THE ACROSOME REACTION IN *MYTILUS EDULIS*

II. Stages in the Reaction, Observed in Supernumerary and Calcium-Treated Spermatozoa

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ABSTRACT

Suspensions of *Mytilus edulis* eggs were fixed with osmium tetroxide at various intervals between 1 and 10 seconds after heavy insemination, and sectioned for electron microscopy to follow the natural process of acrosome reaction in the spermatozoa around the eggs. Sperm suspensions were also fixed after the addition of 10 per cent by volume of M/3 calcium chloride. Within the first second after the acrosome is stimulated to react, an opening appears at its apex, around which the plasma and acrosomal membranes fuse to each other, and the resulting membrane complex is reflected backward, presumably by the swelling of material lining it. At the same time the other material within the now open vesicle disappears, and the rudiment of the acrosomal process, consisting of a short axial rod loosely surrounded by the invaginated part of the acrosomal membrane, is exposed at the anterior side of the sperm head. Within another second this rudiment is extended by elongation of the axial rod and expansion of the surrounding membrane. If the spermatozoon has reacted close to the egg surface, the elongation may be very slight, whereas in suspended spermatozoa the process may reach a length of 13 μ. Possible mechanisms underlying these changes are suggested.

As the previous paper has demonstrated (10), the intact acrosome of the *Mytilus edulis* spermatozoon has a conspicuous conical acrosomal vesicle applied to the anterior face of the sperm nucleus, directly under the plasma membrane. The membrane of this vesicle is invaginated at the side facing the nucleus, to form a spacious pocket; a slender axial rod originates in a tube passing through the nucleus and extends through the center of the invagination to a length of about 2 μ.

The present report will show that the acrosomal vesicle opens when the spermatozoon comes into contact with an egg, exposing the invaginated portion of the acrosomal membrane, within which the axial rod elongates. Whereas the acrosomal process formed in this way resembles those of the echinoderms (6–8) in being single and having a central core, the intact acrosome of *Mytilus* is much more like that of the annelid *Hydroides* (1, 3) in its general structure and in its mode of reaction, the details of which will be described in this paper.

MATERIAL AND METHODS

Eggs of *Mytilus edulis*, obtained by electrically stimulated spawning (9), were heavily inseminated and

