MORPHOLOGY OF GAMETE MEMBRANE FUSION
AND OF SPERM ENTRY INTO
OOCYTES OF THE SEA URCHIN

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ABSTRACT

Sea urchin gametes predominate in molecular studies of fertilization, yet relatively little is known of the subcellular aspects of sperm entry in this group. Accordingly, it seemed desirable to make a detailed examination of sperm entry phenomena in sea urchins with the electron microscope. Gametes of the sea urchins Arbacia punctulata and Lytechinus variegatus were used in this study. Samples of eggs containing 2 to 8 per cent oocytes were selected and fixed with osmium tetroxide in sea water at various intervals after insemination. Fixed specimens were embedded in Epon 812, sectioned, and examined with an electron microscope. An apical vesicle was observed at the anterior end of the acrosome. The presence of this structure, together with other observations, suggested that initiation of the acrosome reaction in sea urchin sperm involves dehiscence of the acrosomal region with the subsequent release of the acrosomal granule. Contact and initial fusion of gamete membranes was observed in mature eggs and oocytes and invariably involved the extended acrosomal tubule of the spermatozoon. Only one spermatozoon normally enters the mature egg. The probability of locating such a sperm in ultrathin sections is exceedingly low. Several sperm do normally enter oocytes. Consequently, observations of sperm entry were primarily restricted to the latter. The manner of sperm entry into oocytes did not resemble phagocytosis. Organelles of the spermatozoon were progressively divested of their plasma membrane as they entered the ground cytoplasm of the oocyte fertilization cone. Initiation of the acrosome reaction, contact and initial fusion of gamete membranes, and sperm entry into oocytes of sea urchins conform to the Hydroides-Saccoglossus pattern of early fertilization events as described by Colwin and Colwin (13).

INTRODUCTION

Much of our current knowledge of the morphological, biochemical, and physiological aspects of fertilization stems from experiments with sea urchin gametes (see 4, 6, 34, 35, 39, 41, 46, 47). Morphological observations, especially at the submicroscopic level, necessarily complement experimental approaches to elucidation of fertilization mechanisms. Sea urchin gametes have been subjected to considerable examination in the electron microscope. Two important phenomena intimately associated with fertilization have been described, namely, the acrosome reaction by Dan et al. (21), and the cortical response of eggs to insemination by Endo (22) and by Wolpert and
incorporation of sperm into sea urchin eggs have been studied more thoroughly in other material. Manton and Friedmann (33) demonstrated coalescence of gamete cell membranes during oogamous fertilization of the green alga Prasiola stipitata. Colwin and Colwin, using mildly poly-spermic material, made a detailed electron microscope study of many aspects of sperm entry in the polychaete Hydrachis hexagonus (9–11, 16), and more recently in the enteropneust Saccoglossus kowalevskii (12, 17). These studies first demonstrated fusion of the everted acrosomal vesicle of sperm that had undergone the acrosome reaction with the egg cell membrane. Szollosi and Ris (42) also found fusion of gamete cell membranes in an independent electron microscope study of sperm entry in the rat. Their study is of particular value since the events observed were based upon normal conditions (i.e., non-polyspermic conditions). Thus, the phenomenon of gamete membrane fusion has been described in fertilization studies of representatives of three animal phyla and one plant division. Additional observations include spermatozoa in extended fertilization cones of the bivalve Spisula solidissima (Rebhun, 36), and sperm-egg attachment in Nereis (Takashima and Takashima, 44).

Since sea urchins predominate in molecular studies of fertilization, it seemed desirable to make a detailed examination of sperm entry in this group with the electron microscope.

Artificially induced polyspermy must be employed in most organisms to assure a reasonable chance of finding examples of sperm penetration. However, in sea urchins an alternative to artificial treatment of eggs or use of dense sperm suspensions is available. In sea urchins the immature eggs (oocytes) are normally polyspermic after insemination. Sea urchin oocytes are, in a sense, not fertilizable, because union or association of gamete nuclei does not occur and development is not initiated. However, several sperm do enter oocytes, and, with due reservation, the oocytes should serve as a useful model for the sperm entry phase of normal fertilization and have intrinsic interest as well. Accordingly, the major emphasis of the present investigation was on sperm entry into oocytes.

Runnström (40) has described some aspects of oocyte insemination including separation of sea urchin oocyte cytoplasm into three phases. Lönnning (31) has described ultrastructural surface and subsurface changes in oocytes resulting from insemination, and some aspects of sperm entry.

