THE ACROSOME FILAMENT AND SPERM ENTRY IN THYONE BRIAREUS (HOLOTHURIA) AND ASTERIAS [1]

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In a number of sea urchin, starfish and mollusc species, Dan (1952, 1954a and 1954b) and Dan and Wada (1955) have demonstrated that the spermatozoon undergoes a profound reaction when treated in one or another of the following ways: exposure to egg water, alkaline sea water, or the presence of eggs; contact with surfaces such as that of glass, a collodion membrane or the egg. The salient feature of this reaction is the formation of a filament, the *acrosome filament,* which extends from the acrosome region. Among other changes is an altered relationship of principal structures. In the echinoderm species the middle piece tends to become displaced to an excentric position and the base of the flagellum then appears to emerge from between middle piece and head, forming almost a right angle with the long axis of the spermatozoon and hence with the projecting acrosome filament.

Spermatozoa having the acrosome filament and structural relationships of reacted specimens described by Dan have also been observed in *Holothuria atra* (Colwin and Colwin, 1955a), in suspensions of sperm treated with egg water or placed in the presence of eggs. In this species it was demonstrated not only that the acrosome filament becomes associated with the egg proper but also that the filament enters the egg intact, as an integral part of the spermatozoon.

The present paper will show that the spermatozoa of another holothurian, *Thyone briareus,* produce acrosome filaments of striking length when appropriately stimulated. The data are, in addition, quantitative. The behavior of the acrosome filament during sperm entry will be described. Collateral confirmatory information on two species of starfish, *Asterias forbesii* and *Asterias vulgaris,* will be included. Abstracts of some of these observations have appeared previously (Colwin and Colwin, 1955b and 1955c).

Materials and Methods

Thyone briareus. Specimens were kept in running sea water. Eggs and spermatozoa were obtained from 15 females and 18 males which were eviscerated at intervals between May 13th and June 19th, and from 6 females and 6 males which shed naturally between June 16th and 20th, 1955. Evisceration, a common response in *Thyone* (Killie, 1939), was induced by electrical stimulation of individual animals; then when the gonadal areas were compressed by hand, the gonads were shed along with the other organs. All gonads were rinsed at once in filtered

[1] Appreciation is expressed for laboratory facilities during the summer of 1955, provided under a contract of the Office of Naval Research with The Marine Biological Laboratory.

sea water. The testes were then stored "dry." To obtain sperm the tubules were cut once or twice with scissors. The sperm which oozed out was then suspended in filtered sea water and variously diluted. No differences were detected between the active spermatozoa obtained in this manner and those shed naturally. Eggs were gently stripped through the cut ends of individual ovarian tubules. A thin cellular capsule, which did not persist on naturally shed eggs, enclosed the stripped egg and its surrounding jelly. Frequently this capsule could be completely or partially removed by sucking the eggs into a glass syringe through a hypodermic needle of No. 27 bore. Then the needle was detached and the eggs slowly expelled into filtered sea water. Effective egg water was procured from these decapsulated stripped eggs as well as from eggs which had been shed naturally.

The egg of *Thyone* is flattened axially. It appears elliptical in lateral view and somewhat circular in polar view; the diameter of this circle is approximately 250 μ. Near the animal pole a typical holothurian "umbilicus" projects from the egg into the surrounding jelly. The jelly hull is approximately 55 μ thick and contains radial striations, some extending as far as its outer edge. These irregular wavy striations differ markedly from the clearly-defined rigid looking acrosome filaments of entering spermatozoa.

Sections of naturally shed eggs show that the germinal vesicle is not intact, confirming the findings of Ohshima (1925). In the living egg, however, the vesicle often *appears* to remain intact owing probably to persistence of its "residual substance," as noted by Ohshima. Eggs from eviscerated gonads were not sectioned.

Asterias. Germ cells of *A. forbesii* were obtained from freshly collected specimens in May and June, 1955. The gonads were removed from detached arms. Ovaries placed in large dishes of sea water shed copiously. Fresh eggs were removed, rinsed several times and put into large dishes of sea water. Concentrated sperm, collected from testes which had been stored "dry," was suspended and diluted in filtered sea water as needed. A few specimens of *A. vulgaris,* observed on May 14th, were also handled in the manner just described.

Alkaline sea water was prepared by adding sufficient 0.1 N NH_4OH to freshly ~~diluted~~ filtered sea water to bring it to pH 9.2–9.4, as determined by color comparison with thymol blue. In some cases a slightly higher pH was used. The sperm suspensions were then added to this alkaline sea water. The actual pH of these final mixtures was not determined but would obviously be lower than that of the original alkaline sea water.

All photographs and sketches shown were made from living material as viewed with oil immersion objectives. Both bright field and phase contrast microscopes were used.

Observations

I. The acrosome filament

A. *Thyone briareus*

1. *Formation of acrosome filament in treated sperm suspensions.* Spermatozoa were treated with either *egg water* or *alkaline (ammoniated) sea water.* Drops of sea water-suspended sperm were added to drops of either solution and, for controls, to filtered sea water in the same proportions. Some typical results as found in

living preparations are shown in Table I. In all these experiments appropriate treatment of the sperm resulted in the production of acrosome filaments in percentages significantly greater than the controls. The alkaline sea water was a much more effective stimulant than the egg water within the concentrations of the latter used. This is shown strikingly in experiments 6 and 7 in which in each case one original sperm suspension was used with the egg water and the alkaline sea water; in both experiments the egg water was ineffective (too weak?) whereas the alkaline sea water caused 90% or more of the spermatozoa to form acrosome filaments.