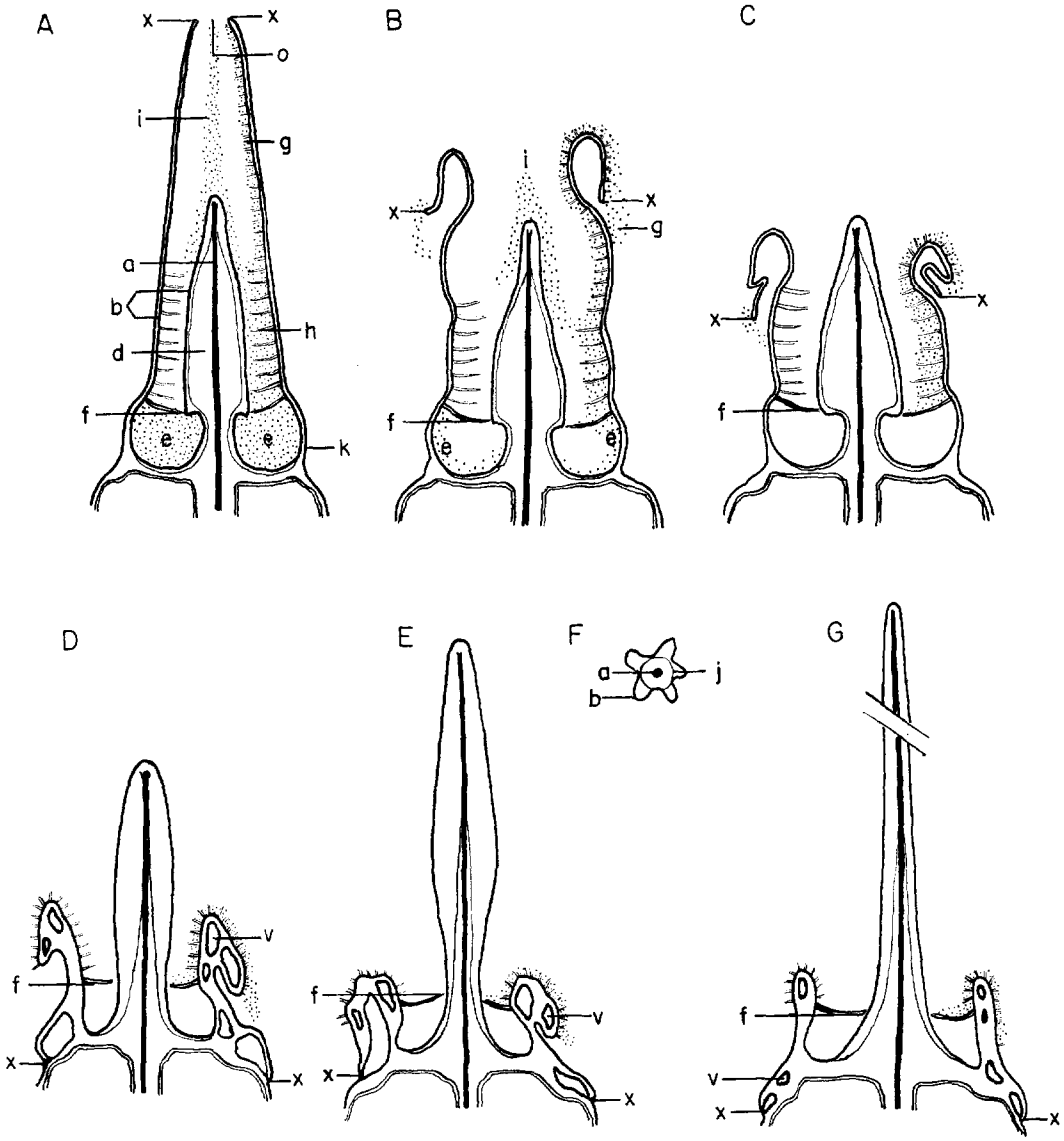


FIGURE 1 Steps in reaction of *Mytilus* acrosome. *A* to *E*, diagrammatic longitudinal sections showing changes occurring within 1 second after stimulus; *F*, transverse section through base of completed acrosomal process; *G*, elongated acrosomal process, as formed by spermatozoa not in close contact with an egg surface or on treatment with excess calcium. *a*, axial rod; *b*, acrosomal membrane; *d*, lumen within invagination of acrosomal membrane; *e*, basal ring material (lysin?); *f*, partition bounding basal ring; *g*, *h*, material lining acrosomal membrane; *i*, axial strand material (lysin?); *j*, membranoid sleeve surrounding axial rod; *k*, plasma membrane; *o*, opening at tip of acrosome; *v*, vesicles formed by apposed areas of plasma membrane; *x–x*, ring of fusion between plasma and acrosomal membranes.

A. Plasma membrane and acrosomal membrane open at acrosomal apex and fused to each other around opening (*x–x*).

B. Fused rim of membrane complex (*x–x*) turned outward and reflected backward toward base of swollen acrosome, preceded by material of lining layer (*g*). Substances *e* and *i* dissolving out of acrosomal vesicle, exposing inner surface of acrosomal membrane invagination (= rudiment of acrosomal process).

fixed at brief intervals (1, 2.5, 3, 5, 8, and 10 seconds) and at 3 minutes thereafter. The acrosome reaction was also induced by adding M/3 $CaCl_2$ to a sea water suspension of spermatozoa in the ratio of 1 to 9.

The material was fixed for 1 hour with 1 per cent OsO_4 in sea water, embedded in a 4:6 mixture of styrene and butyl methacrylate, sectioned with a Porter-Blum microtome,[1] and observed with a JEM T-5 electron microscope.

RESULTS

Phase Contrast Observation of Living Spermatozoa

The acrosome of reacted spermatozoa (9) is represented by a straight, slender process which may reach a length of 13 μ, inserted centrally in the anterior face of the nucleus. Around its insertion can be seen what appears to be an extremely tenuous remnant of the base of the original acrosome.

Electron Microscopy: Stages in Acrosome Reaction

Sections of spermatozoa exposed to fixative 1 second after they had been added to an egg suspension show that the acrosome undergoes an extremely rapid change, in which the apical part of the conical vesicle opens (Figs. 1 *A*; 2), its outer wall is rolled back, and all the particulate material within the vesicle dissipates, exposing the inner surface of the invaginated membrane (Figs. 1 *B* to *D*; 3 to 6, and 8). This membrane then expands and within its lumen the axial rod elongates, to form the acrosomal process (Figs. 1 *E*; 7 and 9).

The first observable step in this series of changes takes place at the tip of the acrosome (Figs. 1 *A*, *o*; 2 and 3), where the two membranes (plasma and acrosomal) break and fuse with each other around the rim of the opening (Fig. 1 *A*, *x–x*). The whole acrosome swells rapidly (Figs. 1 *B*; 4), and the plasma-acrosomal membrane complex is turned outward at its tip and carried backward over its own outer surface, apparently by the swelling of the material lining it. Some of this material (*g* in Figs. 1 *B*, 3, and 4) can be seen spreading over the plasma membrane covering the still intact part of the acrosome in advance of the reflected rim of the membrane complex (Figs. 3 and 4).

As the vesicular wall is rolled back, the material of the axial strand (*i* in Figs. 1 *B* and 4) disappears, as well as that (*e* in Figs. 1 *A*, *B*, and 4) of the basal ring (Figs. 1 *B*, *C*; 4 to 7). The invaginated portion of the acrosomal membrane is now the only structure separating the axial rod from the external medium.

