The Changes in Structural Organization of Actin in the Sea Urchin Egg Cortex in Response to Hydrostatic Pressure

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ABSTRACT We have used hydrostatic pressure to study the structural organization of actin in the sea urchin egg cortex and the role of cortical actin in early development. Pressurization of *Arbacia punctulata* eggs to 6,000 psi at the first cleavage division caused the regression of the cleavage furrow and the disappearance of actin filament bundles from the microvilli. Within 30 s to 1 min of decompression these bundles reformed and furrowing resumed. Pressurization of dividing eggs to 7,500 psi caused both the regression of the cleavage furrow and the complete loss of microvilli from the egg surface. Following release from this higher pressure, the eggs underwent extensive, uncoordinated surface contractions, but failed to cleave. The eggs gradually regained their spherical shape and cleaved directly into four cells at the second cleavage division. Microvilli reformed on the egg surface over a period of time corresponding to that required for the recovery of normal egg shape and stability. During the initial stages of their regrowth the microvilli contained a network of actin filaments that began to transform into bundles when the microvilli had reached ~⅔ of their final length. These results demonstrate that moderate levels of hydrostatic pressure cause the reversible disruption of cortical actin organization, and suggest that this network of actin stabilizes the egg surface and participates in the formation of the contractile ring during cytokinesis. The results also demonstrate that actin filament bundles are not required for the regrowth of microvilli after their removal by pressurization. Preliminary experiments demonstrate that F-actin is not depolymerized in vitro by pressures up to 10,000 psi and suggest that pressure may act indirectly in vivo, either by changing the intracellular ionic environment or by altering the interaction of actin binding proteins with actin.

A highly organized array of actin filaments develops in the sea urchin egg cortex during the first few minutes after fertilization. The formation of this actin network is coupled to a generalized restructuring of the egg's surface that results in the generation of large numbers of microvilli (1, 14, 17, 50, 56). Recent evidence indicates that these cortical actin filaments are derived from both a nonfilamentous storage form of actin that is associated with the unfertilized egg cortex (2, 28, 29, 51) and a pool of soluble cytoplasmic actin (28, 29, 38, 57).

The reorganization of cortical actin following fertilization appears to be regulated by both Ca^{++} and cytoplasmic pH. The transient increase in intracellular free Ca^{++}concentration that occurs at fertilization induces the formation of broad, pseudopod-like surface protrusions containing a network of actin filaments (1, 4, 11). Subsequent cytoplasmic alkalinization transforms this network into bundles of filaments, with the concomitant conversion of the surface protrusions into normal microvilli (1, 4, 11).

Although the actual molecular mechanism of cortical actin reorganization is not known, it is likely to involve actin-binding proteins similar to those that have been described in other systems (see 16, 49, 58 for reviews). Recent evidence suggests that actin is prevented from polymerizing in the unfertilized egg by a profilin-like protein (22, 27). To date however, fascin is the only actin-binding protein that has been demonstrated to be a component of the fertilized egg cortex (38). Since fascin-actin interactions are not Ca^{++} or pH sensitive (7), it appears likely that other as yet unidentified actin-binding proteins may also be involved in establishing the

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structural organization of actin in the fertilized egg cortex.

In addition to participating in the restructuring of the egg cortex at fertilization, cortical actin has also been implicated in the formation of the fertilization cone (56) and the incorporation of the sperm into the egg (10, 47, 48). It is also likely that the array of cortical actin filaments provides the structural basis for many of the mechanical properties of the fertilized egg cortex (20, 21) and is the source of actin from which the contractile ring is formed during cytokinesis (39).

We have used hydrostatic pressure to study the dynamics of actin organization in the fertilized sea urchin egg cortex and the role of cortical actin in early development. Hydrostatic pressure is an ideal probe for these types of studies since it can be rapidly applied and released, and is completely reversible when used in the range of 3,500–10,000 psi (43, 46).

In a series of classic papers, Marsland and his colleagues demonstrated that hydrostatic pressure reduced the gel strength of the fertilized egg cortex as measured by the ability of an applied centrifugal field to dislodge cortical pigment granules (26, 32, 33, 36). This pressure-induced solation of the cortex correlated with an inhibition of cytokinesis (31, 32, 33). On the basis of these observations Marsland (31, 33) proposed the gel contraction theory of cytokinesis, which postulates that cleavage results from the active contraction of a band of cortical gel within the region of the furrow. In light of our present knowledge of the structural organization of actin in the fertilized egg cortex, it appears likely that the pressure induced gel-sol transformation of the cortex results from the disruption of cortical actin filaments. This conclusion is supported by the observation that deuterium oxide, which favors the associated state of self-assembly systems (24, 53), increases the gel strength of the cortex and antagonizes the effects of hydrostatic pressure (34, 35, 37).

Tilney and Cardell (54) have previously demonstrated that hydrostatic pressure disrupts the organization of actin in the brush border of intestinal epithelial cells. Pressurization of segments of salamander small intestine to 6,500 psi resulted in the loss of microvilli and the disappearance of the terminal web. Upon decompression, microvilli containing core bundles of actin filaments gradually reformed, and the normal structure of the terminal web was reestablished. Hydrostatic pressure has also been shown to cause the disappearance of microvilli from the ectodermal cells of sea urchin gastrulae (55), but their subsequent reformation was not studied.

In this report we describe the differential effects of 6,000- and 7,500-psi pressure on cleavage and on the structure of microvilli and their core bundles of actin filaments. A subsequent paper will discuss the effects of hydrostatic pressure on the organization of actin in the contractile ring. A preliminary report of these results was presented previously (3).

MATERIALS AND METHODS

Arbacia punctulata were obtained either from the supply department of the Marine Biological Laboratory (Woods Hole, MA) or from G. W. Nobel (Panacea, FL). The shedding of gametes was induced by injecting 0.5 M KCl into the coelomic cavity. Eggs were washed three times in either filtered sea water (FSW) or artificial sea water (ASW,[1] MBL formula, [12]) and stored in FSW or ASW at 15–18°C. Sperm were collected "dry" and stored at 4°C. Eggs were fertilized by the addition of 2–3 drops of a dilute sperm suspension. Fertilization envelopes and the hyaline layer were removed by suspending eggs in 1 M urea containing 2 mM HEPES buffer, pH 8.0, for 4 min at 4°C. Demembranated eggs were washed three times in Ca^{++}-free ASW (8) containing 10 mM HEPES and 1 mM EGTA, pH 8.0, at 20–22°C. The eggs were allowed to develop as a monolayer in a culture dish containing Ca^{++}-free ASW at 20–22°C. For experiments in which the fertilization envelopes were not removed, fertilized eggs were incubated in culture dishes containing either FSW or ASW at 20–22°C.

