. 2 and 23 A, *SM*) is really composed of a thread or sheet of spermatozoa spirally coiled around a core of mucilaginous secretion, with the secretion extending between the loops of the sperm thread to the surface. The sperm forms a sort of elongated thin plate, coiled edgewise so one edge rests against the core and the other comes to the surface. The plate does not meet the surface and core at right angles, but is tipped slightly so the surface edge lags behind the core edge. By means of the secretion the plate composed of individual spermatozoa is consolidated into a very flexible cylindrical rod that has the character of a unit body. The secretion is tenacious and penetrates in between the spermatozoa, but the core and secretion between the loops of the sperm thread have very few spermatozoa embedded. Because of the appearance the sperm mass thus formed is frequently called the sperm rope. In the squid the loops of the spiral in the completed spermatophore are not very easily seen and, as a rope does not consist of a single strand coiled in this way, the term has no significance and may be misleading. The secretion mixes freely with sea-water, so the spermatozoa obtain their individual liberty promptly when the time comes for them to perform their function.

Just oral to the sperm mass is the cement body. This is more or less definitely attached to the sperm mass by the core of mucilaginous material, which extends orally from the sperm mass as a definite cylindrical filament, and by the inner tunic which continues from one to the other. The connection between the two structures is slight, but sufficient to connect them definitely together.

The cement body (fig. 2, *CB*) the function of which was not determined by Racovitza and was called by him the 'faux boyaux'—is a somewhat elongated pear-shaped body with the base somewhat less in diameter than that of the sperm mass

and the narrower elongated oral portion in contact with the end of the conspicuous spiral filament of the ejaculatory apparatus (figs. 2 and 23 A *CB*).

In the forming spermatophore the narrow end of the cement body, the hyaline core, (*HC*) is continued to the oral end of the spermatophore as a narrow cylindrical rod, which stains about the same as the material of the cement body. This fills the space inside the loops of the spiral filament and, oral to the spiral filament, the continuation of the lumen of the ejaculatory apparatus. I take it from Racovitza's figures and descriptions that this, which he calls the hyaline core, is persistent in the fully formed spermatophore of *Rossia*. In *Loligo* it is transient, disappearing, evidently by liquification, almost simultaneously with the completion of the formation of the spermatophore. The space it occupied remains as the lumen of the ejaculatory apparatus, which is probably filled with the liquid in the completed spermatophore.

In spermatophores in process of formation and frequently in freshly stained fully formed specimens, the cement material can be seen to be spirally wound. There is a central core, evidently a continuation of the cylinder extending between the sperm mass and the cement body, which is continued as a narrow cord the whole length of the hyaline core which extends orally from the cement body (figs. 23 and 23 A). In fully formed specimens the cement material usually does not show the spiral character plainly and the inner core may not be visible.

The cement body is probably covered entirely by the inner membrane, but over the large aboral end, where it is likewise covered by the outer membrane and the inner tunic, and where these membranes are fused tightly together, it cannot be traced as a separate membrane.

Over the narrow oral end of the cement body it is visible as a thin membrane which continues orally after leaving the cement body over the outer surface of the spiral filament. Still further orally, where there is no spiral filament, this membrane lies next to the lumen of the ejaculatory apparatus (fig. 2, *IM*). Formerly it was in contact at this point with the hyaline core.

The spiral filament (fig. 2, *SF*), while formed as a separate structure, evidently sticks to the inner membrane which covers it. The filament is brown, more or less granular, and is not of equal size and shape throughout. It is heaviest, with the coils most open, midway in its course, with both ends rather crowded. The loops of the spiral on the end next the cement body are very closely crowded, flattened, and the extreme end sometimes has the continuity of the thread broken so it is made up of consecutive fragments. The loops of the oral end of the filament become closely crowded, then more open and finally fade away so gradually that it is hard to determine where the filament ends. The function of the filament is not easily determined. It is evidently not a coiled spring and it seems to have very little elastic value. It seems probable that its chief function is to hold the lumen of the ejaculatory apparatus freely open so that evagination, to be described later, can be accomplished without tearing the membranes concerned. The rapidity of the ejaculation must be slowed somewhat to allow time for the oncoming cement body and sperm mass. The resistance caused by breaking the spiral filament into small fragments probably accomplishes this purpose.

Between the inner and the outer membranes is the middle membrane (fig. 2, *MM*). This is very transparent and frequently shows longitudinal striations, indicating the position of the successive windings of the sheet of which it is composed. It is much thicker than the other membranes and, while capable of much stretching, is evidently tough. It extends from the point where the outer membrane, together with the inner tunic, leaves the cement body, to the oral end of the spermatophore. At the aboral end, the tube formed by this membrane is closed by the oral end of the cement body. At the oral end this tube, which was open in formation (fig. 23, *MM*), is closed and closely applied to the inside of the cap where it spreads out laterally and is fastened by its lateral margin to the ridge of the outer tunic (fig. 2, *MM*). In this spreading and flattening process the lumen of the tube is also pressed out laterally so that in form the end is something like a pressed-in hollow rubber ball with

the tube formed by the middle membrane extending back from the concave side of the ball.

The term 'ejaculatory apparatus' has been applied to the inner membrane, together with the spiral filament, the middle membrane, and the outer membrane and inner tunic to their junction with the cement body. This is not a very satisfactory term as the spermatophore acts as a unit in ejaculation. That is, there is no one part that is active while the remainder are passive. The ejaculatory apparatus could not possibly deliver the sperm mass in position were it not for the elastic outer and middle tunics and their relations to liquids and structures. The term may, however, stand for want of a better one, since this portion is mostly concerned in ejaculation.

It may aid somewhat in understanding the arrangement of the parts of a spermatophore if we consider what is present in optical cross-sections through: 1, the region of the sperm mass; 2, the region of the aboral end of the cement body; 3, the region of the oral end of the cement body; 4, the region of the spiral filament, 5, the region just posterior to the cap. The parts cut will be mentioned in turn from the outside to the median axis (figs. 21 to 18).

1. The region of the sperm mass: 1, Outer tunic; 2, middle tunic; 3, space (actual or potential) filled with liquid; 4, inner tunic; 5, sperm mass.

2. The region of the aboral end of the cement body: 1, Outer tunic; 2, middle tunic; 3, space (usually actual) filled with liquid; 4, inner tunic; 5, outer membrane (if actually present fusee with the inner tunic), 6, inner membrane (probably); 7, cement body.

3. The region of the oral end of the cement body. 1, Outer tunic; 2, middle tunic; 3, space (usually actual) filled with liquid; 4, inner tunic; 5, outer membrane; 6, middle membrane; 7, inner membrane; 8, cement body.

4. The region of the spiral filament: 1, Outer tunic; 2, middle tunic; 3, space (actual) filled with liquid; 4, inner tunic; 5, outer membrane; 6, middle membrane; 7, inner membrane; 8, spiral filament; 9, lumen, probably filled with liquid, formerly filled with hyaline core.

5. The region just aboral to the cap: 1, Outer tunic; 2, middle tunic; 3, space (actual) filled with liquid; 4, middle membrane (the inner tunic and outer membranes do not extend this far); 5, inner membrane; 6, lumen (the spiral filament does not extend this far).

If each structure is considered in turn in their longitudinal relations we find:

1. The outer tunic is continuous over the whole spermatophore except at the oral end where there is a modification, the cap, which is spirally wound around the otherwise open tunic to form a closing mechanism. The cap has attached to it a long thread which, when pulled, serves to loosen the cap and thus liberate the enclosed mechanisms.

2. The middle tunic is continuous throughout the length of the spermatophore up to the thickened ridge on the outer tunic near the oral end, which it joins.

3. The inner tunic is continuous over the region of the sperm mass as a closely investing, thin membrane. It becomes thicker over the posterior end of the cement body. After leaving the cement body as an investing membrane it becomes a little thicker and closely covers the outer membrane. Near the region of the anterior extremity of the spiral filament this tunic ends with open mouth closely associated with the outer membrane.

4. The outer membrane probably begins at the aboral end of the cement body, but cannot be definitely distinguished from the inner tunic until near the place where both of these structures leave the cement body and, together, give the appearance of a double membrane. After leaving the cement body the outer membrane is applied to the middle membrane. A short distance orally from the end of the inner tunic the outer membrane also ends with an open mouth. It is important to understand that the oral portions of the inner tunic and the outer membrane together form a tube, closed at the aboral end where they are united to the cement body, and open at the oral end. The opening is of course closed by the other structures. There is, however, no organic union between these structures and the other membranes from the point where the outer membrane and

the inner tunic leave the cement body to invest the middle membrane.

5. The space, potential or actual, between the middle tunic on the outside, and the inner tunic, outer membrane and middle membrane on the inside, is continuous throughout the spermatophore. The liquid enclosed in this space serves the mechanical purpose of a lubricant and at the same time an easily flowing substance to which pressure is applied. The elastic force of the outer and middle tunics is transmitted through this liquid to the sperm mass and other structures during the act of ejaculation.

6. The sperm mass extends through the aboral two-thirds of the spermatophore, inside the inner tunic, which is closely applied and united to it.

7. The cement body is just oral to the sperm mass and attached to it by a connecting cylinder. The aboral end of the cement body is covered by the inner tunic, part of it at least by the outer membrane and possibly also by the inner membrane. If all are present, they are closely fused so they are hard to distinguish. The oral end of the cement body is covered by the inner membrane, outside of which come, in order, the middle membrane, outer membrane, and inner tunic. These are all easily distinguished from one another at this point.

8. The middle membrane forms a tube extending from the position where the inner tunic and the outer membrane leave the cement body to the oral end of the spermatophore. Just beneath the cap the oral end, which, although formed as an open tube, is now closed, becomes closely applied to the inside of the cap and is spread out laterally to the ridge on the outer tunic to which it is firmly attached. The open aboral end of the tube formed by the middle membrane is plugged by the small oral end of the cement body which is covered by the inner membrane. It has no organic connection with the outer membrane or with any part of the cement body, except for about one-third of the length of the surface that is applied to the inner membrane where it covers the cement body (fig. 23 A, *PA*). The portion next to the open mouth of the middle membrane ad-

heres to the inner membrane covering the cement body firmly, and in ejaculation is liberated only by the rupture of this membrane. It is important to understand that the open mouth of the middle membrane is directed aborally and the open mouths of the inner tunic and the outer membrane are directed orally. The one fits inside the other. The oral end of the middle membrane is a closed structure, like an indented hollow rubber ball, attached by its margin to the outer tunic and with the thin convex side (fig. 2, *MM*¹) applied to the under surface of the cap which closes the outer tunic. This part ruptures when the cap is loosened, so the lumen of the ejaculatory apparatus is opened to the outside (fig. 5, *MM*¹).

