A PARTIAL CHARACTERIZATION OF THE CYTOSKELETON IN THE EGGS OF *URECHIS CAUPO*

by

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ABSTRACT

I have partially characterized the previously undescribed cytoskeleton of *Urechis caupo* (Fisher and MacGinitie, 1928) eggs by fluorescence microscopy and electron microscopy. Actin in fixed cells was made visible by labelling with NBD-Phallacidin (specific for F-actin) and by indirect immunofluorescence. NBD-Phallacidin fluorescence is high in oocytes, but after fertilization the amount diminishes nearly to the level of the control by 2 minutes post-insemination (PI). By four minutes PI the amount increases, but not to the original level. The fluorescence of NBD-Phallacidin-labelled eggs is not eliminated by treatment with cytochalasin B, a drug which disrupts actin filaments, but the staining pattern is altered. This change in F-actin may be related to the change in shape which occurs during fertilization as the eggs lose their characteristic indentation and round out.

A continuous, occasionally filamentous, electron-dense band immediately subjacent to the plasma membrane is visible in transmission electron micrographs of the oocytes. This dense band may also be represented as a peripheral substructure of the cytoskeleton in detergent-extracted eggs.

I examined eggs extracted with the non-ionic detergent Triton X-100 with scanning electron microscopy and found an extensive detergent-resistant cytoskeleton. The nucleus and cytoplasm contain an extensive array of filaments, possibly including the F-actin which is visible in NBD-Phallacidin treated eggs. The outer boundary of the cytoskeleton is an anastomosing web which is connected to the internal cytoskeleton. In thin sections of extracted cells, the peripheral component of the cytoskeleton is strikingly similar to the dense band of unextracted eggs, and they may represent the same cell structure.

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I INTRODUCTION

Unfertilized eggs[1] are quiescent cells with a low metabolism. After fertilization the cell cycle is reinitiated, macromolecular synthesis is increased, and cytoplasmic reorganization may occur. In spite of considerable investigation into the processes of fertilization, the role of egg cytoskeleton is poorly understood.

The cytoskeleton has recently been recognized as an important cellular component of all eukaryotic cells studied so far, and at least one prokaryote (Gobel *et al.*, 1981). The term "cytoskeleton" generally refers to a network of filamentous proteins composed mainly of actin, tubulin and intermediate filaments, with up to 200 or more other proteins associated with the main framework (Schliwa and Blerkom, 1981). It is involved in contractility, motility and cell structure (Allen, 1980; Pollard, 1981; Fey *et al.*, 1984). For reviews of cytoskeleton and cytomusculature of non-muscle cells, see Brinkley (1982) and Goldman, *et al.*, (1981). Because of the physical stability of this network it resists detergent extraction, and the term "cytoskeleton"

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[1] I will use the general term "egg" to refer to any of the stages properly called "oocyte", "ovum" or "zygote", and use the specific terms when necessary.

has also been defined as the portion of the cell remaining after extraction (Moon *et al.*, 1983).

By virtue of their amenability to manipulation and availability, sea urchin eggs have been studied most intensively. Immediately after fertilization sea urchin eggs undergo an increase in metabolic activity. Intracellular pH rises as H^+ is released from the cell as "fertilization acid" (Johnson *et al.*, 1976). The change in pH triggers an increase in protein synthesis and regulates a massive polymerization of actin in the cortex and the elongating microvilli (Begg and Rebhun, 1979; Shen and Steinhardt, 1978; Spudich and Spudich, 1979; Wang and Taylor, 1979). For a review of actin polymerization in the sea urchin egg see Kane, (1979). The surface topography changes greatly as a local increase in Ca^{2+} concentration triggers cortical granule exocytosis. This creates a large increase in the surface area of the plasma membrane. Part of this excess of membrane is accommodated in the increased surface area of the elongated microvilli, but some is also taken up by coated vesicles and recycled (Fisher and Rebhun, 1983). The stiffness of the cortex increases up to fivefold by one minute after fertilization, and may be related to the polymerization of actin. These phenomena are reviewed by Vacquier (1981).

Sperm penetration cones (localized extensions of egg cytoplasm which surround the fertilizing sperm) are rich in newly polymerized F-actin, and sperm incorporation is sensitive to cytochalasin B, a drug which disrupts actin filaments (Cline *et al.*, 1983; Schroeder, 1969; Tilney and Jaffe, 1982; Wessells *et al.*, 1971). Later, cortical actin in the contractile ring of the cleavage furrow is involved in cell division (Schroeder, 1976). The relationship of cytoskeleton to cleavage in sea urchin eggs is reviewed by Schroeder (1981).

During early development of sea urchin microtubules do not play a major cytoskeletal role other than in aster and spindle formation (Anstrom and Summers, 1983; Balczon and Schatten, 1983; and Bestor and Schatten, 1981). Although the sea urchin egg is spherical, Presnov and Isaeva (1983) showed that the cytoskeleton is important in the maintenance of the cell's shape.

Sand dollar eggs also contain a cortical F-actin network (Hamaguchi and Mabuchi, 1982). In starfish eggs as well there is burst of actin polymerization, and a rise in Ca^{2+} concentration in the cortex after fertilization (Schroeder and Stricker, 1983). This is followed by a period of actin depolymerization, which may be mediated by depactin, a protein which depolymerizes F-actin (Mabuchi, 1981).

Cortical F-actin is also found in eggs of other phyla. *Tealia crassicornis* (Cnidaria) eggs have no investing layers, but are covered with protective spires of microvilli containing dense bundles of actin filaments (Schroeder, 1982). Cortical actin can be detected in vertebrate eggs. Rat and mouse oocytes contain cortical microfilaments which may be associated with an underlying cytoskeleton (Amsterdam *et al.*, 1977; Battaglia and Gaddum-Rosse, 1984; Lehtonen and Badley, 1980). The cytoskeleton may be involved in the mechanism of meiotic maturation in goldfish and leopard frog eggs, since microfilaments are associated with the migrating pronuclei (Habibi and Lessman, 1984). The cytoskeleton changes through the first cell cycle of frog embryos and probably plays a role in ooplasmic segregation (Elinson, 1983).

The cytoskeleton of ascidian eggs contains two major components, an internal filamentous network, and a peripheral layer revealed after detergent extraction. After fertilization, these cytoskeletal substructures change position during ooplasmic segregation. The cytoskeleton, and the mRNA attached to it, may represent respectively, the "ground substance" and the "cytoplasmic determinants", proposed by Wilson (1925) (Jeffery, 1982, 1983).

The detergent-resistant cytoskeleton of sea urchin eggs has only recently been described in detail by Moon *et al.* (1983). The unfertilized eggs of at least three species (of different genera) have an internal filamentous network of cytoskeletal fibers, similar to that found in ascidian eggs. There is a cortical cytoskeletal sub-structure probably composed of remnants of cortical granules and pigment granules. After fertilization, the cytoskeleton increases in complexity. A peripheral layer of filaments appears in the cortex, probably newly polymerized actin, and more mRNA becomes associated with the inner cytoskeleton. Our ideas of the internal structure of eggs in general and of sea urchin ova in particular are changing rapidly. Only two years ago Schatten (1983) stated, "Unfertilized eggs are remarkable among eukaryotes in lacking any formally organized cytoskeletal array". It has now become clear that this is not the case.

Although sea urchins are the most popular choice for studies of fertilization, they do not represent the whole of the animal kingdom. In fact, they are unusual in that they are shed and fertilized as haploid ova while most other animal eggs are fertilized as oocytes which have not completed meiosis. It is important to study a wide variety of species so that general characteristics can be recognized and specific differences compared.

The eggs of *Urechis caupo*, the fat innkeeper worm (also weenie worm or penis worm), share some advantages with sea urchin eggs and may be superior for some experiments. *U. caupo* gametes are available throughout the year and require no difficult techniques for handling them. They can be easily fertilized *in vitro* and develop synchronously. *U. caupo* is a representative of the protostome phyla and therefore provides information for comparative phylogenetic studies. There are similarities between sea urchin and *U. caupo* that are interesting in terms of comparative fertilization studies. They are both small, non-yolky eggs, and *U. caupo* eggs fall within the size range of the species of sea urchin eggs used most often in developmental biology. They both exhibit a rapid polyspermy block which is related to a change in membrane potential and they release a similar amount of fertilization acid (Gould-Somero *et al.*, 1979; Jaffe, L.A., 1976). *U. caupo* eggs offer an opportunity to test, in another phylum, hypotheses developed from the study of sea urchin eggs in an egg which has both similarities and differences.

U. caupo eggs are characteristically indented with one, two or occasionally three depressions (fig.1). After fertilization a change in cell shape occurs as the egg rounds out, and by four minutes post-insemination (PI), the egg is spherical.

I hypothesized that (1) there might be differences in cytoskeletal structures between the dented and undented regions and (2) that the change in shape might be related to a change in the cytoskeleton. In thin sections of *U. caupo* eggs there is a band of electron-dense material beneath the plasma membrane (M. Paul, unpublished observations). This structure might represent a cytoskeleton.

The objectives of this study were to determine whether (1) a cytoskeleton is present in *U. caupo* eggs, (2) the dense band seen in thin sections of *U. caupo* eggs is a part of a cytoskeletal network, (3) there is an F-actin component in the cytoskeleton and whether it is related to the maintenance of cell shape or the change in cell shape that occurs as a response to fertilization.

II MATERIALS AND METHODS

Collection and storage of Animals and Gametes

Living *U. caupo* were collected from Elkhorn Slough, Moss Landing, California. The worms were kept in aquaria with a few centimeters of mud in which they could burrow.

I collected gametes by gently stimulating the genital pores with a blunt glass probe, allowing the oocytes to be released below the surface into filtered sea water (FSW). Sea water was collected from Haro Straight (20m) and filtered through Whatman's #1 filter paper, then adjusted to the desired pH. The oocytes were filtered through 70 mesh bolting cloth then rinsed at least twice in FSW, all at 15°C. Sperm were collected without exposure to sea water with a Pasteur pipette, stored at 4°C in 0.5 ml Eppendorf tubes then diluted 1:1000 in FSW before insemination.

Transmission Electron Microscopy (TEM)

Eggs were fixed by one of three methods: I. Fixation at 4°C for 3 hours in 3% glutaraldehyde, 1% $K_2Cr_2O_7$ (adjusted to pH 7.4 with 1M KOH) with 0.6M NaCl, pH 7.4. Fresh fixative was added after 15 minutes. Three rinses, fifteen minutes each, at 4° in 1% $K_2Cr_2O_7$--KOH, 0.6M NaCl, pH 7.4 were followed by postfixation for 1 hour at 0°C in 1%OsO_4 in 1% $K_2Cr_2O_7$--KOH, 0.8M NaCl, pH 7.4. The eggs were rinsed in double distilled water to remove fixatives before dehydration. II. I followed the same procedure outlined above, but rinsed the eggs briefly in 0.2% tannic acid before postfixation. III. This method was a modification of the "Eisenman cocktail" (Eisenman and Alfert, 1981). I prefixed the eggs in 0.25 ml fresh postfixative, plus 5.0 ml fixative, pH 7.0 for 5 minutes at $4^{O}C$ just before primary fixation (fig. 8). Otherwise, the procedure was the same as outlined in method I.

Eggs were dehydrated through a series of 30%, 50%, 70%, 2 X 95% and 2 X 100% ethanol at room temperature, then rinsed three times (ten minutes each) in propylene oxide to remove the alcohol.

I embedded dehydrated material in Epon 812, or Polybed 812, by standard methods (Luft, 1961), and cut silver-grey sections (approximately 50-60 nm thick) using a Diatome diamond knife on a Reichert Om U2 ultra microtome. Sections were stained with uranyl acetate and lead citrate, then examined and photographed with a Phillips 300 transmission electron microscope, at 60 or 80 kV.

Scanning Electron Microscopy (SEM)

For SEM, cells were fixed and dehydrated as for TEM, method I, except that fixation and postfixation times were one hour each. Eggs were critical point dried in $C0_2$, and dropped onto stubs covered with double sided tape. They were sputter coated with gold-palladium in a Technics Hummer sputter coater, then viewed and photographed with a Jeol JSM 35 scanning electron microscope.

NBD-Phallacidin Labelling

Oocytes were suspended in phosphate buffered saline (PBS); Dulbecco's PBS = 0.14M NaCl, 2.4mM KCl, 8mM Na_2HPO_4, 1.6mM KH_2PO_4, 1mM $CaCl_2$ and 0.5mM $MgCl_2$, pH 7.8, Schloss *et al.* (1977). At various times postinsemination I removed 50 µl of eggs and added them to 50 µl of a 2X concentrated stain solution. The final stain solution consisted of 3.7% formaldehyde, 100 U/ml NBD-Phallacidin, a stain specific for F-actin (Barak and Yocum, 1981), 0.1 mg/ml palmitoyl lysolecithin (palmitoyl phosphatidyl choline) in PBS. The eggs were stained overnight at 4°C, washed once in PBS and mounted in PBS-glycerol, 1:1. The slides were kept at 4°C until immediately before viewing.

Actin-Antibody Labelling

Eggs were fixed in 3.7% formaldehyde with 0.05 mg/ml lysolecithin for one hour, then rinsed three times in PBS, all at 4°C. I added polyclonal anti-actin from rabbit, diluted 1:10 in PBS, (Miles Laboratories) and anti-starfish gonad-stimulating substance from rabbit (isolated by G. Caine), diluted 1:10 in PBS, to controls. The samples were kept at $4^{O}C$ overnight, and rinsed three times in cold PBS the next morning. All the samples were then incubated for two hours in monoclonal fluorescein-conjugated anti-rabbit IgG from goat (Sigma), diluted 1:50 in PBS, for two hours then rinsed three times in PBS, and mounted in PBS-glycerol, 1:1, all at $4^{O}C$. All of the antibodies were generous gifts from Robert D. Burke.

Photography

Fluorescence micrography was accomplished with a Zeiss Universal microscope fitted with epifluorescence apparatus and a 100W mercury burner. An excitation filter with peak transmittance at 364 nm was used in conjunction with a chromatic beam splitter (FT420) and a barrier filter (LP418) reflecting light below 418 nm.

Using 400 ASA black and white or colour negative film, I photographed fluorescent cells through a 40X objective (numerical aperture = 0.75) or a 100X objective (numerical aperture = 1.3), both Zeiss Neofluar lenses. Exposure time was fifteen seconds with a reciprocity of 12. For the series of photographs showing change in fluorescence over time, (fig 4), all the printing was done at the same time, under identical conditions. Light micrographs were also taken with this microscope, on 100 or 400 ASA film.

Cytochalasin B

Eggs were treated with cytochalasin B (CB) (Sigma), a drug which disrupts actin filaments, (Schroeder, 1969; Wessells *et al.*, 1971), before staining with NBD-Phallacidin to determine whether it disrupted the pattern of stained actin filaments. A stock solution of CB in DMSO (2 mg/ml) was added to egg suspensions for a final concentration of 2X 10^{-6}M in FSW. After incubating the eggs for 10 minutes, I washed out the cytochalasin with three rinses in filtered sea water, then immediately fixed and labelled the eggs with the NBD-Phallacidin stain solution described above. This concentration of CB blocks sperm nucleus penetration in *U. caupo* eggs, but does not block sperm induced egg activation (Gould-Somero *et al.*, 1977).

Surface Coat Removal

I rinsed fertilized or unfertilized eggs three times in a solution consisting of equal parts of 0.5M glucose with 10mM EGTA and 0.87M sucrose with 5mM EDTA, pH 7.8 (modified from Gould-Somero and Holland, 1975 and Engstrom, 1971), sedimenting the eggs during each rinse in 15 ml conical tubes by hand centrifugation. I monitored the progress of surface coat dissolution by examining samples taken every few minutes, and when the outer layer appeared broken I gently forced the cells in and out of a pipette to break and remove both the surface coat layers. By this time the solution was warmed somewhat, and this appeared to improve the results.

When the surface coat was removed, I rinsed the eggs three times in warmed FSW, then slowly cooled the suspension back down to fifteen degrees. It was important to ensure that all the surface coat was dissolved, for returning the eggs to sea water (which contains calcium) caused the inner layer to herden again. However, extended incubation in the sugar solution caused cell lysis.

Different batches of eggs responded differently to this treatment. In 1982 the glucose solution alone worked best, but the next year the combination of glucose and sucrose was superior (which I used most often). There was one batch of worms whose eggs lost their surface coat quickly and completely after three washes in Mg-free SW (Cavanaugh, 1956) with 10mM EGTA, if the suspension was allowed to reach room temperature (fig 15c). However, this was not repeatable with later batches.

I suspect that the age of the worms, the time of year they were collected, and the length of their residence in aquaria all affect the properties of the surface coat and the ease with which it may be removed.