Light Microscopic Observations: Small samples of eggs at metaphase of the first cleavage division were placed on the lower window of the miniature optical hydrostatic pressure chamber of Salmon and Ellis (45). The chamber was filled with sea water, sealed, and placed in its holder (45) on the stage of a Zeiss universal microscope. Eggs were observed with a Zeiss 10 × (NA 0.22) phase-contrast objective and model IS long working distance phase condenser (Carl Zeiss, Inc., Thornwood, NY). When the majority of eggs in the field had begun to cleave, the chamber pressure was rapidly raised to the desired level and held for 5 min. This 5-min time period was chosen to reproduce the conditions used by Marsland (31). At the end of this time, pressure was returned to normal. Photographic records were made on Kodak Plus-X 35-mm film using a Zeiss MC-63 automatic camera.

Electron Microscopic Observations: Suspensions of eggs were fixed under pressure at first cleavage in the pressure fixation apparatus of Landau and Thibodeau (25). The apparatus consists of two stainless steel cylinders: an outer pressure bomb and an inner culture-fixation chamber. The inner cylinder is composed of two 7-ml compartments, separated by a circular glass coverslip. The ends of the two compartments contain rubber diaphragms that transmit pressure equally to the two chambers. The lower chamber also contains a stainless steel ball. Double strength fixative was added to the lower compartment, a coverslip was inserted, and the upper chamber was attached. After filling the upper compartment with a suspension of eggs, the chamber was sealed, placed in the pressure bomb that had previously been filled with water at 20°C, and the entire apparatus attached to an Enerpac hydraulic pump (Boston Hydraulics, Inc., Boston, MA). The pressure was raised to the desired level and the bomb valved off and separated from the pressure line. After pressurization for 5 min, the pressure vessel was inverted and shaken vigorously, causing the steel ball to break the glass coverslip and mix the fixative with the egg suspension. Eggs were fixed for 10 min under pressure and then removed from the pressure apparatus and fixed for an additional 20 min at atmospheric pressure. Double strength fixative consisted of 2% glutaraldehyde (8% stock; EMS, Inc., Fort Washington, PA) and 2% paraformaldehyde in Ca^{++}-free ASW, pH 7.2.

For experiments in which the recovery of eggs from pressurization was followed, the culture chamber was used without a coverslip, and the entire inner chamber was filled with egg suspension. Eggs were pressurized at first cleavage as described above and held under pressure for 5 min. The pressure was then released, the chamber rapidly opened, and the egg suspension transferred to a small beaker. Aliquots of eggs were fixed at various times after pressure release by mixing with an equal volume of double strength fixative. The earliest time point that could reliably be taken was at 30 s after pressure release. Fixation was carried out for 30 min at 20–22°C.

After glutaraldehyde fixation, the eggs were washed three times in Ca^{++}-free ASW, pH 7.2, and postfixed in 0.5% OsO_4 in 0.1 M sodium phosphate buffer, pH 6.0 on ice for 30 min. The eggs were subsequently washed three times in deionized water and incubated at room temperature in unbuffered aqueous 1% uranyl acetate for 45 min before being dehydrated in ethanol and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and were examined with either a Philips 300 electron microscope operated at an accelerating voltage of 60 kV or a JEOL 100CX electron microscope operated at an accelerating voltage of 80 kV.

Pressurization of Purified Actin: Actin was purified from an acetone powder of chicken skeletal muscle according to the method of Spudich and Watt (52). The concentration of actin was determined by absorbance at 280 nm, using an extinction coefficient of 1.09 for a 1 mg/ml solution of G-actin (30). Polymerization was induced by the addition of 100 mM KCl, 10 mM imidazole-Cl and 2 mM $MgCl_2$ to a solution of G-actin at pH 7.2. The polymerization state of actin was monitored by viscometry at 24°C, using Ostwald capillary viscometers (type A150; Cannon Instrument Co., University Park, PA), with a sample volume of 0.6 ml and a buffer flow time of ~30 s. The specific viscosity in centistokes (cs) was calculated as (sample flow time/buffer flow time) − 1, assuming a sample density of 1.

F-actin was pressurized at a concentration of 14 μm in the pressure fixation chamber for 5 min and fixed under pressure in 0.2% glutaraldehyde in polymerization buffer as described above. Fixation was carried out to prevent repolymerization upon decompression. Following fixation, the pressure was released, the viscosity of the actin solution was measured, and samples were diluted to a concentration of 1.2 μM and negatively stained for electron microscopy. Negative staining was carried out on formvar-coated copper grids, stabilized with a thin carbon film. Grids were glow discharged immediately

[1] *Abbreviations used in this paper*: ASW, artificial sea water; FSW, filtered sea water.

before use to improve wetting. Samples were stained with unbuffered 1% uranyl acetate and examined with a Phillips 300 electron microscope as described above.

RESULTS

Effect of 6,000-psi Pressure

Pressurization of fertilized eggs to 6,000 psi during the first cleavage division caused an immediate cessation of cytokinesis and a gradual regression of the cleavage furrow over a period of 3–4 min (Fig. 1). Cytokinesis was completely inhibited at a pressure of 6,000 psi, while at lower pressures the percentage of eggs completing cleavage increased with decreasing pressure. We have therefore routinely used 6,000 psi in the experiments reported here. Cleavage furrows reformed within 30 s to 1 min of pressure release, and the eggs went on to complete division (Fig. 1). Identical results were observed in eggs with and without the hyaline layer.

Exposure of eggs to 6,000-psi pressure disrupted the normal organization of the cortex and altered the structure of the microvilli (Fig. 2). In unpressurized eggs pigment granules were closely associated with the plasma membrane, and long straight microvilli projected from the egg surface (Fig 2, *a* and *c*). The majority of microvilli contained core bundles of 6–7-nm filaments that have previously been shown to be composed of actin (4, 5, 8, 51). During pressurization the pigment granules dissociated from the plasma membrane and drifted into the underlying cytoplasm (Fig. 2*b*). The microvilli became irregular in shape and frequently appeared constricted at their bases (Fig. 2*b*).

Bundles of actin filaments were no longer visible within the cores of the microvilli, which instead contained large numbers of ribosome-like particles (Fig. 2*d*). Since these particles are normally excluded from the microvilli, their presence further indicates the solation of the core structure.

Upon decompression, bundles of core filaments rapidly reformed within the microvilli. Filament bundles began to develop at the tips of the microvilli within 30 s of pressure release (Fig. 3, *a* and *b*) and were completely reformed by 1 min (Fig. 3, *c* and *d*). As the filament bundles reformed the microvilli regained their normal shape, and ribosome-like particles were once again excluded from their cores. Within 2–3 min of pressure release pigment granules became reassociated with the plasma membrane. The time required for the reestablishment of normal cortical actin organization corresponded to the time required for the reinitiation of cleavage.

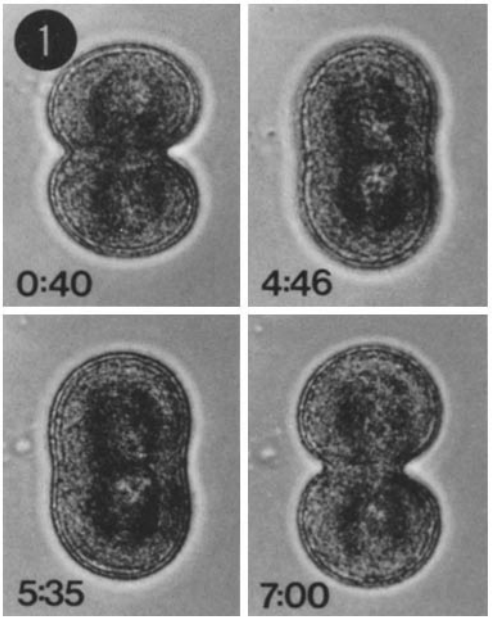

FIGURE 1 Pressurization of eggs to 6,000 psi during first cleavage division. Time in minutes with respect to initial observation is given in lower left of each frame. Pressure was applied at 1 min and released at 5 min. The cleavage furrow regresses under pressure and rapidly reforms after decompression. × 315.