9. The inner membrane and spiral filament are united to the middle membrane. Their positions and relations are better shown by figures than by description. In ejaculation the inner membrane ruptures at the point where it joins the cement body (fig. 9, *PR*).

With these points in structure in mind we can now proceed with the method of ejaculation.

EJACULATION

In delivering the spermatophores the male grasps a bundle of them with the tip of the left ventral arm and quickly passes them into position (Drew, '11). The spermatophores leave the sexual duct of the male aboral ends first and the long threads connected with their caps, embedded in the secretions of the spermatophoric sac, drag behind. The sharp pull occasioned by the movement of the arm pulls on these threads and causes the caps to loosen. This starts the process of ejaculation. Under normal conditions the process is very rapid, occupying about ten seconds. This very rapid action of course makes it impossible to follow the ejaculation under a microscope even though the spermatophore is held in position and the thread pulled when all is ready. It was accordingly necessary to devise some method to slow down the movements.

Evidently the great tension of the elastic outer tunic has much to do with the process of ejaculation. Inasmuch as spermatophores

carefully removed from the spermatophoric sac without pulling the cap thread and placed in sea-water are very likely to ejaculate soon, and placed in fresh water will either ejaculate or burst very promptly, it is evident that osmotic action, in which the middle tunic is probably involved, plays an important part.

Elasticity and osmotic action accordingly have to be considered in searching for some method to slow down the action. It was found that formaldehyde affects probably both the elasticity and the osmotic properties, but that it evidently hardens the cement holding the cap, so it is very difficult to open it when needed, and that the various membranes of the spermatophore are soon so changed, possibly by hardening certain colloids, that ejaculation is not likely to be completed. These very properties were, however of the utmost value in the studies, for full-strength formaldehyde thrown on an ejaculating spermatophore will cause it to stop all action rather promptly. After some experience it became possible to allow the required amount of time to stop a spermatophore at the required stage of ejaculation by squirting full-strength formaldehyde on it just before it reaches the stage wanted. Such a spermatophore may then be stained in aqueous stains and mounted in glycerin jelly for study at leisure. It, of course, requires a great deal of time and patience to get some of the stages, but, as the figures showing the stages of ejaculation accompanying this paper are camera-lucida drawings of such specimens, it will be seen that it is possible to get the stages by this method.

Very many chemicals were tried to get the required slowing effects. Sugar solutions were good, but the membranes were soon weakened so the elasticity was destroyed. Magnesium chloride solutions have given the best results. The strength of the solution that works best seems to differ with spermatophores from different individuals, but a one-fourth saturated solution in sea-water has been very good.

The spermatophores are received from the spermatophoric sac directly into this solution, and in two or three minutes they will be ready for use. The turgidity is evidently effected, and where the spermatophores are left several hours, there may be

changes in elasticity and the freedom with which membranes will move on each other may be disturbed. After the spermatophores have been in the solution some minutes, if they are to be used for work for a long period, more sea-water may be used to dilute the solution. Generally it is best to use material that has recently been put into the solution.

The method used in studying ejaculation was to remove the spermatophore from the magnesium chloride solution to a watch-glass with a little sea-water, placed on a black background. The cap thread was then grasped with forceps and the whole spermatophore shaken. With a reasonably powerful engraver's glass held on the head with a spring the process of ejaculation may be watched, and with a large-mouthed pipette filled with formaldehyde the process can be stopped when desired. The time for ejaculation may be slowed down to take from a minute to two minutes, so it is possible to supplement observations made on the fixed material by observations on the ejaculating spermatophores.

It is necessary to concentrate attention on one portion at a time, but there is no difficulty in following movements of parts under the lens of a compound microscope. The chief trouble is in focusing attention on particular parts, for everything is moving at the same time and the mechanism is too complicated to be taken in at a glance and too large for all to be under a lens of sufficient power at one time.

As the process of ejaculation is somewhat complicated, a series of diagrams are given on plate 6, from which all portions not essential to understanding the process have been eliminated. By referring to these diagrams at this time it will be easier to follow the processes of ejaculation as they are given in other figures and descriptions.

The cap end of the cap thread is flattened and is apparently applied and cemented to the outer tunic in a somewhat spiral manner so the otherwise open end of the tunic is held shut. When the thread is pulled it loosens where cemented (fig. 4), and the end of the outer tunic is allowed to open. There is evidently some tearing, but not much.

With the opening of the tunic the portion of the middle membrane applied to the inside of the cap ruptures so that the lumen of the ejaculatory apparatus is opened to the outside of the spermatophore and the ejaculatory apparatus immediately begins to evaginate because of the pressure on the inside of the spermatophore (fig. 5). The evagination of that portion of the ejaculatory apparatus which is oral to the spiral filament is so rapid in the untreated spermatophore that the eye cannot follow it, but in the specimens treated with magnesium chloride it may be slowed down so the gradual evagination can be followed easily. As the ejaculatory apparatus evaginates, the diameter of the tube is greatly increased and the walls are correspondingly thinned.

There is a distinct pause in the evagination when the region of the spiral filament is reached (fig. 6). This is probably largely due to the stiffness of the filament itself, but may be influenced by the fact that other membranes are involved at about the same point.

Evidently the evagination of the first part of the ejaculatory apparatus is due to the pressure of the liquid between the middle and inner tunics that is in the oral end of the spermatophore. This is shown by the fact that the action is so rapid and by the further fact that the cement body and sperm mass are drawn away from each other (fig. 6). The sperm mass lags behind, so the connection between it and the cement body is stretched to its full extent.

After an instant's delay when the region of the spiral filament is reached, the tube continues to evaginate. The evagination here is continuous, but not nearly so rapid as the first part. The evaginated portion of the tube increases greatly in diameter, the walls become correspondingly thinner and the spiral filament is broken into minute fragments which continue to adhere to the outside of the evaginated tube, (fig. 7). As this process goes on, the free edge of the outer membrane adheres to what is now the inside of the evaginated middle membrane and is reflected so that this membrane, together with the inner tunic with which it is associated, is turned inside out (figs. 7 and 8, *OM* and *IT*).

Evidently the force that causes the evagination is still the elastic and osmotic force in the outer and middle tunics of the spermatophore acting through the liquid which fills the space between the walls of the evaginating tube.

The part played by the spiral filament seems to be largely, if not wholly, that of keeping the tube from collapsing with the pressure, but there may be some elastic force that aids in the evagination. The torsion that would be caused by the turning of a spiral spring might aid in the evagination, when once started, but there is no evidence that the filament is particularly elastic or partakes of the nature of a spring. The very fact that it is broken into minute fragments during the process of the evagination of the tube indicates that it can have no very great elastic properties, and probably indicates that it retards rather than accelerates the evagination of this part. It is necessary that evagination of this portion shall not be too rapid as the sperm mass must gain momentum and move along at a corresponding rate.

That such a filament may serve a very useful purpose in keeping the tube from collapsing or folding is evident. The freedom of the movements of the membranes concerned would be seriously interfered with if the tube were allowed to collapse or kink. The oral end of the tube does not need such a mechanism, as it is short and simple in construction and would naturally evaginate quickly with the pressure of the liquid between it and the outer wall. The same condition would not hold true for the much longer and more complicated tube that has the spiral filament.

It is of passing interest to note that there is a very general impression among zoologists who have no personal acquaintance with Cephalopod spermatophores that this spiral filament is really a spring, that it is used in discharging the sperm mass in the same mechanical way that a spring gun discharges its projectile, and that the discharge is through the end of the spermatophore farthest from the spring. There is, of course, no foundation of fact whatever for such an impression. It is simply arriving at conclusions from superficial appearances rather than by study and experimentation.

When evagination has proceeded as far as the oral end of the cement body, so that the end of this body begins to project through to the outside, the inner membrane is ruptured, so that this membrane, with the remnants of the spiral filament, is separated from the cement body (fig. 9, *PR*).

As evagination now proceeds, the oral end of the cement body projects into the sea-water. At this point ejaculation is retarded until the very great pressure behind the sperm mass forces this mass against the aboral end of the cement body, to which the inner surface of the middle membrane is attached, and so causes the middle portion of the cement body to be drawn out and around the sides of the aboral portion of the cement body in the form of a cap (fig. 11). The extreme oral end of the cement body, from which the inner membrane and spiral filament have been torn, appears as a knob or button on the otherwise smooth surface of the cement body.

It may be well, before proceeding with the other changes that are taking place, to call attention to the position of the oral portions of the inner tunic and outer membrane. In the evagination that has taken place the free ends of the inner tunic and outer membrane which originally enclosed the middle membrane have been turned back by the evaginating tube, so that the opening is directed toward the aboral end of the spermatophore, and, together, they form the inner lining of the evaginated tube as it now appears. In this process the sperm mass is being carried through the opening of the inner tunic and outer membrane and is being forced into the sac formed by them (figs. 9, 12, and 39).

As has already been pointed out, the inner tunic and outer membrane are firmly attached to the sides of the aboral end of the cement body so, when ejaculation has proceeded to this point, the membrane cannot be stripped further aborally. The part which has been turned back with the evaginating middle membrane thus forms a sac, with the cement body firmly attached to the closed end, and the pressure from behind forces the sperm mass into this sac.

The adhesion of the end of the middle membrane to the middle portion of the cement body not only serves to draw this cement body around the end of the sperm mass, but holds the sac in position to have the spermatozoa thoroughly and completely forced into it by pressure behind. It will be noticed that during this process the diameter of the aboral end of the spermatophore is greatly reduced, due to the elasticity of the outer tunic, and that the middle tunic swells, loses its granular appearance, and comes to occupy the space vacated by the sperm mass. At the same time the outer end of the evaginated ejaculatory apparatus becomes considerably expanded as the sperm mass is crowded into it (figs. 12, 13, and 14).

Continued pressure causes the walls of the cement body to burst (fig. 15). The end of the middle membrane is thus released and the sperm mass enclosed in the reflected inner tunic and outer membrane, smeared with cement from the ruptured cement body on its larger closed end, glides rapidly through the middle membrane and is free from all other mechanisms.

The covered sperm mass, which may be called a sperm reservoir (fig. 17), is usually somewhat coiled. The closed end is large and covered with cement, and the open end is small, with a thickened portion just behind the opening. The thickened portion seems to correspond to the thickened portion of the inner tunic and outer membrane, described in connection with the structure of the spermatophore, that lies a little aboral to the free end of this membrane. The thickened walls probably tend to prevent too rapid escape of the spermatozoa.

From the open mouth of this sperm reservoir of untreated specimens the spermatozoa escape in a constant cloud which reminds one of the smoke from an evenly discharging factory chimney. The discharge may go on for hours. When care is taken to provide an abundance of sea-water, such reservoirs will still be discharging twenty-four hours and more after they were liberated from the spermatophores.