Detergent Extraction

The method for extraction was adapted from Jefferey and Meier (1983). Eggs were rinsed twice in extraction buffer (EB) consisting of 10mM piperazine-N,N'-bis [2-ethane sulfonic acid] (PIPES), 300mM sucrose, 100mM KCl, 100mM NaCl, 5mM MgAcetate, 1mM EGTA, and 10^{-6}M Leupeptin, pH 6.8 at 15^{o}C, then incubated for one hour, in EB with 0.5% Triton X-100, at either 15 or 4^{o}C. The addition of bovine trypsin inhibitor (2 x 10^{-6}g/ml), as a second enzyme inhibitor had no more effect than the leupeptin alone. During this time the cells rounded out and after 45 minutes, they became pale pinkish-orange in colour and somewhat transparent, indicating that extraction was complete. The extracted cells at this point were extremely fragile and required careful handling. The cells were then washed twice in EB without detergent and processed for electron microscopy.

III RESULTS

A band of electron-dense material, averaging 100 nm thick, is visible just beneath the plasma membrane in thin sections of *U. caupo* oocytes (fig 2,3). This band appears in fertilized and unfertilized eggs throughout the cortex, in both indented and unindented regions. The band is discontinuous in the plane of thin section (fig 2), ending abruptly, or fading away gradually. Serial sections show that this band is prominent in one section and nearly absent from the next (fig 3). These observations show that the dense band is not a continuous sheet, or sphere. In some sections (fig 2) there are filamentous structures in the dense band.

Fluorescent Labelling

To determine whether *U. caupo* eggs had F-actin as a component of the cytoskeleton I stained the cells with NBD-Phallacidin. There was intense fluorescence in unfertilized eggs compared to controls (fig 4a,b). There is a decrease in the amount of F-actin between 30 sec and one min after insemination or artificial activation with KCl, and by one minute the level of fluorescence decreased to nearly the level of the control (fig 4d). This demonstrated a decrease in the amount of F-actin between 30 sec and one minute PI (fig 4c,d). By two minutes PI fluorescence increases and continues to increase until four minutes PI, but does not return to the original level.

After ten minutes there is little change until 15-30 minutes when there is a higher level of staining in the cortex than in the rest of the cytoplasm. This appears as a ring of fluorescence in optical equatorial section (fig 5a,b). Intense localized staining is also apparant in the region of the cleavage furrow and polar body of the first division, 90 min PI (fig 5c,d).

Sperm penetration cones fluoresce brightly, indicating a high concentration of F-actin (fig 6). Before sperm penetration actually begins, contact of the sperm acrosomal tubule triggers a localized polymerization in a ring-shaped region of the egg cortex (fig 6). While viewing these cells with the fluorescence microscope, I observed that the acrosomal tubules were fluorescent, but they were so thin that their image did not appear on film.

Focussing near the surface of the egg reveals that the cortical staining is uniform in unfertilized eggs but after 30 minutes PI, the fluorescent pattern is filamentous (fig 7). Microvilli do not completely penetrate the surface coat, but extend approximately half way through (fig 8). They are not apparent in optical equatorial sections of fluorescent eggs (fig 5b).

The intense staining of the unfertilized egg is not eliminated by incubation in cytochalasin B, but the cortical staining pattern is different from that of the untreated cell (compare fig 9c to fig 7b). Cytochalasin caused the cortical fluorescence to appear blotchy in unfertilized eggs (fig 8b,c).

Indirect immunofluorescence of anti-actin reveals that actin is located throughout the cytoplasm (fig 10). Anti-actin recognizes both filamentous and monomeric actin and therefore binds to more protein than NBD-Phallacidin does. Since the two stains have different fluorescent conjugates, direct quantitative comparison between the two is impossible.

Detergent Extraction

To examine the cytoskeleton I extracted eggs in Triton X-100 (Brown *et al.*, 1976; Buckley and Raju, 1976; Lenk *et al.*, 1977: Small and Celis, 1978) which depletes the cell of most of its carbohydrate and lipid (Jeffery and Meier, 1983).

Extracted *U. caupo* eggs have an extensive cytoskeleton. The cells retain their shape, and regions such as the cortex and germinal vesicle remain recognizable in eggs cut in half (fig 11a). Extraction is complete after one hour at 4°C. When these cells are torn open to expose the interior, an extensive array of fibers can be seen throughout the cytoplasm (fig 11).

Surface Coat Removal

In order to examine the surface of extracted cells with SEM I had to first remove the surface coat. The surface coat collapses during extraction and fixation, but remains intact. In some cases I physically tore the surface coat off the egg after critical point drying, but more often I dissolved the surface coat from the living cells by washing them in a non-ionic sugar solution that is a modification of recipes used by Engstrom (1971) and Gould-Somero (1975b). When I tried Gould-Somero's method (0.5M glucose with EGTA), I could see the surface coat swell, become thinner and, after about 5 minutes, break in one or more places. At this point gentle agitation could free the eggs from their envelopes. When these cells were processed for SEM, however, I found there was surface coat material still adhering to the plasma membrane (fig 12b). With Engstrom's method (0.5M sucrose with EDTA) the surface coat would swell, but not dissolve completely (fig 12c). I found that a mixture of equal parts of the two solutions dissolved the entire surface coat quickly and completely (fig 12d). Figure 13 is a series of light micrographs showing the stages of dissolution of the two layers of surface coat. Its bilaminar nature is apparent in transmission electron micrographs (TEMs) after a fixation in which tannic acid was added to the first rinse (fig 14). Temin (1956) found the vitelline layer (surface coat) to consist of an outer proteinaceous layer soluble in non-electrolytes (therefore it dissolves in sugar solutions) and an inner calcium-proteinate layer (which dissolves in calcium-free sea water containing divalent ion chelators).

As the surface coat swells and is dissolved into the sugar solution the microvilli are pulled and stretched (fig 15a). Some of them appear to have broken and snapped back (fig 15b). On one occasion only was I able to remove the surface coat by rinsing eggs three times in Mg-free sea water, allowing them to reach room temperature in this medium. This method of surface coat removal was gentle and did not distort the microvilli, which remained long and numerous after fixation for SEM, (fig 15c).

PML

After fifteen minutes of extraction, the plasma membrane becomes distorted and microvilli become shorter (fig 16a). After thirty minutes, microvilli are reduced to bumps and the plasma membrane develops holes as lipids are dissolved away. Soon after exposure to detergent, the unfertilized eggs always rounded out, but did not undergo germinal vesicle breakdown. Whenever the unfertilized eggs were exposed to anything that dissolved membrane lipids, they rounded out, but did not necessarily activate.

When extraction is complete, the surface appears as an anastamosing network of fibers ranging from 50 to 200 nm in diameter, and averaging 100 nm (fig 16c). The appearance of the surface coat changes during extraction, making it difficult to recognize. To distinguish the two layers of surface coat from the plasma membrane cytoskeleton, referred to hereafter as the plasma membrane lamina (PML), I compared cells to which remnants of the surface coat remained attached throughout extraction (fig 15d).

Figure 17 shows an egg which has some inner layer of surface coat remaining. Higher magnification shows the fibrous web of the PML, or outer layer of the cytoskeleton to which is attached some surface coat material (fig 17b). Higher magnification of the cell in fig 17a reveals that the PML is continuous in some places with the cytoplasmic cytoskeleton (fig 17c).

TEM of Extracted Eggs

After removing the surface coat and extracting the unfertilized eggs for one hour, I fixed and dehydrated them as for SEM and then embedded them in Epon. Thin, grey (50 nm thick) sections were so lacking in substance that there was little to see with TEM[2]. Thicker, gold-purple (120 nm) sections were more informative (fig 18). The germinal vesicle was visible as an electron-lucent, 'empty' space. Throughout the cytoplasm there are many dense spheres, about 2 µm in diameter, which appear to be attached to the array of fibers and filaments criss-crossing the section. The surface of the cell appears as a continuous layer of electron-dense material which is filamentous in some places and granular in others. At the thinnest points the layer is 100 nm thick. I examined eggs from this batch with SEM to check that the plasma membrane was gone. There are connections between the outer layer (PML) and the fibrous elements of the cytoplasm which also appear to attach some of the dense granules to the PML.

The PML is present at the two-cell stage (fig 19) and appears to still cover the entire cleavula. Connections between the PMLs of the two separating blastomeres can be seen in the cleavage furrow region (fig 19b).

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2. I will use the acronym TEM to refer to either transmission electron microscopy or micrograph. Similarly, SEM will refer to scanning electron microscopy or micrograph.

IV DISCUSSION

The two main results of my work are 1) the demonstration of a change in F-actin distribution after fertilization detected by NBD-Pallacidin staining, and 2) the discovery of an extensive detergent-resistant cytoskeleton in fertilized and unfertilized eggs. First I will discuss the changes in F-actin distribution and compare them to what is known about F-actin changes in other eggs.

General

Actin is a major protein found in virtually all eukaryotic cells (Allen, 1980; Korn, 1982; Pollard and Weihing, 1974) and at least some prokaryotes (Gobel *et al.*, 1981). It is one of the most abundant proteins in sea urchin eggs (Durica and Crain, 1982; Miki-Noumura and Kondo, 1970). There is little F-actin in unfertilized sea urchin eggs (Cline *et al.*, 1983; Coffee *et al.*, 1982; Schatten, 1983; Spudich and Spudich, 1979), but after fertilization there is a massive polymerization of G-actin to F-actin, mainly in the cortical region and in microvilli (Kane, 1979; Otto, Kane and Bryan, 1980; Tilney and Jaffe, 1982; Wang and Taylor, 1979).

Cortical G-actin polymerizes to form filaments in the microvilli as they elongate, and in the cortex, causing it to become transiently stiffer (Begg, Rebhun and Hyatt, 1982; Burgess and Schroeder, 1977; Longo, 1980; Mabuchi and Sakai, 1972; Vacquier, 1981). Sea urchin eggs stained with NBD-Phallacidin, or injected with fluorescence labelled actin, show an increase in cortical fluorescence after fertilization, most intensely in sperm penetration cones and in the cleavage furrow region, as F-actin is polymerized there (Cline *et al.*, 1983). These changes may have an important role in early development. It was therefore surprising to find a decrease in the amount of F-actin after fertilization in *U. caupo* oocytes. This finding does not support the hypothesis I proposed at the beginning of this study, that polymerization of F-actin is responsible for the rounding out of *U. caupo* eggs.

My results show that, unlike sea urchin eggs, *U. caupo* oocytes have a considerable amount of F-actin throughout the cytoplasm. This extensive F-actin component to the cytoskeleton may contribute to the maintenance of the cell's indented shape. *U. caupo* eggs exhibit a decrease in viscosity after fertilization, which may be related to this (Tyler, 1932). Starfish eggs undergo a change in shape during germinal vesicle break-down, which is also accompanied by an actin-mediated decrease in viscosity, or cortical rigidity (Vacquier, 1981). The hypothesis that F-actin contributes to the maintenance of cell shape is supported by the finding that rounding out, which occurs as a response to fertilization, is coincident with the decrease in NBD-Phallacidin staining. Rounding out begins after fertilization and is complete by 4 minutes PI, when other cortical changes (altered cortical granule and pigment granule distribution patterns in the cortex) are apparent (Gould-Somero and Holland, 1975a). Also coincident with these changes is a decrease in light scattering which may reflect an internal structural change. This decrease in light scattering occurs during the first minute PI, (when the amount of F-actin also begins to decrease), then levels off by one minute PI (Paul, 1975). Fertilization in *U. caupo* is accompanied by the release of fertilization acid as also occurs in sea urchin eggs (Paul, 1975). The rise in internal pH in sea urchin eggs triggers actin polymerization (Begg and Rebhun, 1979), and small changes in pH cause actin polymerization in many *in vitro* systems (Nunnally and Craig, 1980). In *U. caupo* eggs, the rise in pH either must have the opposite effect on actin polymerization, or is unrelated to it.

Effects of Cytochalasin B

Sperm incorporation in sea urchin eggs is an actin filament-mediated event (Tilney and Jaffe, 1982). CB inhibits sperm penetration cone formation and microvillar elongation in *Arbacia punctulata* eggs (Longo, 1980), which are transiently sensitive to CB during the first six minutes PI (Banzhaf *et al., 1980*). Cytochalasin resistant F-actin has been reported in the cortex of rat oocytes, which become progressively sensitive to CB after 15 minutes PI, when CB treated eggs show a punctate staining pattern after fluorescent labelling with NBD-Phallacidin (Amsterdam *et al.*, 1977; Battaglia and Gaddum-Rosse, 1984). A patchy NBD-Phallacidin staining pattern has also been found in CB-treated mouse 3T3 cells, in which CB causes "rearrangement" of microfilament bundles into "stellate patches" (Weber *et al.*, 1976).

U. caupo eggs treated with concentrations of CB sufficient to inhibit sperm penetration without preventing sperm induced egg activation do not round out. This does not rule out the hypothesis that actin-depolymerization is responsible for rounding out, since *U. caupo* eggs contain a significant amount of CB-resistant F-actin. Cytochalasin treated *U. caupo* oocytes display a punctate, or blotchy pattern of fluorescence after NBD-Phallacidin staining, while in untreated oocytes the staining pattern is uniform. This result could reflect surface changes responsible for the irregular outline of eggs exposed to CB for long periods, which activate and round out at the normal time, even though sperm incorporation is blocked (Gould-Somero *et al.*, 1977).

Microfilaments in Thin Sections

If F-actin is so abundant within the oocyte, why is it not apparent in thin sections? Although 5-7 nm filaments are occasionally visible in the dense band, typically, no filaments are obvious in TEMs. This is not unusual, however, since only large bundles or parallel arrays of filaments are easy to detect in thin sections, and usually microfilaments are difficult to see or not visible at all (Tilney, 1983). Conventional fixations, especially exposure to OsO_4 and alcohol, disrupt or destroy actin networks (Hirokawa and Tilney, 1982; Maupin and Pollard, 1978; Small, 1981). Prolonged exposure to osmium destroys microfilaments (Porter *et al.*, 1983). Embedding media have electron scattering properties similar to those of microfilaments, making them difficult to detect in conventional thin sections (Capco *et al.*, 1982; Fey *et al.*, 1984; Penman *et al.*, 1983; Woolosowick and Porter, 1979), or the actin filaments may appear as "granular cytoplasm" (Maupin and Pollard, 1983). Actin destruction is dependent on osmium concentration, temperature, type and concentration of buffer and pH (Maupin-Szamier and Pollard, 1978). Unfortunately the optimum conditions for actin preservation are not optimum for the fixation of *U. caupo* eggs, which, at best, are difficult to fix (Gould-Somero and Holland, 1975a; Eisenman and Alfert, 1981), and compromises must be made.

Tannic acid in the primary fixative will improve F-actin preservation (Maupin and Pollard, 1983; Seagull and Heath, 1979) but must be added to the first rinse during *U. caupo* egg fixation since tannic acid in the primary fixative causes a chunky black precipitate. This method is probably inferior to the addition of tannic acid directly to the fixative.

Another possible explanation for the paucity of filaments in thin sections is that NBD-Phallacidin may stain oligomers too small to be recognized in conventional thin sections (Cline *et al.*, 1983). *In vitro*, some phalloidins bind to oligomeric and even dimeric actin, but to what phallicidin, a related molecule, binds *in vivo* is not known (Barak and Yocum, 1981; Weiland and Faulstich, 1978).

Biochemical assays show that sea urchin cortices are rich in actin and detergent extracted cytoskeletons of cortices show "abundant 7nm arrays", but conventional electron microscopy reveals very few actin or intermediate filaments (Spudich and Spudich, 1979; Vacquier and Moy, 1980). *Mycoplasma pnumoniae* has a detergent-resistant cytoskeleton that contains microfilaments and stains brightly with actin-antibody staining. The cytoskeleton, however, does not show up at all in thin sections (Meng and Pfister, 1980). This phenomenon seems to be widespread, since actin filaments are difficult to preserve and detect with TEM in general.

The Dense Band

The dense band beneath the plasma membrane may be a cytoskeletal element that is relatively easy to preserve. It is a discontinuous structure which appears as an electron-dense band subjacent to the plasma membrane which occasionally appears to contain filaments. The band is discontinuous in the plane of thin section and through serial sections and is therefore not a continuous sheet or sphere. It is strikingly similar to a structure found in thin sections of ascidian eggs which may represent a cytoskeletal element responsible for contraction and ooplasmic segregation (Jefferey, 1984). Most of what is known about membrane-associated cytoskeletons, other than the spectrin network found in erythrocytes and the "terminal web" of intestinal epithelial cells, has been learned in the past ten years (Hauser, 1978; Hirokawa and Heuser, 1981; Hirokawa *et al.*, 1982). For a review see Branton *et al.*, (1981).