Effects of 7,500 psi-Pressure

Fertilized eggs that were pressurized to 7,500 psi or higher during first division also stopped cleaving and showed a gradual regression of the cleavage furrow (Fig. 4). However, in contrast to eggs pressurized to 6,000 psi, furrowing did not resume upon decompression. A pressure of 7,500 psi was used routinely because it was the minimum pressure that completely inhibited cleavage after decompression. After release from 7,500 psi pressure the eggs either gradually rounded up over a period of 10–15 min or exhibited rapid, uncoordinated surface contractions (Fig. 4). This motility can take various forms including the formation of ring-shaped constrictions, the rapid undulation of large areas of the surface, or the extrusion of pseudopod-like processes. In the example shown in Fig. 4, the egg forms a series of unstable constrictions that move toward the base of the cell, forcing the upper region of the cell to bulge outward (Fig. 4, *5:14–5:50* min). A lobopod-like structure then formed and propagated around the cell (Fig. 4, *6:40*). These contractions gradually subsided, and the egg regained its spherical shape (Fig. 4, *16:10*). The number of eggs showing this type of motility varied considerably between batches of eggs and with time in the breeding season. In addition, the induction of amoeboid movement was more apparent in eggs that had their hyaline layer removed, suggesting that this extracellular coat may somehow help to stabilize the surface. The majority of eggs cleaved directly into four cells at the time of second division, although some irregular cleavages were observed.

Pressurization of eggs to 7,500 psi had a dramatic effect on the organization of the cortex and the structure of the cell surface. At this pressure essentially all microvilli were lost from the surface of the egg (Fig. 5). While we cannot rule out the possibility that some of the microvilli may be retracted, the majority appeared to pinch off at their bases and could be found floating free in the surrounding sea water (Fig. 5*a*). These detached microvilli remained embedded in the hyaline layer when it was left intact. In some regions of the egg, a layer of clear granular cytoplasm occupied a zone up to 5 μm thick just beneath the plasma membrane (Fig. 5*a*), while in other regions yolk platelets and mitochondria extended all the way to the plasma membrane (Fig. 5*b*). As in the case of 6,000-psi pressure, the pigment granules lost their association with the cortex and became scattered throughout the cytoplasm (Fig. 5, *a* and *b*).

Upon decompression from 7,500 psi pressure, the cells regrew microvilli and reestablished the normal organization of the cortex. Short protrusions of the egg surface began to form within 30 s of pressure release (Fig. 6*a*). By 2 min these protrusions had developed into finger-like projections with an average length of 0.7 μm (Fig. 6*b*). Also by this time many of the pigment granules had become reassociated with the cortex. While these protrusions were very irregular in shape at first, they gradually became more uniform as they lengthened and ultimately reformed normal appearing microvilli (Fig. 6, *c*

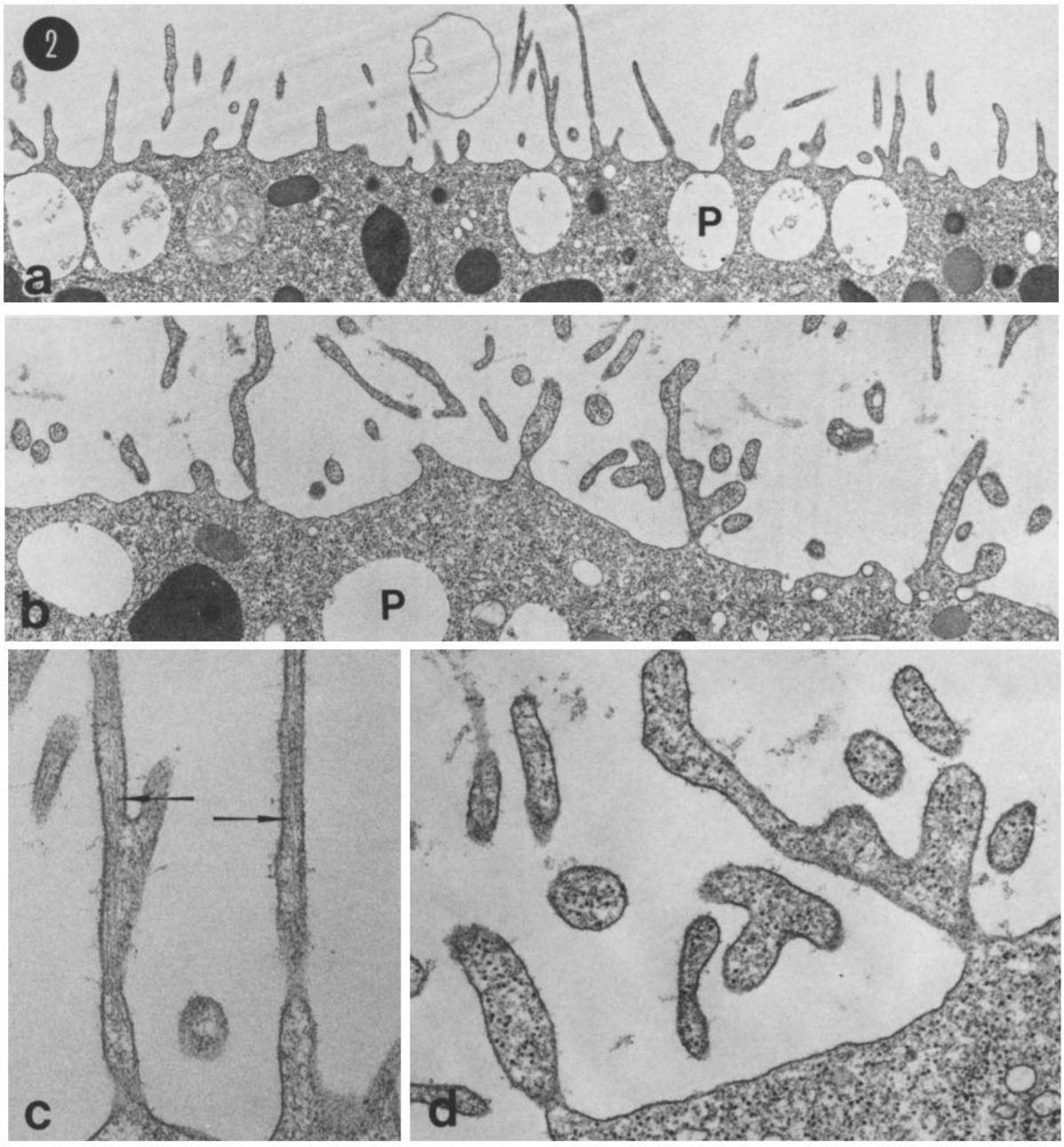

FIGURE 2 Effects of 6,000 psi-pressure on the structure of cortical actin. (*a*) Fertilized egg fixed in pressure chamber at atmospheric pressure. Note the numerous long, straight microvilli. Pigment granules (*P*) are closely associated with the plasma membrane. (*b*) Fertilized egg fixed under 6,000-psi pressure during the first cleavage division. The microvilli are irregular in shape and constricted at their bases. Association of pigment granules (*P*) with egg surface has been disrupted. (*c*) Higher magnification view of microvilli than *a*, showing core bundles of filaments (arrows). (*d*) Higher magnification view of microvilli than *b*, showing absence of filament bundles and presence of ribosome-like particles. (*a*) × 7,900; (*b*) × 14,000; (*c*) × 39,000; (*d*) × 34,000.