Referring to the methods of copulation of the squid, given in a former paper (Drew, '11), it will be seen that when the spermatophores are carried to the mantle chamber of the female

they are held in position by the male long enough for them to discharge and to have the sperm reservoirs fixed by the cement on them to the tissue near the oviduct of the female. In this position each gives out its small cloud of sperm for some hours. If the female lays her eggs within the time they are active, insemination is assured. On the other hand, if the spermatophores are transferred to the region of the buccal membrane of the female, they are held in position by the male until they discharge and the sperm reservoirs are attached to the walls arranged for them. Here, as they discharge, the spermatozoa are directed, evidently by ciliary action, into the sperm receptacle where they are stored for future use.

The discharged empty case (fig. 16) is much smaller, especially in diameter, than it was before ejaculation. The outer tunic appears about as it did. The middle tunic is clear, not granular, and occupies most of the space inside the outer tunic. The evaginated tube that adheres to the oral end of the outer tunic is likewise less in diameter than it was at the time of evagination when there was pressure inside. The end of the tube attached to the outer tunic is clear and corresponds to the oral unornamented portion of the tube in the spermatophore. The region of the spiral filament is shown by the broken fragments adhering to the tube, and the outer end of this marked portion represents the end of the spiral filament and inner membrane that was attached to the oral end of the cement body. This is the point of rupture (*PR*). The remaining unornamented flaring tube is the part of the middle membrane which was in contact with the cement body. The outer third of this portion was firmly attached to the inner membrane that covered the cement body. By the breaking of this attachment the sperm reservoir, with the cement at the closed end, became free to be forced out of the case by the pressure behind it.

FORMATION

At first thought it is very difficult to understand how so complicated a structure as a spermatophore, with its numerous coats and structures, can be formed as a secretion inside the lumen of a glandular duct. To make the process clear it is necessary to know the structure of the duct in some detail.

The parts of the duct have received different names by different writers, and, inasmuch as the functions of the parts were not well understood at the time, the names that have been applied to them are generally not significant and should, I think, be abandoned as misleading.

A recent writer (Marchand, '07), who has covered this subject much more fully than has previously been done and who has made careful comparisons of the male ducts of a large number of Cephalopods, had analyzed the names previously given and made selections that suit his purpose, but as these names are applied without definite knowledge of the functions of the parts receiving the names and as more than one function is performed by a part to which he gives a single name, following the names he gives would seem to lead to even more confusion than to again change them.

The male genital organs of the squid are asymmetrical, only the testis and duct on the left side being present. The testis lies far posteriorly and dorsally (the terms posterior, anterior, dorsal, and ventral are used in the apparent rather than the true morphological sense). Just beyond the testis capsule the vas deferens shows a slight swelling, the ampulla of the vas deferens. From this point the vas deferens, at first a wavy and then a closely plaited tube, extends around the left side of the visceral mass to a point just posterior to the left branchial heart. Here the sexual duct enlarges to form a complicated, folded gland in which the spermatophores are formed.

The whole mass is frequently referred to as the spermatophoric gland. This is proper in the sense that the spermatophores are formed here, and it is not proper, inasmuch as it is not a single gland, but a series of glands and mechanical contrivances,

each portion of which has a definite individual function in the formation of spermatophores. For convenience we will call it the spermatophoric organ. This name will be applied to the whole structure, consisting of various glands and mechanisms, which extend from the vas deferens to the duct that carries the completed spermatophores to the spermatophoric (Needham's) sac. The spermatophoric organ is rather transparent, like most tissues of the squid. The forming spermatophores may be easily seen in the different parts of the organ. It is possible to cut the organ away from the visceral mass, place it in a watch-glass of sea-water and, under a compound microscope, see somewhat clearly the structures and positions of a forming spermatophore. By keeping the water changed on such an organ, its movements, which are very vigorous, will be kept up for nearly an hour and the forming spermatophores during the interval will move some distance. Within this organ the spermatophores are formed and completed.

The duct leading from the spermatophoric organ to the spermatophoric sac, which will be called the spermatophoric duct (frequently called the vas efferens, and by Marchand the distal vas deferens) carries the completed spermatophores for storage in the spermatophoric sac.

The vas deferens (figs. 29 and 30, *VD*) passes dorsal to the spermatophoric organ (between it and the general visceral mass) for about three-quarters of the length of the organ, where it joins the first of a series of structures that together form this organ (fig. 32, *VDO*).

The portion of the spermatophoric organ joined by the vas deferens I will call the mucilaginous gland (figs. 29 to 36). It secretes a sticky substance which is mixed with the spermatozoa and forms the material in which the sperm thread is imbedded, the cement body, and the hyaline core around which the spiral filament is wound. This gland is composed of two parts. One part (*MG*²) extends from the vas deferens to the next portion of the spermatophoric organ. This is referred to by Marchand as the second division of the spermatophoric gland (*vesicula seminalis*). Marchand uses the term spermatophoric gland

for three parts of what is here called the spermatophoric organ. The term does not serve my purpose, for the portion is more than a mere gland or indeed a series of glands. There are more parts to be described than the three divisions given by Marchand, and the term spermatophoric gland would indicate that the spermatophores are formed here, while they are only partly formed here.

The other portion of the mucilaginous gland (MG^1) forms a large outgrowth from the side of the portion just described. The opening of this portion is near the opening of the vas deferens and a considerable portion of the gland extends back between the viscera and the portion just described. This is called the first part of the spermatophoric gland by Marchand, and will be called the first part of the mucilaginous gland here.

In structure the two parts are much alike. Both have thick walls, thrown into folds on the inside. These folds are frequently joined by bridges, and in many places the deeper portions of the depressions between the ridges form pouches or sometimes tubules (Williams '08). The whole is, however, too open to form a true racemose or tubular gland. The cavity of each portion of the gland is extensive, forming a pelvis or basin in which the secretion is poured. The whole interior of the gland is ciliated, but the pelvis is particularly well supplied with cilia. The spermatozoa, entering from the vas deferens, pass into the pelvis of the second part of the mucilaginous gland (fig. 32), where they are mixed with secretion and the moving thread of sperm is covered with it. The spermatozoa do not enter part one of the mucilaginous gland, but are passed along a groove in part two past, but a little to one side of, the opening of part one. In the region of the groove, and for some distance along the side, especially along the side nearest part one, the cilia are large and numerous and serve to move the mixed sperm and secretion continuously toward and along this groove through this portion of the mucilaginous gland.

Possibly one-third of the distance from the vas deferens to the distal end of part two the walls of the groove are thrown into a few spiral ridges (fig. 34, *F*), between which the spermatozoa

are passed. The sperm thread is here flattened between the ridges and wound edgewise so one edge becomes the center and the other edge of the surface of the spirally wound sperm mass. As the spermatozoa are covered by the secretion from the gland, the secretion along that edge which forms the center becomes a continuous core in which there are few spermatozoa, and the secretion on the flat applied sides of the sperm thread stick the successive loops of the coil together. The sperm mass, as coiled, does not lie with flat applied surfaces of the loops at right angles to the central core, but the edge applied to the surface lags a little behind the edge at the central core. The surface edge is accordingly nearer the cement body than the core edge.

In longitudinal section the sperm mass thus appears like a series of small open funnels with the small ends directed toward the aboral end of the spermatophore. The spaces between the funnels, together with the core that would occupy the small open ends of the funnels is filled with sticky material furnished by the mucilaginous gland. From the location of the ridges which serve to coil the thread, through the remainder of the mucilaginous gland and through succeeding glands, the sperm mass is rotated on its longitudinal axis by the action of the cilia in the groove in which it lies. It is through this longitudinal rotation that the sperm mass, molded by the ridges between which it passes, is coiled into the form described. The sperm mass, coiled in this way, is usually called the sperm rope. It should be borne in mind that the coil consists of a single flattened strand and not a number of strands as is the case with a rope (fig. 23 A, *SM*).

Spermatozoa continue to issue from the vas deferens and the sperm mass continues to form until the end first formed reaches some distance past the limit of the mucilaginous gland to a point about opposite the notch (fig. 32, *C*¹). The sphincter muscle around the opening of the vas deferens then contracts and no more spermatozoa are allowed to enter. When the free spermatozoa are wound into the coil the charge of sperm for one spermatophore is complete and the sperm mass is in final form.

As completed the sperm mass is cylindrical, with slightly tapering ends. The surface is smooth, the coiled thread being visible, but the coils are not prominent. The free surface is covered by a small amount of the mucilaginous material. In staining, the spermatozoa take haematoxylin, or other nuclear stains, and the mucilaginous material eosin. Scattered spermatozoa are found in the mucilaginous material, but there are not many of them. In this condition the sperm mass appears much as it does in the completed spermatophore, except that the coils of the sperm thread are a little more open and more easily seen. The change is to be accounted for by the pressure applied in the completed spermatophore by the elastic outer tunic.

As the sperm mass passes back through the mucilaginous gland, the groove in which it lies is formed by an overhanging ridge, an arrangement that becomes very prominent in the succeeding part of the spermatophoric organ (fig. 36, *GR*).

As the sperm mass passes out of the mucilaginous gland the cement body is attached to the end which leaves the gland last. This body is evidently formed by the mucilaginous gland, but I have not observed the actual process of formation. It has been seen immediately after it has left this gland, and, as it must be formed before the coiled filament is laid down, and the coiled filament is formed just beyond the mucilaginous gland, there can be no alternative as to its place of formation.

So far, I have not been able to determine whether parts one and two of the mucilaginous gland have the same function. Possibly one of these portions is concerned in the formation of the cement body alone, but I have not been able to find evidence on the point. With various stains these glands appear alike and the secretion in the sperm mass and the material of the cement body have similar affinities for stains. There seems to be a difference in composition however, for the cement hardens so as to stick permanently to bodies in sea-water, while the mucilaginous material mingled with the sperm mixes freely with sea-water and liberates the spermatozoa. This difference in composition has led me to search diligently for the exact

place and method of formation of the cement body, but thus far I have not been successful.

As the sperm mass and cement body leave the mucilaginous gland and are passed along the spermatophoric organ, a thin thread of mucilaginous material is formed which is continuous with the cement body. This continues to be formed as the forming spermatophore passes on, and becomes the hyaline core (fig. 23 *HC*) around which the spiral filament is wound. Racovitza, ('94) calls this the hyaline core in his description of the spermatophore of *Rossia*, where it evidently persists in the fully formed spermatophore. In the squid it is present only during the formation of the spermatophore and disappears before the spermatophore becomes functional.