The present study provides a more complete account of initial stages of sperm entry into sea urchin oocytes, including attachment, membrane fusion, and incorporation of sperm organelles. In addition, some new features of sperm acrosome structure are described.

MATERIALS AND METHODS

Biological Material

Gametes were obtained from the sea urchins Arbacia punctulata and Lytechinus variegatus collected through facilities of the Alligator Harbor Marine Laboratory of the Oceanographic Institute, Florida State University. Arbacia punctulata were also collected at St. Andrews Park, Panama City, Florida, and through facilities of the Marine Biological Laboratory, Woods Hole, Massachusetts. Sperm of the sand dollar Melitella quinquiesperforata collected at Alligator Harbor were used in one experiment to inseminate mature eggs and oocytes of A. punctulata.

Arbacia punctulata specimens were induced to spawn by application of low voltage electric current (cf. Harvey, 25; Iwata, 28). Gametes of L. variegatus and M. quinquiesperforata were obtained by introduction
Abbreviations For Figures

All figures represent longitudinal sections of spermatozoa unless otherwise specified.

av, apical vesicle
c, centriole
cg, cortical granule
epm, egg plasma membrane
F, sperm flagellum
FC, fertilization cone
f, fibrillar elements within acrosomal tubule
fp, precursor substance of fibrillar elements (f)
g, acrosomal granule
M, mitochondrial material of sperm middle piece
m, mitochondrion
N, sperm nucleus
P, pigment granule
pv, periacrosomal vesicles
spm, sperm plasma membrane
T, acrosomal tubule
ti, tubular invaginations of basal region of acrosomal
granule membrane
vm, vitelline membrane
Y, yolk granule

Figure 1. A. Diagram of an unreacted sea urchin acrosome. A plasma membrane (spm) envelops the
entire spermatozoon. The acrosomal granule (g) is also
enclosed by a membrane, and the basal region of this
membrane contains tubular invaginations (ti). Sandwiched
between the acrosomal granule and the plasma
membrane is a small apical vesicle (av). Precursor ma-
terial of the fibrillar elements of the reacted acrosome
(fp) is located posterior to the acrosomal granule in an
anterior nuclear depression. Periacrosomal vesicles (pv)
are arranged in a ring around the rim of the nuclear de-
pression.

B. Diagram of a reacted sea urchin acrosome. The
acrosomal granule (g) has been forced to the exterior of
the spermatozoon through eversion of the membrane
which encloses the acrosomal granule. This membrane
now limits the acrosomal tubule (T), and has become
continuous with the plasma membrane. Fibrillar ele-
ments (f) have formed within the acrosomal tubule.

Figure 2. Unreacted acrosome of Lytechinus variegatus
sperm, illustrating the apical vesicle (av), periacrosomal
vesicles (pv), and precursor material (fp) of the fibrillar
elements of the acrosomal tubule. × 100,000.

Electron Microscope Preparative Methods

To obtain a sequence of stages of sperm penetra-
tion, mature eggs and oocytes were fixed at intervals
after insemination, the maximum interval being 10
minutes.

A variety of fixation, staining, and embedding
media and techniques were employed to prepare

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specimens for thin sectioning. Most samples were fixed in 1 per cent OsO₄ in sea water (at 4°C for 45 minutes to 1 hour), dehydrated in ethanol, and embedded in Epon 812 according to Luft's (32) recommendations. The water-soluble epoxy resin Durcupan A was used instead of alcohol or acetone for dehydration in a few cases to facilitate staining with aqueous solutions of phosphotungstic acid (1 per cent) or uranyl acetate (saturated) during dehydration. Contrast was increased in many other specimens by staining sections with the same heavy metals or, in a few instances, with lead plumbite using Karnovsky's (29) method A.

The large germinal vesicle of sea urchin oocytes is conspicuous in cured blocks of mixed eggs and oocytes when viewed at a magnification of 50 to 100X with transmitted light. Individual oocytes were selected for sectioning by this means.