and *d*, and Fig. 7). The entire process of microvillar regrowth required 20–30 min. Within 1 min of decompression coated pits began to form at the bases of developing microvilli, and were abundant by 30 min (arrows, Fig. 6*d*). Coated pits were less apparent in eggs after decompression from 6,000 psi.

The initial stages of microvillar regrowth took place in the absence of actin filament bundles. During the first 10–15 min after pressure release, the developing protrusions contained a loose meshwork of filaments (Fig. 7, *a* and *b*). Bundles began to form within this meshwork by 15 min after decompression (Fig. 7*c*), but did not reach their normal degree of organization until shortly before second cleavage (Fig. 7*d*). The reformation of actin filament bundles coincided with the recovery of normal microvillar shape and with the cessation of surface contractile activity (Fig. 4).

Tangentially oriented bundles of filaments were frequently observed just beneath the plasma membrane during the first 10 min after decompression (Fig. 8). These filament bundles disappeared by the time that microvillar core filaments had begun to reform. The appearance of these structures coincided with the onset of surface contractions and their disappearance with the reestablishment of normal egg shape. They were never observed in normal cells or in cells that had been subjected to 6,000-psi pressure.

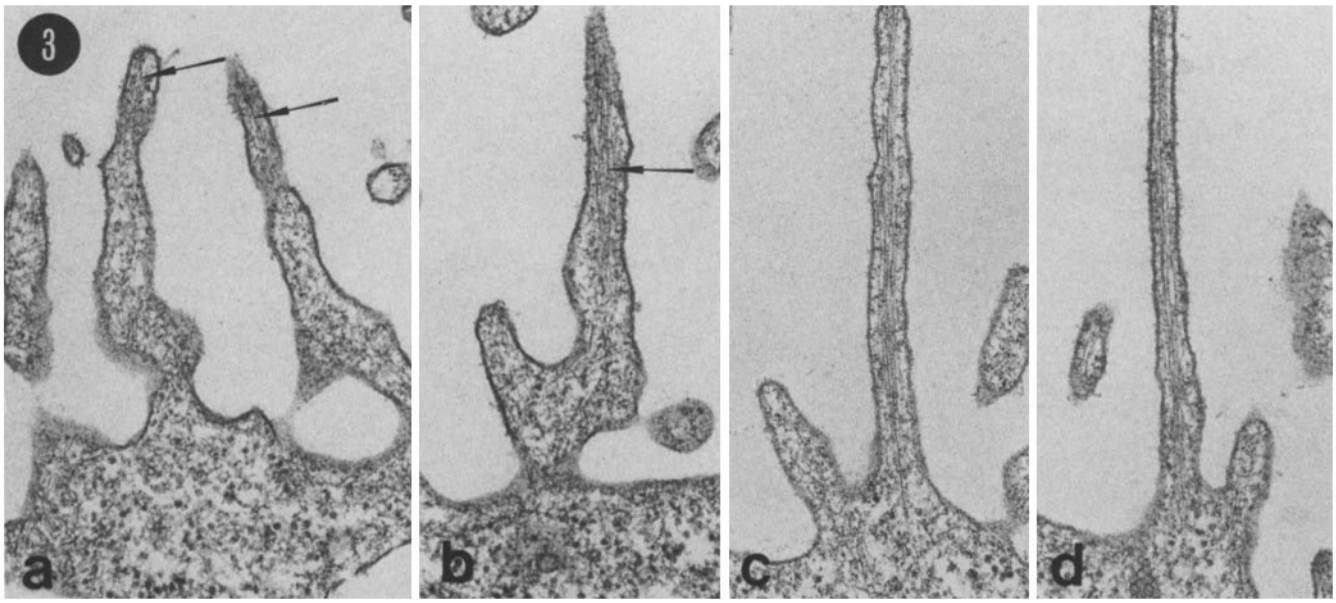

FIGURE 3 Reformation of filament bundles after pressurization to 6,000 psi. (*a* and *b*) 30 s after decompression. Filament bundles are beginning to reform (arrows). (c and *d*) Aliquots of eggs from same experiment 1 min after decompression. Bundles of actin filaments have completely reformed and microvilli have recovered their normal shape. × 39,000.

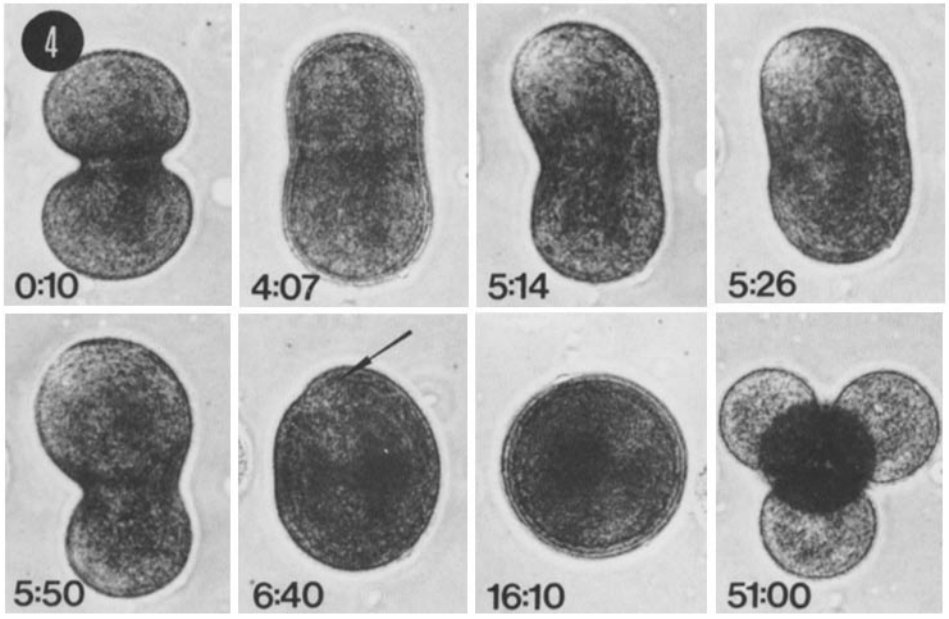

FIGURE 4 Pressurization of eggs to 7,500 psi during first cleavage division. Time in minutes with respect to initial observation is given in the lower left of each frame. Pressure was applied at 30 s and released at 4:30 min. The cleavage furrow regresses under pressure (*4:07*) and does not reform after decompression. Instead the cell undergoes a series of rapid surface contractions that cause the upper end of the cell to bulge out (*5:14–5:50*). A lobopod-like structure then forms and propagates around the cell (arrow, *6:40*). This contractile activity gradually subsides, until by 12 min after decompression the egg has resumed its spherical shape (*16:10*). At the time the controls undergo second division the egg divides directly into four cells. × 315.