TABLE I

Acrosome reaction of spermatozoa of Thyone briareus. Production of acrosome filaments evoked by adding sea water sperm suspensions to egg water or to sea water made alkaline by addition of 0.1 N NH_4OH

Number of experiment	Number spermatozoa counted	% with filaments	Number spermatozoa counted	% with filaments
	Egg water		sea water control	
†EG, SN–1	100	21	100	2
EG, SN–2	52	21	>100	0
EG, SG–3	100	18	100	3
EN, SN–4	104	12.6	100	1
EG, SG–5	73	6.8	100	0
EG, SG–6(a)	111	1.8	††sweep	0
EG, SG–7(a)	100	0	sweep	<1
	Alkaline sea water		sea water control	
EG, SG–6(b)	sweep	>90*	sweep	0
EG, SG–7(b)	sweep	>99*	sweep	<1
EG, SG–7(c)	97	92**	sweep	<1
EG, SG–8	sweep	>90**	sweep	0

Experiments bearing the same number were made simultaneously from the same sperm suspension and have the same control.

† E, eggs; S, sperm; N, naturally shed; G, obtained by removal from gonad.

†† Sweep: extensive examination of area of coverslip by back and forth movement with a mechanical stage.

* pH of alkaline sea water to which sperm was added was slightly higher than 9.2–9.4.

** pH of alkaline sea water to which sperm was added was 9.2–9.4.

2. *Acrosome filaments in untreated sperm suspensions.* Examination of the control figures of Table I makes it clear that spermatozoa with acrosome filaments are sometimes found in a very low percentage in untreated sperm suspensions. Perhaps these result from "contact" stimulation, as Dan has suggested for other species, or from as yet unknown factors.

3. *Acrosome filaments in inseminated cultures of eggs.* The acrosome filament is always found on spermatozoa which are entering eggs. It is also seen on spermatozoa which lie in the jelly but fail to enter the egg; it may (Figs. 2, a and 8) or may not extend as far as the egg surface; its position in the jelly is usually radial, sometimes tangential and occasionally even directed away from the egg surface.

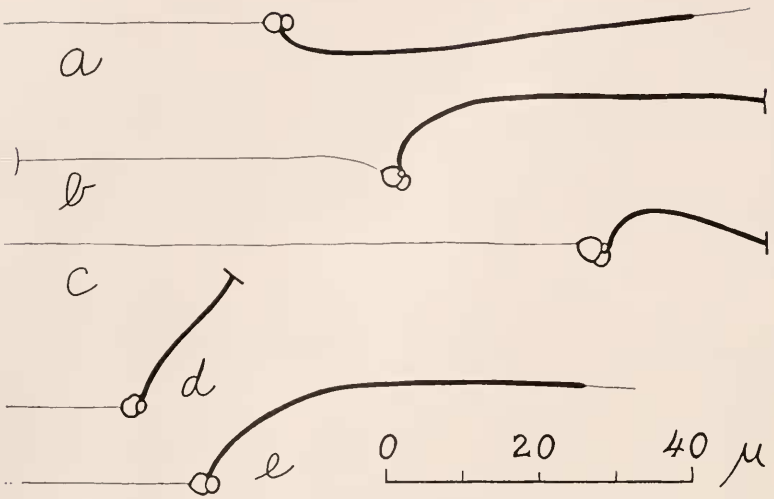


FIGURE 1. Reacted spermatozoa showing acrosome filament, from photographs of living specimens. a–c, *Thyone briareus;* d–e, *Asterias forbesii.* a, in egg water (35 μ); b, at egg surface (48 μ); c, in alkaline sea water (75 μ) (position of distal part of flagellum modified for reasons of space); d, in inseminated culture but directed away from egg (15 μ); e, at egg surface (22 μ).

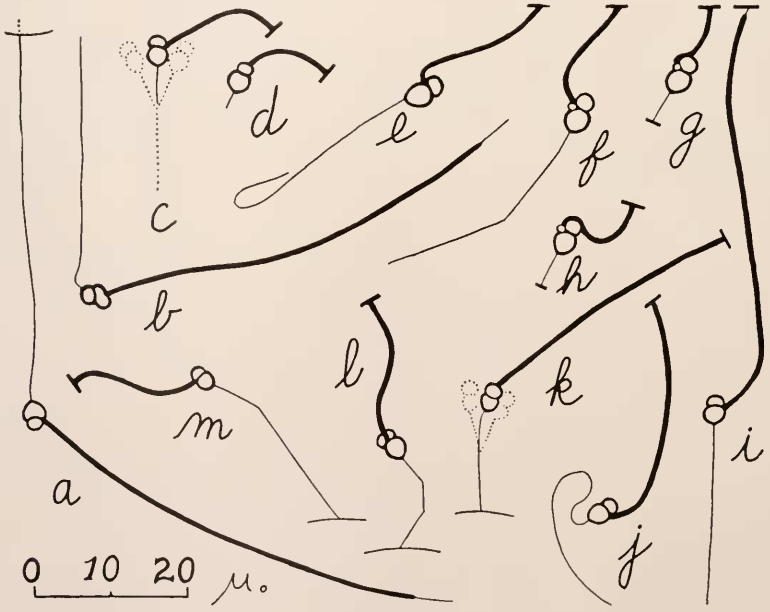


FIGURE 2. Reacted spermatozoa: bending and curving of acrosome filament. From sketches and photographs of living specimens. a–h, *Thyone briareus;* i–l, *Asterias forbesii;* m, *A. vulgaris.* a, at egg (presumed distal portion in dotted line); b, in egg water; c–h, in alkaline sea water. c, oscillating about fixed point (presumed distal part of acrosome filament dotted); d, same specimen as in c, after separating from fixed point; g–h, successive positions of flagellum shown in f; i–j, successive views of one specimen, in inseminated culture but not at egg; k–m, at surface of eggs (k, oscillating specimen).

