8-1-1982

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G. Schatten
Florida State University

H. Schatten
Florida State University

T. H. Bestor
Florida State University

R. Balczon
Florida State University

Recommended Citation

Schatten, G., Schatten, H., Bestor, T. H., & Balczon, R. (1982). Taxol inhibits the nuclear movements during fertilization and induces asters in unfertilized sea urchin eggs. The Journal of cell biology, 94(2), 455-465.

Taxol Inhibits the Nuclear Movements during Fertilization and Induces Asters in Unfertilized Sea Urchin Eggs

G. SCHATTEN, H. SCHATTEN, T. H. BESTOR, and R. BALCZON
Department of Biological Science, Florida State University, Tallahassee, Florida 32306

ABSTRACT Taxol blocks the migrations of the sperm and egg nuclei in fertilized eggs and induces asters in unfertilized eggs of the sea urchins *Lytechinus variegatus* and *Arbacia punctulata*. Video recordings of eggs inseminated in 10 μM taxol demonstrate that sperm incorporation and sperm tail motility are unaffected, that the sperm aster formed is unusually pronounced, and that the migration of the egg nucleus and pronuclear centration are inhibited. The huge monopolar aster persists for at least 6 h; cleavage attempts and nuclear cycles are observed. Colcemid (10 μM) disassembles both the large taxol-stabilized sperm aster in fertilized eggs and the numerous asters induced in unfertilized eggs. Antitubulin immunofluorescence microscopy demonstrates that in fertilized eggs all microtubules are within the prominent sperm aster. Within 15 min of treatment with 10 μM taxol, unfertilized eggs develop numerous (>25) asters *de novo*. Transmission electron microscopy of unfertilized eggs reveals the presence of microtubule bundles that do not emanate from centrioles but rather from osmiophilic foci or, at times, the nuclear envelope. Taxol-treated eggs are not activated as judged by the lack of DNA synthesis, nuclear or chromosome cycles, and the cortical reaction. These results indicate that: (*a*) taxol prevents the normal cycles of microtubule assembly and disassembly observed during development; (*b*) microtubule disassembly is required for the nuclear movements during fertilization; (*c*) taxol induces microtubules in unfertilized eggs; and (*d*) nucleation centers other than centrioles and kinetochores exist within unfertilized eggs; these presumptive microtubule organizing centers appear idle in the presence of the sperm centrioles.

The nuclear movements during fertilization are a remarkable model in which to study microtubule-mediated motility. Unlike virtually any other animal cell, the unfertilized egg contains neither an array of cytoplasmic microtubules nor centrioles. After insemination, several microtubule-associated events and their consequences may be investigated: microtubules emanate from the newly introduced sperm centrioles to form an initially radial structure, the sperm aster, whose proper functioning appears responsible for pushing the sperm nucleus (male pronucleus) centripetally as well as for drawing the egg nucleus (female pronucleus) to its center (reviewed in reference 1). In the absence of any morphologically apparent centrioles in unfertilized eggs (2), certain parthenogenetic treatments, such as hypertonic seawater (3, 4) and heavy water (5, 6), can result in the *de novo* formation of centrioles. Additionally, microtubules not associated with centrioles can be observed following artificial activation (7, 8).

Here we report the effects of taxol, a drug that has been shown to inhibit microtubule depolymerization in vitro (9) and in vivo (10), on sea urchin eggs during fertilization. Taxol induces aster formation in unfertilized eggs in the absence of any centrioles, but does not itself induce artificial activation. At fertilization, the sperm aster enlarges abnormally and persists for several cell cycles, the cytoplasmic migrations of the sperm and egg nuclei are inhibited and syngamy does not occur. Taxol, then, by blocking microtubule disassembly, prevents the normal movements of the nuclei during fertilization, stabilizes the fertilized egg's sperm aster, and induces asters *de novo* in unfertilized eggs.

