STUDIES ON THE ACROSOME. II. ACROSOME REACTION IN STARFISH SPERMATOZOA[1]

JEAN C. DAN

Misaki Marine Biological Station, Misaki, Kanagawa-ken, Japan

With the technical assistance of A. KITAHARA and T. KOHRI

After it had been found that sea urchin spermatozoa react to species egg-water by undergoing a change in the region of the acrosome simultaneously with the agglutination reaction, the possibility became evident that some such reaction might also be occurring in starfish spermatozoa. Fertilization in this form holds a peculiar interest, both because it was in the eggs of the starfish, *Asterias,* that Fol for the first time, in 1877, observed the entrance of a spermatozoan into an egg cell, and because his description of the process, which has never been superseded, contains certain points which are rather difficult to reconcile with usual concepts of the relationship between egg and spermatozoan.

Current ideas concerning the exact details of the process by which fertilization takes place in starfish eggs are in a somewhat surprising state of confusion, considering the number of embryologists who have observed and reported the phenomenon, and the extent to which this material has been used in various studies during the past seventy-five years.

Fol (1877) and Chambers (1923) observed spermatozoa being drawn toward a cone on the egg surface by a long, slender filament, which they believed to be an extension of the cone. Just (1929) repudiated this interpretation, and maintained that the much shorter filament which he observed originates in the sperm head. Hörstadius (1939) reported that a tubular "Empfängnishügel" grows out from the egg surface and takes possession of a spermatozoan at the outer edge of the jelly layer.

Similarly, although workers from the time of Lillie and Loeb have attempted to observe agglutination of starfish sperm in homologous egg-water, their results do not agree. Glaser (1914) and Woodward (1918) reported agglutination in *Asterias forbesii,* and Nomura (1926), in *Asterina pectinifera,* but Just (1930) was unable to confirm the *Asterias* results, and Loeb (1914) and recently Tyler (1941) have also reported lack of success with *Asterias ochraceus* and *Patiria miniata,* respectively. However, in 1944, Metz discovered that the addition of lobster serum caused an unequivocal agglutination reaction in the presence of homologous egg-water. Metz (1945) has further studied the phenomenon in four species of starfish, and found other substances which also act as adjuvants, particularly isotonic white of hen's egg.

The work reported in this paper was planned to test the effect of egg-water (plus an adjuvant) on the acrosome of the starfish spermatozoan.

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Material and Methods

The starfish species used for the majority of the observations were *Asterina pectinifera* and *Asterias amurensis;* the main points were corroborated in *Astropecten scoparius.* Since the oocytes of *Asterina* and *Astropecten* in general do not begin the maturation divisions on suspension in sea water,[2] the egg-water in experiments with these forms was obtained from the immature eggs. In the case of *Asterias,* however, the egg-water was obtained from maturing eggs. No difference in effectiveness was found between the two solutions.

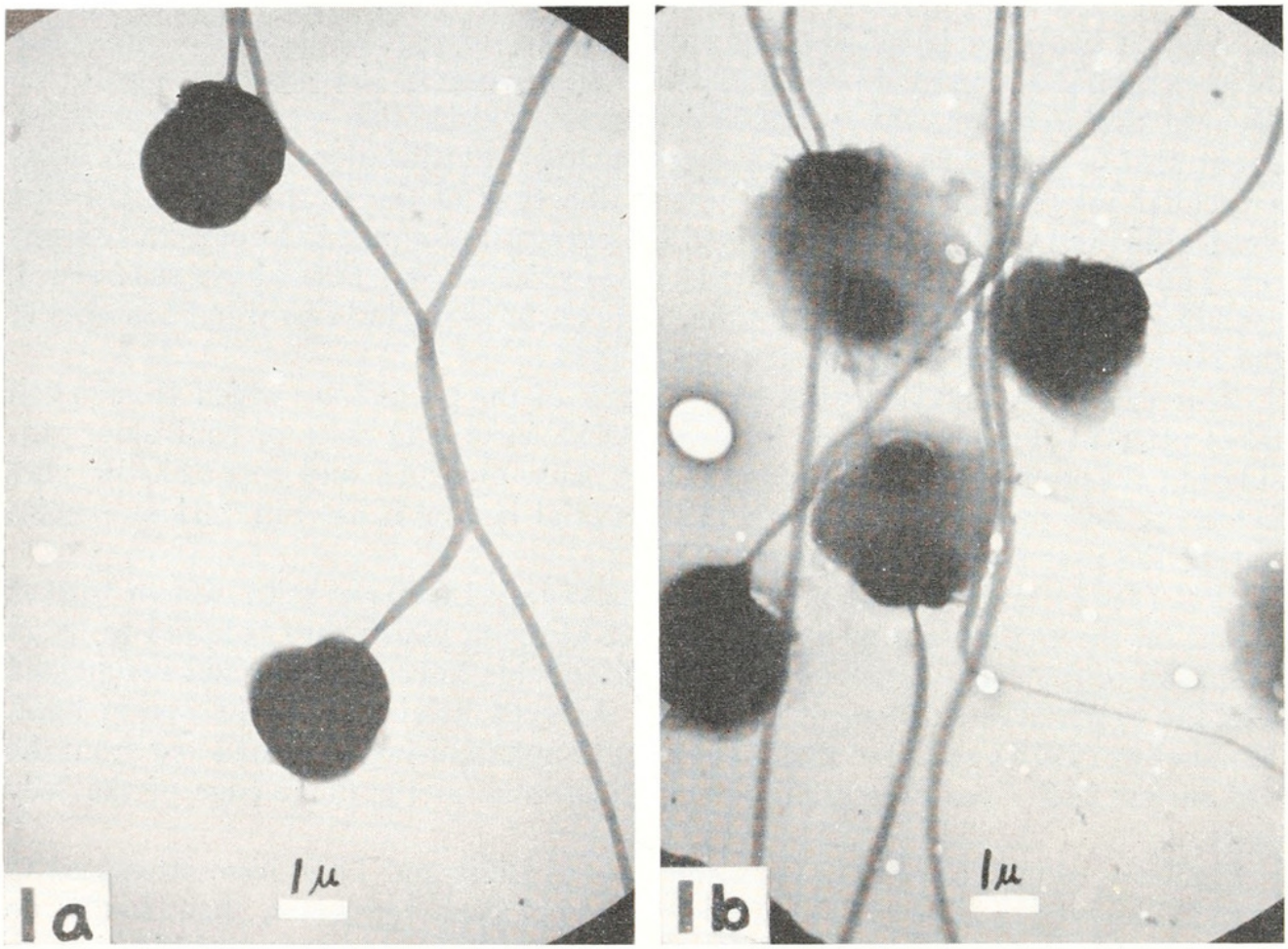


Figure 1. Electron micrographs of *Asterias* spermatozoa fixed in pure sea water, (a) with neutralized formalin; (b) with OsO_4 vapor.

In practice it was found that the jelly of *Asterina* oocytes swells considerably in Ca-low artificial sea water; after having stood for about 30 minutes, such suspensions were centrifuged sufficiently to remove at least part of the jelly, and 4% of 0.36 *M* $CaCl_2$ was added to bring the calcium content approximately to that of normal sea water. In the case of *Asterias,* sufficiently potent egg water was obtained by simply removing and filtering the supernatant fluid from a concentrated suspension of eggs which had stood for at least an hour.

[2] However, there are always a few (less than 10%) of the oocytes in which the germinal vesicles do break down. These fertilize and develop normally.

Spermatozoa were obtained by extirpating the testes, which were kept "dry" in a covered glass container, and the sperm exuding from them was freshly suspended, immediately before use, in the adjuvant solution. Throughout the experiments, crystalline egg albumin, dissolved in sea water and filtered, was used as the adjuvant, in concentrations ranging between 0.1 and 1.0%.