Effects of Pressure on Purified F-Actin

To investigate the molecular mechanism of pressure action, we examined the effect of hydrostatic pressure on purified F-actin. F-actin was pressurized in the pressure-fixation chamber, fixed under pressure to prevent repolymerization upon decompression, and its polymerization state assayed by viscometry and negative staining. Fixation was carried out in 0.2% glutaraldehyde since this concentration inhibited polymerization (Fig. 9*A*), and also did not significantly alter the viscosity of an F-actin solution (Fig. 9*B*).

Repeated measurement of viscosity with Ostwald-type viscometers causes a breakage of actin filaments due to the high shearing forces generated by this technique (15). However a decrease in viscosity is normally not observed due to the rapid reannealing of the filaments ([15] and Fig. 9*B*). Since glutaraldehyde fixed actin is unable to reanneal, it showed a gradual decrease in viscosity with repeated measurement (Fig. 9*B*). This viscosity decay is characteristic of fixed actin and can be used to determine whether or not fixation has occurred.

The results presented in Fig. 9*B* demonstrate that pressures up to 10,000 psi exert surprisingly little effect on the viscosity of purified F-actin. However, a very slight but consistent decrease in viscosity of 0.02 cs was observed at pressures of 8,000 and 10,000 psi. Samples of F-actin fixed under pressures of 6,000, 8,000, and 10,000 psi showed an essentially identical rate of viscosity decay with repeated measurements as actin fixed at atmospheric pressure (Fig. 9*B*). Negatively stained samples of pressurized F-actin demonstrate that normal appearing actin filaments were abundant even at pressures of 10,000 psi (Fig. 10).

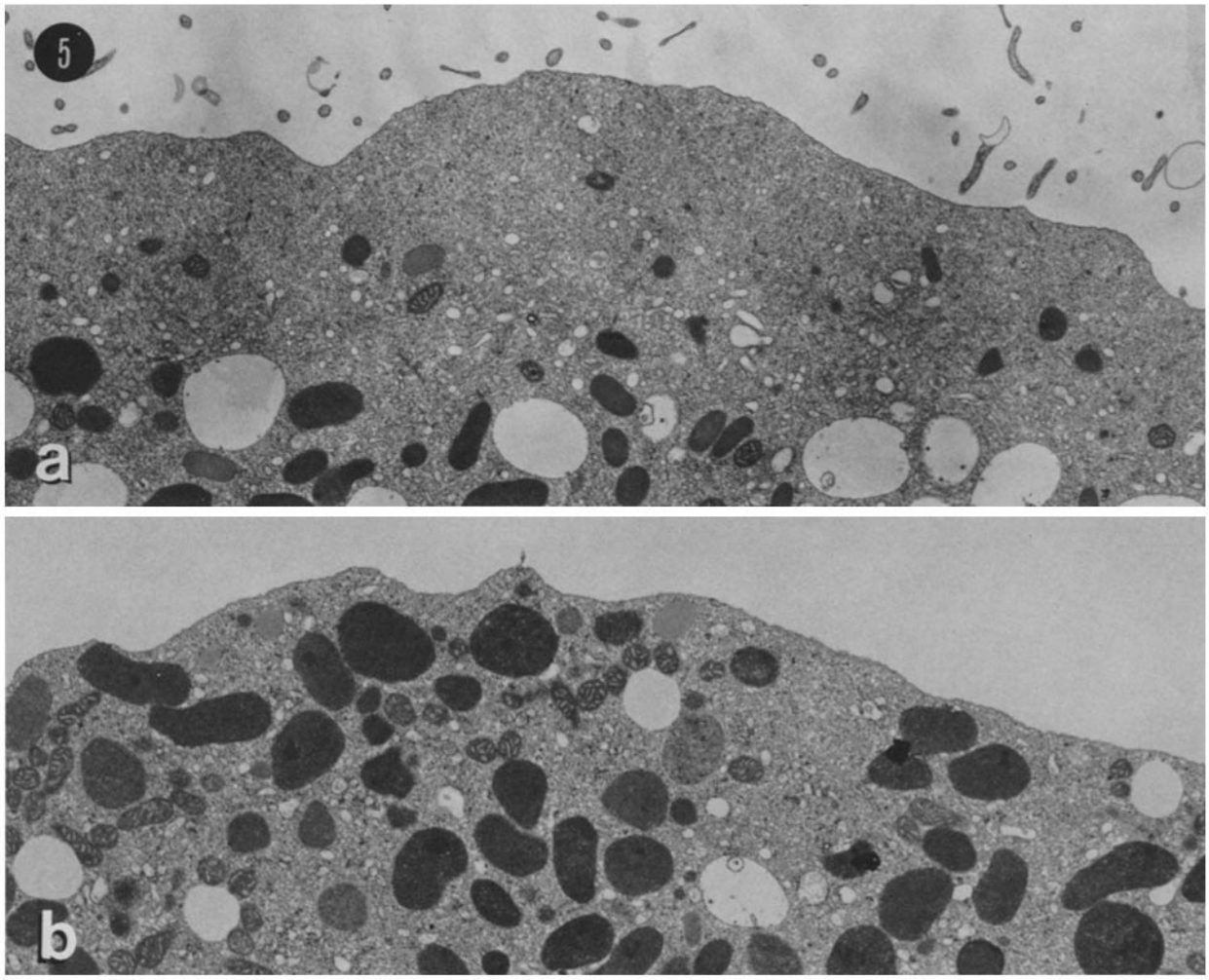

FIGURE 5 Effect of 7,500-psi pressure on the structure of the fertilized egg cortex. Eggs fixed under pressure. The egg surface is devoid of microvilli which in *a* can be seen floating free in the surrounding sea water. The pigment granules have dissociated from the cortex and are found lying in the deeper cytoplasm. In *a* a zone of organelle-free granular cytoplasm underlies the plasma membrane, while in *b* mitochondria and yolk platelets extend all the way to the plasma membrane. (*a*) × 11,000; (*b*) × 10,000.

DISCUSSION

Disruption of Cortical Actin Organization

These results demonstrate that hydrostatic pressures of 6,000 psi or greater reversibly disrupt the organization of actin in the fertilized sea urchin egg cortex and suggest that this disruption provides the structural basis for the pressure-induced gel-sol transformation reported by Brown (6) and Marsland (32). However the actual molecular mechanism of pressure action is not clear from the present data. Actin has previously been reported to undergo a large increase in molar volume upon polymerization (18, 23). Le Chatelier's principle predicts that increased hydrostatic pressure should therefore cause the direct depolymerization of actin filaments, as has been demonstrated for microtubules both in vivo (41) and in vitro (44). However, the results reported here demonstrate that pressures of up to 10,000 psi do not induce a significant depolymerization of purified F-actin, suggesting that the actual volume increase upon polymerization is less than that previously reported.