The part into which the sperm mass and cement body is passed from the mucilaginous gland is thick-walled and granular, but the inside is smooth, not thrown into ridges and grooves as in the mucilaginous gland, nor are there sacules or tubules in its structure. The inner surfaces are smooth and strongly ciliated. The upper surface of the wall (the surface toward the visceral mass) is thrown into a very prominent ridge (figs. 32 and 34, *GR*) very similar in appearance to the typhlosole in the intestine of an earthworm, except that it is not bilaterally symmetrical. One margin of the ridge is drawn to the side and overhangs to form a very definite ciliated groove (fig. 34, *G*), along which the forming spermatophore is passed, moved by the cilia and by movements of the organ, and kept constantly rotating on its longitudinal axis.

The general structure of this portion of the spermatophoric organ is essentially the same from the mucilaginous gland to the narrow duct near the anterior end of the organ, but at least two divisions may be recognized in it. Externally the boundaries of these divisions are roughly marked by constrictions, the first of which (figs. 29 and 32, *C*¹) may be taken as the boundary of the mucilaginous gland and the second (*C*²) the boundary between two functional parts which show very similar structure. Marchand refers to these two divisions jointly as the third part of the spermatophoric gland. As the two parts are functionally

quite different it will be convenient to refer to them by different names.

The first part (figs. 29 and 32, *EG*) is slightly swollen and in it are formed the membranes of the ejaculatory apparatus. I therefore call it the ejaculatory apparatus gland. It is true that one of the membranes, the inner tunic, continues over the sperm mass so this portion actually forms more than the ejaculatory apparatus, but this term answers very well. The remaining portion (figs. 29 and 32, *MTG*) forms the middle tunic and will be called the middle tunic gland.

The sperm mass and cement body enter the ejaculatory apparatus gland, with the hyaline core still being formed in the mucilaginous gland, and moves slowly through it, receiving the inner tunic at the distal end of this gland, near the notch which separates this gland from the middle tunic gland. The material secreted by the glandular walls of this portion of the organ is moved by the cilia over the edge of the ridge. The slowly rotating sperm mass thus has this material wound around it as a thin sheet. Parts of the gland between this point and the mucilaginous gland are at the same time secreting materials that are being wound into other parts. Bear in mind that after the cement body leaves the mucilaginous gland, the hyaline core continues to be secreted by it.

The ridge, under the edge of which the sperm mass and cement body have passed, has a groove across its convex surface at a point about opposite the notch marking the boundary between the mucilaginous and the ejaculatory apparatus glands. This groove (fig. 32, *SFG*) is not very deep, but is easily seen in dissections of spermatophoric organs which have been preserved in formalin. It extends diagonally from one side of the ridge to the other and, on the side where the forming spermatophore passes, is deep enough to join the groove in which it lies. Just after the cement body passes its end the material that forms the spiral filament passes along this diagonal groove and, because of the rotation of the forming spermatophore, is wound around the hyaline core.

Immediately beyond this groove the material for the inner membrane is secreted and wound on as a sheet. The inner

membrane thus covers the cement body and the outside of the spiral filament, to both of which it adheres firmly. Orally to the spiral filament, the inner membrane covers the hyaline core.

A little further on the middle membrane is formed. The sheet of which it is formed is thin, but is wound around many times in building up this comparatively thick membrane. What causes the aboral end to be so definitely limited has not been determined.

The portion of the gland immediately following that which forms the middle membrane forms the outer membrane and that which follows, as already stated, forms the inner tunic.

All of these structures (figs 23 and 23 A), the inner membrane (*IM*), middle membrane (*MM*), outer membrane (*OM*), and inner tunic (*IT*), are formed in the same manner and the gland in which they are formed shows no definite change in structure from one part to the other. Apparently all are being formed at the same time and the formation of each part stops when it is completed. There seems to be nothing visible that limits the extent of the formation of each structure.

Some membranes adhere to others with which they come in contact and some do not. Thus the inner membrane forms a covering for the cement and adheres to the spiral filament and middle membrane. The outer membrane adheres to the inner membrane over the cement body, where they come in contact, and to the inner tunic, but not to the middle membrane.

It is perhaps as well to call attention to certain peculiarities in forming structures here as anywhere. The core of mucilaginous material in the sperm mass seems to be continued forward into and through the cement body (fig. 23 A). The connecting cylinder between the two parts is very prominent. The cement is seen to be spirally wound around this core in the partially formed spermatophore and the core is continued on throughout the length of the hyaline core as a much smaller core. The hyaline core is evidently continuous with the cement material.

The inner core does not stain heavily with any of the stains and seems to be distinct in composition from the cement material.

It is much more like the mucilaginous material in the sperm mass, but it has not just the same staining properties.

How these parts are formed is not known. Possibly the mucilaginous substance binding the sperm mass is continuous as a core and the cement substance and hyaline core are similar substances wound around the central core. If this be the case the mucilaginous gland must consist of two functional parts.

A second point has to do with the spiral filament. This seems to lie directly against the hyaline core, with the inner membrane covering it. The space between the loops of the spiral filament which extends from the inner membrane to the hyaline core is evidently filled with some substance that never stains and is apparently liquid. The hyaline core never bulges much between the loops of the spiral filament, and the inner membrane is never pressed in much between these loops. With the pressure that is put upon the contents of the spermatophore when it is completed—by the elastic outer tunic, even before the hyaline core disappears—there would be distortions were there not a supporting liquid in this space.

It is not difficult to understand how each of the layers described are formed when we bear in mind that each is wound around the slowly rotating mass as it proceeds through the duct. The invisible part, the part connected with the nervous mechanism that sees to it that each secretion is started and stopped at the proper time to make the whole a complete, well-formed complicated machine, is not more remarkable than many other nervously controlled mechanisms.

The forming spermatophore has now passed well back into the middle tunic gland, and by the time the structures described have been completed the first formed end of the sperm mass lies near the distal end of this gland.

As previously stated, the structure of the middle tunic gland (figs. 29 to 36, *MTG*) is essentially the same as that of the ejaculatory apparatus gland. The middle tunic is formed by winding a sheet of secretion around the rotating mass as in the membrane described. There is a little liquid between the middle

and inner tunics so the two do not adhere at any place. The middle tunic is of about uniform thickness over the part occupied by the sperm mass. It is thinner and less granular from this point to the oral end (figs. 23 and 23A, *MT*).

The forming spermatophore apparently remains in this part of the organ for some time; the sperm mass, cement body and part of the ejaculatory apparatus lying in the middle tunic gland, and the forward part of the ejaculatory apparatus lying in the ejaculatory apparatus gland. At this time the forming spermatophore is very much larger than the completed structure. It is sticky and soft so that when it is removed from the organ it remains bent in any shape in which it is placed, provided the bends be not abrupt. Before it is completed and functional, the forward end becomes much folded so the length is greatly decreased and all is shrunken so it is much less in diameter. The shrinking must effect length as well as diameter. All these changes are associated with putting on the outer tunic.

Before leaving the middle tunic gland, mention should be made of a narrow tube that joins its distal end (figs. 31, 32, and 34, X). The lumen of this duct is lined with epithelium lying directly on connective tissue. The walls are not glandular and the epithelium, which is ciliated, is evidently not composed of actively secreting cells. I am unable to assign any function to this tube. It has been suggested, by Marchand, that it may represent a degenerated part of the originally paired sexual ducts, only the left of which is functional. I have no information that throws light on this subject, but the point of junction in the course of a highly modified section of the duct is not what might be expected if this were the case.

I have not been able to observe the actual formation of the outer tunic. In the specimens I have examined the outer tunic is never present while the forming spermatophore is in the middle tunic gland. The outer tunic is always present in a spermatophore that has reached the next large division, which, though I am not entirely sure of its function, I call the hardening gland (figs. 29 to 36, *HG*). Marchand speaks of this gland as the accessory gland (*prostata*), a term with no functional

meaning. It can in no way be compared with the prostate gland of vertebrates. The inner walls of the gland are marked by various connecting ridges which project into the large cavity of the gland. The gland forms a blind sac with only one duct.

Only the end of the spermatophore that contains the sperm mass is pushed back into the hardening gland, and, as has been said, when the spermatophore is pushed into this gland the outer tunic is always present.

The connecting duct between the middle tunic gland and the hardening gland is relatively small, but the walls are highly glandular. The duct forms a bent cylindrical tube with a lumen that corresponds pretty well with the diameter of the spermatophore. I have not succeeded in removing spermatophores passing through this portion without injuring them and in sections the injury of the spermatophores is usually considerable. I find, however, that the material of the outer tunic is present on parts of the spermatophore that have not reached the hardening gland, so this narrow gland must be responsible for its formation. It may therefore be called the outer tunic gland (figs. 29 to 33, *OTG*). Inasmuch as spermatophores are seldom found in this gland, they probably pass through it rather rapidly.

The end containing the sperm mass is passed back into the hardening gland to about the level of the cement body. The region of the cement body and ejaculatory apparatus never enter this gland. The aboral end of a spermatophore, when present in this gland, projects into its lumen from the narrow outer tunic gland without touching its walls. The spermatophore has definite outlines, the outer tunic is fully formed and not sticky, and the liquid in the lumen of the gland is transparent and not noticeably viscid. When the gland is opened in sea-water, the secretion that mixes with the water is visible only because it has a different refractive index. It mixes readily with the water and disappears. In sections of the organ the contents of this gland frequently show some coagulated and stained material which probably comes from the secretion.

While the aboral end of the spermatophore is in the hardening gland the oral end is passed along the outer tunic gland to

the position of the opening from the side of this gland. This opening communicates with a complicated portion in which the spermatophore is completed. Marchand calls this (appendix) the blind sac of the distal vas deferens. It is not a true blind sac, as it has two openings, and the term appendix, which has been applied by other writers, has no meaning. I will call this (figs. 29 to 32, *FG*) the finishing gland. In passing the oral end of the spermatophore from the outer tunic gland into the opening of the duct leading to the finishing gland (fig. 32, *FD*) this end of the spermatophore is considerably folded, and, as the further movement is now with this end directed forward, the folds are held and compressed while the outer tunic hardens around them.

Just how much of the gland is responsible for the formation of the outer tunic is uncertain, but judging from the structure of the gland, the appearance of the tunic as seen in sections, and the appearance of spermatophores removed from the gland, I am inclined to think that the whole structure, from the end of the middle tunic gland to the end which extends into the hardening gland, is very active and that the duct leading from this portion to the finishing gland, the finishing gland duct, and the finishing gland itself, adds to the outer tunic over the oral end of the spermatophore.

Spermatophores taken from this position in the organ exhibit great differences in the appearance of the oral ends, and, as spermatophores are common in this position, the meaning probably is that the spermatophore is held here until the oral end is shaped and covered. It is then passed, oral end first, down the duct to the pointed end of the finishing gland.