When the rolling-back process has proceeded some distance along the acrosome, a secondary folding of the doubled membranes takes place, as though the rate of reversion exceeded the rate at which the membrane complex slips over the acrosomal surface (Figs. 1 *C*; 6 and 7). This effect is particularly marked in the thick part (*h* in Fig. 1 *A*) of the vesicular wall (Figs. 1 *D*; 7 to 9), where the tubular elements of the lining layer persist after the particulate component has dispersed. The fused rim of the membrane complex nevertheless continues to move backward (Figs. 1 *D*, *E*, *G*, *x–x*; 5, 7, and 9), eventually extending past the base of the acrosome to the anterior part of the nucleus, where it usually is closely applied to the plasma membrane, although it may occasionally lie free alongside it (Fig. 12).

Only after the outer wall of the acrosomal vesicle has been removed does the axial rod begin to elongate under the invaginated portion of the acrosomal membrane to form the definitive acro-

[1] The Porter-Blum microtome used in this study was purchased with part of a Rockefeller Foundation grant to Tokyo Metropolitan University, administered by Professor Katsuma Dan.

C. Secondary folding in reflected membrane complex. Process rudiment slightly distended but not yet elongating. Substances *e* and *i* completely dissipated.

D. Membrane complex turned inside out, fused rim (*x–x*) now found surrounding anterior part of nucleus. Secondary folding of membrane complex has moved basally, now seen as ridge encircling process; fine tubules of lining layer persist on surface of this ridge. Partition (*f*) which separated contents of basal ring (*e*) from lining material (*h*) now clearly visible. Apposed layers of plasma membrane broken up into vesicles (*v*) under acrosomal membrane. Axial rod has elongated within distended process membrane.

E. Fully elongated acrosomal process formed by spermatozoon in contact with egg surface.

F. Cross-section through base of acrosomal process, composed of axial rod (*a*), longitudinally folded acrosomal membrane (*b*), and sleevelike membrane (*j*).

G. Elongated process formed by spermatozoon not in immediate contact with egg surface.

somal process (Figs. 1 *D*, *E*, *G*; 7, 9, 11 to 13). In longitudinal sections of the completely formed process, the membrane lies at some distance from the rod, indicating that it is distended by some means while it is also being stretched by the elongating rod.

As is shown most clearly in Fig. 9, in the collar-like remnant of the membrane complex, as well as around the anterolateral part of the nucleus, the two apposed layers of plasma membrane have ceased to exist as membranes; in their place are many small vesicles. In sections of spermatozoa fixed after 3 minutes these vesicles, as well as the layers of plasma membrane which apparently give rise to them, are lacking; in general there is a tendency for the vesicles to be larger and more numerous in earlier fixations, and smaller and fewer as more time elapses between insemination and fixation.

The sleevelike membranoid structure persists apparently unchanged, except that its distal portion becomes closely applied to the axial rod (Figs. 4, 9, 11, and 13). In cross-sections through the base of the acrosomal process (Figs. 1 *F*; 10), it is seen as a regular circle, against which rest the inner sides of several longitudinal folds which have appeared in the acrosomal membrane at this level.

Spermatozoa exposed to excess calcium (Figs. 8 and 9), like those which react at some distance from the egg, form longer processes but in all other respects resemble spermatozoa reacting in close contact with the egg surface.

DISCUSSION

In the absence of contractile elements associated with the acrosomal vesicle of the *Mytilus* spermatozoon, it is necessary to find some other kind of mechanism which would explain its extremely rapid change in form. It seems possible that an abrupt change in state of an appropriately located material could exert enough force against the vesicular membrane to cause its eversion. The observations described above suggest that the material lining the outer wall of the acrosomal vesicle is qualified to perform this work. The following attempt to visualize the mechanism underlying these structural changes is presented as a hypothesis, which appears to the authors to provide the most plausible explanation of the observations, although other interpretations are not precluded.

It is proposed that the "trigger" of the acrosome is located at its apical tip; although no special structure similar to the apical vesicle of the *Hydroides* spermatozoon (1) has been detected here, this is certainly the site of the earliest observable change, as the result of which the plasma and acrosomal membranes break and fuse with each other, opening the apex of the acrosome. Presumably sea water enters through this opening, rapidly dissolving the substance of the basal ring and axial strand (Fig. 1 *A*, *e* and *i*); at least one of these substances (if they are not identical) is believed to be the lysin which is known to attack the vitelline coat of the egg (5, 13; see also 3).