In the last few years the spectrin-associated "membrane-cytoskeleton" of erythrocytes and the terminal web, once considered unique, have been thought of more as one type of "surface lamina" found in many cells. There is a membrane-associated cytoskeleton in *Dictyosteleum discoideum* (Clarke *et al.*, 1975; Goodloe-Holland and Luna, 1984; Luna *et al.*, 1981), lymph node cells (Allore and Barber, 1982) and other cultured cells (Boyles and Bainton, 1979a,b; Mesland, *et al.*, 1981; Moore *et al.*, 1978). In many types of cultured cells, the outer boundary of the cytoskeleton appears as an electron-dense band beneath the plasma membrane, very similar to the dense band in *U. caupo* eggs (Ben-Ze'ev *et al.*, 1979). This is also true of ascidian eggs (Tsukita *et al.*, 1980). In some instances this band is known to be a lattice of actin filaments that interacts with the plasma membrane (Condeelis, 1982). In *Amoeba proteus* this band is discontinuous, as it is in *U. caupo* (Hull and Staehelin, 1979). A band of microfilaments in the cortex of fertilized sea urchin eggs may contribute to the cleavage furrow filament network (Usui and Yoneda, 1982). A dense band has been identified in intestinal cells of *Xenopus laevis* and may be a prominent band of microfilaments (Hull and Staehelin, 1979). This structure, called the terminal web (a term specific for intestinal epithelial cells) contains microfilaments and myosin and is connected to the plasma membrane by cross-linking proteins (Mooseker and Tilney, 1975). In cells of mouse embryos, actin and actin binding proteins are found in a dense band in the cortex and are considered part of the cytoskeleton (Lehtonen and Badley, 1980).

Some annelid eggs have similar sub-membranous cortical structures; in *Tubifex* there is a 150 nm electron-dense layer (the band in *U. caupo* eggs averages 100 nm) which is "rich in microfilaments" (Shimuzu, 1982, 1983a, b) and in *Serpula*, a band of similar dimensions occurs (Krogstad, 1982). A cortical dense band has been well documented in ascidian eggs (Jeffery, 1984; Sawada and Osanai, 1981) and in *Ilyanassa* eggs (Burgess, 1977; Schmidt *et al.*, 1981). For reviews of the possible roles of membrane-associated cytoskeletons, see Schroeder, (1976, 1981), Weatherbee (1981), Vacquier,(1981) and Jacobson (1983).

The cytoskeleton has been implicated in the role of regulating cell shape (Henson and Schatten, 1983; Penman *et al.*, 1983; Stossel, 1983; Usui and Yoneda, 1982), and may participate in the maintenance of the indented shape of the *U. caupo* oocyte. I could not positively identify actin as a component of the dense band, since I was unable to get ferritin-conjugated antibodies or S-1 myosin subfragments into the cell. The surface coat is a major obstacle to stain penetration in *U. caupo* oocytes, but removing it, coupled with the necessary permeabilization of the the plasma membrane proved to be extremely traumatic to the cells. This may be related to the fact that the surface coat of *U. caupo* eggs is never shed, as it is in sea urchin embryos, but becomes incorporated into the adult cuticle (Engstrom, 1971).

Detergent-Resistant Cytoskeleton

For another view of the cytoskeleton, I removed the lipid and other soluble components of the cytoplasm with a non-ionic detergent, Triton X-100 (TX-100). After extraction of vertebrate cells from culture, a branched network of filaments remains (Loftus *et al.*, 1984; Schliwa and Blerkom, 1981). Roughly 20-25% of the total protein and RNA, including some mRNA, remains associated with the cytoskeleton of ascidian eggs (Jeffery, 1982; Jeffery and Meier, 1983). In a variety of cells studied, the cytoskeleton after extraction consists of F-actin, microtubules, intermediate filaments and material adhering to them such as mRNA, polyribosomes, some lipid, low concentrations of soluble proteins, glycoproteins and actin binding proteins (Branton *et al.*, 1981; Bravo *et al.*, 1982; Fulton *et al.*, 1980; Jeffery, 1983; Penman *et al.*, 1983). It is reasonable to expect that the detergent-insoluble cytoskeleton of *U. caupo* eggs is composed of similar elements.

After one hour of detergent extraction, *U. caupo* eggs are paler in colour, due to the loss of soluble components of the cytoplasm, and they are less dense (they settle more slowly). There is a considerable amount of detergent-resistant material left in the cytoplasm and the nucleus. There are many filaments, ranging from 7 to 300 nm in diameter. It is difficult to positively identify 7nm filaments (microfilaments, or F-actin filaments) in SEM images because the gold-palladium coating can add 20% to a filament's thickness, or an extra 1.4nm (F. Morrill, personal communication, 1984). Non-soluble components, such as ribosomes or mRNA, may adhere to the filaments and increase their diameter (Parysek and Eckert, 1980). In HeLa cells, all polyribomes, as well as the protein substructure of the endoplasmic reticulum, remain associated with the cytoskeleton after extraction (Cervero *et al.* 1981), and certainly these must add to the thickness of the cytoskeletal filaments.

During extraction, as the plasma membrane dissolves, holes in the surface develop, and after one hour only a meshwork of filaments averaging 100-150 nm in diameter remains. This layer (PML) is connected to the detergent-resistant cytoskeleton in the underlying cytoplasm. The PML must be immediately subjacent to, or part of the plasma membrane. Ascidian eggs contain a PML, similar in appearance to that in *U. caupo* eggs, which is found throughout the unfertilized egg surface. After fertilization, a cytoplasmic contraction (of the PML) causes a rearrangement of embryonic plasms (ooplasmic segregation). The PML remains with the yellow-coloured myoplasm (Jeffery and Meier, 1983). For a review of this phenomenon, see Jeffery, 1984.

Many vertebrate cells in culture contain a cortical network forming the outer layer of the extracted cytoskeleton. Platelets contain peripheral webs connected to branched networks of discrete and bundled filaments (Loftus *et al.*, 1983). The cytoskeleton of extracted erythrocytes has an outer shell consisting of the integral membrane proteins band 4.1 and band 4.9, as well as actin, spectrin and possible ankyrin (Sheetz, 1979; Tsukita, *et al.*, 1980; Branton, Cohen and Tyler, 1981; Nermut, 1981). Many integral membrane proteins are attached to the underlying "membrane" skeleton' or PML, and some remain after detergent extraction. Tannenbaum *et al.*, (1977) found that for HEp-2 cells, 25% of membrane proteins resisted

TX-100 extraction and 60% of tritium-labelled membrane-bound cytochalasin D binding sites remained also. Extracted muscle cells retain the surface receptor for acetylcholine, as well as virus and and lectin binding sites (Fulton *et al.*, 1981; Prives *et al.*, 1982). Plasma membrane proteins become part of the PML after the lipid and soluble portions of the membrane is removed. It may be that the plasma membrane proteins form a sheet after extraction (Ben Ze'ev *et al.*, 1979). At least 20% of membrane proteins remain after extraction (Allore and Barber, 1982; Moore *et al.*, 1978) and therefore must add to the PML seen with SEM. For a review of membrane-cytoskeleton interactions, see Weatherbee (1981).

The PML of *U. caupo* eggs may be similar to these other "membrane cytoskeletons" described above, a part of the cytoskeleton with attached detergent-resistant plasma membrane proteins. Since this is the outer-most part of the cytoskeleton and is closely associated with the plasma membrane, it is probably the same structure represented by the dense band in thin sections. The thickness of the two structures is similar, even though there may be considerable differences in shrinkage and contamination by other components between the two preparatory techniques.

The thin sections of extracted eggs I studied revealed that the PML is remarkably like the dense band, (bearing in mind that the sections of the extracted eggs are necessarily much thicker than the sections of intact eggs). The cytoplasm in thin sections (silver-grey) is "empty"; only fine granular material and few filaments are apparant. Thicker sections (gold-purple) have more material and revealed a cross-linked network of filaments (comparable to SEM images) throughout the cytoplasm, and a more continuous, thicker PML. At its thinnest, the PML is 100 nm thick, the same average thickness of the dense band. In these areas the PML must be cut at an angle most nearly perpendicular to its surface and therefore its thickness in these regions must most closely represent the true value. At these points the PML appears filamentous, as the dense band occasionally does.

If the PML and the dense band represent the same structure, then the SEM images reveal why the dense band is discontinuous in thin sections. It is a web, or network, and thin sections would only rarely cut a longitudinal section of the webbing itself. More often oblique or cross-sections and sections through the holes would be cut. Longitudinal sections through the webbing would be the only ones to expose longitudinally aligned filaments running through them, making the occurrence of filaments in the dense band rare, which it is.

NBD-Phallacidin Staining of the Fertilized Egg Cortex

The cortex of NBD-Phallacidin stained eggs 30 min PI contains fluorescent filaments approximately 100 nm in diameter in a network near the cell surface. These filaments may represent actin in the PML, but may also be microvilli which are flattened on the egg surface by the pressure of the coverslip necessary for fluorescent microscopy with a 100X objective. In equatorial optical sections, the microvilli are visible with standard or Nomarski optics, but are not visibly labelled with NBD-Phallacidin when viewed by fluorescence microscopy. This suggests that microvilli are not responsible for the fluorescent filamentous pattern at the surface of the egg, but it is not proof, since microvilli of NBD-Phallacidin labelled sea urchin eggs are not visible either in optical equatorial section, but are apparent on the surface when the cells are flattened for fluorescence microscopy using a 100X objective (Schroeder, 1984, personal communication).

Conclusions and Summary

I found no changes in the PML or dense band during fertilization, and did not detect any differences in these structures between the indented and unindented regions of the oocyte. The fact that the PML appears the same in fertilized and unfertlized eggs may be misleading since the addition of any permeabilization agent (without a fixative) immediately causes the cells to round out. After extraction, even the unfertilized eggs have been rounded out for one hour, and it would be foolish to suppose this does not affect the PML. Perhaps a technique for detergent extraction under high hydrostatic or osmotic pressure can be devised in which the eggs would not round out, and comparisons between indented and unindented eggs could be made.

In summary, it appears that the depolymerization of F-actin in the newly fertilized eggs of *U. caupo* may contribute to or cause the rounding out process. The changes in NBD-Phallacidin staining coincide temporally with rounding out and other early responses to fertilization. F-actin is responsible for the maintenance or change in shape in other cells, and it may have a role in *U. caupo* egg shape changes. The F-actin visible in the cortex of NBD-Ph-stained zygotes may be located in a structure which is represented by a dense band in thin sections and the PML in SEMs of extracted eggs. The PML and the dense band both represent the peripheral layer of the cytoskeleton which is attached to the network of filaments in the cytoplasmic cytoskeleton and may also be attached to membrane proteins. This type of peripheral cytoskeletal substructure has been found in many cell types in the last three years and may prove to be a ubiquitous, important and basic part of the cell's inner architecture.

V LITERATURE CITED

Allen, R.D. 1980. Cytoskeletal ultrastructure and motility of cells. Can. J. Bot. *58*:745-749.

Allore, R.J. and B.H. Barber. 1982. Structural studies of purified pig lymph node plasma membrane; association of cytoskeletal components with the plasma membrane and the effect of detergent solubilization. Can. J. Bioch. *60*:57-70.

Amsterdam, A, H.R Lindner and U. Groschel-Stewart. 1977. Localization of actin and myosin in the rat oocyte and follicular wall by immuno-fluorescence. Anat. Rec. *187*:311-328

Anstrom, J.A. and R.G. Summers. 1983. A morphological analysis of the first cleavage mitotic cytoskeleton isolated from sea urchin embryos. (*Strongylocentrotus purpuratus*). J. Morphol. *177*:329-344.

Balczon, R. and G. Schatten. 1983. Microtubule-containing detergent-extracted cytoskeletons in sea urchin eggs from fertilization through cell division: anti-tubulin fluorescence microscopy. Cell Motil. *3*:213-226.

Banzhaf, W.C., R.H. Warren and D.R. McClay. 1980. Cortical reorganization following fertilization of sea urchin eggs: sensitivity to cytochalasin B. Develop. Biol. *80*:506-515.

Barak, L.S. and R.R. Yocum. 1981. 7-Nitrobenz-2-oxa-1,3-diazole(NBD)-Phallacidin: Synthesis of a fluorescent actin probe. Anal. Bioch. *110*:31-38.

Battaglia, D.E. and P. Gaddum-Rosse. 1984. The distribution of F-actin in the rat egg and its sensitivity to cytochalasin during fertilization. J. Cell Biol. *99*: 246a.

Begg, D.A. and L.I Rebhun. 1979. pH regulates the polymerization of actin in the sea urchin egg cortex. J. Cell Biol. *83*:241-248.

Begg, D.A., L.I. Rebhun and H. Hyatt. 1982. Structural organization of actin in the sea urchin egg cortex: microvillar elongation in the absence of actin filament bundle formation. J. Cell Biol. *93*:24-32.

Ben-Ze'ev, A., A. Duerr, F. Solomon and S. Penaman. 1979. The outer boundary of the cytoskeleton: a lamina derived from plasma membrane proteins. Cell *17*:859-865.

Bestor, T.H. and G. Schatten. 1981. Anti-tubulin immunofluorescence microscopy of microtubules present during the pronuclear movements of sea urchin fertilization. Develop. Biol. *88*:80-91.

Boyles, J. and D.F. Bainton. 1979a. Changing patterns of plasma membrane-associated filaments during the initial phases of polymorphonuclear leukocyte adherance. J. Cell Biol. *82*:347-368.

Boyles, J and D F. Bainton. 1979b. Changes in plasma membrane associated filaments during endocytosis and exocytosis in polymorphonuclear leukocytes. Cell. *24*:905-914.

Branton, D., C.M. Cohen and J. Tyler. 1981. Interaction of cytoskeletal proteins on the human erythrocyte membrane. Cell. *24*:24-32.

Bravo, R., J.V. Small, S.J. Fey, P.M. Larson and J.E. Celis. 1982. Architecture and polypeptide composition of HeLa cytoskeletons; modification of cytoarchitectural polypeptides during mitosis. J. Mol. Biol. *154*:121-143.

Brinkley, B.R. 1982. The cytoskeleton. A perspective. *In* Methods in cell biology: the cytoskeleton. Part A. L. Wilson, ed. *24*:1-8.

Brown, S., W. Levinson and J.A. Spudich. 1976. Cytoskeletal elements of chick embryo fibroblasts revealed by detergent extraction. J. Supramol. Struct. *5*:119-130.

Buckley, I.K., and T.R. Raju. 1976. Form and distribution of actin and myosin in non-muscle cells: a study using chick embro fibroblasts. J. Microscopy. *107*:129-149.

Burgess, D.R. 1977. Ultrastructure of meiosis and polar body formation in the egg of the mud snail, *Ilyanassa obsoleta*. *In* Cell shape and surface architecture. Revel, Henning and Fox, eds. pp. 569-579.

Burgess, D.R. and T.E. Schroeder. 1977. Polarized bundles of actin filaments within microvilli of sea urchin eggs. J. Cell Biol. *74*:1032-1037.

Capco, D.G., K.M. Wan and S. Penman. 1982. The nuclear matrix: 3-dimensional architecture and protein composition. Cell. *29*:847-858.

Cavanaugh, G.M. 1956. Formulae and methods V of the Marine Biological Laboratory. Marine Biological Laboratory, Woods Hole Mass.

Cervero, M., G. Dreyfuss and S. Penman. 1981. Messenger RNA is translated when associated with the cytoskeletal framework in normal and VSV-infected HeLa cells. Cell. *23*:113-120.

Clarke, M., G. Schatten, D. Mazia and J.A. Spudich. 1975. Visualization of actin fibres associated with the cell membrane in amoeba of *Dictyosteleum discoideum*. Proc. Natnl. Acad. Sci. U.S.A. *72*:1758-1762.

Cline, C.A., H. Schatten, R. Balczon and G. Schatten. 1983. Actin mediated surface motility during sea urchin fertilization. Cell Motil. *3*:513-524.

Coffee, G., G. Foucault, M.O. Soyer, F. DeBilly and J. Pulles. 1982. State of actin during the cycle of cohesiveness of the cytoplasm in parthenogenetically activated sea urchin eggs. Exp. Cell Res. *142*:365-372.

Condeelis, J.S. 1982. Reciprocal interactions between the actin lattice and the cell membrane. Neurosci. Res. Prog. Bull. *19*:83-99.

Durica, D.S. and W.R. Crain. 1982. Analysis of actin synthesis in early sea urchin development. Develop. Biol. *92*:428-439.

Eisenman, E.A. and M. Alfert. 1981. A new fixation procedure for preserving the ultrastructure of marine invertebrate tissues. J. Microsc. *125*:117-120.

Eisenman, E.A., N.K. Das and M. Alfert. 1977. Concanavalin A induced changes in the submembrane contractile structures in fertilized eggs of *Urechis caupo*. J. Cell Biol. *75*:264a.