The duct to the finishing gland is much larger than the lumen of the outer tunic gland and has a very definite groove along the side away from the visceral mass, which ends on the side of the finishing gland in a pouch (figs. 29 to 32, *PF*). The spermatophore usually lies in the part of the duct away from this groove and pouch, but in a few cases I have found the oral end of the spermatophore in this pouch. This position may not have been normal, for, in opened animals, the mechanism con-

trolling the movements of the parts of the spermatophoric organ, and accordingly the forming spermatophores, must be badly interfered with.

In a spermatophore removed from this position the outer tunic over the aboral end is well formed and normal in appearance. That over the oral end is thin, somewhat opaque, and adheres to a needle. It is most difficult to get specimens at this stage of formation free without injury. Figure 24 shows the oral end of the only really perfect specimen I have been able to remove.

In passing into the finishing gland the oral end of the spermatophore is pressed forward into the pointed end of the gland and evidently receives further additions to the outer tunic. The aboral end of the spermatophore is now free from the hardening gland and the secretions from this gland are free to find their way into the finishing gland. Whether this actually takes place I do not know, but the oral end, which just before was covered by a thin, somewhat opaque and sticky outer tunic, changes in form and appearance to that of the completed spermatophore.

The last processes in the change have to do with the formation of the cap. As shrinkage takes place, the oral end of the ejaculatory apparatus becomes further coiled and the cap region is bulged outward by the end of the tube formed by the inner and middle membrane (fig. 25). The cap thickens and the tube in question is forced over and finally pressed out sidewise so the lumen of the tube is spread to correspond to the shape of the cap (figs. 26 and 27). The margins of the tube that come in contact with the margins of the swollen cap are fastened to the ridge of the outer tunic along the borders of the cap, and further shrinkage brings the spermatophore into functional form.

It is while in the finishing gland that the spermatophore shrinks into the finished size and the outer tunic becomes normally turgid and elastic. Here the hyaline core disappears probably becoming liquid. When the spermatophore starts down the spermatophoric duct it is completed in form and capable of normal ejaculation. A slight shrinkage, especially in

the region of the cap (fig. 28) will take place, but otherwise all is completed.

I have not been able to determine just how the thread that extends free from the cap is formed. It was first seen shortly before the oral end of the spermatophore enters the spermatophoric duct. A small glandular tube (figs. 31 to 33, Y) lies along the spermatophoric duct, and opens into the finishing gland near where this gland opens into the spermatophoric duct. The lumen of this duct is flattened in cross-section and the position of its opening is so near the point where the thread is first seen that I have been inclined to the belief that secretions from this gland form the thread. I have, however, no real evidence.

The spermatophore passes down the spermatophoric duct and enters the spermatophoric sac oral end first, with the cap thread lying by its side. Here it reverses ends again as the spermatophoric sac extends posteriorly beyond the spermatophoric duct a distance equivalent to the length of a spermatophore.

Each successive spermatophore crowds its predecessor side-wise and by forcing its oral end into the posterior pointed end of the spermatophoric sac causes the preceding spermatophore to move, aboral end forward, further into the spermatophore sac. Successive spermatophores are thus arranged in a spiral manner inside the sac, and the cap threads trail back from their oral ends. The last spermatophore to enter the sac has its oral end slightly posterior to the oral end of the spermatophore that preceded it into the sac.

The walls of the spermatophoric sac are muscular, and spiral lamellae, extending into its interior (fig. 33 to 36, SS), keep the spermatophores in position, practically parallel to each other, but spirally arranged, with the aboral ends moving forward. The muscular action of the spermatophoric sac is evidently responsible for the most of the movements of the spermatophores it contains.

Where the spermatophoric sac joins the outer muscular duct, commonly called the penis (a term somewhat misleading as to function), the spermatophores largely lose their spiral arrange-

ment and become arranged in groups of from twenty to forty or more, parallel to each other and filling the lumen of the duct. Thus, when they are ejected from the penis and grasped by the hectocotylized arm, an even group, with their aboral ends forward and the threads still embedded in the secretion of the penis, is presented to the grasping arm.

SUMMARY

Spermatophore structure. The contents of the spermatophore are referred to as the sperm mass, cement body, and ejaculatory apparatus.

The sperm mass consists of the spermatozoa surrounded by and mixed with a mucilaginous material which mixes readily with water. It is the proper delivery of the sperm mass that is the essential action of the spermatophore.

The cement body contains the sticky material that finally sticks the reservoir, into which the sperm mass is forced, in position on the female.

The ejaculatory apparatus consists of membranous tubes and structures that together form by their evagination, the conducting tube through which the cement body and sperm mass are forced, and the sperm reservoir into which the sperm mass is forced.

The contents of the spermatophore, as described, are enclosed inside a very elastic outer tunic and a middle tunic that is elastic and capable of taking up water rapidly. Together these tunics supply the power necessary for ejaculation of the spermatophore.

The outer tunic is closed by a cap which is cemented in position and may be loosened by pulling the thread connected with the cap.

Spermatophore ejaculation. When the cap loosens the force supplied by the elastic outer tunic and osmotic middle tunic causes the ejaculatory apparatus to evaginate. In doing so the two outer coats of this apparatus, the inner tunic and the outer membrane, are reflected to form the sperm reservoir into which the sperm mass is forced. The continued action of the outer

and inner tunics forces the reservoir containing the sperm mass out, ruptures the cement body and smears the cement over the closed end of the sperm reservoir.

In this condition this body is freed from the remainder of the spermatophore and is normally stuck in position on the female by the cement.

Reference to the diagrams on plate 6 will aid in understanding the essential processes of ejaculation.

Spermatophore formation. The spermatophore is completely formed inside of that portion of the sexual duct called the spermatophoric organ. This is a complicated series of continuous glands, in the lumens of which the forming mass is kept rotating on its longitudinal axis. By this rotation the sperm mass, cement body, spiral filament, and the various enclosing membranes are spirally twisted and wrapped into position as the mass moves along the lumen of the organ.

When fully formed, the whole spermatophore undergoes a shrinking process by which the elastic outer membrane is left in a state of high tension which makes the whole spermatophore turgid and ready to ejaculate.

LITERATURE CITED

- Papers that mention Cephalopod spermatophores and spermatophoric organs are very numerous, but only a few have been cited in this paper.
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- WILLIAMS, LEONARD W. 1908 (?) The anatomy of the common squid, *Loligo pealii* (Les.). Amer. Mus. Nat. Hist.

EXPLANATION OF FIGURES

With the exception of figures 32 and 37 to 41, all of the figures were outlined with the aid of a camera lucida. Where cut surfaces are shown on spermatophores and spermatophoric organs the relations were worked out by study and shown for convenience of those interested in the paper. It is hardly necessary to say these were added after the camera-lucida sketches were made.

The sizes of spermatophores and spermatophoric organs differ with the sizes of the individuals from which they were obtained. This explains the differences in the size of the figures. The spermatophores were all drawn with the same magnification except figures 1, 12, 16, and 17, which are not so highly magnified. The sections of the spermatophores shown in figures 18 and 22 were considerably broken in preparation. While their outlines were obtained with the aid of a camera lucida, the damage was repaired by study. The figures are not from sections of the same spermatophore.

Large spermatophores measure 16 mm. in length, but 13 mm. is more common. During formation they are larger, but they shrink to their final size after they are otherwise fully formed.

ABBREVIATIONS

- C*, cap covering the oral end of the spermatophore.
*C*¹, constriction separating the mucilaginous gland from the ejaculatory apparatus gland.
*C*², constriction separating the ejaculatory apparatus gland from the middle tunic gland.
CB, cement body.
*CB*¹, cement liberated by rupture of cement body.
CC, connecting cylinder between the sperm mass and the cement body. In forming specimens the connecting cylinder is continuous with material which extends the length of the sperm mass, cement body, and hyaline core. In fully formed specimens this material may sometimes be distinguished in places.
CT, cap thread. When this is pulled the cap is normally loosened and the spermatophore ejaculates.
EA, ejaculatory apparatus. This term is slightly misleading, as the process of ejaculation is not confined to this part.
EG, ejaculatory apparatus gland. This term is not quite accurate, as other portions than the so-called ejaculatory apparatus are formed by this gland.
F, folds that serve to wind the sperm thread into a spiral. In the position shown in figure 34 they are much smaller than they are a little further along in the groove.
FD, finishing gland duct, connecting the finishing gland with the outer tunic gland.
FG, finishing gland. Where the cap and cap thread are formed and where the shrinking of the spermatophores is completed.
G, groove along which forming spermatophores pass. In some figures the forming spermatophores are present.

- GR*, gland ridge; typhlosole-like in appearance. Under one edge of this ridge is the groove along which the forming spermatophores are passed.
- HC*, hyaline core. Present in forming spermatophores, but later disappears, probably by liquefaction, possibly by withdrawal to the cement body.
- HG*, hardening gland. This may not be properly named. Only the aboral end of the spermatophore is thrust into this gland. In this position the aboral end of the spermatophore is always covered by the outer tunic, which is smooth elastic, and not sticky. The hardening of the oral end of the spermatophore takes place in the finishing gland, possibly by secretions delivered with the spermatophore from the hardening gland, possibly by secretions furnished by the finishing gland itself.
- IM*, inner membrane. A membrane of the ejaculatory apparatus and a covering for at least a portion of the cement body. On its inner surface it bears the spiral filament. It is so thin it has been represented by a line.
- IT*, inner tunic. Inconspicuous and represented by a line over the sperm mass and connecting cylinder, becoming thicker and more conspicuous over the ejaculatory apparatus, where, with the outer membrane, a double membrane is formed. This becomes part of the covering of the sperm reservoir when this is discharged from the spermatophore.
- MM*, middle membrane. A conspicuous membrane of the ejaculatory apparatus. The tube formed by it is firmly attached to the outer tunic at the oral end and has its open mouth applied to the shoulder of the cement body beneath the outer membrane.
- MM*¹, middle membrane, cap end. This portion ruptures when ejaculation of the spermatophore begins.
- MT*, middle tunic. Probably of a highly osmotic material that furnishes part of the power which causes ejaculation of the spermatophore.
- MTG*, middle tunic gland.
- MG*¹, mucilaginous gland, part one.
- MG*², mucilaginous gland, part two. The separate functions of these two parts have not been determined, but together they form the secretions with which the spermatozoa are mixed, and which form the connecting cylinder, the hyaline core, and the cement body.
- OM*, outer membrane. A portion of the ejaculatory apparatus. For most of its length it is intimately associated with the inner tunic so the two appear as a double membrane. The tube which it forms is applied to the middle membrane and ends with a free opening near the oral end of the spermatophore. With the inner tunic it forms the sperm reservoir.
- OT*, outer tunic. A highly elastic tough outer covering. This, together with the middle tunic, furnishes the power that causes ejaculation. When the spermatophore nears completion this tunic shrinks until it is under great tension and the spermatophore becomes very turgid as the result.
- OTG*, outer tunic gland. It is possible this may not be responsible for the formation of the outer tunic, but it probably is.
- PA*, point where adhesion between the middle and inner membranes covering the cement body becomes strong. From this point to the end of the middle membrane they adhere firmly. As the spermatophore nears completion, the point of adhesion is not so easily seen, but during ejaculation the adhesion is seen to be strong.