While it is clear from the present results that pressures up to 10,000 psi exert relatively little effect on the polymerization state of actin in vitro, it is difficult to extrapolate from this result to the effects of pressure on the state of actin in the living cell. In vivo the ionic conditions, pH, nucleotide concentrations, etc., as well as the association of actin binding proteins with actin, may alter the effects of pressure on the monomer-polymer equilibrium of actin. Nevertheless, the results reported here suggest that pressure may act indirectly in vivo, either by altering the interaction of actin with actin-binding proteins or by changing the intracellular ionic environment, which could in turn affect actin-actin-binding protein interactions. For example, the pressure-induced release of sequestered stores of Ca^{++} could inhibit filament cross-linking by Ca^{++} sensitive α-actinin-like proteins (9) or could induce the fragmentation of actin filaments by Ca^{++}-activated fragmenting proteins (16, 58).

Alternatively, the slight decrease in viscosity observed at 8,000 and 10,000 psi may reflect a small pressure dependent decrease in either filament length or total amount of polymer. Since the steady state conditions of actin in vivo are not known, a slight change in the monomer-polymer equilibrium might have a profound effect on the organizational state of actin in the cell.

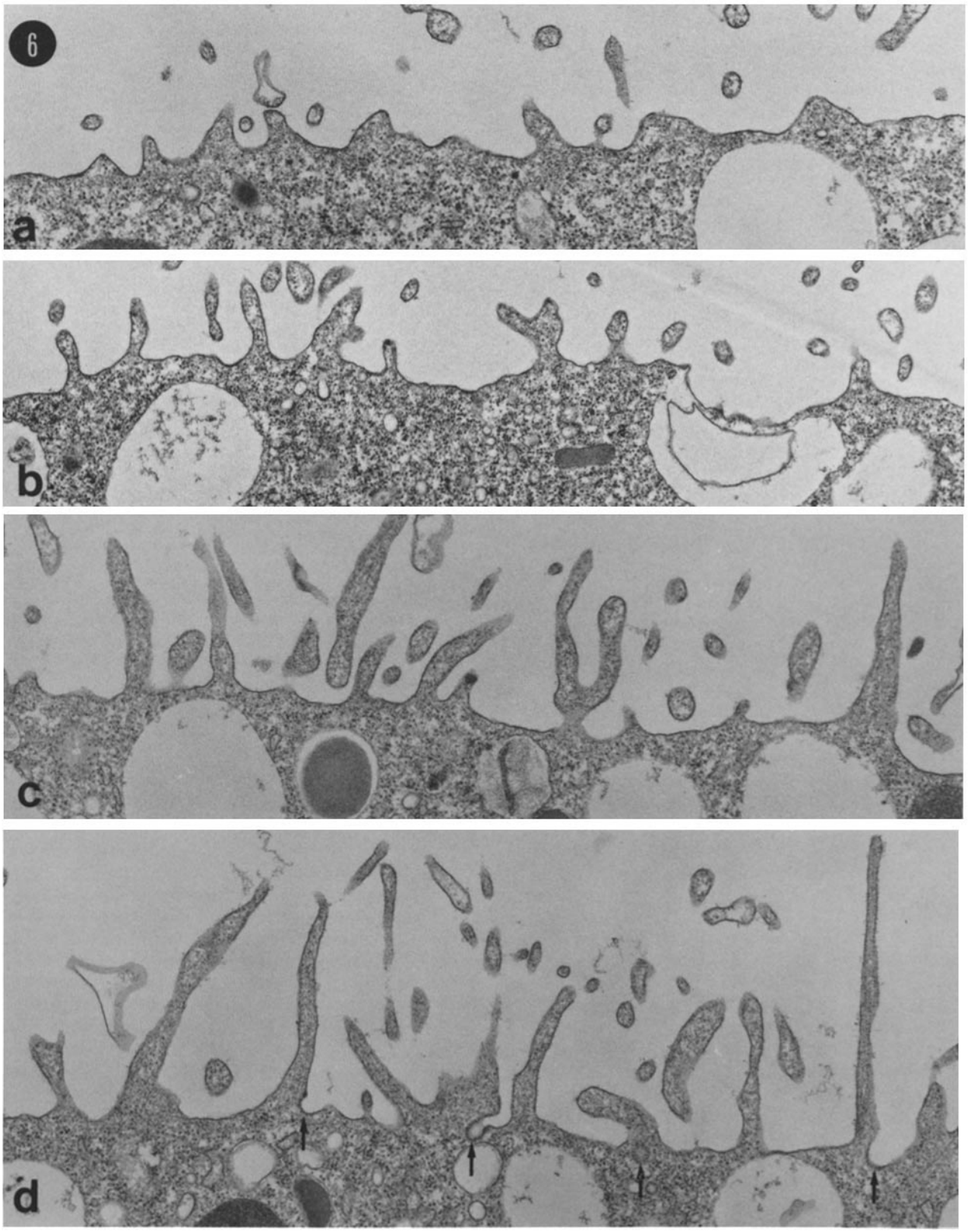

FIGURE 6 Microvillar reformation after pressurization to 7,500 psi. (*a*) 30 s after decompression. Short protrusions of the egg surface have begun to form. (*b*) 2 min after decompression. Protrusions have elongated to ~0.7 μm. (*c*) 10 min after decompression. Protrusions have reached an average length of 1.5 μm. They appear more microvillar-like, but have highly variable diameters. (*d*) 30 min after decompression. Microvilli have reached an average length of 2.5–3 μm. Bundles of filaments are evident in many of the microvilli. Numerous coated pits are visible (arrows). (*a*) × 22,300; (*b*) × 15,300; (*c*) × 21,800; (*d*) × 20,000.