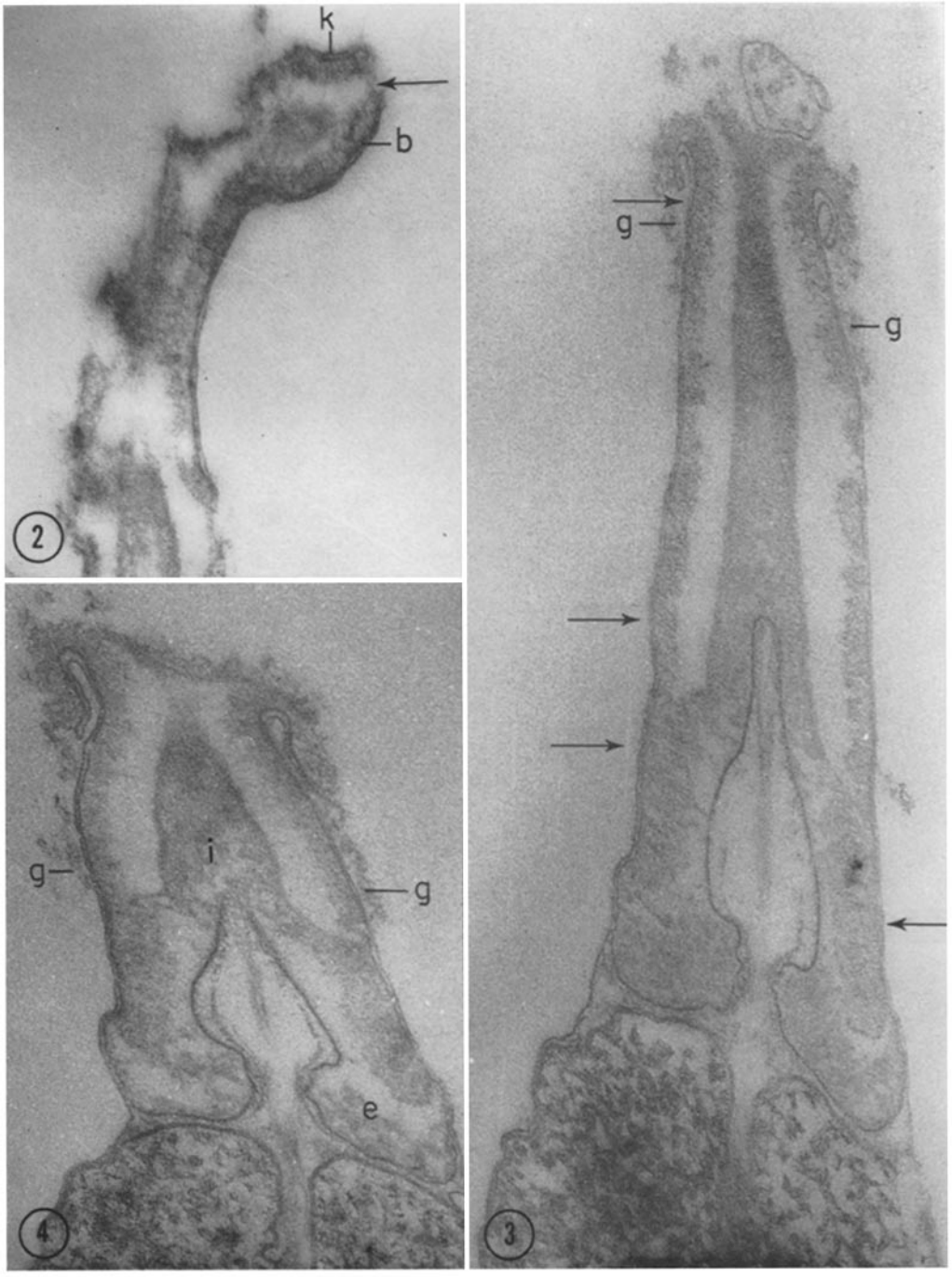
The explosive rolling outward and backward of the vesicle wall appears to depend on a characteristic mode of swelling and dissolution of the material making up its lining layer. Since this layer disappears within a very brief time after the tip of the acrosome opens, it is reasonable to suppose that its material undergoes a preliminary phase of rapid swelling as the result of exposure to

FIGURES 2 TO 4 Longitudinal sections of *Mytilus* acrosomes in early stages of reaction.

FIGURE 2 Apex of acrosome immediately after beginning of reaction. Plasma membrane (*k*) and acrosomal membrane (*b*) have opened (arrow) and their edges have fused to each other. × 40,000.

FIGURE 3 Fused membrane complex beginning to be turned outward and backward, presumably by swelling of lining material, some of which has spread over intact surface of acrosome (*g*). Tubular elements of lining layer visible in vicinity of arrows. × 50,000.

FIGURE 4 Acrosomal vesicle has swollen; amounts of axial strand (*i*) and basal ring (*e*) substances (lysin?) diminished; reflection of membrane complex continues, with dissolving lining material (*g*) spreading ahead of reflected edge. No change seen in structures within invagination (process rudiment). × 50,000.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sea water. If it is assumed that this swelling begins at the exposed inner surface of the layer, the resulting unequal expansion would cause it to flare outward. In the micrographs of this stage of the reaction, the partly dissolved lining material shows a marked tendency to spread over the outer surface of the still intact part of the acrosome; the everted edge of the acrosomal-plasma membrane complex is apparently caught under this layer of dissolving material and carried rapidly toward the base of the acrosome as the swelling involves successively more proximal levels of the acrosomal wall.

For this mechanism to work effectively, the condition inducing swelling should be localized at the apical edge of the vesicular wall. If this condition consists in exposure of the lining material to a certain concentration of some component of sea water, it will automatically be maintained by the progressive eversion of the vesicular wall. Furthermore, the efficiency of this mechanism will be greater if the lining material is not free to slip over the inner surface of the vesicular membrane. It is suggestive that this layer includes what appear to be extremely delicate tubular elements or arrangements of the lining material, attached perpendicularly to the acrosomal membrane (*e.g.*, Fig. 3). These may well correspond to the "granules . . . disposed in irregular lines in positions approximately normal to the acrosomal membrane" observed by the Colwins (3, p. 238) in the lining material of the *Hydroides* acrosome.