Most of the observations were made on living sperm suspensions, with the oil immersion objective of a phase contrast microscope. For photographing and electron microscopic preparations, the suspensions were fixed with osmium vapor. Because of the presence of the dissolved albumin, however, it proved very difficult to obtain satisfactory preparations for direct electron microscopic observation. This difficulty was partially resolved by making replicas. The electron microscope was a Hitachi Standard, operating on 50 KV.

Results

The head and middle piece of the starfish spermatozoan make up an approximately spherical structure, slightly flattened anteriorly (Fig. 1a). In the living spermatozoan a bluntly cone-shaped acrosome can be seen imbedded in the nuclear

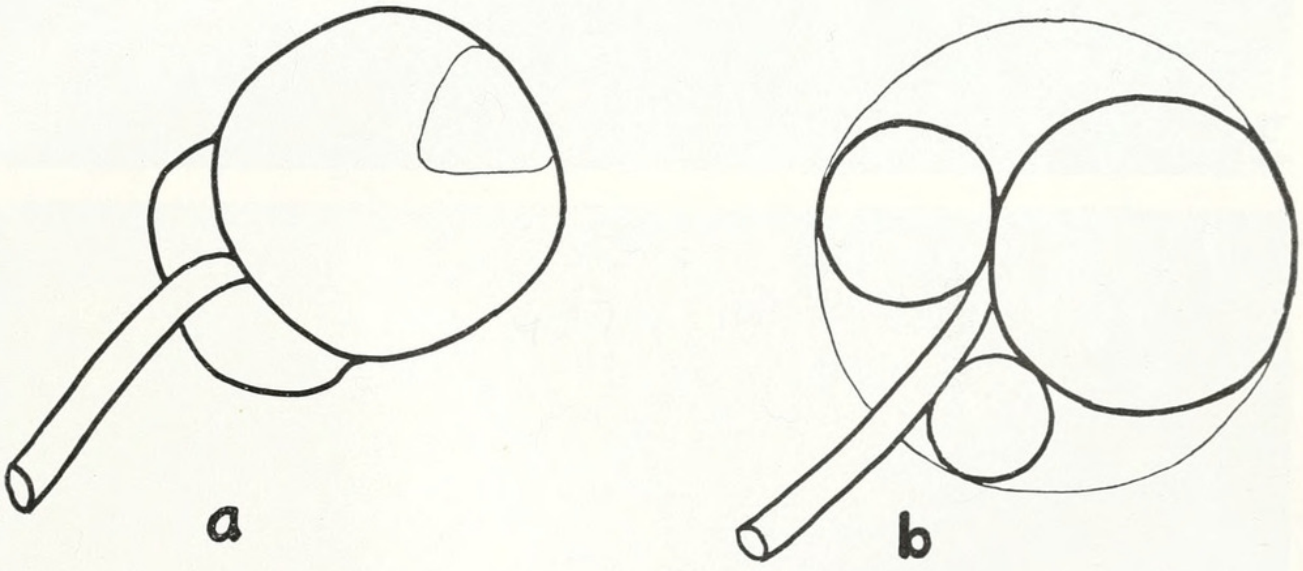
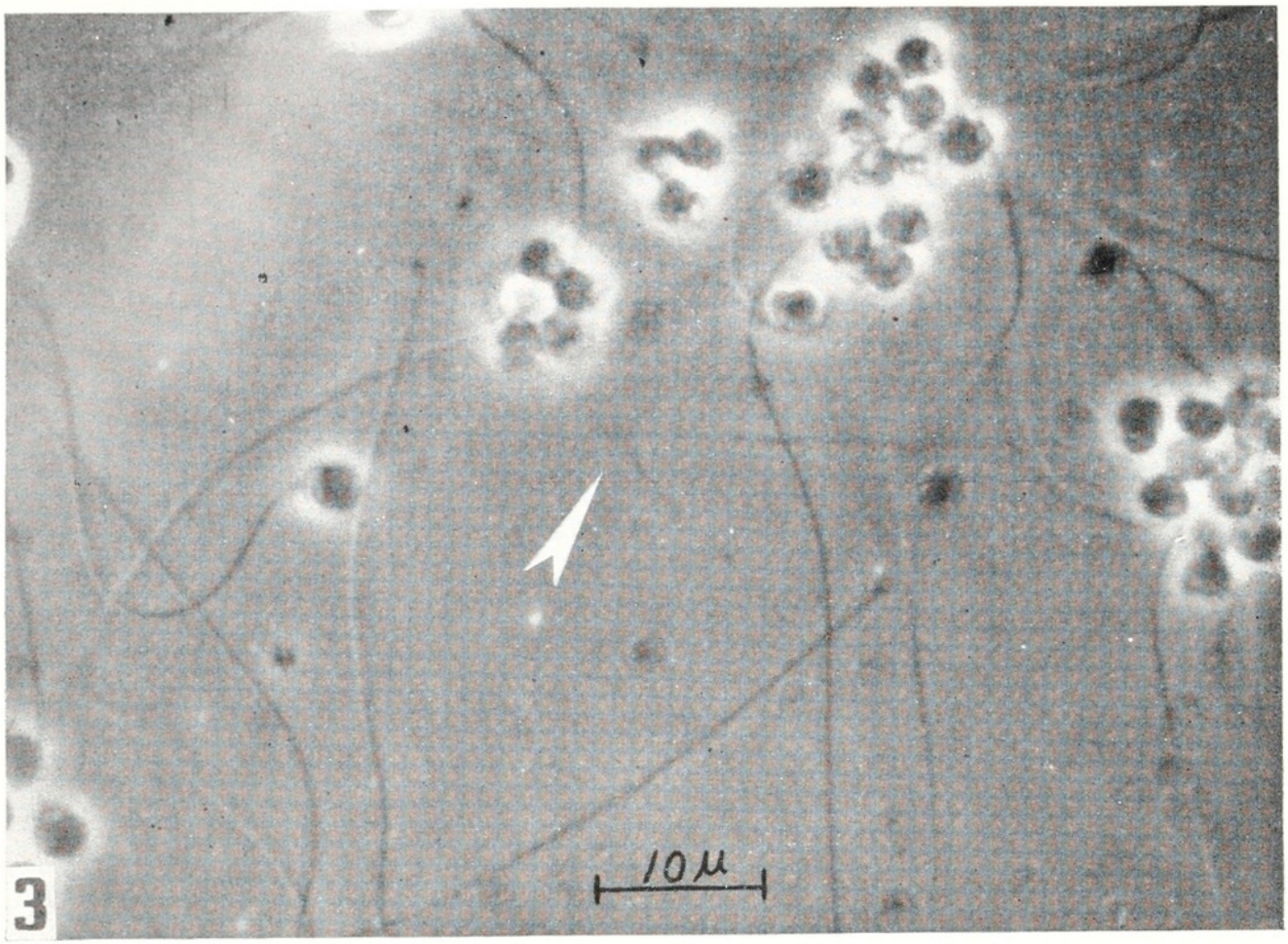


FIGURE 2. (a) Diagram of starfish spermatozoan in sea water. Middle piece is closely applied to posterior part of head; acrosome appears as a rounded cone imbedded in nuclear portion of head. Tail curves around middle piece and extends more or less directly backward. (b) Diagram of moribund starfish spermatozoan, showing nucleus, acrosome and middle piece rounded up separately within inflated membrane.

material so that the apex of the cone points backward, toward the middle piece (Fig. 2a). With phase contrast the acrosome material appears strongly refringent, in contrast to the material of the nucleus. The middle piece is relatively large, and is closely applied to the base of the head. While the combined length of the starfish sperm head and middle piece is only about half that of a moderately-sized sea urchin spermatozoan (*i.e., Hemicentrotus*), the tail is fully as long as any of the sea urchin sperm tails (50 μ). The head and middle piece are enclosed in a single membrane which is not apparent in normal specimens, but becomes visible