MATERIALS AND METHODS

Gamete Collection and Handling

Gametes of the local Gulf coast sea urchins *Lytechinus variegatus* and *Arbacia punctulata* were obtained by intracoelomic stimulation with 0.5 M KCl. Eggs

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were shed into millipore-filtered natural seawater (0.22 μm); sperm were collected "dry" on ice and diluted immediately before insemination. Both species were examined with time-lapse video and immunofluorescence microscopy, and *A. punctulata* was studied with transmission electron microscopy (TEM).

Video Recording

Time-lapse video tape recording of fertilization was performed as described by Schatten (11). Basically, a half-inch time-lapse recording unit (Panasonic NV8030) with a time date generator (Panasonic WJ 800) was interfaced with a Zeiss Universal microscope with differential interference contrast and water immersion optics. Still images were photographed from a high-resolution video monitor with underscan features (Panasonic WV5316) using a 35-mm Olympus camera (OM-2).

Immunofluorescence Staining

Monospecific antitubulin immunofluorescence staining was performed as described by Bestor and Schatten (12), following the methods of Brinkley et al. (13). Eggs denuded with 10 mM DTT and glued onto polylysine-coated glass slides (14) were fixed in 90% methanol, 50 mM EGTA, pH 6.0, at −20°C (15). Staining was performed by incubating the samples in 50 μg/ml monospecific column-purified antibody to porcine brain tubulin in borate saline buffer at 37°C. Fluorescein-labeled goat anti-rabbit immunoglobulin (Miles Laboratories, Elkhart, IN) was used as a second antibody. Stained cells were observed with a Zeiss WL microscope equipped with a Zeiss epiilluminator and the appropriate filters, photographed with Tri-X 35 mm film at an ASA of 1,600 (Kodak, Inc., Rochester), and developed in Diafine (Accufine, Inc., Chicago).

DNA Synthesis

Duplicate taxol-treated, unfertilized and fertilized cultures (1.5% egg concentration) were incubated in [^{3}H]thymidine ([^{3}H]methyl; 18.4 Ci/mmol) at a final concentration of 1 μCi/ml of egg suspension. The cultures were stirred continuously throughout the experiments. The incorporation of radioactive [^{3}H]thymidine into DNA was determined by the methods of Hinegardner et al. (16). At each time point 5-ml samples of each of the three cultures were taken, centrifuged, fixed in 10% trichloroacetic acid (TCA) on ice, washed twice with 5% TCA, washed with ethanol, and dissolved in NCS tissue solubilizer (Amersham/Searle Corp., Arlington Heights, IL) for 1 h at 60°C. After solubilizing, samples were counted in a Packard scintillation counter (Packards Instrument Co., Inc., Downers Grove, IL).

TEM

Unfertilized eggs were incubated in 10 μM taxol for 60 min and processed for TEM. Fertilized eggs were incubated in 10 μM taxol for 15 min before fertilization and processed for TEM at 15 and 45 min after insemination. Eggs were fixed in 1% OsO_4 in 0.4 M sodium acetate at pH 6.1 (17) for 90 min. Two washes in 0.4 M sodium acetate at pH 6.1 were followed by dehydration in increasing series of acetone. Cells were embedded in Spurr's embedding medium. Thin sections (Porter-Blum Ultra-Microtome MT-2) were stained in 3% aqueous uranyl acetate followed by lead citrate and viewed in the TEM (Phillips 201).

RESULTS

Effects on Unfertilized Eggs

Unfertilized eggs exposed to 10 μM taxol for up to 6 h do not display any of the normal morphological consequences of artificial activation. The addition of taxol to an unfertilized egg does not induce the cortical reaction or resultant elevation of the fertilization coat (Fig. 1 *a* and *b*; 18). The egg nucleus does not undergo centration (Fig. 1 *c*), nor are cycles of nuclear breakdown and reformation (Fig. 1 *c–f*), chromosome condensation (19), or cleavage attempts observed, as in the case of artificial activation (20–22). In Fig. 2, the incorporation of [^{3}H]thymidine indicates a lack of the initiation of DNA synthesis, another sign that taxol does not artificially activate unfertilized sea urchin eggs.