Spermatozoa with acrosome filaments are also found near but entirely outside the jelly hull of the egg; in these no particular orientation with reference to the egg has been noted.

4. *Appearance of the unreacted spermatozoon.* Fresh sea water suspensions of sperm were spread thinly between slide and coverslip and individual spermatozoa examined as soon as they became sufficiently quiet. As seen in outline (Figs. 3, a–c and 5) the head is nearly circular. Closely applied to it is a narrow curved structure containing the middle piece and, apparently, a highly refringent circular body. The flagellum projects posteriorly as though from the middle piece but its actual point of origin is obscure. The flagellum is about 60 μ in length; its terminal

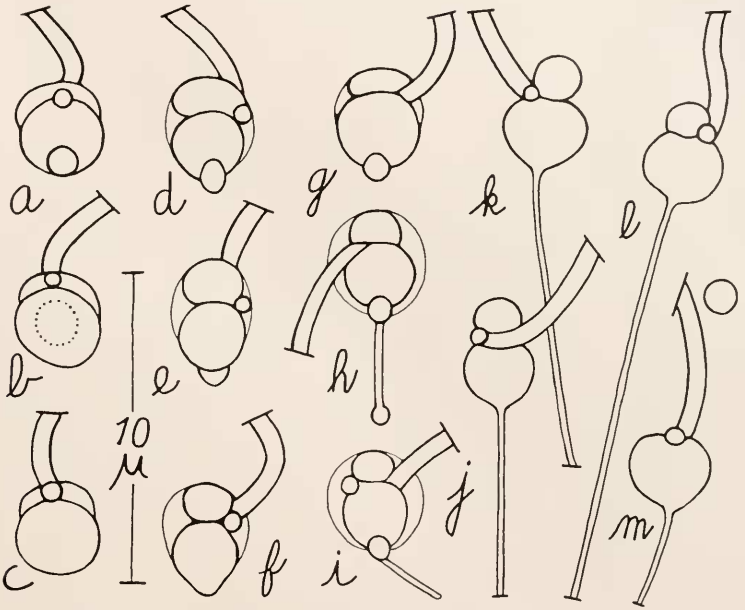
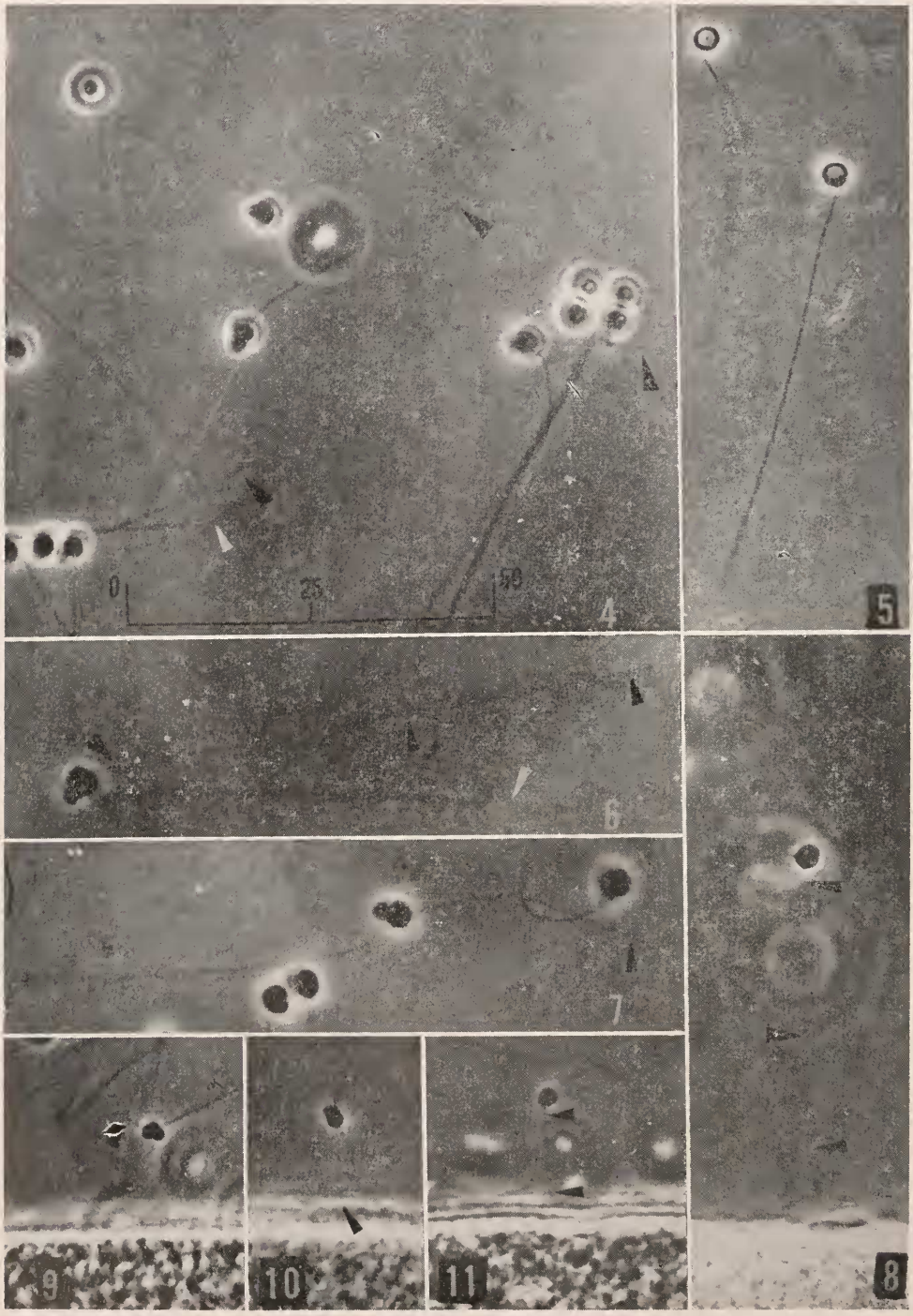