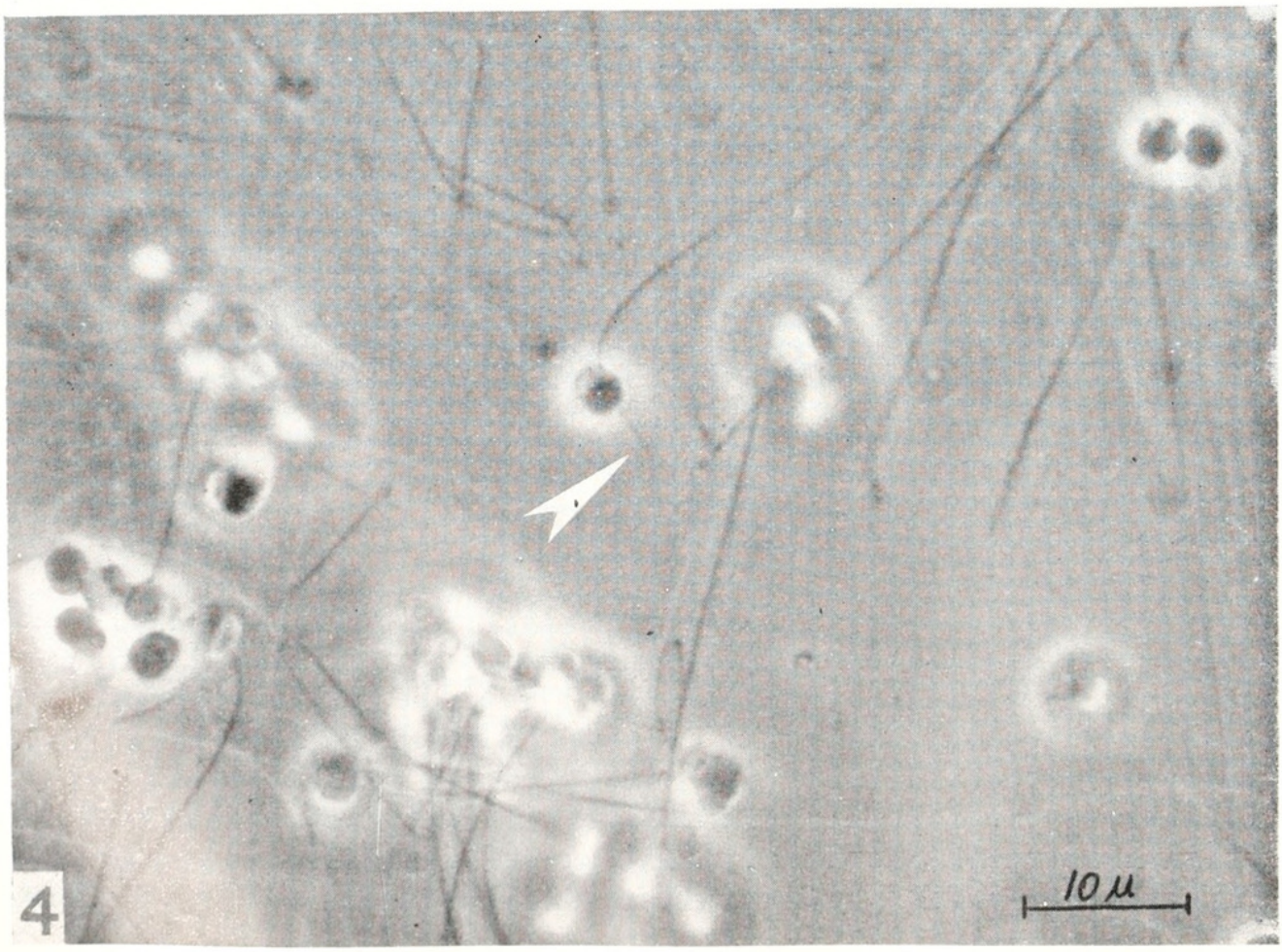


FIG. 3–4.

in moribund sperm or following various types of treatment. In an aged suspension, moribund individuals are frequently seen in which the membrane has separated and become somewhat inflated, and within this spherical membrane the nucleus, middle piece and a refringent body, presumably the acrosome, are all to be seen as discrete spheres (Fig. 2b). The nucleus, middle piece and tail apparently retain their original connection with each other at one point, but the acrosome has moved out of place entirely and seems to be free within the membrane.

As Metz has reported, although starfish sperm are usually not appreciably activated either by suspension in sea water or by addition of homologous egg water, they become intensely active when they are suspended in egg albumin-sea water.