Sections were cut with glass knives (scribed angle less than 45°, clearance angle usually 4°) on an LKB Ultratome with the 1 mm/sec. cutting rate modification. Most specimens were viewed with a Philips model 100B electron microscope with Ladd anode and a 25-μ objective aperture. The JEM 6B (courtesy of Fisher Scientific Instruments, Inc., at the Fertilization and Gamete Physiology Training Program, Marine Biological Laboratory, Woods Hole) and Philips model 75A electron microscopes were also used.
OBSERVATIONS

The Acrosome

The acrosomal region of sea urchin sperm is structurally complex. Under appropriate stimulation, as first shown by Dan (18, 19), the acrosome undergoes a striking change in structural organization called the acrosome reaction. The unreacted and reacted acrosome of Arbacia punctulata and Lytechinus variegatus sperm are illustrated.

Figure 6 Diagrammatic representation of successive stages of gamete contact and fusion, and of sperm entry into oocytes. Details are given in the text. A, contact of everted acrosomal tubule of the spermatozoon with the mature egg surface. B, fusion of the acrosomal tubule membrane with the egg plasma membrane. C, early stage of incorporation of the sperm nucleus into an oocyte. D, incorporation of the sperm middle piece and tail into an oocyte. E, migration of the incorporated spermatozoon to the organelle region of oocyte cytoplasm. F, migration of the spermatozoon into the organelle region of oocyte cytoplasm. The oocyte cytoplasm exhibits three phases at this time (cf. Löning, 31; Rünnström, 40).
diagrammatically in Fig. 1 and are described below, following the terminology of Colwin and Colwin (13). No significant interspecies differences in the morphology of the acrosomal region were observed. Several structural features of sea urchin acrosomes have been described by Afzelius (1, 2), Afzelius and Murray (3), Bernstein (5), Dan et al. (21), and Rothschild (37). However, some new features are revealed in the present study.

The acrosome consists of a membrane-bounded vesicle (cf. Bernstein, 5) together with its contents, the acrosomal granule. The acrosomal region, in addition, includes that portion of the sperm cell membrane which overlies the acrosome, and any periacrosomal material or structures within the immediate vicinity of the acrosome. Posterior to the acrosome is a nuclear depression (cf. Afzelius, 1). This depression contains a granular periacrosomal substance that apparently forms fibrillar elements in the reacted spermatozoon (Figs. 2 and 3). This substance was observed by Afzelius (1) and by Dan et al. (21). A small apical vesicle sandwiched between the acrosome and the cell membrane at the sperm apex was seen in a few specimens (Fig. 2). High magnification of favorable specimens also revealed tubular invaginations at the base of the acrosomal granule (Fig. 3). The apical vesicle and the tubular invaginations of the base of the acrosomal vesicle have been reported previously by the Colwins (10) in sperm of the polychaete Hydroides hexagonus, but not in sea urchin sperm. These structures may have particular significance in the acrosome reaction and initial stages of fertilization in sea urchins.

Elongate periacrosomal vesicles (Fig. 2) have been observed by Dan et al. (21) and can be seen in Afzelius' micrographs (1). These vesicles are stacked around the rim of the depression at the acrosomal end of the nucleus and they ring the acrosome. Fig. 4 represents non-median, longitudinal sections that pass through these periacrosomal vesicles.

![Figure 7](image-url) Contact of the acrosomal tubule of a spermatozoon with a mature egg surface. Note fibrils (f) within the acrosomal tubule, and the thin vitelline membrane (vm) of the egg, Arbacia punctulata. X 36,000.
The Acrosome Reaction

As stated in the Introduction, the acrosome reaction of sea urchin sperm has been described by Dan et al. (21). Their observations are generally confirmed for *A. punctulata* and *L. variegatus* by the present investigation.

During the acrosome reaction the membrane of the acrosomal vesicle everts to form a fingerlike projection, the acrosomal tubule (Figs. 5, 7 to 10). As the acrosomal vesicle everts, material of the acrosomal granule is forced to the outside of the sperm (Fig. 5), and in fully reacted acrosomes this material is retained as a membrane-free ring around the basal region of the everted tubule (Fig. 8). During elongation of the acrosomal tubule, granular matter formerly situated posterior to the acrosomal granule reorganizes into long fibrillar elements (Fig. 7). At the completion of the reaction the wall of the acrosomal tubule is continuous with the sperm cell membrane and is noticeably thicker in the region surrounded by acrosomal granule material (Figs. 8 to 10). It should be emphasized that the acrosome reaction results in exposure of a new surface membrane at the sperm apex, the acrosomal tubule membrane. This membrane formerly bounded the acrosomal vesicle. Thus the inner surface of the membrane of the acrosomal vesicle becomes the new cell surface at the sperm apex. This event has been demonstrated in sperm of *Hydroides hexagonus* and *Saccoglossus kowalevskii* by the Colwins (12, 13, 16).