FIGURE 3. Spermatozoa of *Thyone briareus*, from sketches and photographs of living specimens. a–c, unreacted, in sea water; d–e, moribund, in aging sea water suspensions; f–g, partial reaction (?), in inseminated culture but not at egg; h–i, partial reaction (?), in alkaline sea water; j–l, reacted, as seen in all media described in text; m, reacted, entering egg, middle piece separated as seen occasionally.

portion or end piece is about 6 μ and much thinner than the main portion. The rounded acrosome, somewhat subterminal to the anterior part of the head, is often not seen, presumably because of the position of the specimen; it seems to intrude into the mass of the head (Fig. 3, a–c).

5. *Appearance of the reacted spermatozoon.* Reacted spermatozoa are morphologically indistinguishable from each other, whether they occur in egg water (Figs. 1, a and 4), in alkaline sea water (Figs. 1, c and 6–7), in association with the egg (Figs. 1, b and 8), or in untreated sperm suspensions; they are, however, markedly different from unreacted spermatozoa. The middle piece is round in outline, forming with the head the configuration of a figure eight (Figs. 1, a–c and 3, j–l). The flagellum emerges posterolaterally from between them, com-



FIGURES 4–11.

monly seeming to arise from the head (Figs. 1, a–c and 3, k–l). The refringent circular body lies near the point of contact between head and middle piece (Fig. 13). Anteriorly, from the acrosome region, projects the acrosome filament, frequently at an approximate right angle with the base of the emerging flagellum (Figs. 1, a–c and 5).

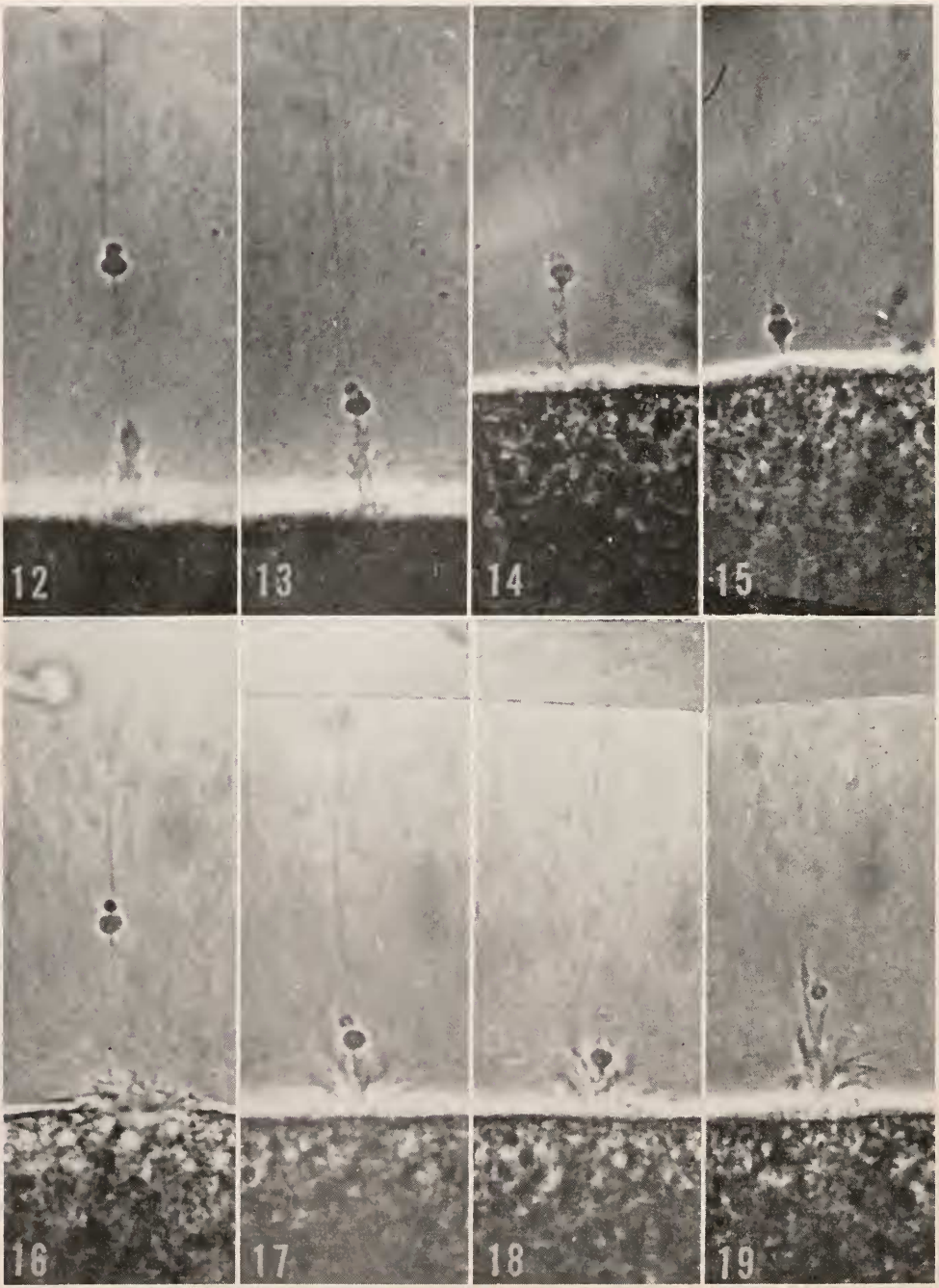
Successive stages of a specific reacting specimen have not been observed. Under certain conditions individuals are found like those shown in Figure 3, d–e (from aging sperm suspensions), Figure 3, f–g (in inseminated cultures) and Figure 3, h–i (in alkaline sea water). Here the enclosing membrane is loosened; the head, middle piece and refringent body are rounded and clearly discrete; and the flagellum emerges laterally, much as reported by Dan (1954a) for moribund spermatozoa of starfishes. The acrosome, however, is an additional balloon-like structure (*e.g.*, in Fig. 3, d) whose contents, under phase contrast, appear pale and diffuse as compared with the darker, seemingly more dense, head. Occasionally there is a short acrosome filament (Fig. 3, i) which is sometimes slightly thicker and ends in a rounded knob (Fig. 3, h). Though enclosing membranes are not prominent in fresh specimens, whether reacted or not (Figs. 3, a–c and j–l), it may be that these spermatozoa with loosened membranes hold clues to the intermediate stages of the acrosome reaction; except for the appearance of the membranes, Figures 3, g–i, for example, might be transitions from Figure 3, a to Figure 3, l.