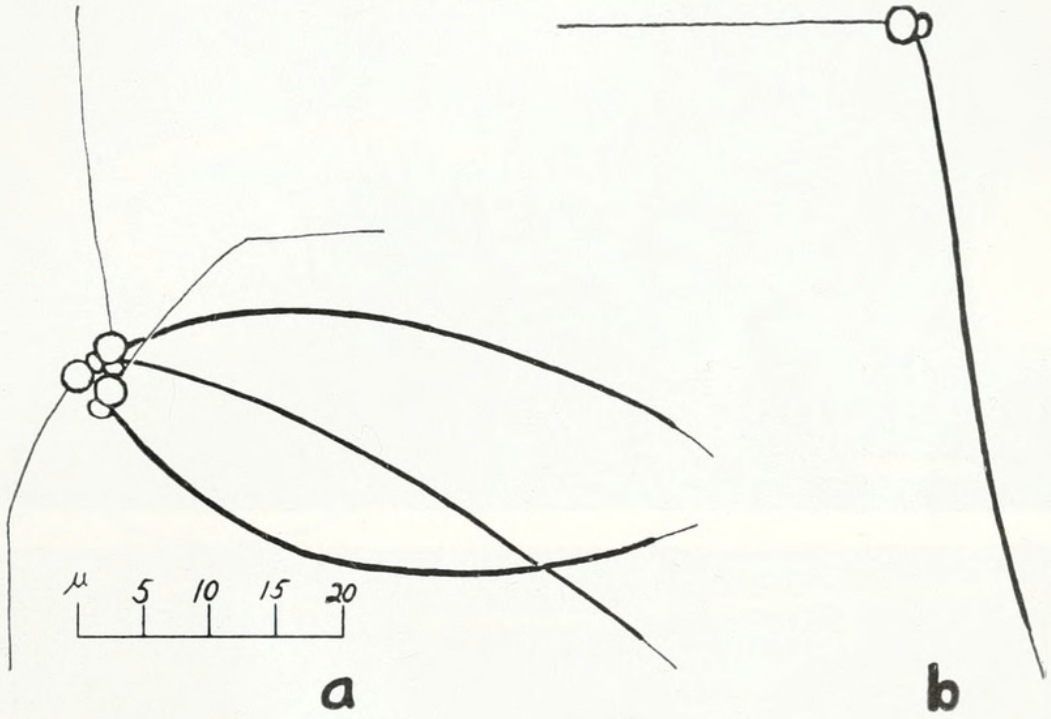


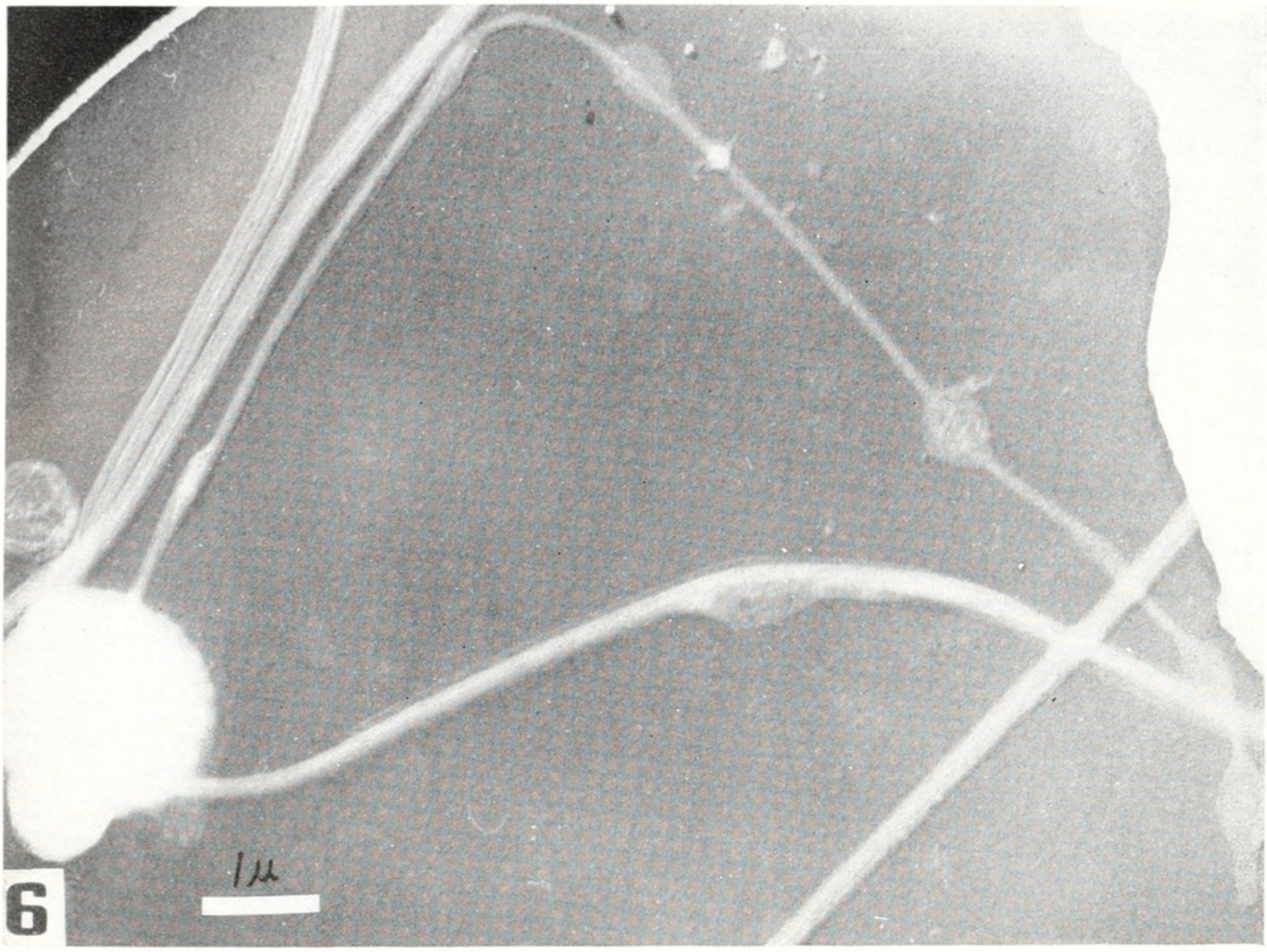
FIGURE 5. Camera lucida drawings of *Asterina* spermatozoa suspended in 0.5% albumin-sea water, after addition of homologous egg-water. (a) Small cluster of agglutinated sperm, showing the acrosome filament on each. A strong current of water was passed across this field from the left; two of the filaments were bent and all the tails swept toward the right side. (b) Single reacted spermatozoan held against cover glass by acrosome filament.

Such activated sperm, however, do not show any change in structure when they are examined either in the living state with phase contrast, or with the electron microscope.

If egg-water is then added to the suspension of spermatozoa in albumin-sea water, and a sample is observed under high magnification, many clumps including from a few to several hundred spermatozoa can be seen, the heads in contact with each

FIGURE 3. Phase contrast micrograph of *Asterina* spermatozoa suspended in 1% egg albumin-sea water, fixed with OsO_4 vapor after addition of homologous egg-water. Most of the spermatozoa are included in small head-to-head clusters; arrow indicates several acrosome filaments stuck to underside of coverglass.

FIGURE 4. Single *Asterina* spermatozoan which has reacted to egg-water focussed to show origin of filament in acrosome region of head.



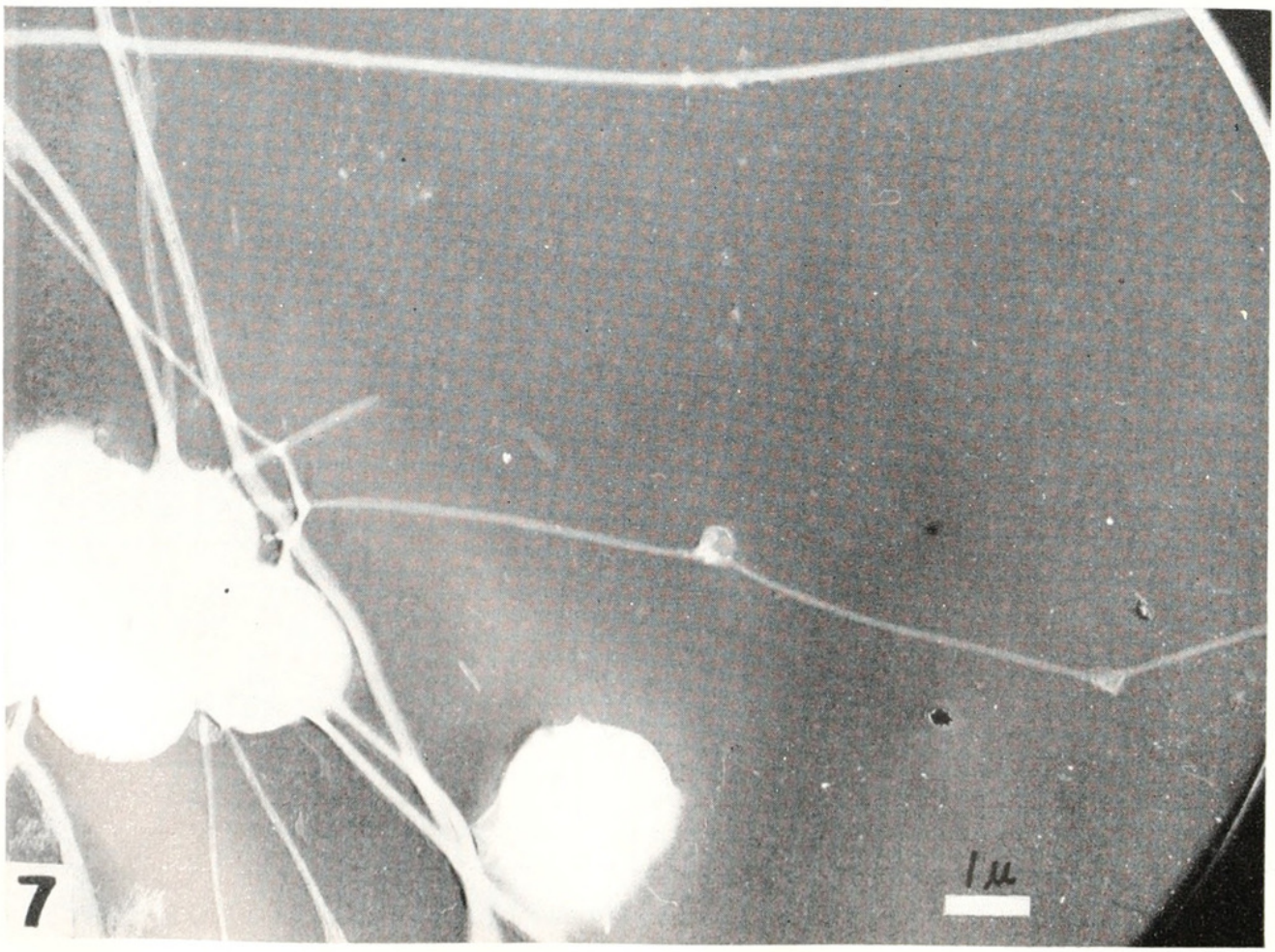


FIG. 6–7.

other, and the tails in vigorous movement (Fig. 3). This agglutination is irreversible.