Contact and Fusion of Sperm and Egg (Fig. 6 A and B)

Spermatozoa found near the egg surface were consistently surrounded by a netlike substance (Fig. 5) that bore some resemblance to egg jelly but was more intensely stained and of coarser

**Figures 8 and 9** Initial fusion of the sperm acrosomal tubule (*T*) with the plasma membrane (*epm*) of the mature egg. The acrosomal tubule membrane is continuous with the sperm plasma membrane, hence is labeled either *T* or *spm*. Sites of fusion are indicated by arrows. *Arbacia punctulata*. Fig. 8, × 57,500; Fig. 9, × 87,500.
consistency. Characterization of this substance was not attempted. Sperm situated in the egg jelly coat usually, but not invariably, had reacted acrosomes.

Contact of sperm and mature egg (or oocyte) prior to membrane fusion was most frequently found in specimens fixed 10 to 20 seconds after insemination. The apex or thin-walled portion of the acrosomal tubule had pressed into the egg surface (between cortical granules, except in one case) so that the thick-walled region of the acrosomal tubule, still surrounded by acrosomal granule material, was level with the egg surface (Fig. 7). Initial stages of fusion of sperm and egg cell membranes were usually found in specimens fixed 20 to 30 seconds after insemination. Such fusion invariably involved the newly exposed sperm surface of the everted acrosomal tubule. Fusion at this region of the spermatozoon allows direct confluence of fibrils and other substances within the acrosomal tubule with the cortical cytoplasm of the mature egg or oocyte (Figs. 8 to 10). Observations of contact and initial fusion of the gametes were made only in *A. punctulata*.

The first visible response of mature sea urchin eggs to insemination (as seen by electron microscopy) is delamination and elevation of the vitelline membrane from the cell surface, accompanied by disruption of cortical granules. These processes constitute part of the "cortical reaction," and have been investigated in detail with the electron microscope in other sea urchins by Endo (22) and Wolpert and Mercer (51). Light microscope observations such as those reported by Wilson and Leaming (50) and reviewed by Harvey (26) have shown that a hyaline protrusion (fertilization cone) forms at the point of sperm attachment to the egg or oocyte surface. Electron microscope observations have confirmed the hyaline (ground substance) composition of the fertilization cone (23, 31, 40, 43). In oocytes, or in mature eggs undergoing experimentally induced refertilization (*cf.* Kojima, 30), fertilization cones are usually numerous and greatly exaggerated in size (Fig. 16). The fertilization cones of sea urchin oocytes are generally resorbed after 10 to 20 minutes, but may occasionally persist beyond 30 minutes (*cf.* Hagström and Lönng, 24; Runnström, 40).

**FIGURE 10** Initial fusion of the sperm acrosomal tubule with the plasma membrane of an oocyte. Sites of fusion are indicated by arrows. *Arbacia punctulata.* X 42,000.
FIGURES 11 AND 12  Early stages of entry of the sperm nucleus. The oocyte and sperm membranes are continuous. Small vesicles are evident along the regions of contact of sperm nucleus and oocyte cytoplasm (arrows). Arbacia punctulata. Fig. 11, × 30,000; Fig. 12, × 30,000.

Incorporation of Sperm into Oocytes (Fig. 6 C and D)

Contact and initial fusion of gamete membranes were observed in both mature eggs and oocytes of A. punctulata. Observations of sperm entry were restricted to oocytes (with one exception) but included specimens of both A. punctulata and L. variegatus. Examination of thin sections of oocytes fixed at intervals of 30 to 40 seconds after insemination revealed several ultrastructural changes in the gametes. Stages of sperm incorporation found during this interval ranged from early entry of the sperm nucleus (Figs. 11 and 12) to entry of the entire nucleus and middle piece (Fig. 13). Even at the early stages the sperm nucleus had expanded and lateral portions of the nuclear membrane had apparently disappeared (Fig. 11). During these early stages of entry small membranous vesicles were evident at the boundary region of the sperm nucleus and oocyte ground substance (Figs. 11 and 12). Whether these vesicles

FIGURE 13  An incorporated sperm nucleus and middle piece. The sperm plasma membrane is clearly absent. Arbacia punctulata. × 35,000.