Indirect immunofluorescence microscopy with monospecific antibody to porcine brain tubulin detects microtubules within these unfertilized eggs. Fig. 3 *a* demonstrates that within 15 min of exposure to taxol multiple punctate sites stain for tubulin. These sites enlarge at a half hour (Fig. 3 *b*) to form discrete astral structures throughout the unfertilized egg cytoplasm which persist for at least 2.5 h.

TEM (Fig. 4) reveals that the tubulin-staining structures observed by immunofluorescence microscopy are microtubules. In unfertilized control eggs, microtubules have not been observed by TEM nor detected by antitubulin immunofluorescence microscopy (12). Microtubules observed following taxol treatment are most prominent within discrete vesicle-rich clear zones in the egg cytoplasm that are devoid of yolk platelets (Fig. 4 *a*), which can be correlated with the antitubulin staining patterns. Though the microtubules tend to emanate radially from these zones, microtubules at oblique angles to the astral radii are frequently observed. Microtubule bundles emanate from a variety of centers, which can be the nuclear envelope (Fig. 4 *b*), running parallel with annulate lamellae (Fig. 4 *c*), or electron transparent regions (Fig. 4 *d*). Centrioles have never been observed in unfertilized taxol-treated eggs. However, the astral centers often contain osmiophilic granules from which the microtubules appear to extend (Fig. 4 *e* and *f*): At times, a centriolelike structure is observed with double and triple microtubule sets (Fig. 4 *e*, arrows).

These taxol-induced asters, isolated in 0.1 M MES, 0.5 mM EGTA, 0.1 mM $MgCl_2$, 0.1% Nonidet P-40, 4% polyethylene glycol (6,000 mol wt), pH 6.8, and processed for antitubulin immunofluorescence microscopy, retain their astral appearance

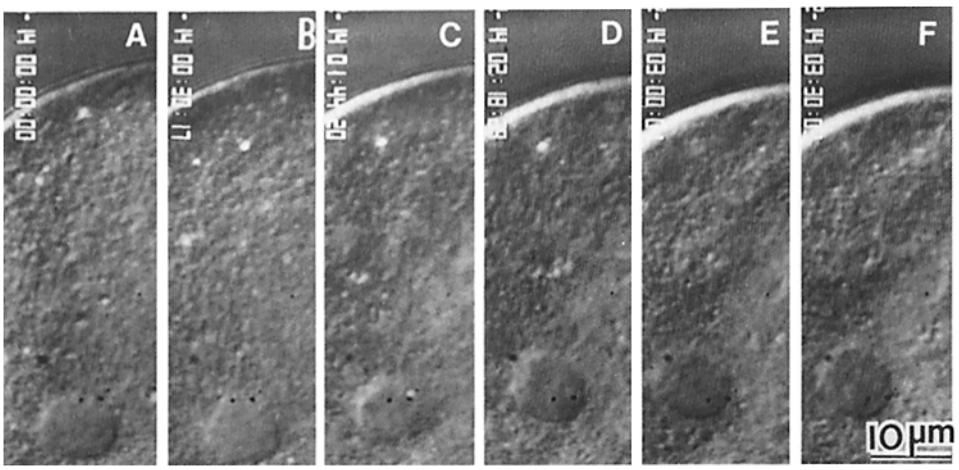


FIGURE 1 Effect of taxol on unfertilized eggs. 10 μM taxol itself does not induce artificial activation as judged by the lack of a cortical reaction (*a* and *b*), centration of the egg nucleus (*c*), or nuclear breakdown cycles or chromosome condensation-decondensation cycles (*c–f*). *Lytechinus variegatus*. Differential interference, water immersion optics videotape recorded in time-lapse. Time (h:min:s); upper left. Bar, 10 μm. × 1,100.