Many other spermatozoa are in rapid solitary motion, and some can be found with their heads stuck against the glass surfaces, while their tails move freely. Close examination shows that these spermatozoa are held affixed to the glass by a long (ca. 25 μ), very slender filament which extends perpendicularly from the center of the acrosome surface (Figs. 3, 4 and 5a and b, 6). These filaments cannot be seen on swimming spermatozoa, but fixation of the suspension shows that this reaction of the acrosome has taken place in a large proportion of the cells. Especially in the case of the small agglutinated clusters, the filament can be seen on each spermatozoan (Figs. 5a, 7).

Measurements of camera lucida drawings of several of these filaments ranged between 22 and 28 μ. In the living state the filaments show considerable rigidity, bending only partially in the direction of a strong current of water passed over

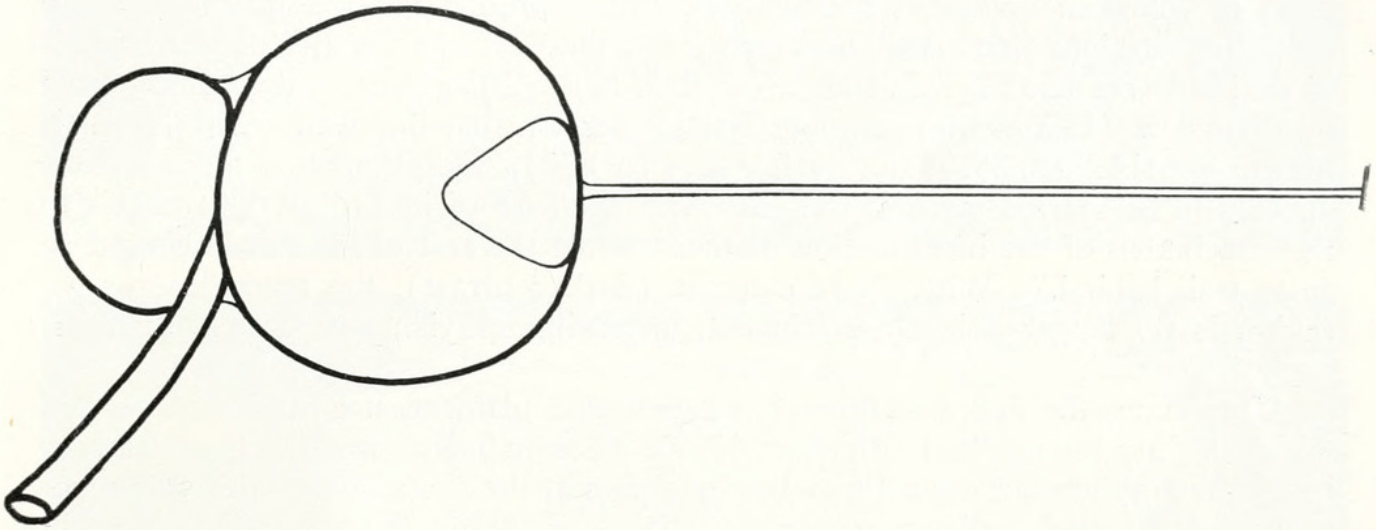


FIGURE 8. Diagram of starfish spermatozoan after acrosome reaction has taken place. Filament extends from center of acrosome surface; middle piece has become nearly spherical, and tail projects laterally, between head and middle piece. Only proximal portion of acrosome filament indicated.

them, and returning to their original position when the current is stopped. They can also be seen to vibrate when hit by a passing spermatozoan, and are sometimes found broken, with part of the filament attached at an angle to the remainder. Some time after the addition of the egg-water, when the actively moving spermatozoa have become sufficiently stationary to permit observation, some of them can be seen to have short fragments of the filaments still projecting from the center of the acrosome surface. Apparently the rest has broken off, during their swimming

FIGURE 6. Shadowed electron micrograph of *Asterina* spermatozoan; suspended in albumin-sea water and mixed with homologous egg-water on collodion membrane; fixed with OsO_4 vapor. Acrosome filament has been bent in handling, and vesicles formed at various points, probably as an effect of the fixative. Note that the membranous sheath of the filament seems to be closed at the tip, and that the central core gives evidence of spiral fibrillar structure.

FIGURE 7. *Asterina;* same treatment as above. Acrosome filaments projecting from agglutinated clump of spermatozoa.

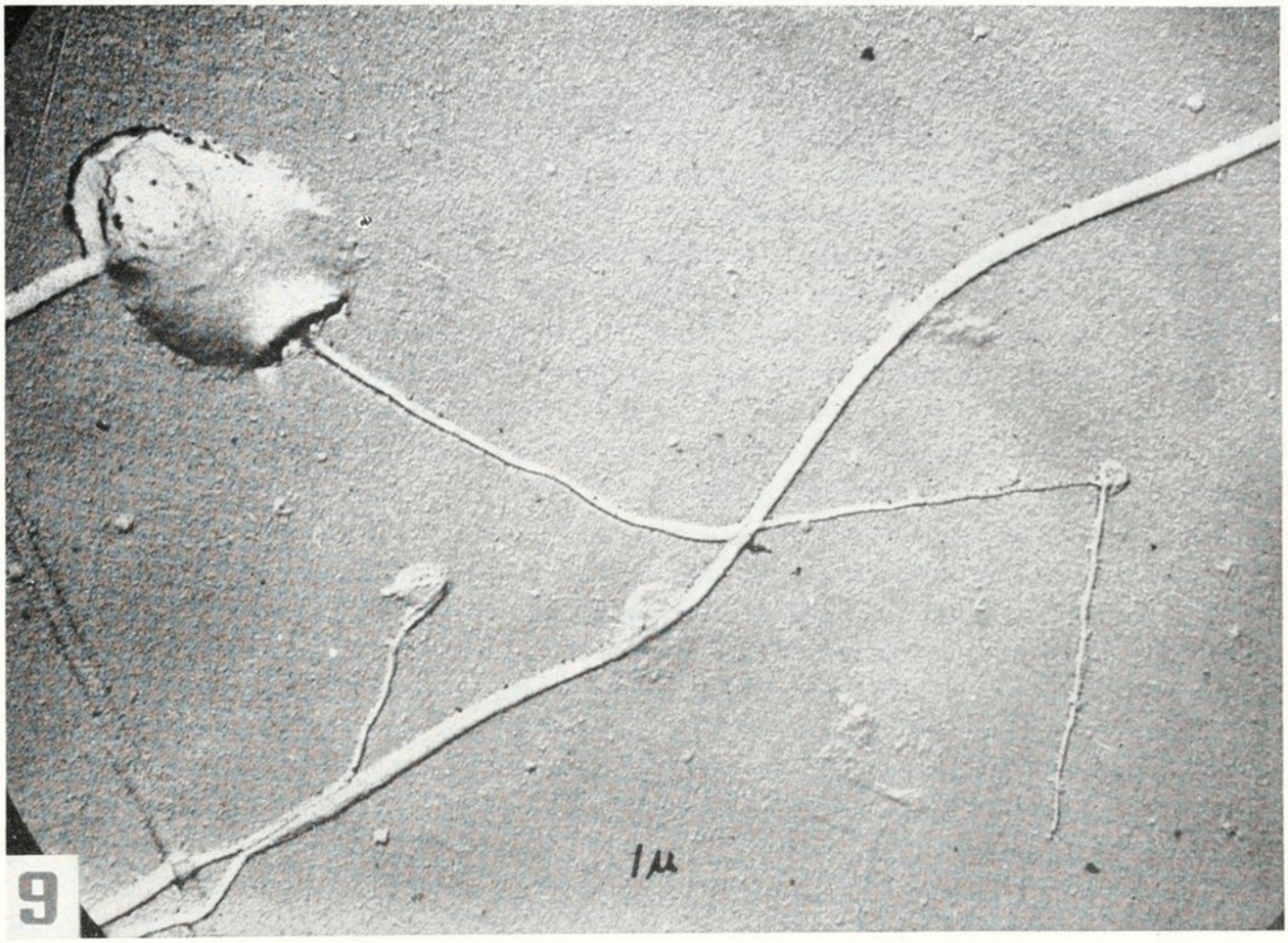
about. Whether the filaments gradually disintegrate in sea water could not be determined definitely for this reason; it is certain that those which have been stuck to the glass from the beginning remain apparently unchanged for at least 30 minutes, but these are not fully exposed to a possible dissolving action of the sea water. No acrosome filaments could be seen on spermatozoa fixed with formalin, although they were well preserved after fixation with osmium vapor. This latter fixative, however, was quite ineffective with respect to the nuclear part of the sperm head, which became extremely flattened and diffuse in outline when the preparation was dried (Figs. 1b, 6, 7, 9, 10).