Elinson, R.P. 1983. Cytoplasmic phases in the first cell cycle of the activated frog (*Rana pipiens*) egg. Develop. Biol. *100*:440-451.

Engstrom, W.S. 1971. Removal of the fertilization membrane of fertilized eggs of *Urechis caupo* and development of "membraneless embryos". Biol. Bull. *140*:369-375.

Fey, E.G., K.M. Wan and S. Penman. 1984. Epithelial cytoskeletal framework and nuclear matrix intermediate filament scaffold: 3-dimensional organization and protein composition. J. Cell Biol. *98*:1973-1984.

Fisher, W.K. and G.E. MacGinitie. 1928. A new echiuroid worm from California. Ann. and Mag. Nat. Hist. 1:199-204.

Fisher, G.W. and L.I. Rebhun. 1983. Sea urchin egg cortical granule exocytosis is followed by a burst of membrane retrieval via uptake in coated vesicles. Develop. Biol. *99*:456-472.

Fulton, A.B., J. Prives, S.R. Farmer and S. Penman. 1981. Developmental reorganization of the skeletal framework and its surface lamina in fusing muscle cells. J. Cell Biol. *91*:103-112.

Fulton, A.B., K.M. Wan and S. Penman. 1980. The spatial distribution of polyribosomes in 3T3 cells and the associated assembly of proteins into the skeletal framework. Cell. *20*:849-857.

Gobel, U., V. Speth and W. Bredt. 1981. Filamentous structures in adherent *Mycoplasma pneumonia* cell treated with nonionic detergents. J. Cell Biol. *91*:537-543.

Goldmand, R.D., B. Chojnacki, A.E. Goldman, J. Starger, P. Steinert, J. Talian, M. Whitman and R. Zackroff. 1981. Aspects of the cytoskeleton and cytomusculature of non-muscle cells. Neurosci. Res. Prog. Bull. *19*:59-82.

Goodloe-Holland, C.M. and E.J. Luna. 1984. A membrane cytoskeleton from *Dictyosteleum discoideum.* III. Plasma membrane fragments bind predominantly to the sides of actin filaments. J. Cell Biol. *99*:71-78.

Gould-Somero, M and L. Holland. 1975a. Oocyte differentiation in *Urechis caupo* (Echiura): a fine structural study. J. Morph. *147*:475-506.

Gould-Somero, M. and L. Holland. 1975b. Fine structural investigation of the insemination response in *Urechis caupo*. Develop. Biol. *466*:358-369.

Gould-Somero, M., L. Holland and M. Paul. 1977. Cytochalasin B inhibits sperm penetration into eggs of *Urechis caupo* (Echiura). Develop. Biol. *58*:11-22.

Gould-Somero, M., Jaffe, L.A. and Holland, L.Z. 1979. Electrically mediated fast polyspermy block in eggs of the marine worm *Urechis caupo*. J. Cell Biol. *82*:426-440.

Habibi, H.R. and C.A. Lessman. 1984. Involvement of cytoskeleton in lower vertebrate oocyte nuclear migration and dissolution: effects of cytochalsin B and demecolcine. J. Cell Biol. *99*:55a.

Hamaguchi, Y. and I. Mabuchi. 1982. Phalloidin microinjection and localization of fluorescein-labelled phalloidin in living sand dollar eggs. Cell Motil. *2*:103-113.

Hauser, M. 1978. Demonstration of membrane-associated and oriented microfilaments in *Amoeba proteus* by means of a Schiff base/glutaraldehyde fixative. Cytobiol. *18*:95-106.

Henson, J.H. and G. Schatten. 1983. Ca^{2+} regulation of the actin-mediated cytoskeletal transformation of sea urchin coelomocytes. Cell Motil. *3*:525-534

Hirokawa, N. and J.E. Heuser. 1981. Quick-freeze, deep-etch visualization of the cytoskeleton beneath surface differentions of epithelial cells. J. Cell Biol. *91*:399-409.

Hirokawa, N. and L.G Tilney. 1982. Interactions between actin filaments and membranes in quick-frozen and deeply etched hair cells of the chick ear. J. Cell Biol. *95*:249-266.

Hirokawa, N., L.G. Tilney, K. Fujiwara and J.E. Heuser. 1982. Organization of actin, myosin and intermediate filaments in the brush border of intestinal epithelial cells. J. Cell Biol. *94*:425-443.

Hull, B.E. and C.B. Staehelin. 1979. The terminal web - a reevaluation of its structure and function. J. Cell Biol. *81*:67-82.

Jacobson, B.S. 1983. Interactions of the plamsa membrane with the cytoskeleton: an overview. Tissue and Cell. *15*:829-852.

Jaffe, L.A. 1976. Fast block to polyspermy in sea urchin eggs is electrically mediated. Nature. *261*:68-71.

Jeffery, W.R. 1982. Messenger RNA in the cytoskeletal framework: analysis by *in situ* hybridization. J. Cell Biol. *95*:1-7.

Jeffery, W.R. 1983. Messenger RNA localization and cytoskeletal domains in ascidian embryos. *In* Time, space and pattern in embryonic development, Alan Liss Inc. p. 241-259.

Jeffery, W.R. 1984. Pattern formation by ooplasmic segregation in ascidian eggs. Biol. Bull. *166*:277-298.

Jeffery, W.R., and S. Meier. 1983. A yellow crescent cytoskeletal domain in ascidian eggs and its role in early development. Develop. Biol. *96*:125-143.

Johnson, J.D., D. Epel and M. Paul. 1976. Intracellular pH and activation of sea urchin eggs after fertilization. Nature. *262*:661-664.

Kane, R.E. 1979. Actin polymerization and function in the sea urchin egg. 1979. *In* Cell motility: molecules and organization. S. Hatano, H. Ishikawa and H. Sato. eds. Univ. of Tokyo Press.

Korn, E.D. 1982. Actin polymerization and its regulation by proteins from nonmuscle cells. Physiol Reviews. *62*:672-737.

Krogstad, H. 1982. An ultrastructural study of the gametes and fertilization in *Serpula vermicularis* L. (Annelida, Polychaeta). MSc. Thesis, University of Victoria.

Lehtonen, E. and R.A. Badley. 1980. Localization of cytoskeletal embryo proteins in pre-implantation mouse embryos. J. Embryol. Exp. Morph. *55*:211-225.

Lenk, R., L. Ransom, Y. Kaufmann and S. Penman. 1977. A cytoskeletal structure with associated polyribosomes obtained from HeLa cells. Cell. *10*:67-78.

Loftus, J.C., J. Choate and R.M. Albrecht. 1984. Platelet activation and cytoskeletal reorganization: high voltage electron microscopical examination of intact and triton-extracted whole mounts. J. Cell Biol. *98*:2019-2025.

Longo, F.J. 1980. Organization of microfilaments in sea urchin (*Arbacia punctulata*) eggs at fertilization, effects of cytochalasin B. Develop. Biol. *74*:422-433.

Luft, J.H. 1961. Improvements in epoxy embedding methods. J. Biophys. Bioch. Cytol. *9*:409-414.

Luna, E.J., V.M. Fowler, J. Swanson, D. Branton and D.L. Taylor. 1981. A membrane cytoskeleton from *Dictyostelеum discoideum* I. Identification and partial characterization of an actin-binding activity. J. Cell Biol. *88*:396-409.

Mabuchi, I. 1973. A myosin-like protein in the cortical layer of the sea urchin egg. J. Cell Biol. *59*:542-547.

Mabuchi, I. 1981. Purification from starfish eggs of a protein that depolymerizes actin. J. Biochem. *89*:1341-1344.

Mabuchi, I and H. Sakai. 1972. Cortex protein of sea urchin eggs I. Its purification and other protein components of the cortex. Develop. Growth and Differ. *14*:247-261.

Maupin-Szamier, P. and T.D. Pollard. 1978. Actin filament destruction by OsO_4. J. Cell Biol. *77*:837-852.

Maupin, P. and T.D. Pollard. 1983. Improved preservation and staining of HeLa cell actin filaments, clathrin coated membranes and other cytoplasmic structures by tannic acid-glutaraldehyde-saponin fixation. J. Cell Biol. *96*:51-52.

Meng, K.E. and R.M. Pfister. 1980. Intracellular structures of *Mycoplasma pneumoniae* revealed after membrane removal. J. Bact. *144*:390-399.

Mesland, D.A.M., H. Spiele and E. Roos. 1981. Membrane-associated cytoskeleton and coated vesicles in cultured hepatocytes visualized by dry-cleaving. Exp. Cell Res. *132*:169-184.

Miki-Nuomoura, T. and H. Kondo. 1970. Polymerization of actin from sea urchin eggs. Exp. Cell Res. *61*:31-41.

Moon, R.T., R.F. Nicosia, C. Olsen, M.B. Hille and W.R. Jeffery. 1983. The cytoskeletal framework of sea urchin eggs and embryos: developmental changes in the association of messenger RNA. Develop. Biol. *95*:447-458.

Moore, P.B., C.L. Owenby and K.L. Carroway. 1978. Interactions of cytoskeletal elements with the plasma membrane of Sarcoma 180 Ascites tumour cells. Expt. Cell Res. *115*:331-342.

Mooseker, M.S. and L.G. Tilney. 1975. Organization of an actin filament-membrane complex. J. Cell Biol. *67*:725-743.

Nermut, M.V. 1981. Visualization of the "membrane skeleton" in human erythrocytes by freeze-etching. Eur. J. of Cell Biol. *25*:265-271.

Nunnally, M.H. and S.W. Craig. 1980. Small changes in pH within the physiological range cause large changes in the consistency of actin filament mixtures. J. Cell Biol. *87*:218a.

Otto, J.J., R.E. Kane and J. Bryan. 1980. Redistribution of actin and fascin in sea urchin eggs after fertilization. Cell Motil. *1*:31-40.

Parysek, L.M. and B.S. Eckert. 1980. Cytoskeletal elements of detergent-extracted human neutrophils. J. Cell Biol. *87*:222a.

Paul, M. 1975. Release of acid and changes in light-scattering properties following fertilization of *Urechis caupo* eggs. Develop. Biol. *43*:299-312.

Penman, S., D.G. Capco, E.G. Fey, P. Chatterjee, T. Reiter, S. Ermish and K. Wan. 1983. The 3-dimensional structural network of cytoplasm and nucleus: function in cells and tissue. *In* Modern cell biology. Allan Liss Inc. *2*:385-415.

Pollard, T.D. 1981. Cytoplasmic contractile proteins. J. Cell Biol. 91:*156s-165s*.

Pollard, T.D. and Weihing, R.R. 1974. Actin and myosin and cell movement. Crit. Rev. Biochem. *2*:1-65.

Porter, K.R., M. Beckerle and M. McNiven. 1983. The cytoplasmic matrix. *In* Modern cell biology. Alan Liss Inc. 2:259-302.

Presnov, E.V. and V.V. Isaeva. 1983. Rearrangements of the cleavage spatial organization in temporarily flattened sea urchin (*Strongylocentrotus nudus*) zygotes. Ontogenez *14*:319-323.

Prives, J., A.B. Fulton, S. Penman, M.P. Daniels and C.N. Christian. 1982. Interaction of the cytoskeletal framework with acetylcholine receptors on the surface of embryonic muscle cells in culture. J. Cell Biol. *92*:231-236.

Sawada, T. and K. Osanai. 1981. The cortical contraction related to the ooplasmic segregation of *Ciona intestinalis* eggs. Wilhelm Roux's Arch. *190*:208-214.

Schatten, G. 1983. Motility during fertilization. Endeavor. *7*:173-175.

Schliwa, M. and J. Blerkom. 1981. Structural interaction of cytoskeletal components. J. Cell Biol. *90*:222-235.

Schloss, J.A., A. Milsted and R.D. Goldman. 1977. Myosin subfragment binding for the localization of actin-filaments in cultured cells. A light and electron microscopic study. J. Cell Biol. *74*:794-815.

Schmidt, H.R., T. Wight, A. Habenicht, J Glomset and R. Ross. 1981. Maintenance of 3T3 cell shape requires mevalonic acid. J. Cell Biol. *91*:297a.

Schroeder, T.E. 1969. The role of contractile ring filaments in dividing *Arbacia* eggs. Biol. Bull. *137*:413-414.

Schroeder, T.E. 1976. Actin in dividing cells: evidence for its role in cleavage, but not in mitosis. *In* Cell motility. Goldman, Pollard and Rosenbaum eds., New York, Cold Spring Harbor.

Schroeder, T.E. 1981. Interrelations between the cell surface and the cytoskeleton in cleaving sea urchin eggs. *In* Plasma membrane organization. Poste and Nicolson eds. Elsevier/North-Holland Biom. Press.

Schroeder, T.E. 1982. Novel surface specialization on a sea anemone egg: "spires" of actin-filled microvilli. J. Morphol. *174*:-207-216.

Schroeder, T.E. and S.A. Stricker. 1983. Morphological changes during maturation of starfish oocytes: surface ultrastructure and cortical actin. Develop. Biol. *98*:373-384.

Seagull, R.W. and I.B. Heath. 1979. The effects of tannic acid on the *in vitro* preservation of microfilaments. Eur. J. Cell Biol. *20*:184-188.

Sheetz, M.P. 1979. Integral membrane protein interaction with triton cytoskeletons of erythrocytes. Bioch. and Biophys. Acta. *557*:122-134.

Shen, S. and R. Steinhardt. 1978. Direct measurement of intracellular pH during metabolic derepression of the sea urchin egg. Nature. *272*:253-254.

Shimuzu, T. 1982. Ooplasmic segregation in the *Tubifex* egg: mode of pole plasm accumulation and possible involvement of microfilaments. Wilhelm Roux's Arch. *191*:246-256.

Shimuzu, T. 1983(a). Cortical differentiation of the animal pole during maturation in fertilized eggs of *Tubifex* (Annelida, Oligochaeta). Develop. Biol. *85*:65-76.

Shimuzu, T. 1983(b). The organization of actin filaments during polar body formation in eggs of *Tubifex* (Annelida, Oligochaeta). Eur J. Cell Biol. *30*:74-82.

Small, J.V. (and G. Langanger). 1981. Organization of actin in the leading edge of cultured cells: influence of OsO_4 and dehydration on the ultrastructure of actin meshworks. J. Cell Biol. *91*:695-705.

Small, J.V. and J.E. Celis. 1978. Direct visualization of the 10-nm (100A)-filament network in whole and enucleated cultured cells. J. Cell Science. *31*:393-409.

Spudich, A. and J.A. Spudich. 1979. Actin in triton treated preparations of unfertilizad and fertilized sea urchin eggs. J. Cell Biol. *82*:214-226.

Stossel, T.P. 1983. The spatial organization of cortical cytoplasm in macrophages. *In* Modern cell biology. Alan Liss Inc. *2*:203-223.

Tannenbaum, J., S.W. Tanenbaum and G.C. Godman. 1977. The binding sites of cytochalasin D. 1. Evidence that they may be peripheral membrane proteins. J. Cell Physiol. *91*:225-238.

Temin, H.M. 1956. Studies on activation in eggs of *Urechis caupo, Nereis limbata* and *Asterias forbesi*. Biol. Bull. *61*:313-314.

Tilney, L.G. 1983. Interactions between actin filaments and membranes give spatial organization to cells. *In* Modern Cell Biology. Alan Liss Inc. *2*:163-199.

Tilney, L.G. and L.A. Jaffe. 1982. Actin, microvilli and the fertilization cone of sea urchin eggs. J. Cell Biol. *87*:771-782.

Tsukita, S., S. Tsukita and H. Ishikawa. 1980. Cytoskeletal network underlying the human erythrocyte membrane. J. Cell Biol. *85*:567-576.

Tyler, A. 1932. Changes in volume and surface of *Urechis* eggs upon fertilization. J. Exp. Zool. *63*:155-173.

Usui, N. and M. Yoneda. 1982. Ultrastructural basis of the tension increase in sea urchin eggs prior to cytokinesis. Dev. Growth and Differ. *24*:453-465.

Vacquier, V.D. 1981. Dynamics of the egg cortex. Develop. Biol. *84*:1-26.

Vacquier, V.D. and G.W. Moy. 1980. The cytolytic isolation of the cortex of the sea urchin (*Lytechinus pictus*) egg. Develop. Biol. *77*: 178-190.

Wang, Y. and D.L. Taylor. 1979. Distribution of fluorescently labelled actin in living sea urchin eggs during early development. J. Cell Biol. *82*:672-679.

Weatherbee, J.A. 1981. Membranes and cell movement: interaction of membranes with the proteins of the cytoskeleton. Int. Rev. of Cytol. Suppl. *12*:113-172.

Weber, K., D.C. Rathke, M. Osborne and W.W. Franke. 1976. Distribution of actin and tubulin in cells and in glycerinated cell models after treatment with cytochalasin B (CB). Exp. Cell Res. *102*:285-297.