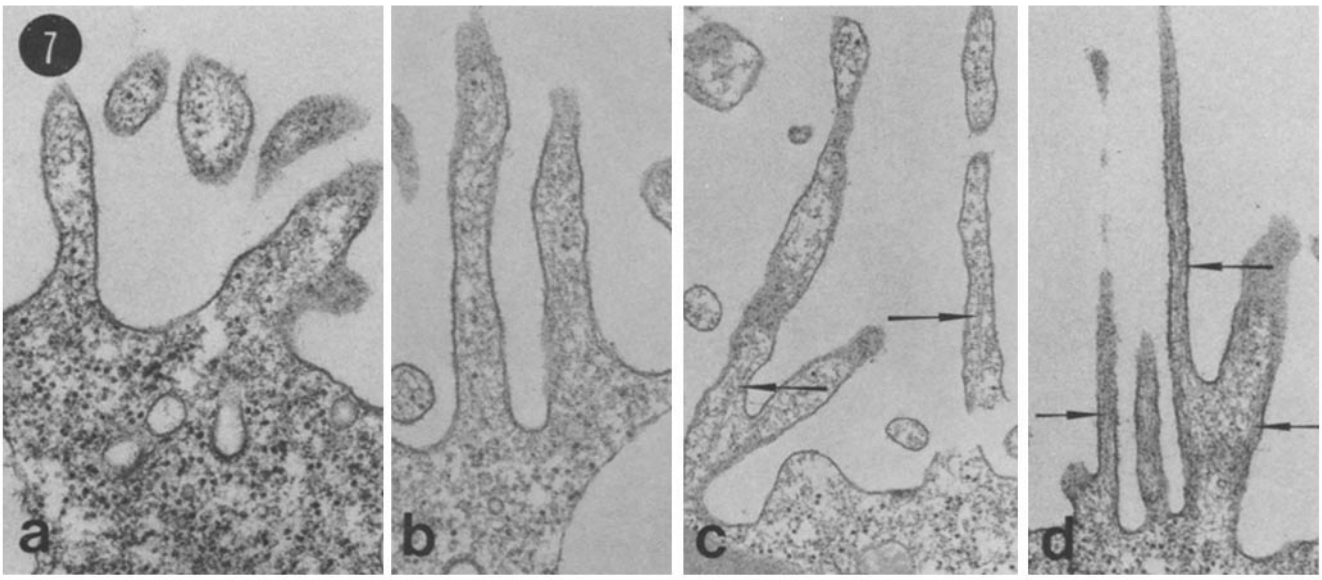

FIGURE 7 Formation of actin filament bundles during regrowth of microvilli. (*a*) 2 min after decompression from 7,500 psi-pressure. Filament bundles are absent. (*b*) 10 min after decompression. Microvilli contain a network of filaments, but bundles have not started to form. (*c*) 15 min after decompression. Filament bundles are beginning to develop (arrows). (*d*) 45 min after decompression. Bundles of filaments have reformed (arrows) and the microvilli have regained their normal shape. (*a* and *b*) × 35,750; (*c*) × 25,200; (*d*) × 28,100.

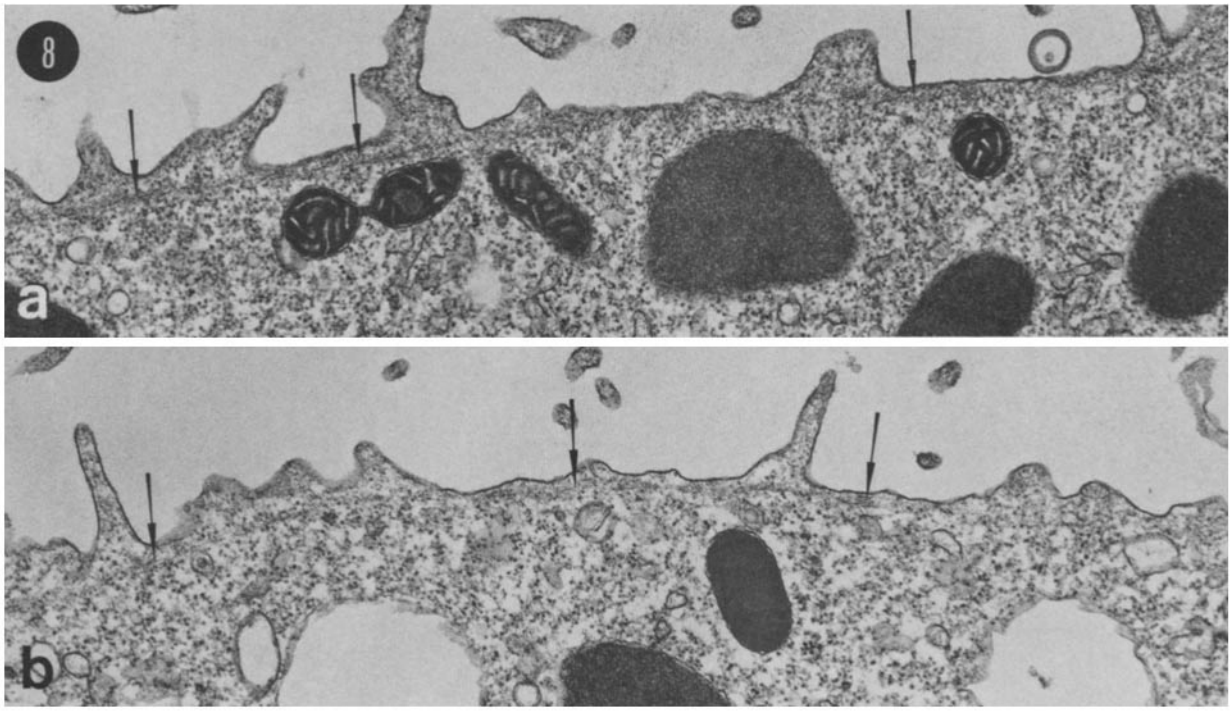

FIGURE 8 Bundles of actin filaments in cortex of motile eggs after decompression from 7,500-psi pressure. (*a*) 5 min after pressure release. (*b*) 2 min after pressure release. Arrows indicate bundles of actin filaments adjacent to the plasma membrane. (*a*) × 25,400. (*b*) × 21,450.

Reorganization of the Cortex Following Pressurization

The normal structural organization of the cortex is reestablished remarkably quickly following decompression from 6,000 psi pressure. Within 30 to 60 s of pressure release bundles of actin filaments reformed, the microvilli regained their normal shape, and ribosome-like particles were excluded from the microvillar cores. This rapid rate of reorganization suggests that the egg cortex may normally be in a highly dynamic state and its actin and associated proteins can exchange with cytoplasmic pools.

Exposure of eggs to pressures of 7,500 psi or higher caused the shedding of essentially all microvilli from the egg surface. As a result, recovery involved the regrowth of microvilli in addition to the reestablishment of normal cortical actin or-