6. *Dimensions of acrosome filament.* It has not been ascertained what length characterizes the average, fully reacted acrosome filament but most of those examined ranged between 35 μ and 65 μ. Several spermatozoa like the one shown in Figure 8 (which had attached but failed to enter the egg) had filaments measuring 46–48 μ. The acrosome filament of the spermatozoon shown in Figure 6 (and schematically in Fig. 1, c) measured 75 μ and exceeded the length of the flagellum. In one exceptional case the filament measured 90 μ. The acrosome filament is extremely tenuous, its diameter being of the general order of magnitude of that of the end piece of the flagellum.

B. *Asterias forbesii and Asterias vulgaris*

In both species reacted spermatozoa were found in fresh living preparations of inseminated eggs. Except that their acrosome filaments were very much shorter, the reacted spermatozoa of both species looked very much like those of *Thyone*. The heads were somewhat smaller but the flagella measured about 55–60 μ in length, including the short filamentous end piece. In *A. forbesii* the maximum length of the acrosome filament was approximately 25 μ; acrosome filaments attached to but failing to enter eggs (Figs. 1, e, 10 and 11) measured from 15–22 μ. The same order of magnitude prevailed in *A. vulgaris* (Fig. 2, m). In both species some reacted spermatozoa were found with their acrosome filaments directed *away* from the eggs (Figs. 1, d and 9).

FIGURES 4–11. Unretouched photographs of living spermatozoa; all to scale shown in Figure 4, representing 50 μ. Black arrows point to acrosome filament, white arrows to end piece of flagellum. Figures 4–8, *Thyone briareus;* Figures 9–11, *Asterias forbesii.* All figures show reacted spermatozoa with acrosome filament except Figure 5, which shows unreacted specimens. Figure 4, in egg water; Figures 6–7, in alkaline sea water; Figures 8, 10–11, at surfaces of eggs but did not subsequently enter; Figure 9, near egg but directed away from it.



FIGURES 12–19. Unretouched photographs of living spermatozoa penetrating egg of *Thyone briareus*. To same scale as in Figure 4. (All Figures except 15 and 16 have had non-relevant pieces spliced in to upper portions.) Figures 12–13, successive views of slender cone with broad

C. *General nature of the acrosome filament*

Some properties of the acrosome filament may now be described. As they are apparently much the same in both *Thyone* and *Asterias,* and whether they occur in egg water, alkaline sea water or in inseminated cultures, reference to species and medium will be omitted here but may be found with the appropriate illustration. Despite its thread-like dimensions the filament is usually straight (Figs. 2, i, 4, 6 and 11) or curved only in very wide arcs (Figs. 2, a and 8). Figure 2, k shows a specimen which was attached to (but did not enter) an egg; the filament curved slightly as the head oscillated with the motion of the flagellum. When not at the

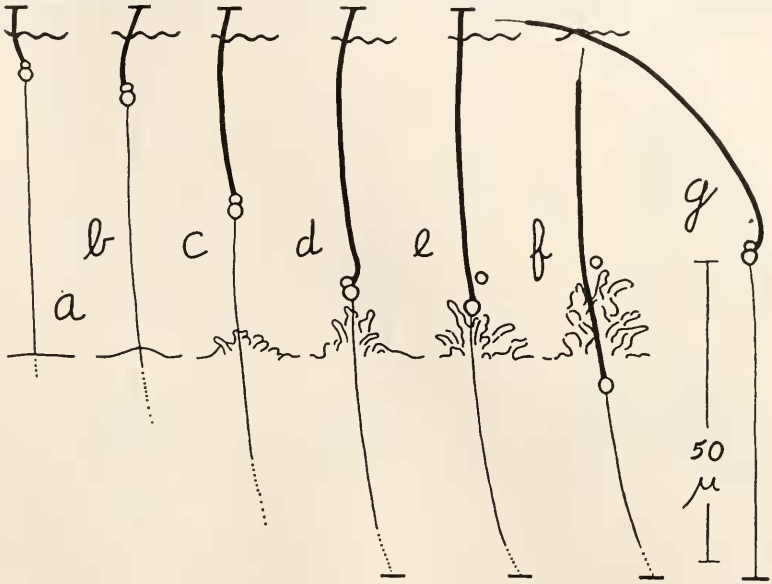


FIGURE 20. a–f, scheme of sperm entry process in *Thyone briareus;* part shown in dotted line represents minimum additional length of acrosome filament presumed but not actually seen to enter egg. g, reacted spermatozoon from alkaline sea water suspension for comparison of length of acrosome filament. a, presumed very early stage based on spermatozoon which failed subsequently to enter; b, low broad incipient cone rises as acrosome filament proceeds into egg proper; c–f, successive stages of sperm entry, acrosome filament enters egg proper as advance integral part of spermatozoon (middle piece, left outside in this case, frequently enters egg). In subsequent stages, entire flagellum passes into egg proper.