Weiland, T. and H. Faulstich. 1978. Amatoxins, phallotoxins, phallolysin and antamanide: the biologically active components of poisonous *Amanita* mushrooms. Crit. Rev. Bioch. *5*:185-260.

Wessells, N.K., B.S. Spooner, J.F. Ash, M.O. Bradley, M.A. Laduena, E.L. Taylor, J.T. Wrenn and K.M. Yamada. 1971. Contractile microfilament machinery of many cell types is reversibly inhibited by cytochalasin B. Science. *171*:135-143.

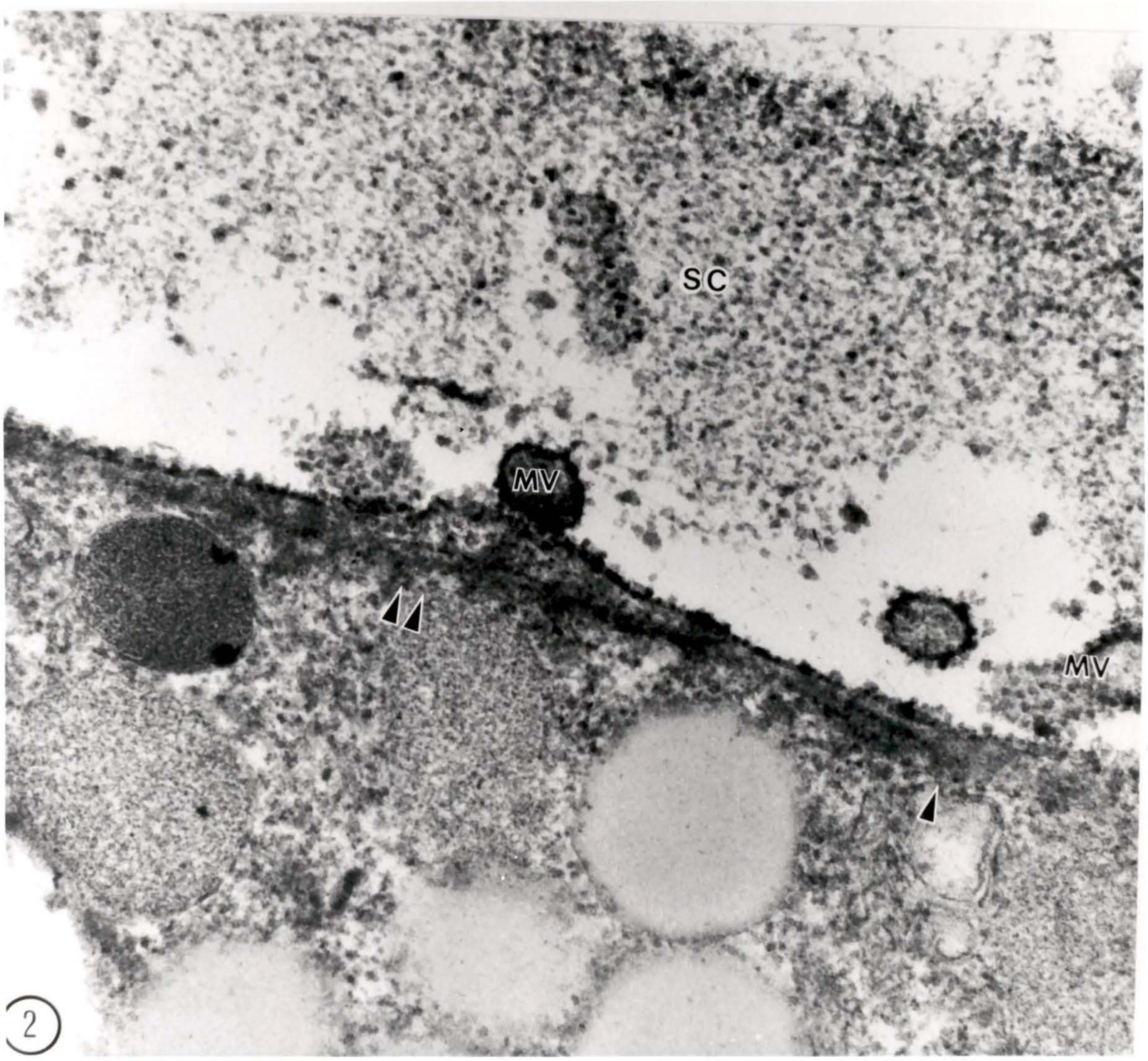
Wilson, E.B. 1925. The cell in development and heredity. McMillan.

Woolosowick, J.J. and K.R. Porter. 1979. Microtrabecular lattice of the cytoplAsmic ground substance. Artifact or reality. J. Cell Biol. *82*:114-139.

Fig 1. Living *Urechis* eggs. Oocytes (O) are characteristically indented while fertilized eggs (F) are spherical. GV, germinal vesicle; N, nucleolus. Photomicrograph, x600.


O
GV
N
F
1

Fig 2. Thin section of unfertilized egg cortex. The dense band is discontinuous (arrow) and occasionally appears filamentous (double arrow). SC, surface coat; MV, microvillus. TEM, x69,000.



2

Fig 3. Three serial sections through a fertilized egg cortex.

a. First of three serial sections. The dense band is discontinuous (arrow). MV, microvillus. TEM, x28,000.

b. Prominent dense band. Arrows, dense band. TEM, x28,000.

c. Reduced dense band in the third serial section. TEM, x28,000.

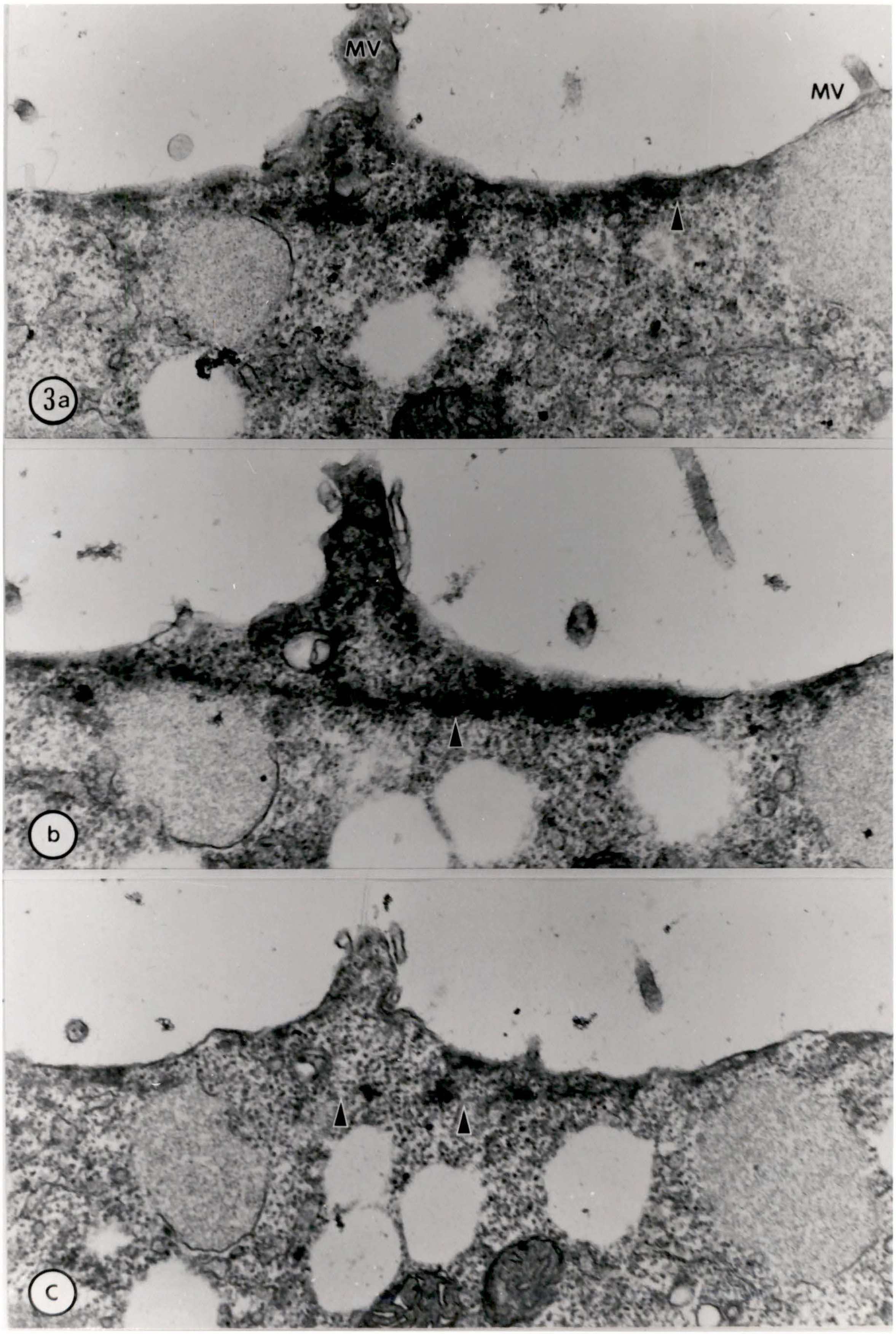

MV
MV
3a
b
c

Fig 4. Series of NBD-Phallacidin labelled eggs. Fluorescence micrographs (FMs), x610.

a. Control oocyte.

b. Oocyte. GV, germinal vesicle.

c. 30 seconds PI.

d. 1 minute PI.

e. 2 minutes PI.

f. 4 minutes PI.

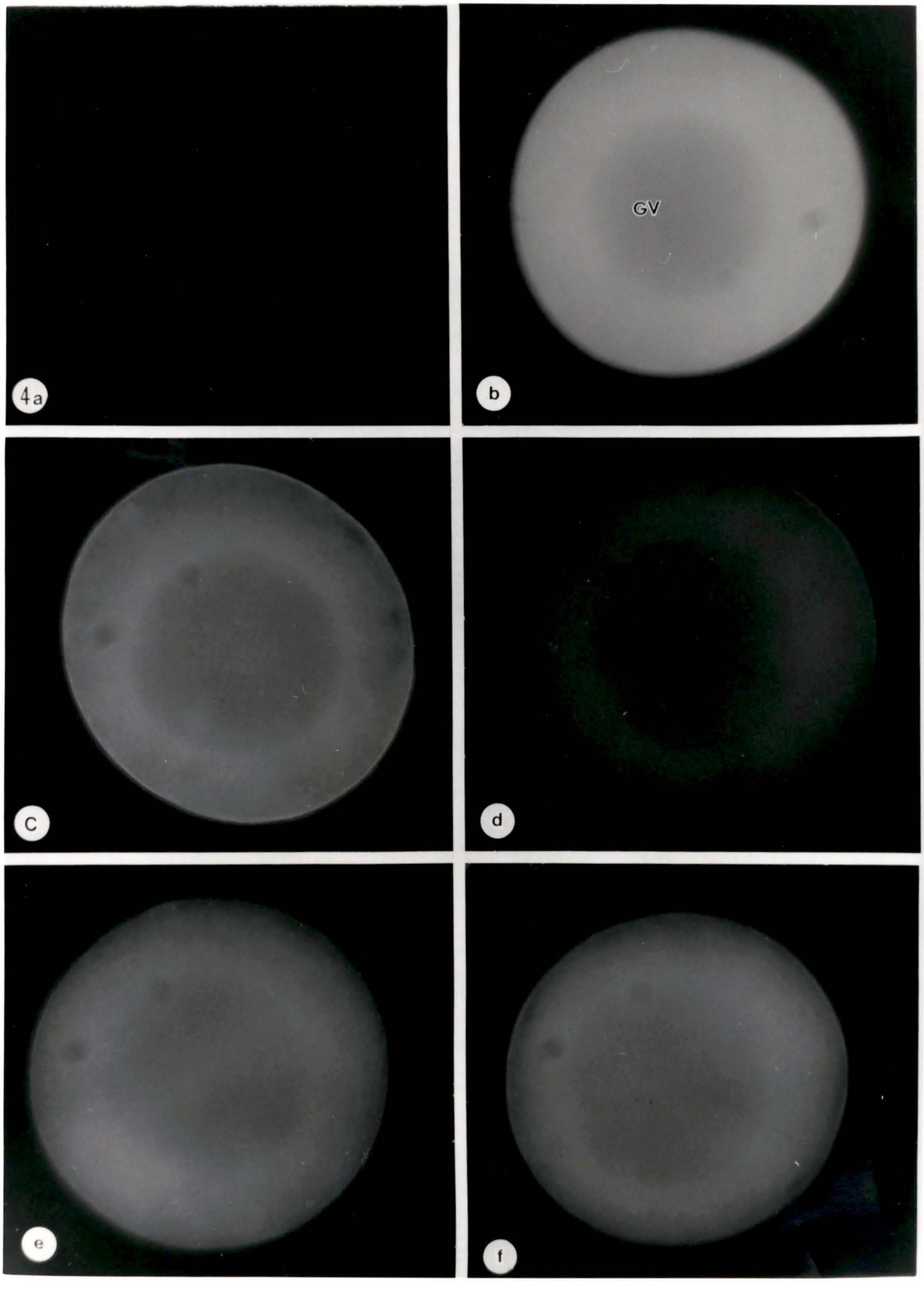

4a
b
GV
c
d
e
f

Fig 5. NBD-Phallacidin-labelled embryos, FM.

a. Optical equatorial section shows cortical "ring" of fluorescence. 30 min PI, x 490.

b. 30 min PI, x1,220.

c. Two-cell stage. The cleavage furrow region stains intensely. 90 minutes PI, FM, x490

d. Polar body of cleavula stains brightly. Same cleavula as in c. 90 min PI, x490.

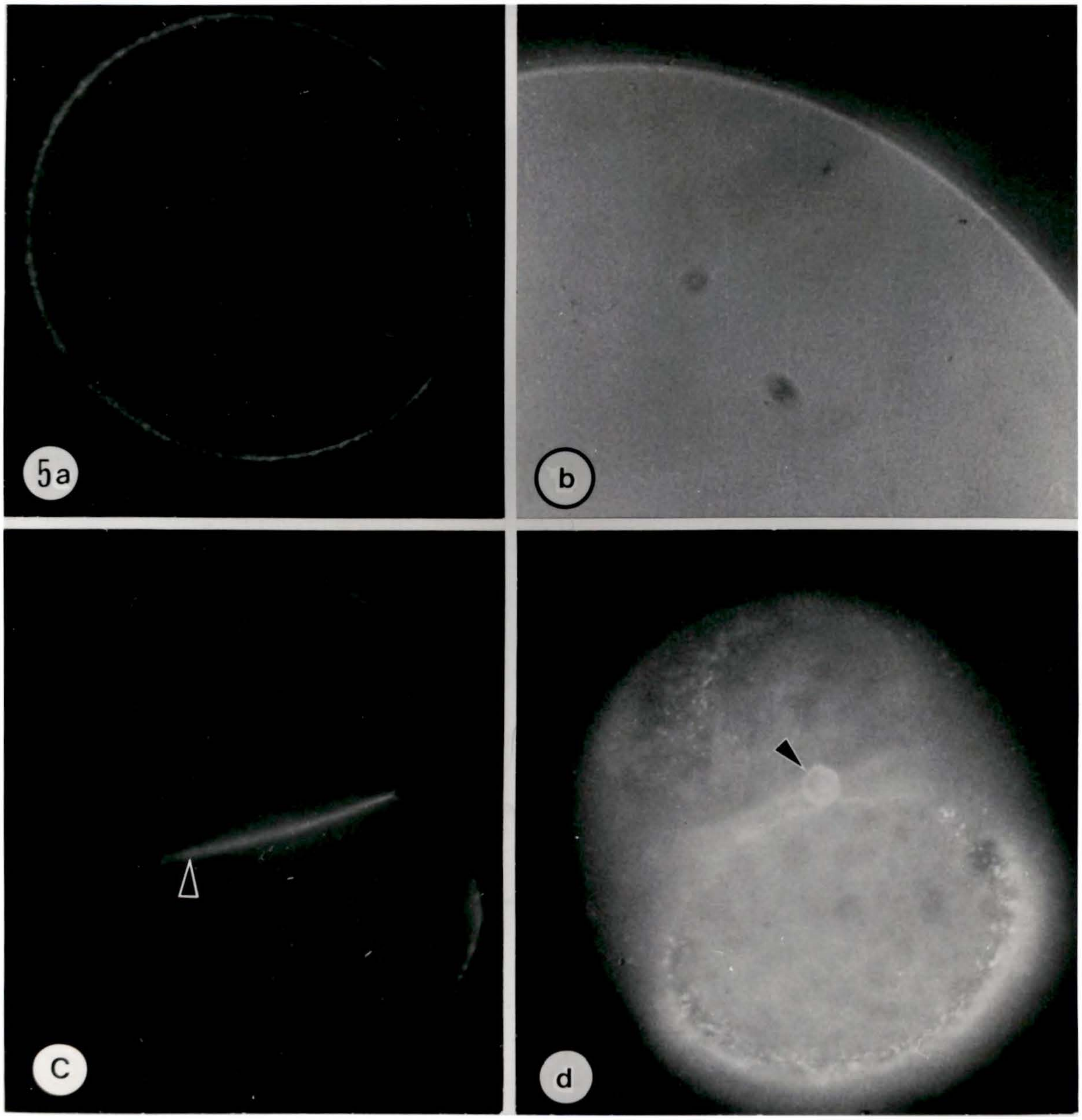

5a
b
c
d

Fig 6. Sperm penetration cones.

a. 3.5 min PI. A ring of fluorescence appears in the cortical cytoplasm where the sperm penetration cone forms (arrow). FM, x1,420.

b. 4 min. PI. The cone of cytoplasm surrounding the fertilizing sperm fluoresces intensely (arrow). FM, x1,420.