FIGURE 14  Incorporation of the sperm tail. The membrane of the tail (spm) is still intact. Lytechinus variegatus. × 18,000.
FIGURE 15  Migration of the spermatozoon to the organelle zone of cytoplasm. The centrioles (c) and the middle piece (M) maintain their original positions relative to the sperm nucleus. Arbacia punctulata. × 29,000.
FIGURE 16  Migration of the spermatozoon into the organelle zone of oocyte cytoplasm. The three cytoplasmic phases (I, II, III) described by Runnström (40) are evident at this time, 8 minutes after insemination. Arbacia punctulata. × 17,000.
represent incorporated portions of the fused sperm-oocyte plasma membrane or a vesiculative degeneration of the sperm nuclear membrane is not clear. Portions of the sperm nuclear membrane at the basal or centriolar region of the nucleus (cf. Rothschild, 38) and at the apex or acrosomal region of the nucleus remain intact during and after entry of the spermatozoon (Figs. 13, 15, and 17).

In later stages of sperm entry found during the same interval (30 to 40 seconds after insemination) the sperm nucleus and middle piece had been incorporated into the ground substance of the fertilization cone, and the sperm plasma membrane was clearly absent from these organelles (Fig. 13).

Early stages of engulfment of the tail were seen in two cases. In the first the sperm tail had partly entered the fertilization cone but the membrane of the tail was still intact (Fig. 14). A micrograph by Takashima and Takashima (45, Plate 6) shows a similar situation in a nicotine-treated mature egg. In the second case, a hybrid cross between *Melitina quinquiesperforata* sperm and *Arbacia punctulata* oocytes, sperm flagella that had just been incorporated were surrounded by small membranous vesicles (Fig. 19). Portions of sperm tails deeper within the same oocyte were also surrounded by such vesicles. Although similar vesicles were present throughout much of the oocyte cytoplasm, observations of a series of sections of this particular specimen revealed a definite aggregation of the vesicles around sperm flagella. These vesicles may consist in part of sperm cell surface. This suggests that the sperm membrane does not remain as a part of the egg surface (cf. Tyler, 48); however, this question has not been tested experimentally.

### Sperm Migration in Oocytes (Fig. 6 E and F)

Within 2 to 21½ minutes after insemination of oocytes the sperm nucleus, middle piece, and sizable portions of the tail had generally migrated as a unit deep into the base of the fertilization cone (which had reached maximum size) to the structureless boundary separating mitochondria, Golgi bodies, yolk, and other cytoplasmic organelles from the ground cytoplasm of the fertilization cone. A degree of rotation of spermatozoa (cf. Wilson and Learning, 50) was sometimes evident in these preparations. The centrioles were still in the same relative positions as in normal, free spermatozoa. Organoids of the deeper cytoplasm were generally more compact than at previous stages. This stage is shown in Fig. 15.

The last stage of sperm entry examined is represented by Figs. 16 and 17. These figures show *A. punctulata* oocytes fixed at 8 minutes after insemination. Observations of this stage were limited to *A. punctulata*. Sperm morphology and positions of sperm components relative to one another were still unchanged from the condition at 21½ minutes after insemination. It is possible, however, that the middle piece and portions of the tail had separated in some specimens. These sperm organelles were now situated well within the organoid-laden zone of the oocyte. Fertilization cones were still present and contained sperm flagellar remnants.

No indication of aster formation was found in these oocytes. Hagström and Lönning (24) have previously observed that sperm asters do not form in inseminated sea urchin oocytes. Harvey (26) reported that aster formation occurs 8 minutes after insemination of mature *A. punctulata* eggs at 23°C (24°C used here).

Fig. 16 demonstrates the three phases of oocyte cytoplasm that have been previously described by Runnström (40), and by Lönning (31). The intermediate phase (Runnström's zone II) was first evident in *A. punctulata* oocytes fixed at 8 minutes after insemination, after sperm had migrated deep into the oocyte.