egg the distal portion and/or tip of the filament often sticks to the slide or coverslip; for example, the specimen shown in Figure 2, i–j, moved suddenly, causing the non-adherent proximal part of the filament to curve sharply. Other partly adhering, sharply curved filaments are shown in Figures 2, b and 2, e. The acrosome filament *may* even break. This could explain the spermatozoon shown in Figure 2,

base partly retreated; note acrosome filament in cone. Figures 14–15, successive views of one specimen, very slender cone; sperm head did not subsequently enter egg proper. Figures 16–19, successive views of specimen in which low broad cone gave rise to filose projections which continued growing outward even after sperm head had entered egg proper; no tall slender cone formed in this specimen; middle piece failed to enter.

c–d; when first seen it was oscillating like a pendulum with a short stem. Suddenly it began to whirl freely in many directions, and its filament seemed even shorter; the presumed adhering distal part of the filament was not observed. A number of spermatozoa have been seen with the filament bent permanently (Figs. 2, f, l, m, 7 and 10).

II. Sperm entry

A. *Thyone briareus*

Many individual eggs were examined, both from natural sheddings and stripped from the gonads. As lightly inseminated cultures of the naturally shed eggs gave 98–99% cleavage and more than 90% active larvae, it was evident that these were

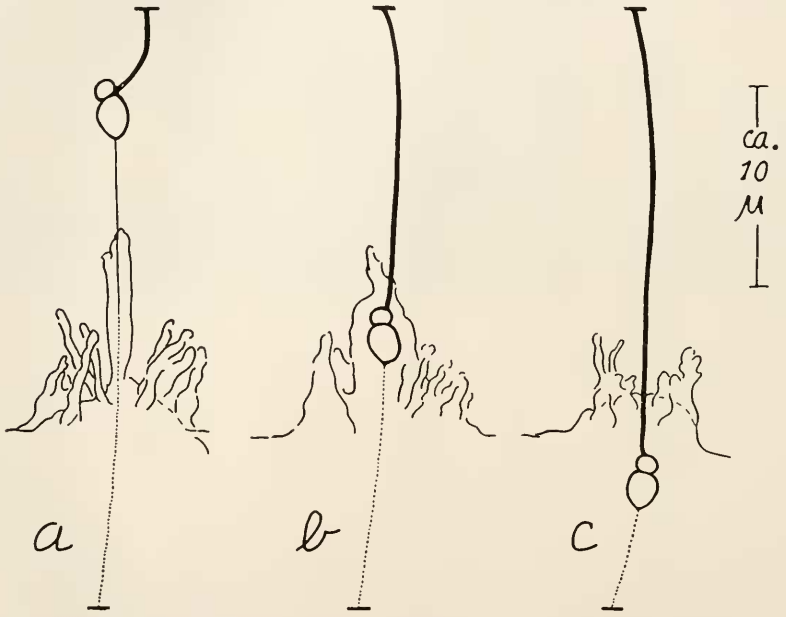


FIGURE 21. Successive stages of sperm entry in *Thyone briareus*, from sketches of a living specimen. A broad cone with filose projections elevated one moderately slender sleeve about the inmoving acrosome filament. Acrosome filament seen in outer part of cone: solid line. Presumed further course of filament: dotted line.

normal eggs. On the other hand, cleavage was never observed in inseminated cultures of the stripped eggs. Yet, whichever the source, a number of spermatozoa entered every egg which was observed individually in slide and coverslip preparations. Generally, the entry was more rapid and the height of the cone less great in the naturally shed eggs.

A scheme of the entry process is shown in Figure 20. The spermatozoon does not swim through the thick jelly hull; its first contact with the egg surface is made by means of its acrosome filament. Presumably a very early stage would appear as in Figure 20, a. At the earliest stage noted frequently in successful entry, the main body of the spermatozoon had already progressed part way through the jelly and its acrosome filament was already associated with the broad low hyaline in-

cipient cone (Fig. 20, b). In some instances, as the spermatozoon continued moving inward, filose projections arose from the cone and continued growing outward even after the sperm head had passed through the cone into the egg proper (Figs. 16–19).

Some common variations in the cone are shown as they appeared in specific living eggs. The cone shown in Figures 16–19 never rose extensively above the egg surface. Sometimes a moderately slender projection would move outward like a sleeve ascending the inmoving acrosome filament, and then retract after the sperm head had entered the cone, as in Figure 21, a–c. In this specimen the cone remained broad at the base throughout the entry process but often an initially