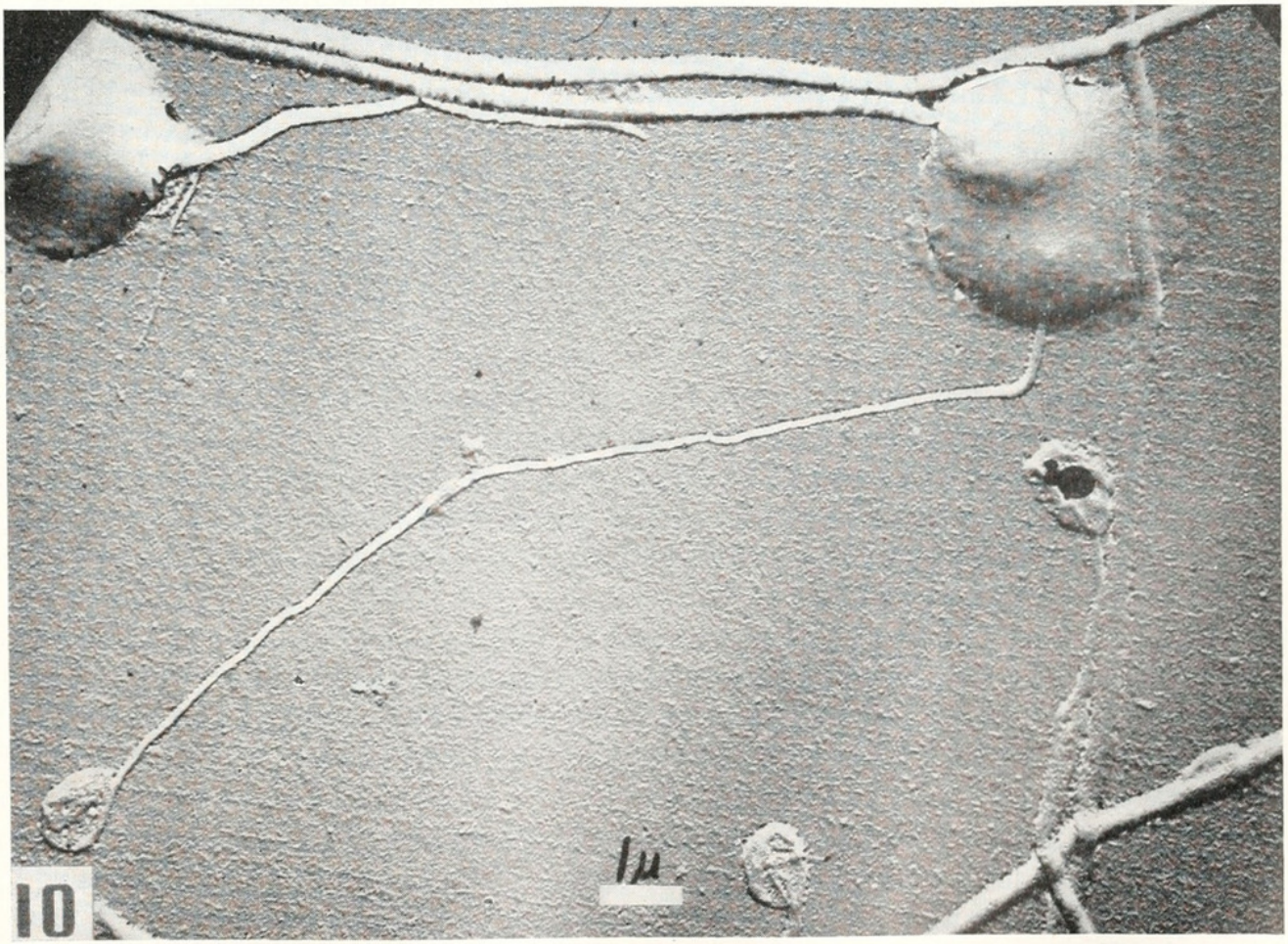
Besides the appearance of the acrosomal filament, there is a structural readjustment which takes place in response to the stimulus of egg-water and makes it possible to differentiate between spermatozoa which have reacted and those which have not. This consists in what appears to be a partial relaxation of the tight enveloping membrane, so that the middle piece rounds up and is less closely applied to the posterior part of the nucleus (Fig. 8; compare with Fig. 2a). Moreover, the tail, which in untreated spermatozoa curves around the middle piece before extending straight backward, now projects laterally from the posterior midpoint of the head (see also Figs. 5a and b, 6, 7, 8, 9, 10). This phenomenon was reported by Chambers (1930), who mentions an "impression that the head of the sperm is bent to one side" (p. 352), and further says (p. 352), "Occasionally a spermatozoan appears to be carried through the jelly with the base of its tail at right angles to the attachment of the insemination filament, while the rest of the tail is curved so as to trail behind." With phase contrast (dark contrast), the reacted acrosome region is no longer brightly refringent, appearing grayish and darker than the nuclear material.

Concerning the fine structure of the acrosome filament, not much can be said with certainty on the basis of the available electron micrographs. It appears to have a slightly greater diameter at the base than at the tip, although this difference cannot be detected in living specimens. There is definitely a central core, probably fibrous, surrounded by a sheath or membrane (Fig. 6). In fixed specimens, local blister-like swellings of this membrane are often found (Figs. 6, 7, 9). The end of the filament apparently has no special structure (Figs. 7, 9), although Figure 6 indicates that the tip is enclosed by the membrane. The knob-like condition of the filament tip in Figure 10 is apparently the result of fixation, and has no particular significance with respect to the acrosome filament itself, since the axial filaments at the ends of the sperm tails in the same preparation also reacted in the same way.[3] This at least suggests that the two slender filaments may have certain properties in common. They are further similar in that both appear to be rather sticky, since spermatozoa are often held firmly to the glass surfaces by the two filaments, while all the other parts—the head, middle piece and sheathed part of the tail—are movable against the glass.