- PF*, pouch on the finishing gland, of unknown function. This pouch is connected with the lumen of the finishing gland duct and with the finishing gland itself as a sort of diverticulum.
- PR*, point of rupture of the inner membrane. During ejaculation the inner membrane and the spiral filament separate from the oral end of the cement body. As ejaculation proceeds they form the outer covering of the tube through which the sperm mass is forced. The extreme outer end of this tube is free from them as the middle membrane, which forms the extreme outer end, extends along the cement body past the point of rupture.
- S*, 18, 19, 20, 21, 22, lines on figure 1 and figure 16 indicating the planes of sections of spermatophores represented by figures bearing the same numbers.
- S*, 33, 34, 35, 36, lines on figure 29 that indicate the planes of sections of the spermatophoric organ represented by figures bearing the same numbers.
- SD*, spermatophoric duct, connecting the finishing gland with the spermatophoric sac. When a spermatophore starts into this duct from the finishing gland it is completely formed, except that a slight shrinking, especially in the region of the oral end, will still take place. By the time the spermatophoric sac is reached the shrinking is complete.
- SF*, spiral filament. This is fastened to the inner membrane and seems to serve to keeping lumen of the ejaculatory apparatus open. The material of which it is composed is brittle and the filament is broken into small fragments as the tubes composing the ejaculatory apparatus are everted. The same letters have been used for the filament while the coils are distinct and for the broken fragments that remain sticking to the outside of the evaginated inner membrane. See figure 7.
- SFG*, spiral filament groove. The material from which the filament is formed is passed along this groove to the forming spermatophore which is passing along the groove underneath the ridge across which the spiral filament groove cuts.
- SL*, space filled with liquid. This liquid originates in the middle tunic gland. It does not stain and evidently has only the double purpose of lubrication and transmission of pressure.
- SM*, sperm mass. In lettering the same letters have been used for the mass of spermatozoa, whether in position in the spermatophore, during ejaculation, or in the sperm reservoir after ejaculation is complete. It should be borne in mind that the arrangement is changed so the original sperm mass is disorganized entirely by the time it reaches the sperm reservoir. As the disorganization is a continuous process in ejaculation, it seems more confusing to attempt to designate it by different letters and names than to use the same letters with this explanation.
- SS*, spermatophoric sac. The receptacle in which the completed spermatophores are stored. Because of size it is shown only in the figures of cross-sections of the spermatophoric organ. It is really not a part of the spermatophoric organ, but a storage receptacle. It receives the spermatophores from the spermatophoric duct which comes from the finishing gland, and delivers them through the penis.
- VD*, vas deferens. This plaited tube joins the testis with the spermatophoric organ and delivers the completely formed spermatozoa to it.

- VDO*, vas deferens opening into the mucilaginous gland. The opening is provided with a sphincter muscle and the spermatozoa are allowed to enter only at definite intervals.
- X*, a duct of unknown function. A ciliated, not glandular, duct which opens into the distal extremity of the middle tunic gland. It has been suggested that this represents the vestige of the right vas deferens, but this seems rather doubtful.
- Y*, a glandular duct of unknown function that joins the finishing gland near the opening to the spermatophoric duct.

PLATE 1

EXPLANATION OF FIGURES

- 1 Spermatophore completely formed as taken from the spermatophoric sac. The cap thread is shown broken at a little less than one-half the normal length. At this magnification the outer tunic is represented by a single line and ejaculatory apparatus details are not shown. $\times 20$ diameters.
- 2 Oral end of a spermatophore. $\times 70$ diameters.
- 3 Oral end of a spermatophore. Represented as cut when fresh so the ejaculatory apparatus has expanded; uncoiled and thrust back through the cut ends of the outer and middle tunics. In this condition the free oral ends of the outer membrane, *OM*, and the inner tunic *IT*, are more easily seen. $\times 70$ diameters.
- 4 Oral end of a spermatophore. Shown with the cap thread loosening. $\times 70$ diameters.
- 5 Oral end of a spermatophore, after the cap has opened and the ejaculatory apparatus had begun to evaginate. $\times 70$ diameters.
- 6 Oral end of a spermatophore, after the oral unornamented portion of the ejaculatory apparatus has evaginated and before the portion bearing the spiral filament has begun to evaginate. There is a slight pause at this stage of ejaculation. $\times 70$ diameters.
- 7 Oral end of a spermatophore, when the portion of the ejaculatory apparatus bearing the spiral filament is evaginating. The spiral filament is broken into small fragments in the act of evagination. The fragments, which remain sticking to the inner membrane (now on the outside), are responsible for the broad, indefinite, spiral ornamentation on the outside of the evaginated tube. $\times 70$ diameters.
- 8 A portion of the evaginating ejaculatory apparatus at a slightly later interval than shown in figure 7. This shows the relation of the membranes in the evaginating position. $\times 70$ diameters.

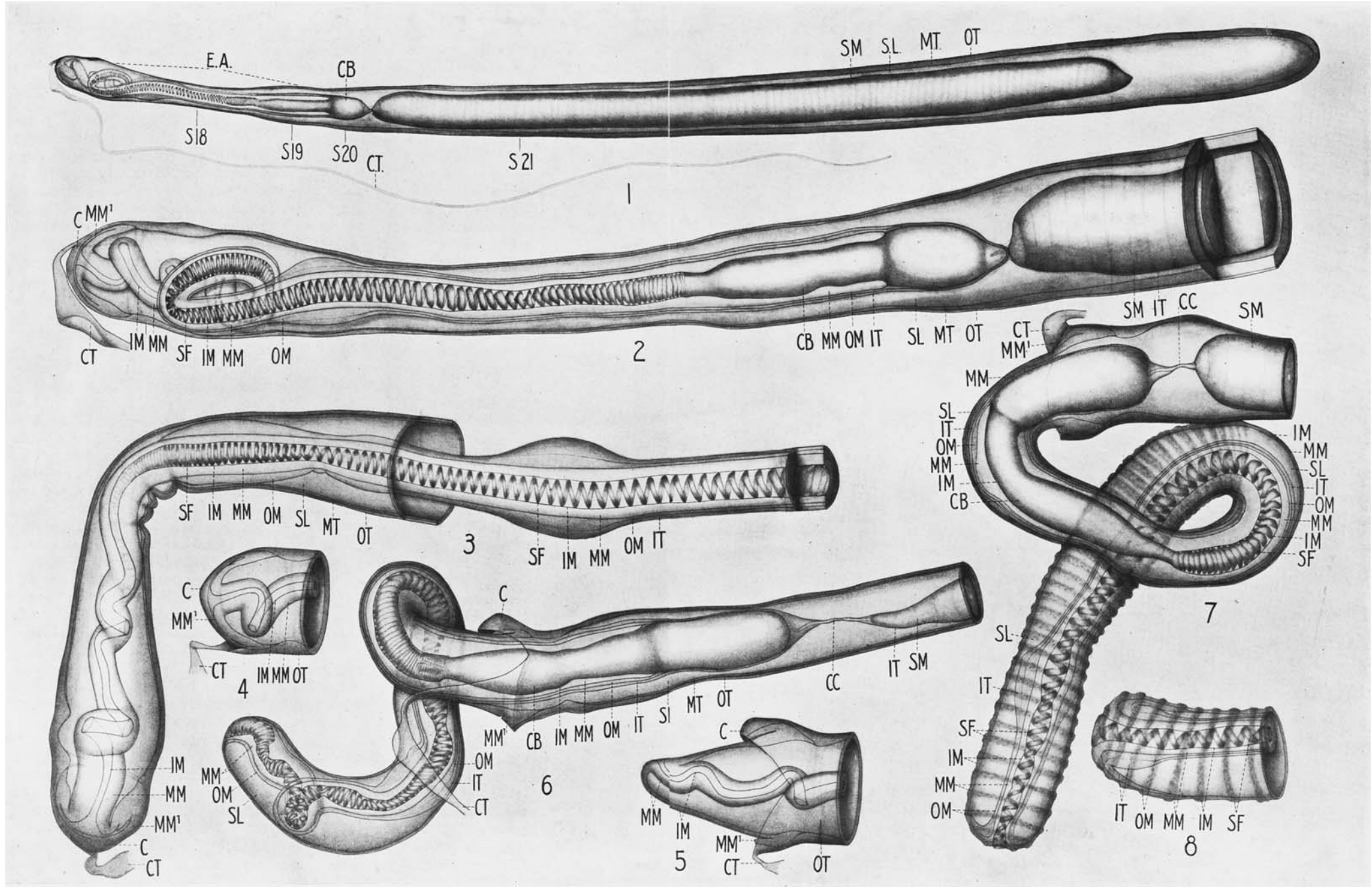


PLATE 2

EXPLANATION OF FIGURES

9 Extremity of the evaginating portion of a spermatophore at the instant the cement body has reached the end of the nearly evaginated ejaculatory apparatus. The inner membrane and spiral filament have broken from the tip of the cement body and are seen at the limit of ornamentation on the outside. The middle membrane adheres closely to the cement body, but the pressure from behind has not yet caused the cement body to change shape. $\times 70$ diameters.

10 A portion of the evaginated ejaculatory apparatus of the same specimen shown in figure 9, taken some distance from the oral extremity, at the point where the thickened portions of the outer membrane and inner tunic now lie. Evagination has turned these membranes back on the inside of the ejaculatory apparatus where they now form a reservoir wall into which the disorganized sperm mass is being forced. $\times 70$ diameters.

11 Extremity of the evaginating portion of the spermatophore an instant later than shown by figure 9. The pressure from behind has caused the adhering middle membrane to draw out the oral portion of the cement body at the sides. $\times 70$ diameters.

12 A whole spermatophore shown at a stage of ejaculation just a little more advanced than shown by figure 11. For convenience in placing on the plate the spermatophore is drawn as if cut in two parts. The position of the sperm mass which is being forced through the evaginated ejaculatory apparatus is shown. $\times 20$ diameters.