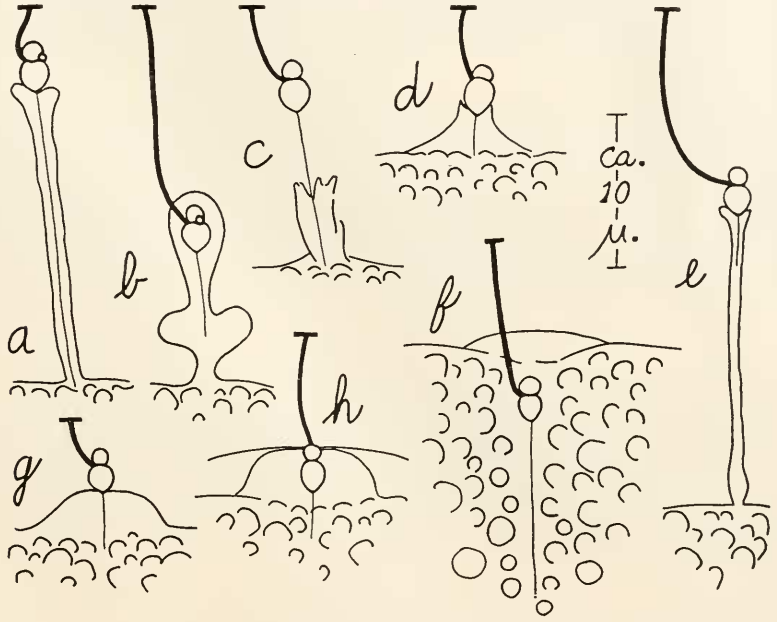


FIGURE 22. Variations in sperm entry, from sketches of living specimens. a–f, *Thyone briareus;* g–h, *Asterias forbesii.* Acrosome filaments shown only to depth actually seen. a–b and c–d, successive views of two specimens, respectively; e, very slender cone embracing acrosome filament; f, acrosome filament within egg proper; g–h, from two specimens of *A. forbesii,* successive stages which preceded stage closely resembling that shown in f.

broad base would retreat partly or wholly so that the only obvious external cone would be a single slender projection (Figs. 12–13, 14 and 22, a, c and e). Transitory changing dilations appeared along such cones (Figs. 12–13 and 14). The cones were sometimes so slender that they might easily have been called "filaments" by an observer not cognizant of the existence of the much more delicate acrosome filaments (Figs. 14, 22, a and e). There was also variation in the distance from the egg proper at which the sperm head entered the cone (*cf.* Figs. 22, b and d). In the specimen shown in Figure 22, c–d a column of hyaline protoplasm continued to grow outward for some time after the sperm head had passed into the egg proper.

Although the middle piece ordinarily entered the egg with the sperm head (Figs. 21, 22, b and f) it sometimes became separated (Fig. 3, m) and remained outside

altogether (Fig. 19). Occasionally an apparently detached middle piece eventually passed into the egg. Several times a detached middle piece was seen to vibrate vigorously with the movement of the flagellum while the head continued moving on into the egg without pronounced vibration.

The entire flagellum passes into the egg. However, it was sometimes not seen or seen only with difficulty within the cone, even in cases in which it was observed continuously until finally the end piece disappeared into the cone.

The much more tenuous acrosome filament was also seen only with difficulty after it had been enveloped by the cone (Figs. 12–14 and 22, c–d). Sometimes only a portion of the filament could be seen (Fig. 22, e). In other cases a part of the filament would be seen and then disappear with the changing condition of the cone (Figs. 22, a–b). Within the egg proper, observation was further hindered by the presence of yolk granules (Fig. 15) and it was only in some specimens that the acrosome filament was seen there with certainty (Fig. 22, f). Occasionally some disturbance of the yolk granules in advance of the entering sperm head suggested the presence of an acrosome filament not actually seen. In the scheme of sperm entry shown in Figure 20 the acrosome filament has been represented by solid lines to the depth within the egg that it has sometimes been seen, and in dotted lines to the depth to which it might be presumed to penetrate, judging by the known lengths of acrosome filaments as seen in alkaline sea water (Fig. 20, g) or attached to but not entering the egg (Fig. 20, a).

B. *Asterias*

A small number of polyspermic eggs of *A. forbesii* were examined. In the earliest stages observed the spermatozoa were already quite close to the egg surface. In some cases the acrosome filament was seen within the cone (Fig. 22, g); in a few it extended into the protoplasm of the egg proper (Fig. 22, h). Several times an appreciable length of the filament was seen within the egg, very much as shown in Figure 22, f representing *Thyone*.

Discussion

A. Acrosome filaments

The acrosome reaction resulting in the production of a filamentous structure, the acrosome filament, first demonstrated by Dan, has been shown here to occur also in the holothurian, *Thyone briareus*. Whereas the longest previously reported acrosome filament, that of starfishes (Dan, 1954a), measured 22–28 μ, the acrosome filament of *Thyone* may reach two to three times this length and can even exceed the length of the flagellum.

Recently Rothschild and Tyler (1955) have questioned that the acrosome filament is formed by means of an acrosome reaction, in the sense of Dan. Their reservations are based on observations of untreated sperm suspensions of two species of mollusc and two species of sea urchin, viewed by both phase contrast and electron microscopy. They state that in such preparations generally two types of spermatozoa are found, one type with a short "acrosomal filament" and one with a long "acrosomal filament." Two such types of sperm are shown, for example, for the sea urchin *Echinocardium cordatum* in their Figures 8 and 9. Their Figure 8

shows a spermatozoon with a fairly long and knobbed acrosome, and it appears as previously described for this species by Vasseur (1947) and by Afzelius (1955); it was the form invariably found in a living sperm suspension. Their Figure 9, however, shows a spermatozoon with a profoundly different appearance; a filament much longer than that of the other type extends from the apex of the head, the middle piece is excentric, and the flagellum and filament lie nearly at right angles to each other. In all these features the spermatozoon resembles the reacted spermatozoon of the sea urchin as described by Dan (1952). Furthermore, Tyler (1952) has previously reported that fertilizin-treated sea urchin spermatozoa show just such a displacement of the middle piece from the normal position to a posterolateral one. There seems little doubt, then, that the spermatozoon shown by Rothschild and Tyler in their Figure 9 represents a reacted spermatozoon and the long filament extending from the apex of the head is an acrosome filament, in the sense of Dan.