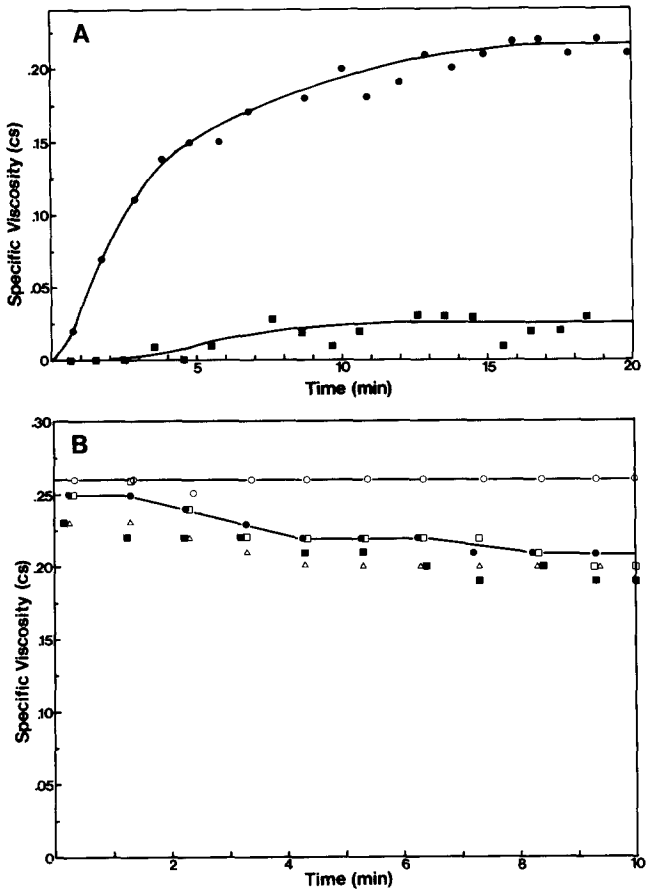

FIGURE 9 Effects of hydrostatic pressure on purified F-actin. (*A*) Polymerization of G-actin (7 μm) in 100 mM KCl, 10 mM imidazole-Cl, 2 mM $MgCl_2$, 0.1 mM ATP, pH 7.2 in the absence (●) and presence (■) of 0.2% glutaraldehyde. (*B*) Viscosity of 7 μm F-actin solution: unfixed (○), and fixed in the pressure-fixation chamber at 1 atm. (●), 6,000 psi (□), 8,000 psi (△), and 10,000 psi (■). The lower line is drawn through points measured for actin fixed at 1 atm.

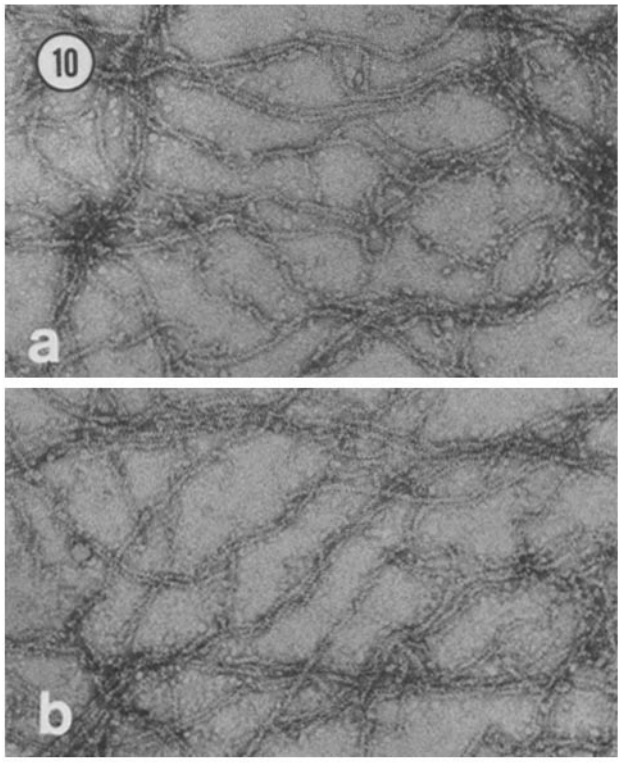

FIGURE 10 Negatively stained F-actin fixed in the pressure-fixation chamber in 0.2% glutaraldehyde at atmospheric pressure (*a*) and at 10,000 psi (*b*). × 125,000.

ganization. In contrast to eggs subjected to 6,000 psi pressure, actin filament bundles did not begin to reform until 10–15 min after decompression, when the microvilli had reached ~⅔ of their final length, and did not regain their normal degree of organization until just before the second cleavage division. This slow rate of recovery suggests that some essential component for filament bundle formation may be lost when the microvilli are shed and that the delay in bundle formation reflects the time required for its resynthesis or reassociation with the developing microvilli. It is unlikely that this rate-limiting component might be actin, since >60% of the egg's actin is cytoplasmic rather than cortical (38). Even if all cortical actin were lost with the microvilli, a substantial cytoplasmic pool would remain. In addition, a network of actin filaments was observed in the developing microvilli well before bundles began to reform. A more likely possibility is that the shedding of the microvilli depletes the egg of actin-binding proteins that are required for bundle formation, or that membrane sites for the anchorage of actin filaments are lost.

Regrowth of Microvilli

The reformation of microvilli following pressurization does not involve the same series of surface shape changes that occurs at fertilization (1, 14). Instead the microvilli developed directly as finger-shaped protrusions of the egg surface that gradually elongated over the next 20–30 min. However, as in normal microvillar formation, the developing protrusions initially contained a network of actin filaments that subsequently transformed into bundles.

Our results differ from those of Tilney and Cardell (54) on the reformation of microvilli by newt intestinal epithelial cells after pressurization. They reported that core bundles of actin filaments were present at all stages of microvillar regrowth and concluded that these bundles are necessary for microvillar formation. The bundles appeared to nucleate on dense plaques at the tips of the developing microvilli and increased in length as the microvilli elongated. However the normal formation of microvilli on intestinal epithelial cells during embryological development occurs by a process similar to that observed in sea urchin eggs. Microvilli develop as irregular protrusions of the apical membrane containing a network of actin filaments and subsequently transform into typical microvilli with core filament bundles (13). In light of this observation and the results reported here, it would be useful to reexamine the process of microvillar reformation in intestinal epithelial cells following pressurization.

Cortical Actin Organization and Cytokinesis

Our results confirm Marsland's earlier reports (31, 32) that pressurization of dividing sea urchin eggs to 6,000 psi reversibly inhibits cytokinesis and suggest that this inhibition is due to the disruption of cortical actin organization. Following decompression from 6,000 psi, the array of cortical actin filaments rapidly reformed, the cleavage furrow was reestablished, and cytokinesis continued normally. The cleavage

furrow always reformed in its previous location, indicating that the egg retained the necessary information for the proper positioning of the furrow under pressure, in spite of the apparently complete dissolution of cortical actin structure.

The more extensive disruption of cortical organization by higher pressures resulted in a much slower reformation of the array of cortical actin following pressure release. In the prolonged absence of normal cortical actin organization the eggs were unable to generate a stable cleavage furrow, and instead underwent a series of rapid contractions that propagated over the cell surface. Eggs that exhibited this type of contractile behavior frequently contained long arcs of actin filament bundles in their cortices that may be involved in generating the force for contraction. Similar actin filament bundles have been observed at the base of motile lobopodia on isolated fish embryonic cells (19).

Surface protrusive activity has also been observed in fertilized eggs from various other marine organisms (42). In each case this motility was induced by some type of perturbation to the egg cortex (42). Fertilized eggs of the surf clam *Spisula* undergo extensive amoeboid-like movements when the vitelline membrane is removed (41), a treatment that also strips the microvilli from the egg surface (40).

The inability of the egg to develop a properly positioned cleavage furrow in the absence of normal cortical organization suggests that the stability of the contractile ring depends upon its anchorage to the array of cortical actin filaments. The results further suggest that this network of cortical actin maintains a balance of forces within the egg surface that determines the equatorial position of the furrow. This conclusion is consistent with a mechanism of contractile ring formation based upon the redistribution of a preexisting network of cortical actin filaments, such as that recently proposed by White and Borisy (59). The loss of this cortical actin array in response to pressurization could alter the normal anchorage of the contractile ring to the cortex and lead to the translocation of the contractile apparatus across the egg surface.

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