13 Extremity of the evaginating portion of the spermatophore shown in figure 12. This is an instant later than the stage shown by figure 11. The adhering middle membrane has drawn the cement body out to form a cap over the end of the sperm mass which is being forced against it. $\times 70$ diameters.

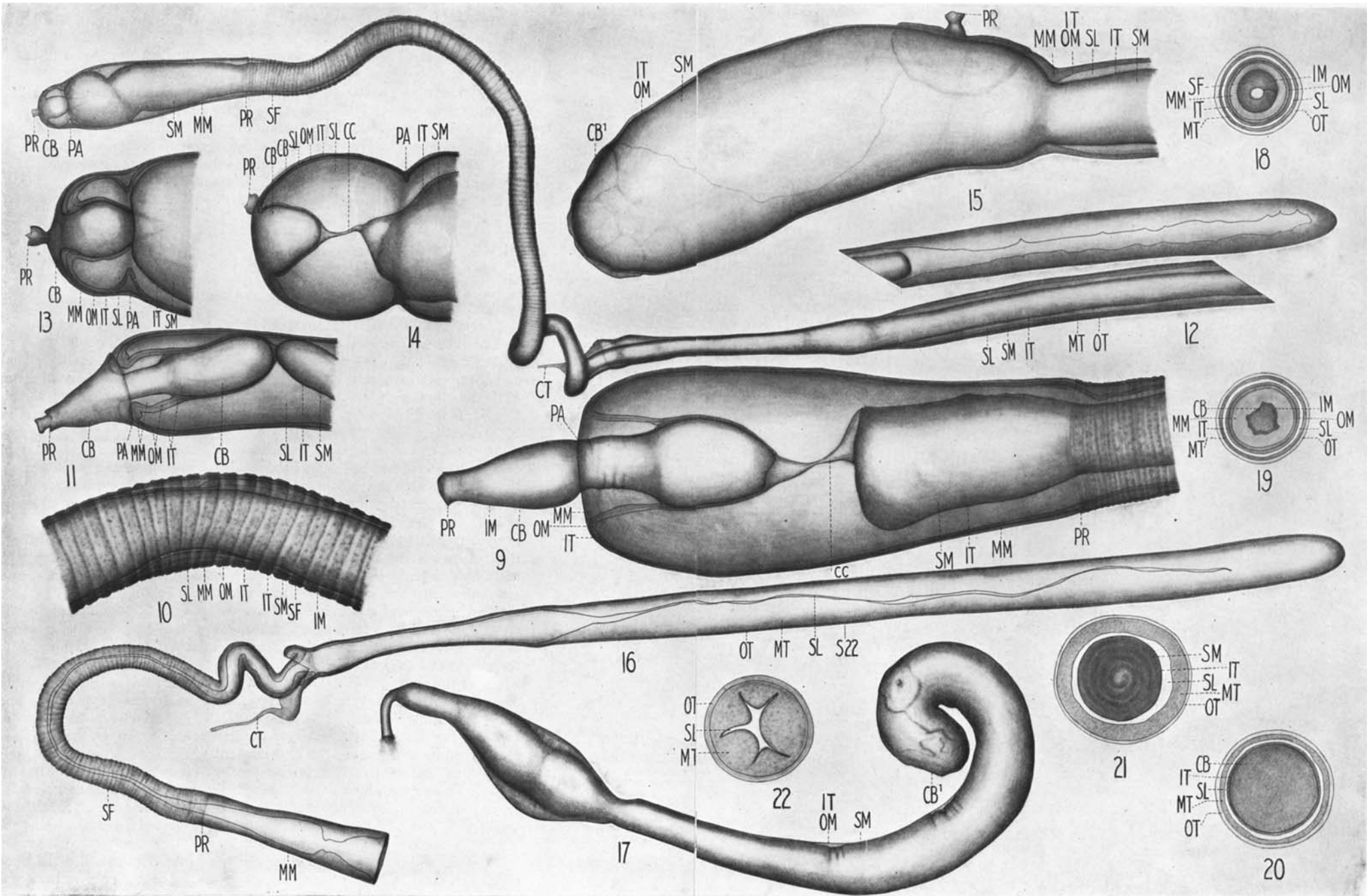
14 A slightly later stage than that shown by figure 13. $\times 70$ diameters.

15 Extremity of the evaginating portion of a spermatophore immediately after the pressure has caused the cement body, to which the middle membrane has adhered, to burst the inner membrane which has confined its viscid cement material. This act at once liberates the cement, which is spread over the end of the reservoir wall that encloses the sperm mass, and frees the reservoir wall, which consists of the outer membrane and inner tunic, now stretched and forced together so their individuality can no longer be distinguished, so it may slip out of the evaginated middle membrane, against which the outer membrane lies. $\times 70$ diameters.

16 The empty case, consisting of the outer and middle tunics, and the evaginated middle and inner membranes with the broken fragments of the spiral filament, after the sperm mass with the enclosing membranes and cement have been discharged. $\times 20$ diameters.

17 The sperm mass with the enclosing membranes and with the cement spread over the closed end, after being ejected from the case. This mass may be called the sperm reservoir. The walls consist of the stretched outer membrane and inner tunic, which are open at the pointed end. Here spermatozoa leaves as they are mixed with sea-water and become active. The cement hardens in sea-water and sticks the reservoir in place. The thickened portion near the opening, with the constricted portion immediately beyond it, is characteristic of the reservoirs. It may have something to do with the thickened portions of

(Continued on page 426)



(Continued from page 422)

the inner tunic and the outer membrane, but I am not certain this is the explanation. $\times 20$ diameters.

18 Transverse section of a spermatophore through the region of the spiral filament. For position see figure 1. $\times 70$ diameters.

19 Transverse section of a spermatophore through the region of the oral end of the cement body. For position see figure 1. $\times 70$ diameters.

20 Transverse section of a spermatophore through the region of the aboral end of the cement body. For position see figure 1. $\times 70$ diameters.

21 Transverse section of a spermatophore through the region of the sperm mass. For position see figure 1. $\times 70$ diameters.

22 Transverse section through the case of an ejaculated spermatophore. For position see figure 16. \times diameters.

PLATE 3

EXPLANATION OF FIGURES

23 and 23A Two continuous portions of the oral end of the same spermatophore dissected from a spermatophoric organ. The oral end of 23 was near the point C^1 , figure 32. The cement body was just beyond the point C^2 . The aboral end of the spermatophore (not represented in the figure) was near the distal end of the middle tunic gland. As the middle tunic is evidently almost completely formed, it would probably soon have been passed on to the outer tunic gland. $\times 70$ diameters.

24 Oral end of a forming spermatophore dissected from a spermatophoric organ. The oral end had passed through that portion of the outer tunic gland that connects with the middle tunic gland, and had passed into the finishing gland duct. The extreme end was near the point where this duct widens into the finishing gland. The aboral end of the specimen (not shown in the figure) was well back in the hardening gland and the cement body region was in that part of the outer tunic gland that extends up into the hardening gland. The outer tunic was elastic and would not adhere to a needle on that portion in the hardening gland, and was soft and sticky over the whole oral extremity. This portion had not been in the hardening gland and would not have entered it. $\times 70$ diameters.

25 Oral end of a forming spermatophore dissected from a spermatophoric organ. The oral extremity had reached the pointed end of the finishing gland and the aboral end (not shown in the figure) was in the finishing gland duct. The whole of the outer tunic, oral as well as aboral end, was elastic and would not adhere to a needle, but the spermatophore was not yet as turgid nor as small as those more fully formed. The shrinking of the oral end, the formation of the cap and the accompanying changes in the ejaculatory apparatus are the chief features to be understood. $\times 70$ diameters.

26 Oral end of a forming spermatophore dissected from a spermatophoric organ. It was contained entirely within the finishing gland and had its extreme oral end very near the opening of the spermatophoric duct. $\times 70$ diameters.

27 Oral end of a forming spermatophore dissected from a spermatophoric organ. The oral end had entered the spermatophoric duct. $\times 70$ diameters.

28 Oral end of a spermatophore dissected from a spermatophoric duct. A slight shrinkage, especially at the oral end, is the only change to take place in completing the spermatophore. $\times 70$ diameters.