A closer look at the membranes during the series of form changes seen in Figs. 5 to 9, 11, and 12 brings to light several points of some interest. For instance, the membrane complex formed by the apical fusion of the plasma and acrosomal membranes shows itself to be viable and functional even after the underlying material has been removed. Moreover, the way it survives being turned inside out and carried back over the nucleus indicates that its component membranes are so highly plastic as to resemble liquid surfaces: the ring of fusion at the tip of the acrosome has a diameter of only about 200 mμ, but within a second or two its component membranes have been stretched and relocated so that the ring encircles the nucleus, with a tenfold increase in diameter

Again, these micrographs demonstrate that the everted acrosomal membrane, after it has been folded back over the anterolateral part of the

FIGURES 5 TO 9 Steps in reaction during which everted membrane complex extends to sides of nucleus, while vesicular invagination expands to form acrosomal process. Figs. 8 and 9 represent spermatozoa induced to react by exposure to excess calcium.

FIGURE 5 Non-median section: partly emptied basal ring region collapsed; tubules in lining layer more apparent (arrows) as material around them dissolves. × 32,000.

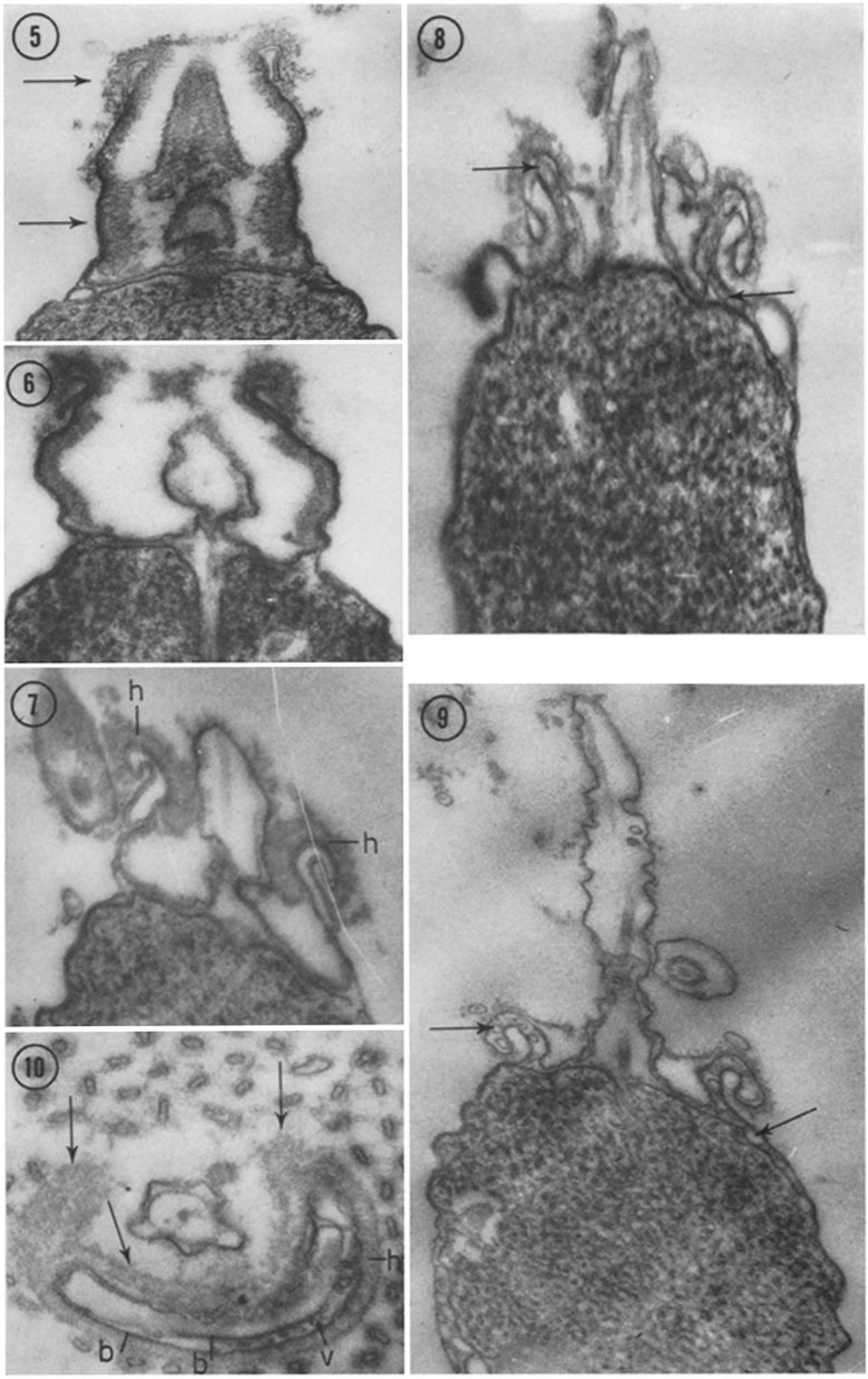
FIGURE 6 Material of axial strand and basal ring dispersed; membrane complex shows beginning of secondary reflection. × 32,000.