6a

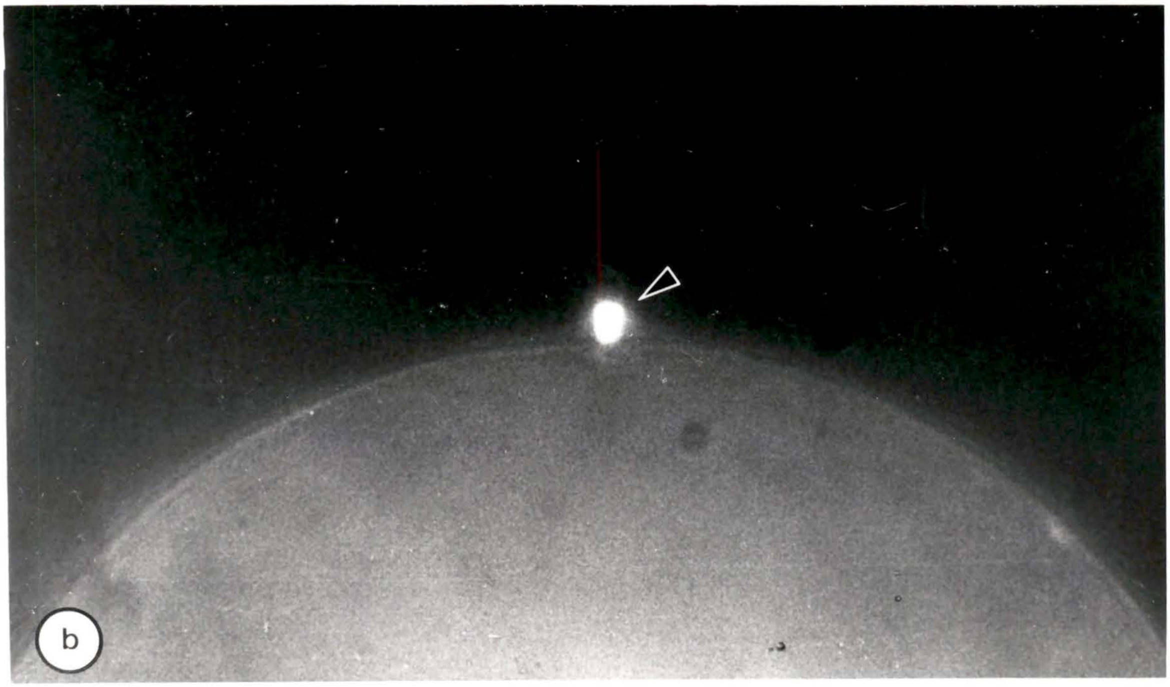
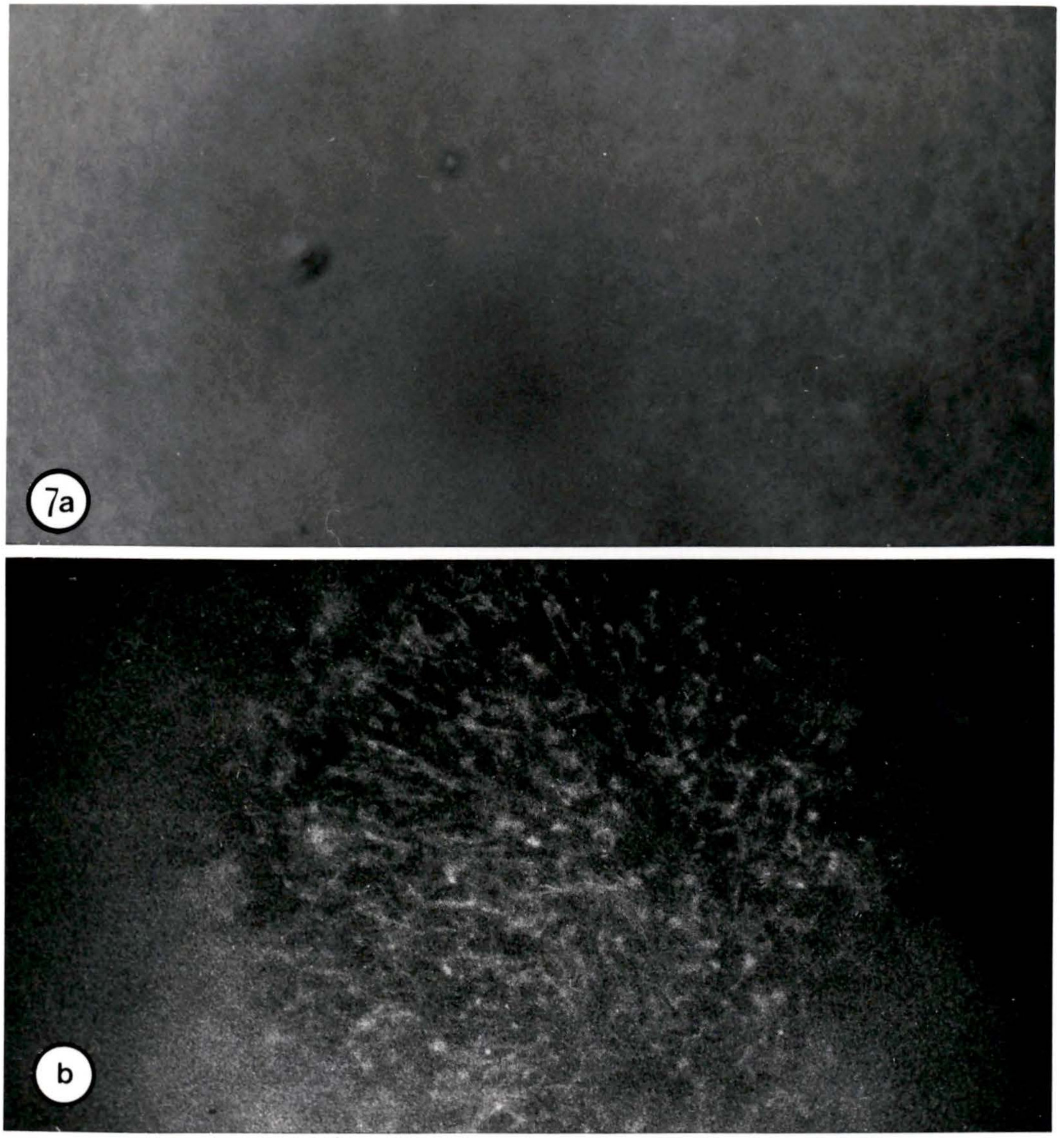

b

Fig 7. Surface of NBD-Phallacidin-labelled eggs.

a. Oocyte. FM, x1,200.

b. 30 min PI. There is a filamentous pattern of fluorescence in the cortex. FM, x1,200.

Fig 8. Microvilli (MV) are embedded in and extend partway through the surface coat (SC). TEM, x55,000.


7a
b

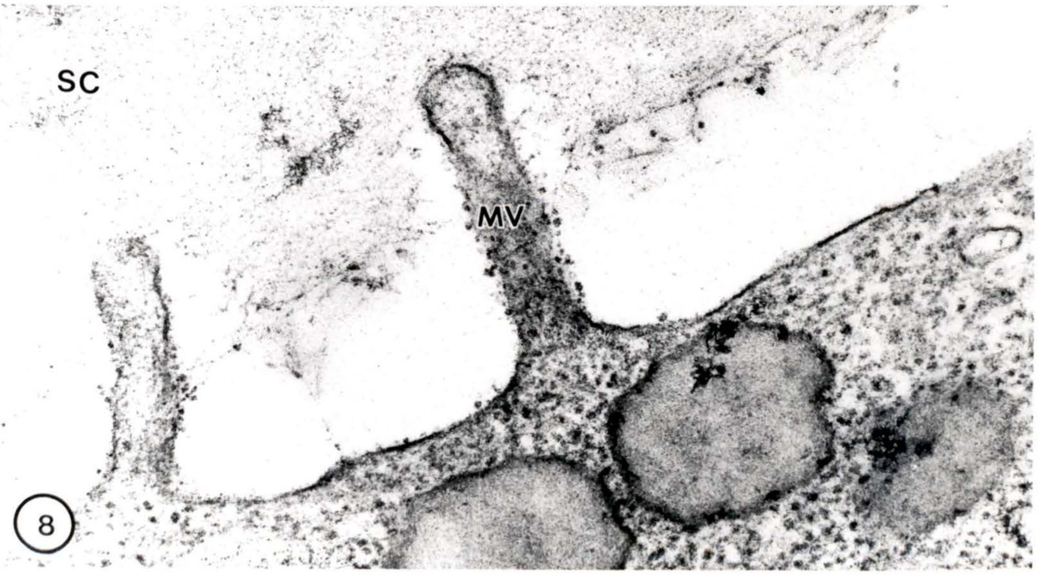

SC
MV
8

Fig 9. Cytochalasin B-treated eggs stained with NBD-Phallacidin.

a. Control oocyte. FM, x540.

b. Oocyte. The cortical region appears blotchy (arrows). GV, germinal vesicle, FM, x540.

c. Higher magnification of oocyte shown b. The cortex contains patches of fluorescence (arrows). FM, x1,200.

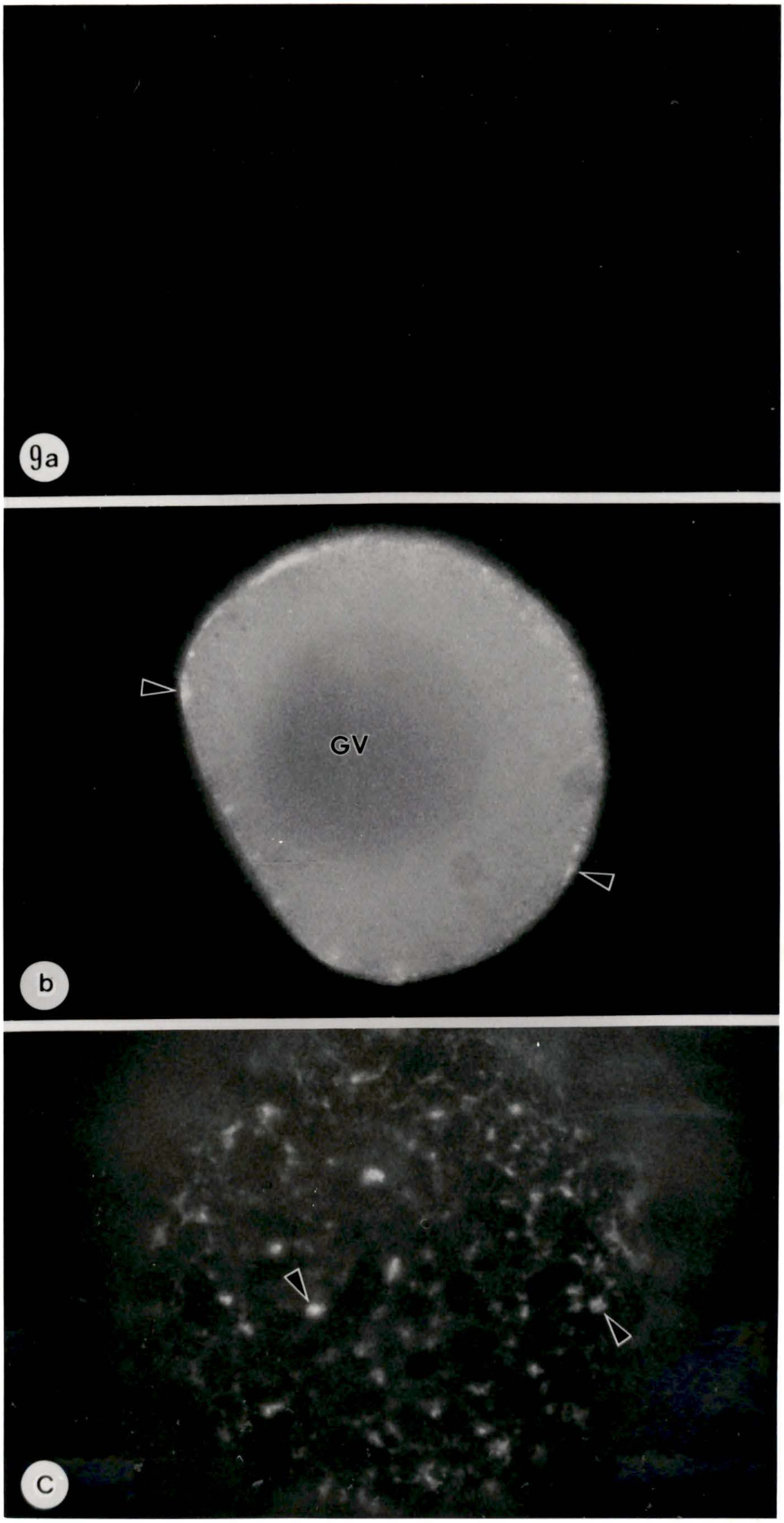

9a
GV
b
c

Fig 10. Florescent anti-actin labelled oocytes.

a. Control. FM, x510.

b. Labelled oocyte. Fluorescence appears throughout the oocyte.

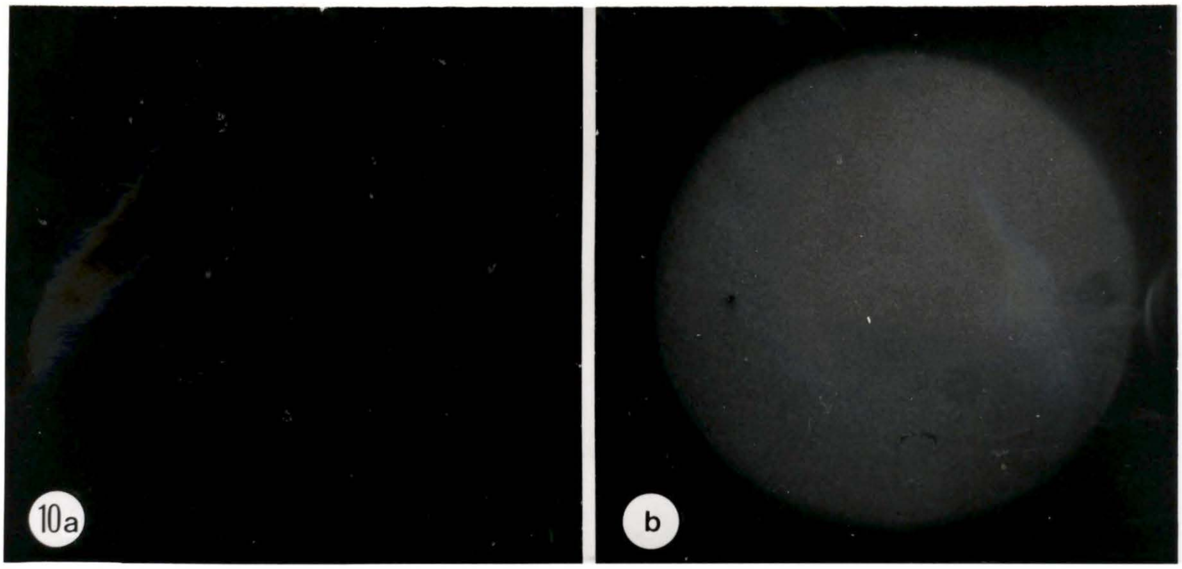

10a
b

Fig 11. Detergent extracted oocytes.

a. Oocyte torn open to reveal interior. Cytoskeletal fibers are numerous throughout (arrows). SC, surface coat; GV, germinal vesicle, SEM, x1,400.

b. Extracted oocyte with part of the surface coat peeled away. Arrows show the fibers of the cytoskeleton. SEM, x900.

c. Higher magnification of fig 11b. Arrows show fibers of cytoskeleton. SC, surface coat, SEM, x3,500.