Perhaps the reservations of Rothschild and Tyler might have been obviated by the presence of quantitative data in the reports by Dan; and similarly it would be very interesting if Rothschild and Tyler had presented quantitative data of *treated* sperm suspensions to compare with their results of the untreated suspensions. Any reservation regarding the existence of the phenomenon of the acrosome reaction should be dissipated by the quantitative data presented here for *Thyone,* in which it is clearly demonstrated that the treated preparations show significantly greater percentages of reacted spermatozoa with acrosome filaments than untreated preparations, *as observed exclusively in living material.* The tremendously long acrosome filaments of *Thyone* make these observations relatively easy.

The occurrence of the two types of spermatozoa observed by Rothschild and Tyler in their untreated preparations may have been caused by "contact" or other as yet unknown factors which supervene during preparation of the sperm suspensions. It is important to note that in *Echinocardium cordatum* Rothschild and Tyler found the long "acrosomal filaments" only in *fixed* material; the possibility exists that in this case the fixative, or some feature of the process en route to fixation, may have been the stimulus for the acrosome reaction. In this connection an observation of Lillie (1912) on *Nereis* now becomes extremely interesting; he noted that in the *living* spermatozoon the perforatorium (acrosome) was shaped like the spike of a helmet and was *shorter* than in the *fixed* spermatozoon, whether the latter was free or attached to an egg; in this fixed material the acrosome had the shape of a filament. What Lillie observed in the fixed material would seem to be an acrosome filament resulting from an acrosome reaction (possibly caused by fixation). Recently Metz and Morrill (1955) have also studied fixed spermatozoa of *Nereis;* the acrosome filament was present in low percentage in normal sea water controls, and in significantly higher percentage in spermatozoa which had been treated with fertilizin. A more detailed discussion of this and related matters is presented elsewhere (Colwin and Colwin, 1956).

B. Sperm entry

In *Holothuria atra* (Colwin and Colwin, 1955a) the acrosome filament behaves as an integral part of the sperm head, enters the egg intact and remains not appreciably altered until at least well within the egg proper. A relatively broad cone rises to surround the entering filament like a sleeve. Frequently a short narrow

projection creeps up the acrosome filament in advance of the main body of the cone. In *Thyone,* although the cones are narrower, and the acrosome filaments a great deal longer, the major aspects of sperm entry are much the same, namely, the acrosome filament enters the egg as an integral part of the spermatozoon and remains essentially intact at least during its initial entry into the egg proper; the substance of the cone moves outward enclosing *the acrosome filament which has already established the first true contact between spermatozoon and egg proper.* But whereas in *H. atra* all the observations were made on eggs which had been stripped from the gonads artificially, in *Thyone* not only stripped eggs but also normally shed eggs, controls of which developed normally, showed essentially the same picture. This strengthens the suggestion, made previously, that sperm entry as described for *H. atra* reflects the essentials of the entry phenomenon in the normal egg of that species, particularly with respect to the behavior of the acrosome filament.

The tall slender type of cone usually seen in *Thyone* strongly suggests the reception cone described by Hörstadius in *Holothuria poli* (1939a) and the starfish *Astropecten aranciacus* (1939b). In *Thyone,* however, since the cones can be seen to embrace the already established inmoving acrosome filament, there is no basis for their interpretation as the agents of first contact between egg and spermatozoon, as has sometimes been held. A more extensive discussion of the mechanism of sperm entry and a reinterpretation of the events of entry as described in echinoderms by earlier workers will be found elsewhere (Colwin and Colwin, 1955a and 1956).

From the measurements in the present observations it is evident that the acrosome reaction in *Thyone* is capable of producing acrosome filaments much longer than the depth of the jelly hull of the egg. The question arises: how long must the acrosome filament be in order to initiate successful sperm entry? As the spermatozoon does not swim through the jelly in *Thyone,* it seems that the minimum length necessary would be that of the thickness of the jelly.

The authors have never witnessed a subsequently entering spermatozoon at the exact moment of its first contact with the egg in *Thyone,* or indeed in *H. atra* and the three species of *Asterias* which have been studied. In all of these it has been considered likely that the earliest stage would resemble that seen when spermatozoa attach to the egg but fail subsequently to enter, as in Figures 8, 11 and 20, a. However, since it is known that the acrosome filament of *Thyone* can be longer than in such attached spermatozoa, the possibility certainly exists that these attached specimens represent a stage later than the earliest one. Inasmuch as the filament of successfully entering spermatozoa moves into the egg, perhaps in these attached non-entering spermatozoa some distal portion of the filament may already extend into the egg.

Summary

1. As shown by quantitative data, spermatozoa of *Thyone briareus* treated with egg water or alkaline (ammoniated) sea water undergo an acrosome reaction resulting in the production of acrosome filaments in a percentage significantly greater than found in untreated controls. Acrosome filaments are also found in inseminated cultures of eggs, either associated or unassociated with the eggs.

2. The acrosome filaments of *Thyone briareus* are exceptionally long and may even exceed the thickness of the jelly hull (55 μ) or even the length of the flagellum (60 μ).

3. Reacted spermatozoa of *Asterias forbesii* and *A. vulgaris* seen in inseminated cultures of eggs have acrosome filaments measuring about 15–22 μ.

4. The general nature of the acrosome filament is much the same in both species of *Asterias* and in *Thyone,* regardless of the stimulating agent. Though thread-like in dimensions it is usually straight, or curved only in a wide arc. However, it is capable of curving sharply, bending and perhaps even breaking. The tip or distal portion often sticks to the glass of the slide.

5. At sperm entry in *Thyone* and *Asterias* the acrosome filament makes the initial contact with the egg and then enters the egg as the first element of the spermatozoon of which it is an integral part. In *Thyone* the cone is sometimes so slender as to appear filamentous itself, even though it contains the acrosome filament.

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