FIGURE 7 Thick part of lining layer (*h*) involved in membrane reflection; process rudiment shows first sign of distension. × 30,000.

FIGURE 8 Calcium-induced reaction. Section through base of extended process showing completely reflected membrane complex. Vesicles (arrows) between layers of acrosomal membrane believed to be formed by vesiculation of apposed plasma membranes. × 34,000.

FIGURE 9 Median section through spermatozoon fixed after treatment with excess calcium. "Membrane complex" now consists of only acrosomal membrane; two underlying layers of plasma membrane have apparently broken up into vesicles (arrows). × 30,000.

FIGURE 10 Somewhat diagonally transverse section through base of completely formed process (see Fig. 1 *F*). Crescent-shaped formation at lower right represents fold of membrane complex; small ovoid profiles at upper left are sections through microvilli of egg surface. Arrows indicate portions of boundary partition (*f*). *b*, acrosomal membrane; *h*, material lining acrosomal membrane; *v*, vesicle. × 55,000.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nucleus, replaces the plasma membrane as the outermost covering of the cell in this region. After a few seconds it is impossible to detect any inequality in the outline of the cell which would indicate the exact line of fusion between the original plasma membrane and the edge of the acrosomal membrane (*e.g.*, Fig. 9; upper side of Fig. 11; left side of Fig. 12). This substitution leads to a final result similar to that observed by the Colwins (3) in *Hydroides; i.e.*, formation of a mosaic outermost covering of the sperm cell, consisting of acrosomal membrane joined to the original plasma membrane.

Finally, the vesiculation observed to take place where the plasma membrane is folded back upon itself by the eversion of the acrosomal vesicle offers material for speculation with respect to the conditions under which the plasma membrane is stable. Comparison of Figs. 8, 9, and 11 with Fig. 12 suggests that this effect results specifically from contact between the apposed areas of plasma membrane. At the left side of Fig. 12, as in the three other sections, the apposed plasma membranes have vesiculated where they were pressed together under the acrosomal membrane, whereas no vesicle formation has occurred at the right side, where the membrane complex lies somewhat atypically free from the nucleus so that the apposing plasma membrane surfaces are not in contact with each other. The Colwins' study of sperm entry in *Hydroides* (2) has already demonstrated that the capacity for disposing in this way of plasma membrane no longer on the surface of the cell is not limited to the sperm acrosomal region, since similar fusion and vesiculation take place where the egg plasma membrane of the rising fertilization cone becomes apposed to the sperm plasma membrane at the sides of the sperm nucleus (see Colwin and Colwin, 2, Figs. 1 *c*, *d*; 12, 14 to 17).

Rosenbluth (11) has found that apposing plasma membranes in toad spinal ganglia give rise to aligned vesicles during or after osmium tetroxide, but not permanganate, fixation. He cites results of Robertson which suggest that the outside and middle layers of a plasma membrane are less well stabilized than the inner layer by osmium tetroxide, and consequently might recombine during the later processing of the tissues to produce such vesicles. However, he also points out that not all paired plasma membranes exhibit this phenomenon, indicating that an explanation based on purely physical factors is inadequate, and suggesting that some membranes are predisposed to break up into vesicles while others are not. The possibility that the vesiculation observed in this study may belong to Rosenbluth's category of osmium tetroxide fixation artifacts cannot be absolutely denied on the basis of the available micrographs, but the fact that the vesicles are conspicuously smaller and fewer in later fixations supports the interpretation presented above, that this vesicle formation constitutes a normal part of sperm cell reorganization during the acrosome reaction.

The present study shows, then, that the *Mytilus* acrosome reaction takes place in two phases, during the first of which a vitelline coat lysin is released and the outer side of the acrosomal vesicle is removed. Both these results follow automatically when the apical region is appropriately affected, and leave exposed the rudiment of the acrosomal process, made up of the invaginated posterior side of the acrosomal membrane and the rod in its lumen.