(Fig. 5). This may indicate some sort of firm attachment site at their nucleation center, rather than a loose netting of interconnecting microtubules.

Effects of Sperm Tail Motility

To determine whether taxol will influence the beating of the sperm tail, "dry" semen was treated for 30 min on ice with either 10 μM taxol or an equivalent amount of dimethyl sulfoxide (DMSO). When cells were suspended in seawater, motility was judged by video recordings with phase-contrast microscopy. No influence either on the percent of swimming sperm or on activity of individual sperm could be detected in the taxol-treated samples.

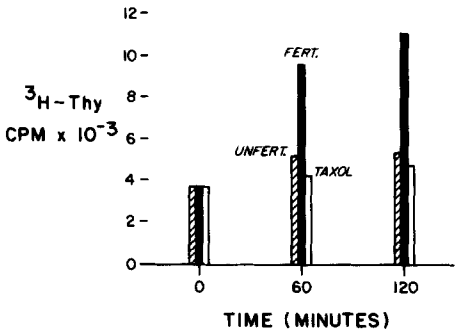


FIGURE 2 DNA synthesis in unfertilized (*Unfert*), fertilized (*Fert*), and taxol-treated unfertilized eggs. 10 μM taxol does not artificially activate DNA synthesis in unfertilized eggs.

Taxol Effects during Fertilization

Unfertilized eggs treated for 15–30 min with 10 μM taxol will incorporate the sperm normally, but are unable to complete the pronuclear migrations. In Fig. 6, the events during fertilization of an egg treated with 10 μM taxol for 30 min before insemination are depicted. Sperm incorporation, which involves the formation of the fertilization cone around the successful sperm and then the rotation and displacement of the sperm at the egg cortex (11), occurs normally in the presence of taxol (Fig. 6 *a–e*). The sperm aster forms on schedule at ~5 min postinsemination (Fig. 6 *f*, arrow) and appears to contact the female pronucleus (Fig. 6 *g*), but the swift migration of the female pronucleus (Fig. 6 *h* and *i*), the disassembly of the sperm aster (Fig. 6 *j*) and syngamy (Fig. 6 *k* and *l*) do not occur. At the time of mitosis, the pronuclei in this taxol-treated egg undergo a cycle of nuclear breakdown and reformation (Fig. 6 *m* and *n*) within the stabilized monopolar sperm aster, as the egg surface undergoes a cleavage attempt.

In Fig. 7, of an egg treated for 30 min with 10 μM taxol, the attempted motility of a taxol-treated sperm aster is depicted. The female pronucleus is contacted (Fig. 7 *a*) and, for the next half hour, the sperm aster distorts the female pronucleus as if slowly pulling it to the astral center (Fig. 7 *b* and *c*); this distortion of the female pronucleus from a sphere to an oblate ovoid is characteristic of the egg nucleus during its migration, which typically occurs within 1 min (11).

Eggs treated for 15 min before insemination, like the half-hour treated samples, are able to incorporate the sperm but are unable to complete the pronuclear migrations because of the