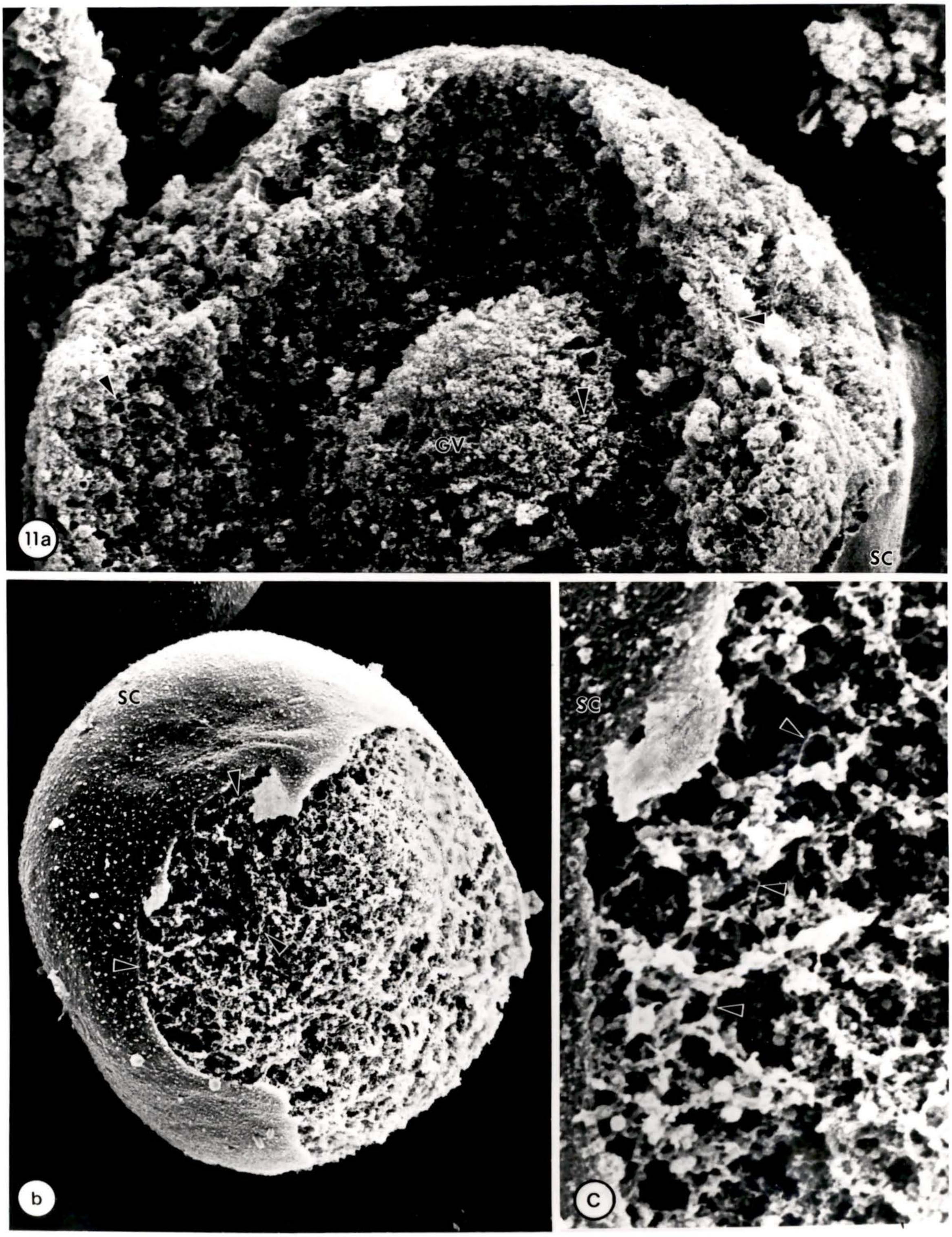

11a
GV
SC
b
SC
c
SC

Fig 12. Montage of stages of surface coat removal.

a. Oocyte before surface coat (SC) removal. SEM, x850.

b. Oocyte with surface coat remaining. The outer layer (arrow) of the surface coat is only partly removed. SEM, x850.

c. Part of inner layer of surface coat (arrow) remaining. SEM, x850.

d. Surface coat completely removed. Arrows indicate microvilli. SEM, x850.

Fig 13. Light micrographs of the progressive dissolution of the surface coat.

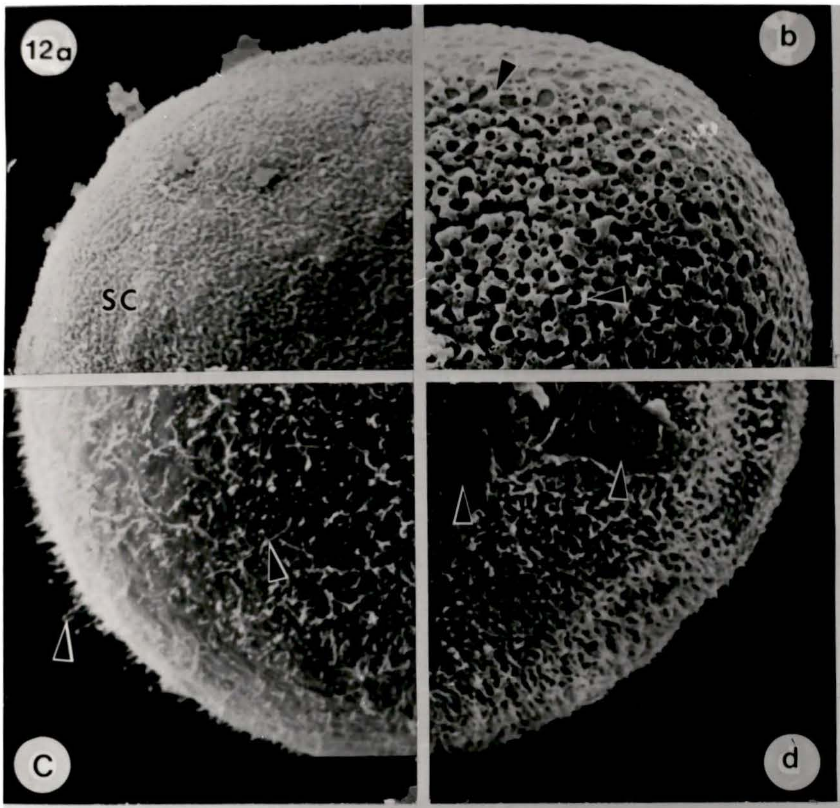
a. Outer layer (arrow) of surface coat becomes wrinkled. GV, germinal vesicle, x330.

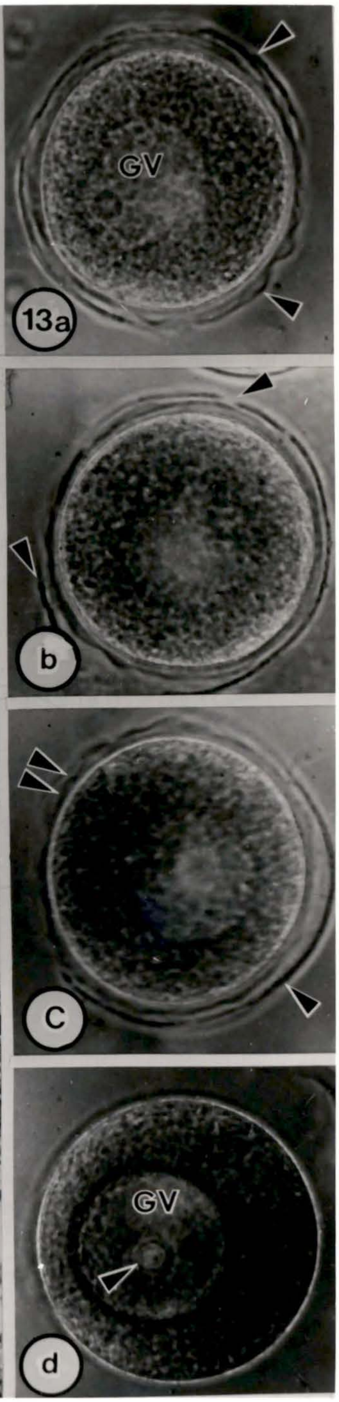
b. The outer layer swells and breaks in one or more places (arrow). x330.

c. The inner layer of the surface coat begins to dissolve (double arrow). The outer layer (arrow) can be removed by gentle agitation. x330.

d. Both layers of surface coat are removed. GV, germinal vesicle; arrow, nucleolus. x330.

Fig 14. Bilaminar nature of the surface coat. Eggs fixed in the presence of tannic acid show an outer layer (OL) and and inner layer (IL) of the surface coat. [Microvillar tips (MVT) are separated from the microvilli during oogenesis and remain as a layer covering the surface coat]. Presence of tannic acid in the fixative causes surface glycoproteins to be densely stained. TEM, x61,600.


12a
SC
b
c
d


GV
13a
b
c
GV
d

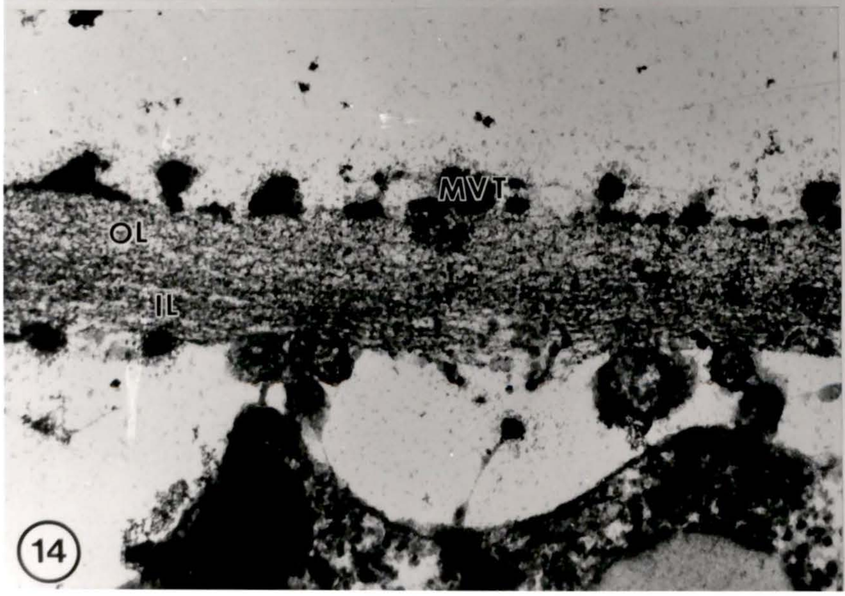

MVT
OL
IL
14

Fig 15. Disruption of microvilli by surface coat removal.

a. Microvilli (arrows) are stretched by the dissolving surface coat (SC). SEM, x5,400.

b. Microvilli (arrows) appear to have snapped back and may be broken when the surface coat (SC) is finally removed. SEM, x5,200.

c. Oocyte with surface coat removed by Mg-free sea water. Microvilli are long and numerous. SEM, x770.

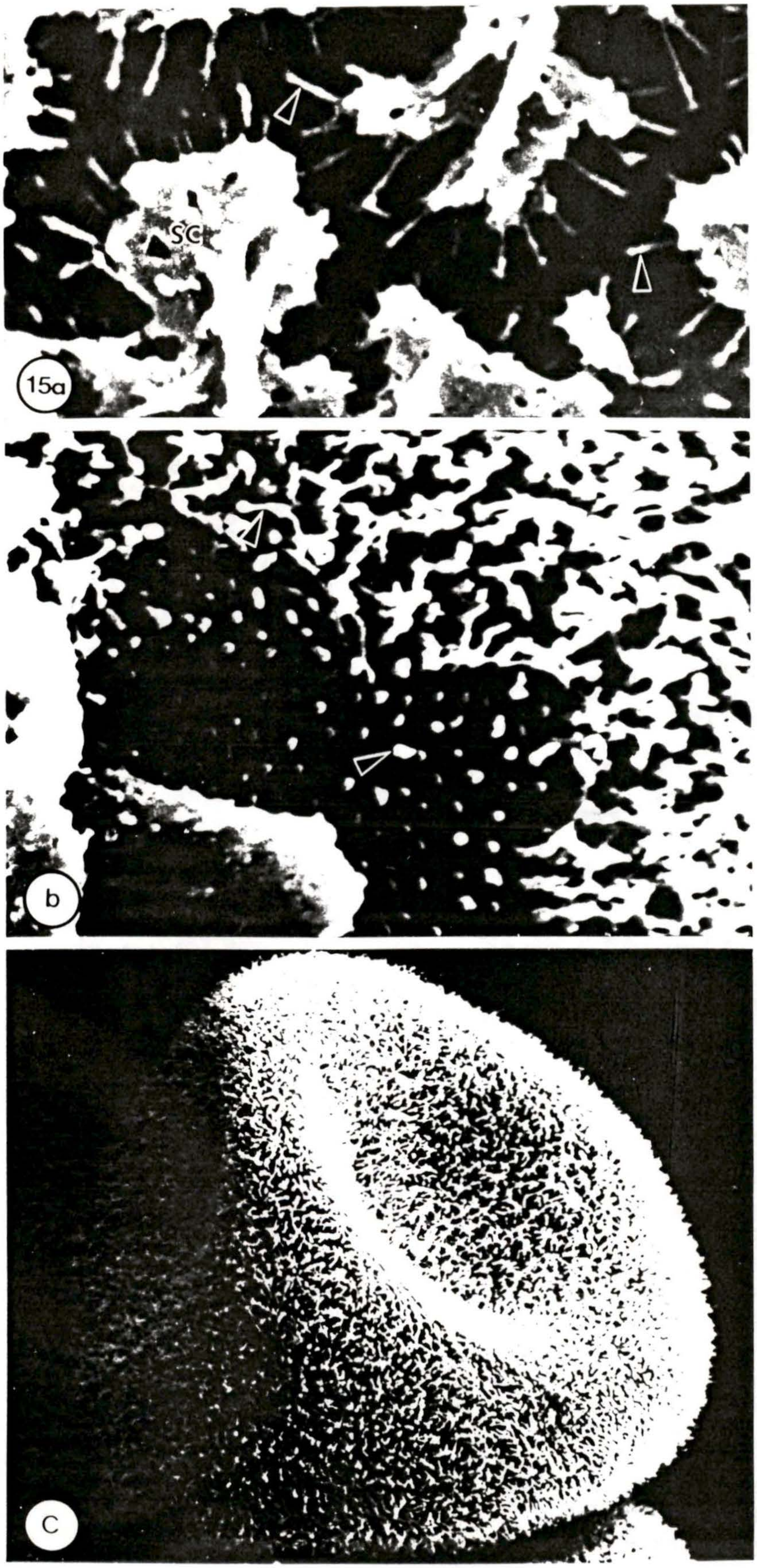

15a
SC
b
c

Fig 16. Extraction of the plasma membrane.

a. Early stage of extraction. After 15 minutes, the plasma membrane becomes convoluted and microvilli (arrows) become shorter and distorted. Some microvilli appear to fuse with one another (double arrows). SC, surface coat, SEM, x2,500.

b. The plasma membrane after 30 minutes of extraction. Holes are visible in the surface (arrows) and the remaining microvilli (double arrows) are reduced to bumps. The surface coat (SC) is also altered by extraction. SEM, x2,000.

c. The PML after one hour of extraction. The surface is an anastamosing web of fibers approximately 100 nm in diameter (arrows). SEM, x6,400.

d. Completely extracted oocyte with patches of surface coat remaining. OL, outer layer or surface coat; IL, inner layer of surface coat, SEM, x1,200.