### "Hybrid" Oocytes

*Arbacia punctulata* oocytes were inseminated with sperm of the sand dollar *Melitina quinquiesperforata* in one experiment to determine whether or not species differences in sperm and oocyte cell membranes would be reflected in the mode of entry of heterologous sperm into oocytes. Only 2 per cent (2:100) of mature eggs in this cross cleaved and developed to the prism stage. Of 52 oocytes from the same female, 85 per cent characteristically formed exaggerated fertilization cones. Oocytes fixed at 10 minutes after cross-insemination were examined in the electron microscope. In one instance an extended acrosomal tubule was seen to be fused with an oocyte surface, in the same manner as with homologous sperm. The process of sperm entry undeniably involved membrane fusion (Fig. 18) as with homologous sperm. As previously stated, sperm flagella, found during or after entry, were surrounded by small vesicles whether deep or near the oocyte surface (Fig. 19).
FIGURE 17  Spermatozoa within the organelle zone of oocyte cytoplasm. Note the extensive portion of a sperm flagellum (F). Arbacia punctulata. X 30,000.
DISCUSSION

Sea urchin sperm structure, both before and after the acrosome reaction, contact and fusion of sperm with mature eggs and oocytes, and penetration of sperm into oocytes, has been examined in this study.

Structure and Reaction of the Sperm Acrosome

Observations of the acrosomal region of sea urchin sperm and the finding of an apical vesicle in the acrosomal region suggest that initiation of the acrosome reaction in this group follows the pattern of dehiscence and fusion of the cell membrane and acrosomal membrane shown in *Hydroides hexagonus* sperm by Colwin and Colwin (10, 16). In other respects the definitive description of the acrosome reaction of sea urchin sperm published by Dan et al. (21) is strongly supported.

The exact role of the acrosomal granule in sea urchin fertilization has not been demonstrated. However, in several other invertebrates (cf. Colwin and Colwin, 8, 15; Dan, 20; Wada, Collier, and Dan, 49) this material evidently contains a lysozine which dissolves or weakens extracellular egg membranes to facilitate contact of the sperm and egg plasma membranes. Echinoid sperm do contain an agent which disperses egg jelly (cf. Brookbank, 7; Hathaway, 27). This agent may be a constituent of the acrosomal granule.

The structural components of the sperm acrosomal region that are of more immediate interest are those that interact directly with the egg and initiate the events of fertilization. These include the evaginated acrosomal tubule of the reacted sperm and the contents of the tubule. A new sperm surface is exposed by the acrosome reaction. This surface, the limiting membrane of the acrosomal vesicle, evaginates and becomes continuous with the plasma membrane of the bulk of the sperm during the acrosome reaction. It is this newly exposed surface, which may differ in composition and properties from the original sperm plasma membrane, that first contacts and fuses with the egg plasma membrane. Moreover, fusion of the acrosomal tubule with the egg plasma membrane affords direct confluence of the contents of the acrosomal tubule (including fibrillar elements) with cortical cytoplasm of the egg. These observations are consistent with previous observations of Colwin and Colwin in *Hydroides hexagonus* and *Saccoglossus kowalevskii* (11, 13, 17).

The major emphasis of this study has been on examination of sperm penetration into oocytes. As previously stated, sperm that enter oocytes do not form asters (cf. Hagström and Lönnings, 24) and do not participate in karyogamy. Furthermore, the propagated cortical response of mature sea urchin eggs to insemination does not occur in oocytes. Formation of a sperm aster and karyogamy have no bearing upon the mode of sperm entry. Both of these events occur after sperm penetration. The propagated cortical response of mature eggs to insemination functions to prevent polyspermy entry in part through formation of an elevated fertilization membrane. There is no evidence that the ability of the egg to undergo a propagated cortical response influences the mode of entry of the spermatozoon. Although insemination of oocytes cannot be considered to represent normal fertilization, the sperm entry events in mature eggs and in oocytes appear to be similar, except for the exaggerated size of the fertilization cone in the latter.

The accumulation of ground substance to form sperm entry cones at sites of successful sperm-egg interaction occurs in sea urchin oocytes, normal mature eggs, and nicotine-pretreated mature eggs (cf. Takashima and Takashima, 43), and, under certain conditions, apparently occurs in

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Figures 18 and 19 Entry of *Melita quinquiesperforata* sperm into *Arbacia punctulata* oocytes.

Fig. 18. A cross-section of a sperm middle piece within a fertilization cone. A second sperm is entering at the right. The membrane of the latter (*spm*) is continuous with the oocyte membrane (*opm*). × 11,500.

Fig. 19. Portions of entering sperm flagella (*F*), surrounded by small vesicles. × 34,000.
reinseminated mature eggs and insenminated mature eggs that have been reactivated by artificial means (cf. Kojima, 30). Formation of everted acro-
oocytes conforms to the pattern of sperm-egg interaction and sperm penetra-
tion as described by Colwin and Colwin (9, 11–13, 16, 17).

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