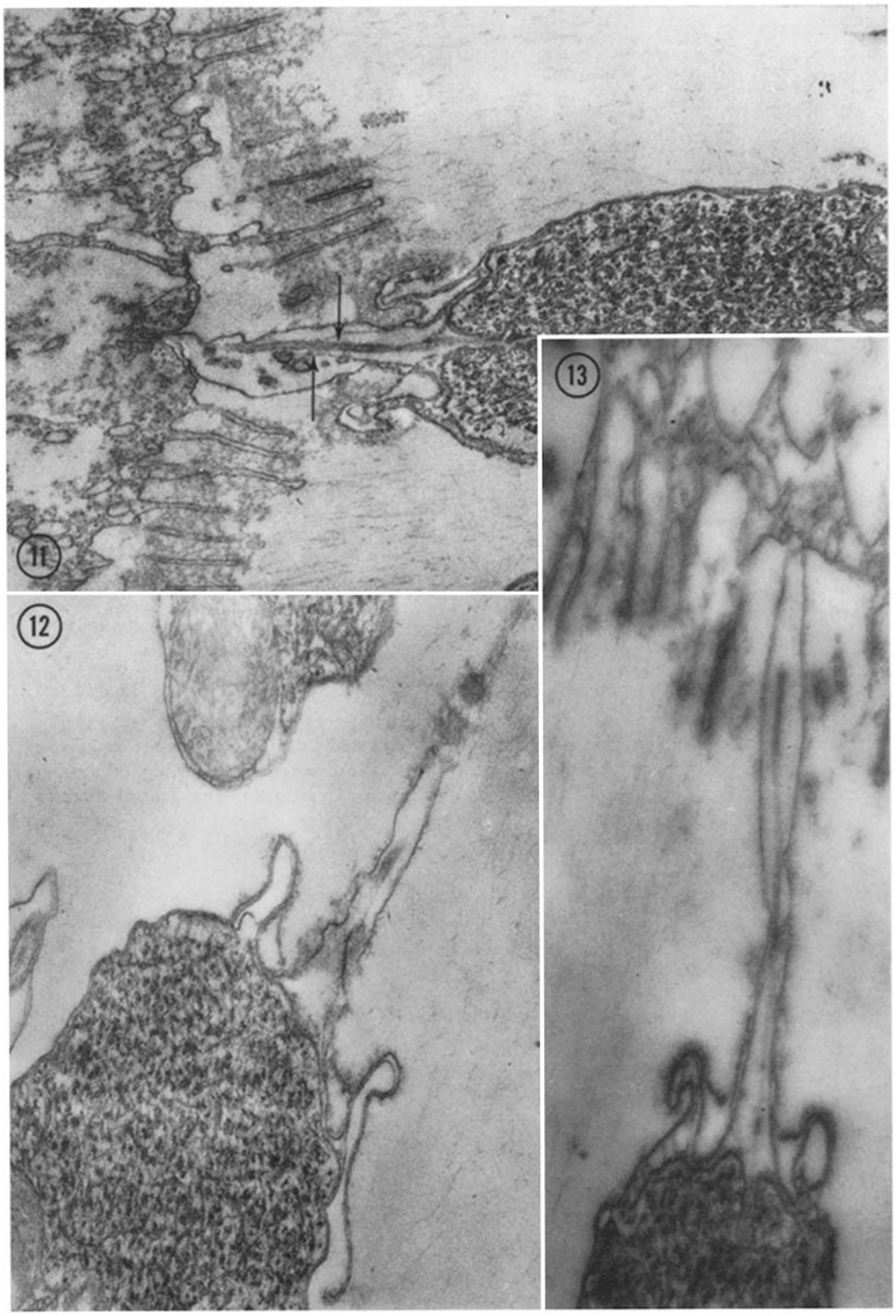
In the second phase of the reaction, the rod elongates and its covering of acrosomal membrane also undergoes some expansion. The first of these changes unquestionably takes place outside the acrosomal vesicle. Unless the invaginated portion

FIGURES 11 TO 13 Longitudinal sections of supernumerary spermatozoa at egg surface.

FIGURE 11 Egg plasma membrane is indented but not broken by tip of process; sleevelike structure now close to axial rod, especially distally (arrows). × 26,000.

FIGURE 12 Reflected membrane complex atypically separate from sperm head at right side of figure. Plasma membrane surfaces have not vesiculated where they are not in contact. × 32,000.

FIGURE 13 Relatively long process of spermatozoon not in immediate contact with egg surface. × 27,000.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of the acrosomal membrane is assumed to expand autonomously, possibly as the result of its exposure to the outer medium, the factors governing its expansion must also operate outside the vesicle. These two changes therefore appear to be different in nature from those which have preceded them, and to depend on a different mechanism. The most likely place to look for such a mechanism is the tubular passage through the nucleus, where, as described above, the basal end of the rod is continuous with a quantity of unoriented material. Further investigation will be required to determine whether the amount of this material is markedly reduced during the formation of the acrosomal process.

Even granted that unorganized precursor material within the nuclear passage is affected so that it forms more rod of a similar density and diameter, and thereby pushes forward the preformed section, it is not obvious how such a change is induced, unless the acrosomal membrane, now the only covering over the anterior side of the cell, is more permeable to sea water components than was the intact plasma membrane.

When the acrosome reaction is induced by excess calcium, the acrosomal process is much longer ($\sim$ 13 μ) than one formed on contact with the surface of an egg (Fig. 11). This suggests that the main function of the rod is to secure firm contact between its covering membrane and the plasma membrane of the egg, and that the extra length attained by the process in suspended spermatozoa is due to overextension in the absence of a resistant opposing surface.

As has been reported previously (4, 9, 12), supernumerary spermatozoa can be seen with the light microscope to be closely affixed to the egg surface immediately after insemination, but within 15 to 20 seconds they become detached by the movements of their flagella. The acrosomal processes of such spermatozoa, as observed with phase contrast, are less than 3 μ in length. This observation, however, applies only to spermatozoa which have been prevented by the "block to polyspermy" from eliciting the reaction on the part of the egg which would lead to incorporation of the sperm cell. Whether the acrosomal rod of a fertilizing spermatozoon reaches a greater length within the egg cytoplasm will have to be determined by sections through actually entering spermatozoa.