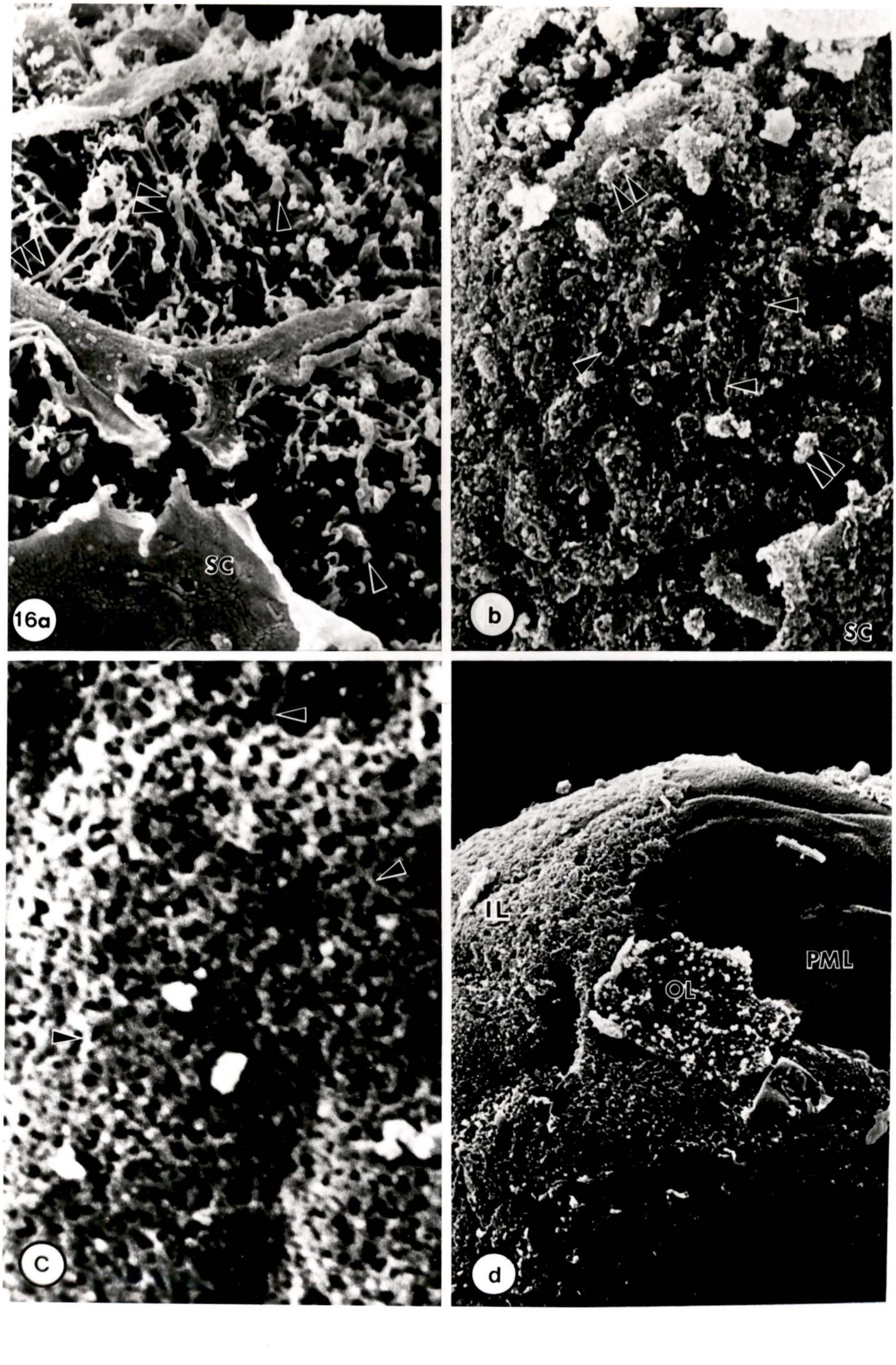

16a
SC
b
SC
c
IL
PML
OL
d

Fig 17. Extracted oocyte showing PML, surface coat and internal cytoskeleton.

a. PML torn to reveal internal cytoskeleton (CSK). SC, surface coat, SEM, x870.

b. Higher magnification of the oocyte shown in fig 17a. SC, surface coat, SEM, x4,500.

c. The PML is continuous with the internal cytoskeleton (CSK). SEM, x10,100.

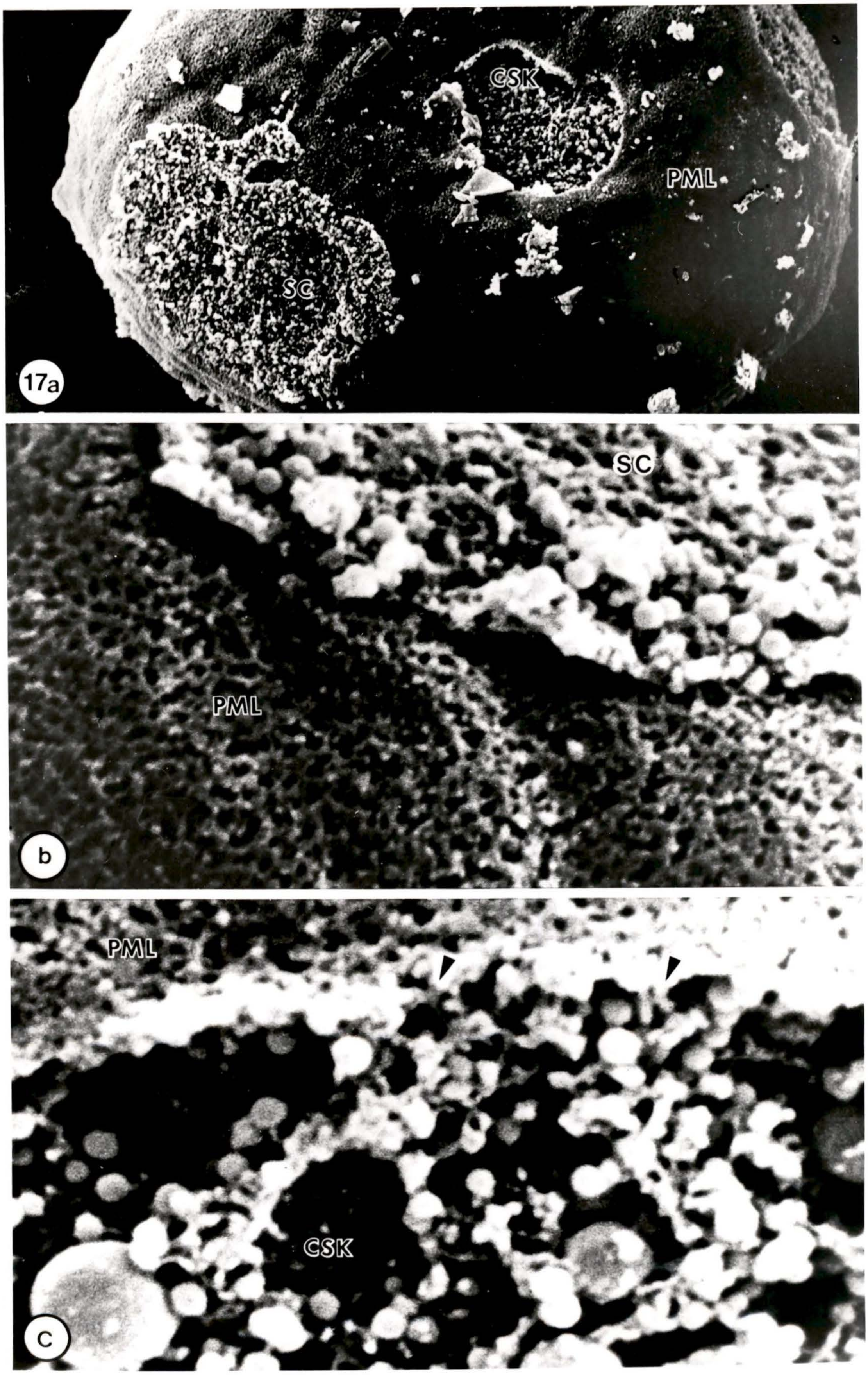

CSK
PML
SC
17a
SC
PML
b
PML
CSK
c

Fig 18. Thin section of extracted oocyte.

a. The area bounded by the square is shown in fig 18b. Arrows, cytoskeletal fibers; double arrow, PML; GV, germinal vesicle, TEM, x2,900.

b. Higher magnification of section shown in fig 18a. The PML at its thinnest points appears filamentous (arrows). TEM, x20,000.

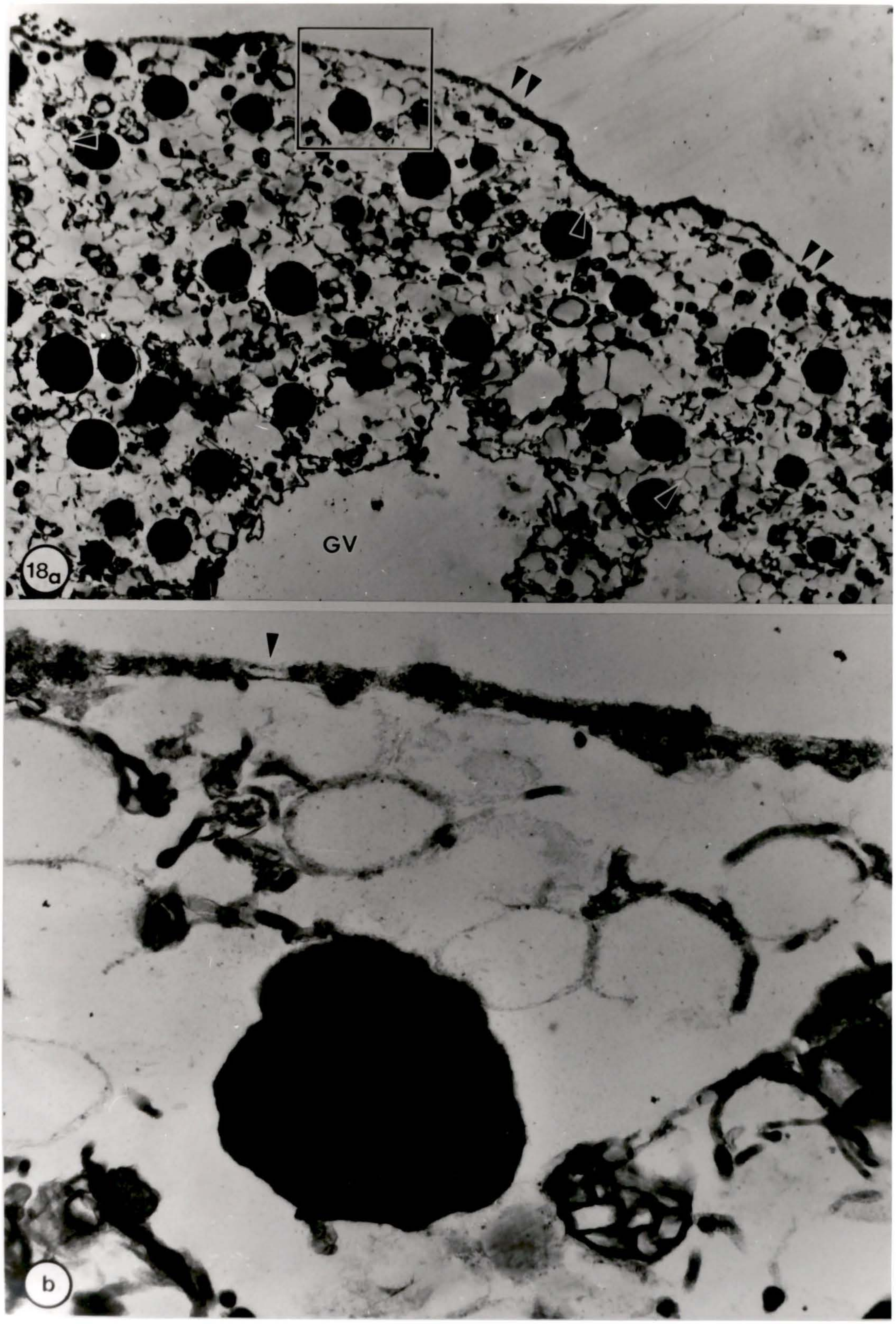
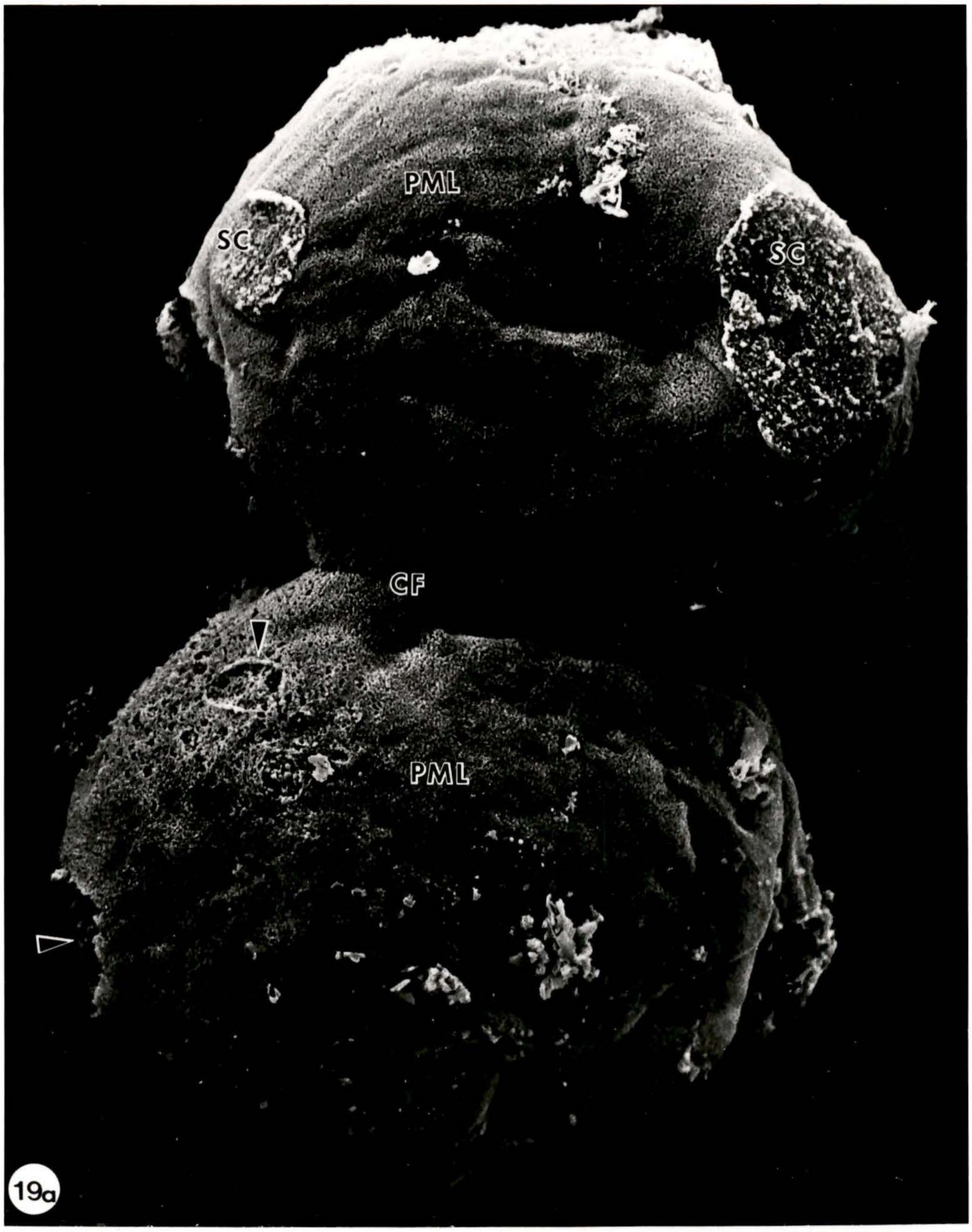
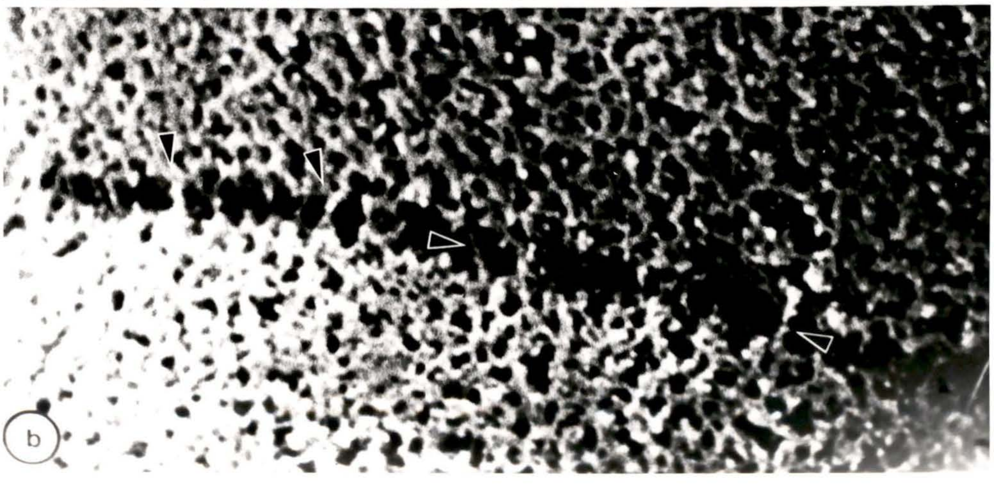

18a
GV
b

Fig 19. Extracted two-cell stage (cleavula).

a. Some patches of surface coat (SC) remain, and the PML is torn in places (arrows). CF, cleavage furrow region, SEM, x1,400.

b. Higher magnification of cleavage furrow region shown in fig 19a. PML is continuous between the two cells (arrows). SEM, x6,500.


PML
SC
SC
CF
PML
19a


b

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PARTIAL CHARACTERIZATION OF THE CYTOSKELETON IN THE EGGS OF *URECHIS CAUPO*

Author [redacted]
Ellen Blythe Nilson

February 28th, 1985
Date