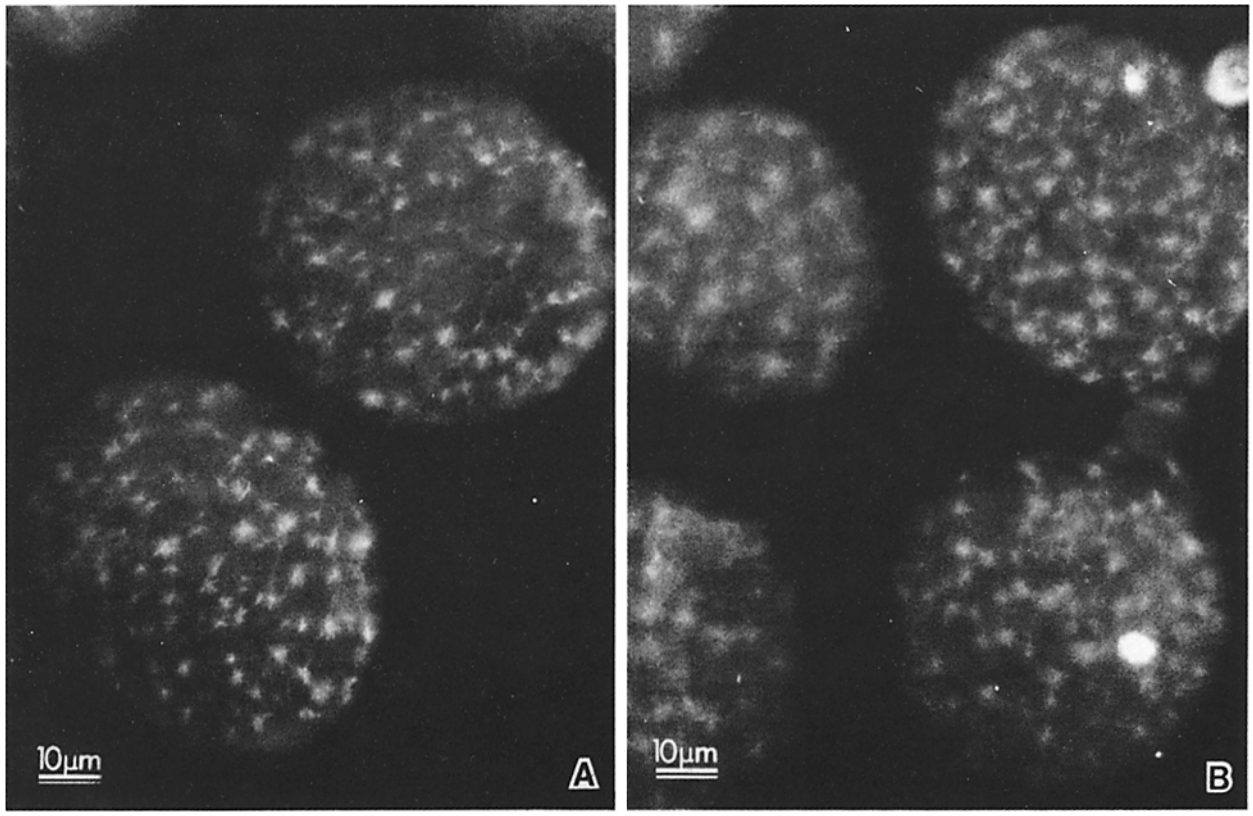


FIGURE 3 Antitubulin immunofluorescence microscopy of taxol-treated unfertilized eggs. Within 15 min of taxol addition, numerous punctate tubulin-containing structures appear throughout the egg cytoplasm (*a*). These structures increase in size by 30 min and appear as discrete asters (*b*), which persist for at least 2.5 h. *Arbacia punctulata*. Bars, 10 μm. × 1,100.