In no case, in either *Asterina* or *Asterias*, has the acrosome reaction been found to take place in the great majority of the spermatozoa, as it does in sea urchins. Most of the sperm which fail to react have small, spherical heads, with the middle piece closely applied to the base of the head; while those which react to egg water

[3] These artifacts are no doubt identical with the vesicles formed at the tips of silver salmon sperm tails on fixation with osmium tetroxide and Zenker's fluid (Lowman, 1954).





FIGURES 9 AND 10. Replicas taken from *Asterias* spermatozoa suspended in 0.1% albumin-sea water, and fixed with OsO_4 vapor after addition of homologous egg-water.

are larger, the anterior part of the head, consisting of the acrosome surface, is flattened, and the middle piece is broad and prominent and its junction with the head is clearly differentiated. It seems possible that the spermatozoa of the smaller type may be non-functional.

Preliminary observations of fertilization in the three starfish species under consideration show that when insemination is carried out soon after the extrusion of the first polar body (see Delage, 1901), the eggs fertilize monospermically and develop normally, at least in the early stages. In all three species, within the minimum of 20–30 seconds which is required for insemination and adjustment of high dry (phase contrast) focus, the inseminating spermatozoan has become attached to the vitelline membrane by a very slender filament and is moving steadily through the dense jelly layer. About the time it reaches the vitelline membrane, this is being pushed away from the egg surface by a low fertilization cone. The spermatozoan traverses the intervening membrane and can be seen momentarily within the hyaline protoplasm of the cone, which becomes somewhat larger but is usually restrained during its developing phase by the very slowly rising fertilization membrane (see Dan, 1950, Fig. 7). By the time the sperm tail is nearly inside the egg, the membrane has lifted away from the degenerating cone, which rather quickly subsides in an irregular fashion and disappears completely within 6–10 minutes after insemination.

Discussion

The fact that starfish spermatozoa can be shown to produce, in response to the stimulus of species egg-water, a long, slender filament which coincides in appearance with the filament supposed by various workers to proceed from the egg surface, necessitates a reconsideration of the evidence on which this supposition was based. Chambers (1930) cites Fol as having rejected the possibility that the filament originated in the spermatozoan because he was unable to detect any diminution in the size of the heads of spermatozoa attached to such filaments. For the following several reasons the decision seems unavoidable that Fol was mistaken in drawing such a conclusion:

1. the heads of starfish sperm are far from uniform in size, as observed in the living state, under phase contrast oil immersion;
2. estimates from observations of living sperm and electron micrographs indicate that the diameter of the living starfish sperm head is about 2 μ, which is too small, even with modern optical equipment, to permit accurate estimation of minor changes in volume;
3. calculations based on measurements of electron micrographs of osmium-vapor-fixed sperm heads and acrosome filaments indicate that loss of the volume of substance contained in a filament 25 μ in length and 0.13 μ in diameter would result in a reduction of the sperm head diameter of only about 2%;
4. the partial separation and rounding-up of the middle piece and main part of the sperm head at the time of the acrosome reaction produce sufficient change to obscure a much greater loss of substance than that necessary to produce the filament.

Fol's description of the fertilization process in echinoderms was so detailed and accurate that, as Chambers pointed out when he undertook a re-examination of the question nearly half a century later, "even recent textbooks comment upon the completeness of his observations" (1923, p. 821). Although Chambers referred to Fol's idea of the mechanism in starfish eggs, by which "the presence of spermatozoa in the immediate vicinity causes the egg to respond by forming on its surface a conical elevation which attracts the spermatozoan from a distance" as "rather extraordinary" (loc. cit.), he followed Fol in this interpretation, apparently in lieu of sufficient evidence to the contrary.

Chambers found that when starfish eggs are in the optimum condition for fertilization, the process takes place so rapidly that the filament is already extending between sperm head and cone tip by the time the earliest observation can be made. Relying on Fol's assumption of the "attraction cone" as the site of origin of the filament, Chambers records a number of observations in this and his later paper (1930) which convincingly serve to demonstrate its existence and show that it is the agency by which the spermatozoan is drawn through the jelly to the egg surface. However, the best evidence which he could muster, in the course of the two studies, for the origin of the filament in the attraction cone depends on the assumption that sperm entrance takes place in the same manner whether the eggs are in the optimum condition or either under- or over-mature.[4]

It is well known that in sea urchin eggs there are highly significant differences between the modes of sperm entrance, and more especially between the reactions of the eggs to insemination, under different conditions of maturity. In immature eggs there is apparently no block except spatial limitation to the entrance of any number of sperm; no fertilization membrane is raised; and the only apparent response of the egg cytoplasm to sperm entrance is the formation of conspicuous, semi-permanent structures on the surface which appear to be bundles of protoplasmic fibrils and bear little resemblance to the small, rounded, temporary cones of normal fertilization. Hobson (1927) found that in the oocytes of *Asterias rubens,* which had the germinal vesicles still intact and were "never observed to cleave or to complete their maturation" (p. 100), the reaction to sperm entrance was so similar to that reported by Seifritz (1926) in oocytes of *Echinarachnius parma* that he did not bother to repeat the description.

Since the optimal condition in sea urchin and sand dollar eggs lasts for a very long period, the various changes in the fertilization reaction associated with the passage of time may equally well be the result of a senescent loss of vitality, as of a condition of "over-maturity" such as that occurring in "post-optimal" starfish eggs, and there is probably little profit in attempting to compare the two sets of phenomena.

Just, on the other hand, attacked the interpretation of Fol and Chambers on the basis of his own observations of starfish eggs, which, he wrote, "indicate that maturated eggs lose the capacity for normal fertilization, and parallel with this loss runs the production of filaments." ". . . the production of filaments by *Asterias* ova . . . as a response to insemination is a phenomenon quite apart from the behavior

[4] The terms "under-" and "over-mature" are used with reference to the "optimum condition for fertilization," which lasts only during the period between the extrusion of the first and second polar bodies.

in the normal fertilization reaction" (Just, 1929, p. 319). "*Fol used stale eggs,* which accounts for the filament formation" (op. cit., p. 322).

The observations of other workers, then, do not support Chambers' primary assumption, that the fertilization reaction follows the same process regardless of the state of the egg with respect to the meiotic divisions. As a result, his conclusion based on this assumption, that since filaments have been observed to arise from the entrance cones in immature eggs and in old eggs, the filament in normal fertilization must necessarily originate in the same way, loses much of its validity.

With respect to eggs in the optimum condition for fertilization, Chambers says, "In fresh maturing eggs I have never been able to see the cone without also seeing the advancing sperm and the filament connecting the two. The formation of the filament is apparently too rapid" (1930, p. 354). This is the fact which remains when we cancel out of Chambers' observations the influence of Fol's dictum that the fertilization filament could not have arisen from the spermatozoan.

On the other hand, while Just insists that a strand which he observed connecting the sperm head with the fertilization cone "is a prolongation of the spermatozoon" (op. cit., p. 321), the fact that he based his observations partly on fixed material automatically raises a question as to their value. It seems obvious that such a process as the approach of a spermatozoan through the jelly to the egg surface cannot be preserved in successive stages by any of the ordinarily used methods of fixation, since they either cause the jelly layer to shrink or dissolve it completely away. In the best case the exact relation of the sperm to the egg surface could only be captured by fixation after the spermatozoan had become firmly attached to the egg cytoplasm.

At least the first part of Just's observation seems to have been made on living material: "The intensely active spermatozoa rush toward the jelly hull; of these, one rapidly moving through it reaches the egg within 5 seconds after insemination" (loc. cit.). In the experience of Chambers, as well as of the writer, it has been found that at least twenty or thirty seconds are required to bring the freshly inseminated eggs into high power focus and discover one with a fertilizing spermatozoan attached exactly in the largest optical section. The sperm is by this time already connected with the egg surface by the filament in question, and according to Chambers' figures (1923, Fig. 2, a-c; 1930, Fig. 2, A-H), about one minute more is required for the sperm to pass through the jelly layer and reach the surface of the vitelline membrane. Preliminary observations by the writer on *Asterias amurensis* showed the sperm head reaching the membrane surface about two minutes after insemination (at 17° C.). In *Asterina pectinifera,* the sperm passed through the narrow jelly layer within 50–60 seconds after insemination (23° C.). *Astropecten scoparius* has a somewhat wider jelly layer, which the sperm crossed in about seventy seconds (at 25° C.).