The most reasonable suggestion which can be made with respect to the part played by the membranoid sleeve surrounding the rod is that it may act as a valve to regulate the distribution of fluid within the elongating process. The folds which appear in the base of the acrosomal membrane (Fig. 10) as it is stretched to the new dimensions of the elongating process indicate that the diameter of this part has been reduced, presumably by withdrawal of the fluid contents to the more distal part of the process. Though this structural change provides evidence that such a shifting of fluid occurs, it does not afford an explanation of its cause.

Various observations indicate that the *Mytilus* "trigger" is in an especially unstable condition: many shed spermatozoa ($\sim$ 25 per cent) are found to have reacted on contact with sea water (4, 10), and reaction can be induced in nearly all cells by exposure to low temperature or calcium-rich sea water (13). Moreover, the low rate of acrosomal reaction obtained with *Mytilus* "egg water," and the presence around the *Mytilus* egg of structural elements, the properties of which are very different from those of the echinoderm jellies (5), suggest that factors other than the chemical influence of the jelly substance may affect the *Mytilus* acrosome trigger. Whether the nature of the inducing condition is chemical or physical or a combination of the two, its efficacy is greatly reduced by removing calcium from the medium.

This study, then, shows that the explosive eversion of the acrosome occurring in the *Mytilus* acrosome leads to the same general result as the apparently more gradual course of events seen in the *Hydroides* acrosome reaction (3). The presence of a single axial rod, on the other hand, links the resulting acrosomal process of *Mytilus* and probably other pelecypods (9) with those of the echinoderms (6–8) which have been studied in detail. In this latter group, however, the rod is formed entirely *de novo* at the time of reaction, whereas in *Mytilus* a preexisting segment either elongates or is pushed forward by new rod formation at its base.

Since the information now available indicates that these variously derived acrosomal processes are engaged in a single function, which the *Hydroides* study (2) has defined as that of mediating the fusion of sperm and egg plasma membranes, any characteristics which they display in common should be regarded as potentially important for an understanding of the way in which this func-

tion is performed. One such characteristic revealed by these morphological studies is that the outer face of the acrosomal process in every case consists of a newly exposed (*Hydroides*, *Mytilus*) or newly formed (sea urchin, starfish (6–8)) membrane surface. What special properties such a surface might be expected to possess as a result of this quality of newness is still wholly a matter of speculation.

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REFERENCES

1. COLWIN, A. L., and COLWIN, L. H., Fine structure of the spermatozoon of *Hydroides hexagonus* (Annelida), with special reference to the acrosomal region, *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, 211.
2. COLWIN, A. L., and COLWIN, L. H., Changes in the spermatozoon during fertilization in *Hydroides hexagonus* (Annelida). II. Incorporation with the egg, *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, 255.
3. COLWIN, L. H., and COLWIN, A. L., Changes in the spermatozoon during fertilization in *Hydroides hexagonus* (Annelida). I. Passage of the acrosomal region through the vitelline membrane, *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, 231.
4. DAN, J. C., The acrosome reaction, *Internat. Rev. Cytol.*, 1956, **5**, 365.
5. DAN, J. C., The vitelline coat of the *Mytilus* egg. I. Normal structure and effect of acrosomal lysin, *Biol. Bull.*, 1962, **123**, 531.
6. DAN, J. C., KUSHIDA, H., and OHORI, Y., Formation of the acrosomal process in echinoderm spermatozoa, Fifth International Congress Electron Microscopy, 1962, **2**, YY-12.
7. DAN, J. C., OHORI, Y., and KUSHIDA, H., Studies on the acrosome. VII. Formation of the acrosomal process in sea urchin spermatozoa, *J. Ultrastruct. Research*, 1964, **11**, 508.
8. DAN, J. C., and HAGIWARA, Y., Studies on the acrosome. VIII. Course of acrosome reaction in starfish spermatozoa, in preparation.
9. DAN, J. C., and WADA, S. K., Studies on the acrosome. IV. The acrosome reaction in some bivalve spermatozoa, *Biol. Bull.*, 1955, **109**, 40.
10. NIIJIMA, L., and DAN J., The acrosome reaction in *Mytilis edulis*. I. Fine structure of the intact acrosome, *J. Cell Biol.*, 1965, **25**, 243.
11. ROSENBLUTH, J., Contrast between osmium-fixed and permanganate-fixed toad spinal ganglia, *J. Cell Biol.*, 1963, **16**, 143.
12. WADA, S. K., Fertilization of *Mytilus edulis*, with special reference to the acrosome reaction of the spermatozoa, *Mem. Fac. Fisheries, Kagoshima Univ.*, 1955, **4**, 105.
13. WADA, S. K., COLLIER, J. R., and DAN, J. C., Studies on the acrosome. V. An egg-membrane lysin from the acrosomes of *Mytilus edulis* spermatozoa, *Exp. Cell Research*, 1956, **10**, 168.