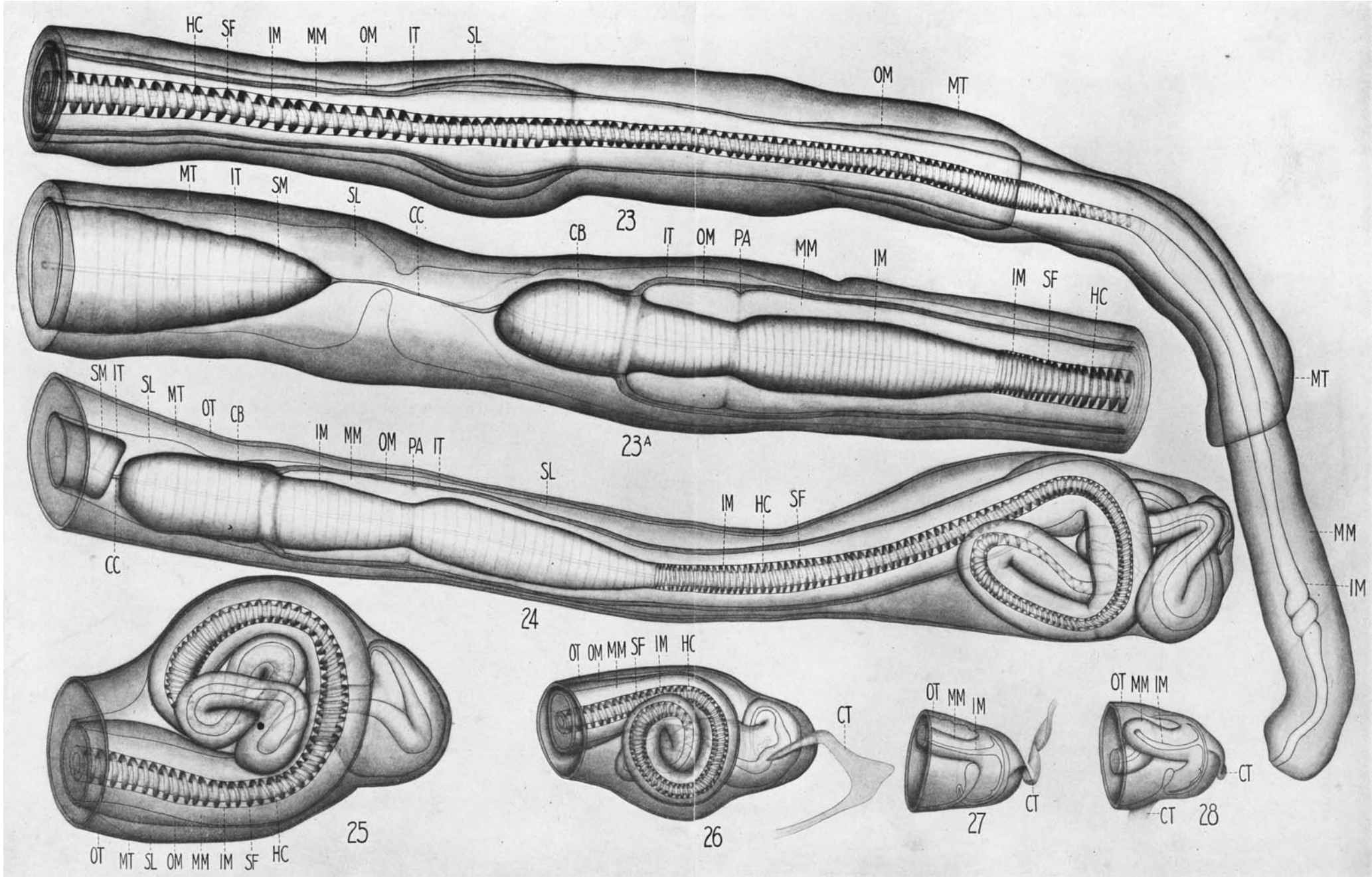


PLATE 4

EXPLANATION OF FIGURES

29 Spermatophoric organ seen from the surface that is free from the visceral mass. $\times 4$ diameters.

30 Spermatophoric organ seen from the surface that is applied to the visceral mass. $\times 4$ diameters.

31 Finishing gland end of a spermatophoric organ seen from the same position as that is figure 29, but showing cut ends of the parts exposed by cross-section. $\times 12$ diameters.

32 Semidiagrammatic view of a spermatophoric organ with the parts separated and the walls cut away to show the internal arrangements of the parts. The second division of the mucilaginous gland has had the wall cut away so as to expose the vas deferens as it approaches its entrance to this gland. The figure is made from the study of many dissections, and reconstructions from the study of sections of the organ.

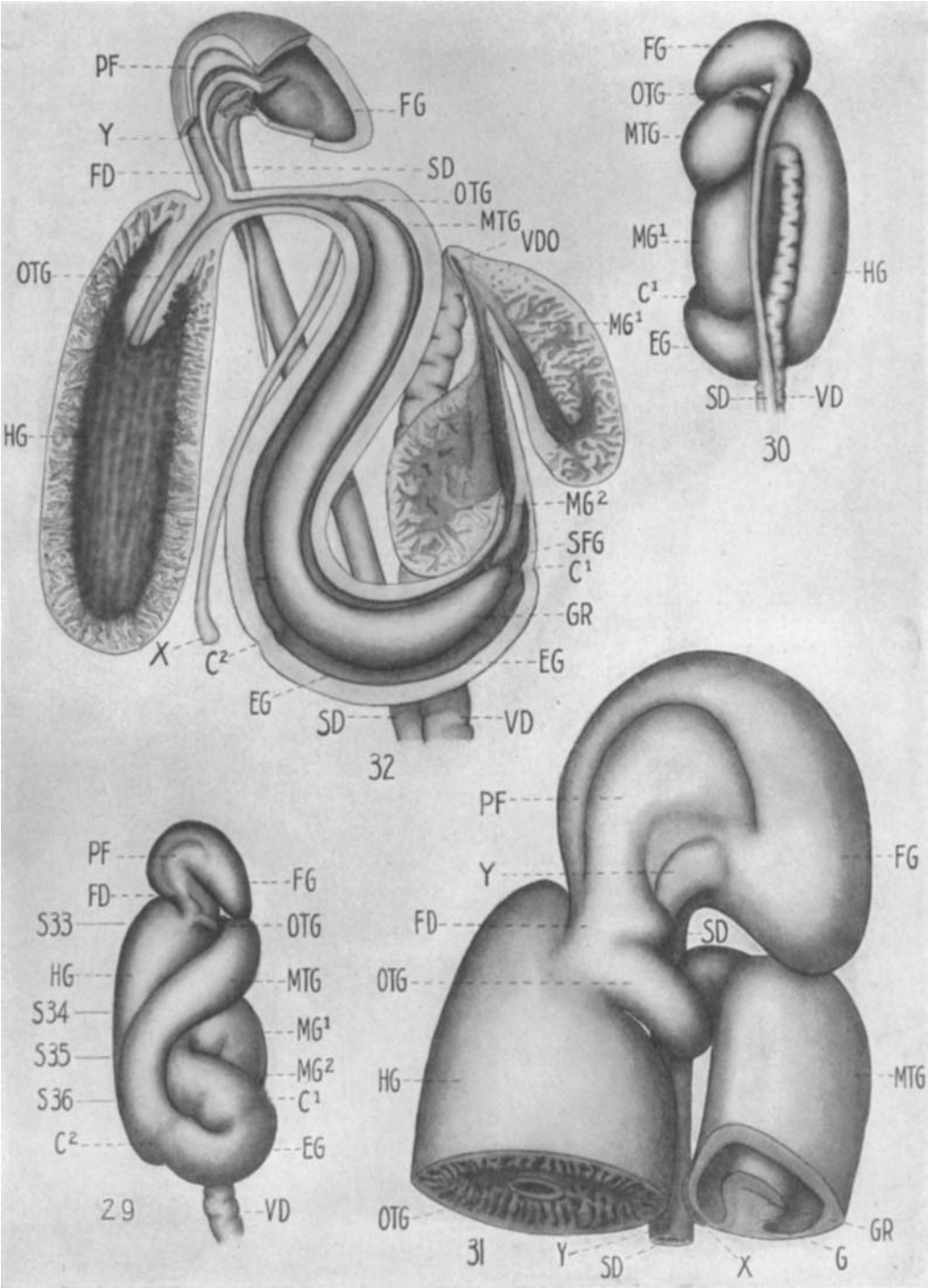


PLATE 5

EXPLANATION OF FIGURES

33, 34, 35, 36 Transverse sections of a spermatophoric organ. For positions of sections see figure 29. The spermatophoric sac, which is not a portion of this organ, is shown in the figures. $\times 12$ diameters.

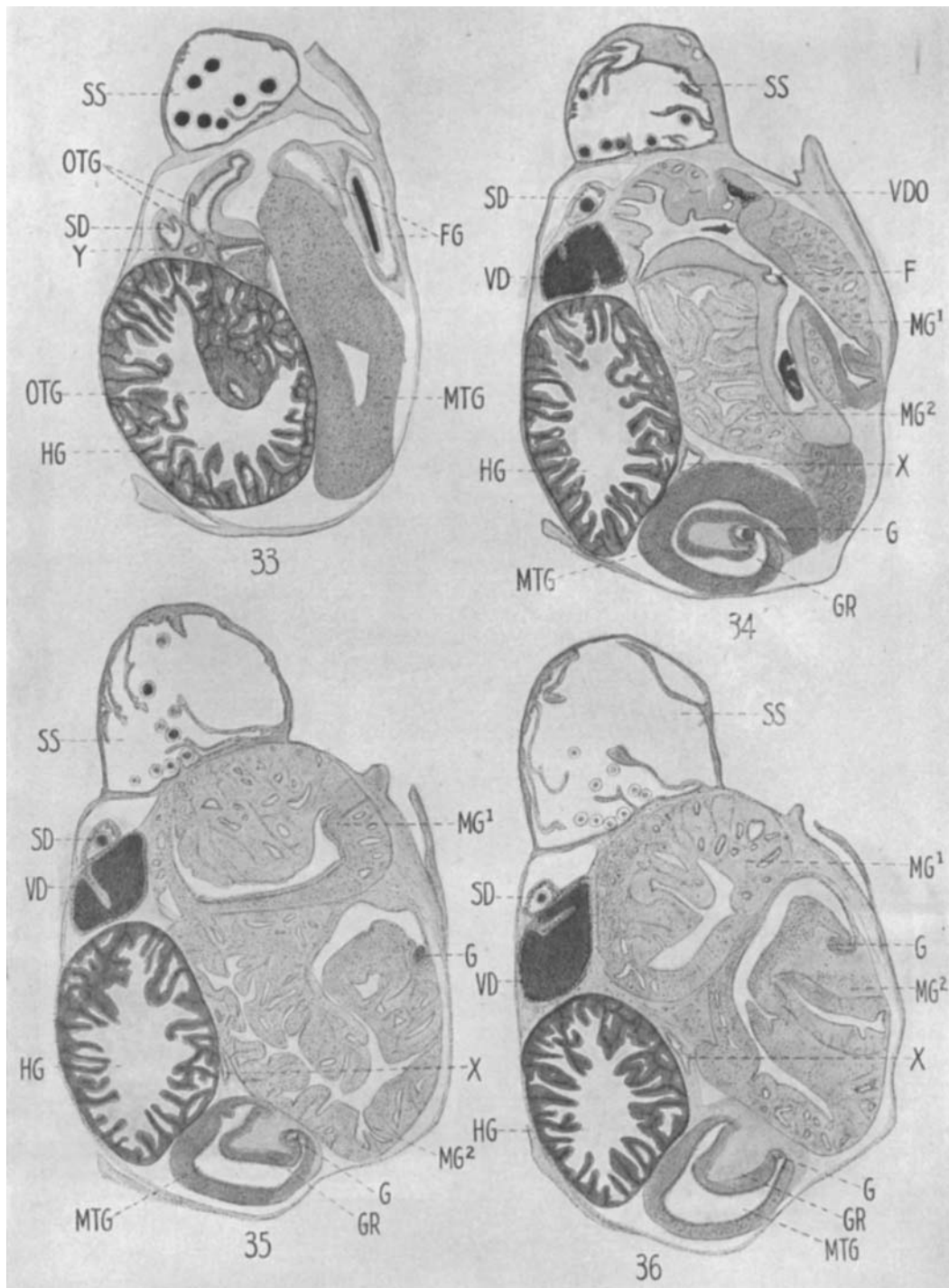


PLATE 6

EXPLANATION OF FIGURES

Diagrams showing the successive stages in the ejaculation of a spermatophore. For simplicity everything not necessary for understanding the process has been omitted from these figures. Thus the cap, the inner membrane and spiral filament, and the inner tunic are not shown.

37 Diagram of the spermatophore ready for ejaculation.

38 Beginning of the process of ejaculation. The evagination of the ejaculatory apparatus, represented here by the middle and outer membranes.

39 Near the end of the evagination of the ejaculatory apparatus, showing the position of cement, sperm reservoir membrane (represented here by the outer membrane), and spermatozoa.

40 Sperm reservoir with filled spermatozoa, and the cement body ruptured.

41 The end of the process. The separation of the discarded case and the filled reservoir.