A more detailed study of the fertilization reaction in these three species will be presented in a later paper; these preliminary data are cited as the basis of the writer's inability to accept Just's figure of five seconds as the time required for a spermatozoan to traverse the jelly layer of the *Asterias* egg.

Just further describes the process as he saw it in both living and fixed preparations: "As the cone grows the spermatozoon is pushed off from the egg, a delicate strand connecting the tip with the apex of the cone. This strand never attains the

length given by Fol, and of course could never therefore be equal to the greater length figured by Chambers" (1929, p. 321). Just does not explain how or why such a structure is produced, beyond stating categorically, *"this strand is a prolongation of the spermatozoon, the tip of which is fixed within the cone"* (Just's italics; op. cit., p. 321). In the conclusion of his paper, however, he draws a comparison which probably reveals the source of his confidence in making the above statement: "This cone in *Asterias* egg closely resembles that in *Nereis* egg," except for the time factor. "Moreover, the strand between the sperm head and the cone in both cases has the same origin, namely, from the spermatozoon itself" (op. cit., p. 324).

It seems doubtful whether this analogy provides a sufficiently firm basis for a flat rejection of Chambers' observations as well as his interpretation.

Since Hörstadius, in describing fertilization in *Astropecten aranciacus,* does not mention filaments of the order of size of those under consideration, a discussion of his interpretation of the process is not strictly appropriate at this time. However, since the writer has clearly observed the formation of acrosome filaments in response to egg-water in the spermatozoa of *Astropecten scoparius,* and also in the fertilization process, it appears likely that in *A. aranciacus,* also, filaments from the sperm acrosome give the stimulus for the elevation of the cylindrical cones observed by Hörstadius. That author himself suggests that the formation of very many large cones is a characteristic response of immature eggs to insemination.

To summarize this discussion concerning the origin of the "fertilization filament"—it appears that, among later workers, only Chambers actually observed the filaments first discovered by Fol. Just's categorical statement quoted above with respect to the length of the filament, as well as the sequence of steps in its formation and their timing, all indicate that he failed completely to see the structure described in the earlier papers. Moreover, since Chambers, in spite of his accurate observation, was unable to establish conclusively the origin of the filament in the "attraction cone," we find the whole weight of this explanation of starfish fertilization resting upon Fol's assumption. This, in turn, depends upon his belief that he should have been able to observe a difference in the size of sperm heads before and after the extrusion of the filament.

If the criticisms of this assumption listed above be allowed, it follows that no incontrovertible evidence has been presented proving that the filament, observed by Fol, Chambers and the writer connecting the sperm head with the egg surface, arises from the egg. There is, moreover, no other case in animal fertilization, so far as the writer is aware, in which an egg has been shown to reach out and capture a spermatozoan.

On the positive side, evidence has been found (unpublished data) to show that the acrosomes of sea urchin and sand dollar spermatozoa undergo a reaction, in response to the stimulus of species egg-water, which can most readily be interpreted as providing a method of penetrating the first serious obstruction [5] which the spermatozoa encounter in their progress toward the egg pronucleus—the vitelline membrane. Since the tip of the sperm head is already in contact with the membrane, the liberation of a lytic substance, together with the reaction of the egg

[5] In these species, the spermatozoa swim through the jelly layer in a radial direction with at most only a slight reduction in speed.

cytoplasm would presumably be sufficient to effect penetration. In the case of the starfish egg, on the other hand, an impenetrable jelly layer intervenes between the spermatozoan and the living egg surface. This necessitates the extrusion of a projection of the acrosome, long enough to cross the jelly layer, and sufficiently rigid to penetrate the vitelline membrane and establish contact with the reactive cytoplasm. It is believed that these conditions are fulfilled by the filament which is extruded from the starfish acrosome in response to the presence of species egg-water.

At present there is no evidence as to how the filament is drawn into the egg cytoplasm. That such a process is entirely possible is shown by the fact that in the sea urchin, *Mespilia globulus*, the entrance of the sperm tail may take place very slowly (Dan, 1950), so that the sperm aster appears and even syngamy is complete before the tail is more than halfway into the egg. In this case it is impossible to imagine that the motionless tail is proceeding under its own motive power; nevertheless, its whole length is taken into the cytoplasm within about 15 minutes.

Tyler (1948) has suggested an explanation of the starfish filament in terms of a fertilizin-antifertilizin interaction, in which active antifertilizin groups on the sperm would combine with active fertilizin groups on the micelles of the jelly, resulting in precipitation, or contraction, of the micelles. Since these are assumed to be anchored to the surface of the egg, their contraction would draw the spermatozoan a slight distance toward the egg and into contact with new micelles, where the reaction would be repeated, until the sperm head finally reached the egg surface.

This is an ingenious attempt to fit the starfish fertilization process into the general echinoderm pattern, and represents a decided advance beyond the appeal to a mysterious force directing an attraction cone filament to a waiting spermatozoan. Observation of the actual fertilization process shows, however, that the filament has already attained its full diameter while the sperm head is still nearly the whole width of the jelly layer away from the egg surface, and no change in this diameter can be detected as the sperm head approaches the egg. Moreover, it seems questionable whether a filament of microscopically observable dimensions could be produced by the mechanism suggested, even given a radial arrangement of the submicroscopical jelly micelles. Finally, it must obviously be quite hopeless to rely on this scheme for an explanation of the filaments found on egg-water-treated spermatozoa.

The abrupt extension of a long, slender filament is a phenomenon already familiar in the discharge of trichocysts by ciliates and nematocysts by the coelenterates. Since the acrosome of the starfish spermatozoan measures only a fraction of a micron, it seems hardly possible that any very complex mechanism could be contained within it. This is one consideration in favor of a trichocyst-like discharge mechanism. However, the problem is within the range of direct experimental attack, and further discussion will be reserved until evidence is available.

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SUMMARY

1. When the spermatozoa of three starfish species, *Asterina pectinifera, Asterias amurensis* and *Astropecten scoparius,* are suspended in a dilute (0.1–1.0%) solution of egg albumin in sea water and mixed with homologous egg-water, three effects can be noted:

a. many of the spermatozoa are agglutinated by their heads, forming permanent clusters;
b. from the center of the acrosome of each agglutinated spermatozoan there has been extended a long (ca. 25 μ), very slender, straight filament which possesses considerable rigidity;
c. in all the spermatozoa which have so reacted, there is a rearrangement of the principal parts, so that the middle piece is less tightly apposed to the head and the tail appears to be inserted laterally, between the head and middle piece.

2. A critical examination of the widely accepted explanation of starfish fertilization—that the effective spermatozoan is drawn through the jelly layer to the egg surface by a filament originating in an "attraction cone"—shows that this depends mainly upon an assumption made by Fol, which various considerations show to be of doubtful validity. In the light of the fact that starfish spermatozoa produce a similar filament from their acrosomes, on contact with dissolved jelly substance, it is proposed that this sperm acrosome reaction is the source of the filament which, extending through the jelly, stimulates the egg cortex. Following this stimulation, the egg cytoplasm draws in the filament with the attached spermatozoan and simultaneously forms a fertilization cone beneath the vitelline membrane, which separates as the fertilization membrane. This sequence of events is the same as that constituting the fertilization reaction in other echinoderms.

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