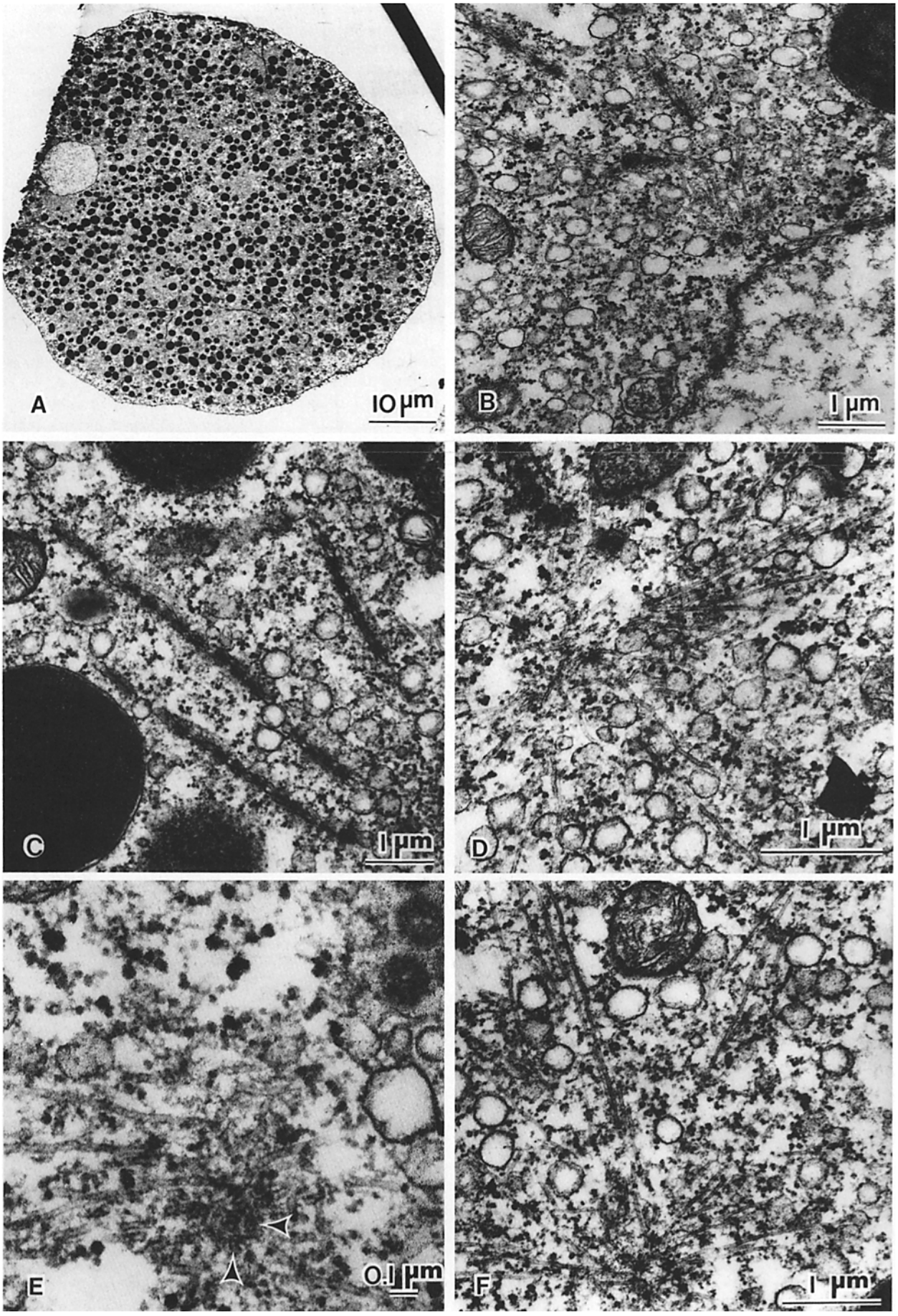

A
10 µm
B
1 µm
C
1 µm
D
1 µm
E
0.1 µm
F
1 µm

FIGURE 5 Isolated asters induced *de novo* by taxol in unfertilized eggs. Antitubulin immunofluorescence microscopy of asters isolated from unfertilized eggs treated for 30 min with 10 µM taxol demonstrates that the isolates retain their astral orientation. *A. punctulata*. Bar, 1 µm. × 900.

unusual stability of the taxol-treated sperm aster. The female pronuclear migration often occurs before the taxol effects are noted (Fig. 8 *a* and *b*). Cycles of nuclear breakdown and reformation and cleavage attempts occur on schedule (Fig. 8 *c*). Eggs treated for an hour will typically form a large sperm aster after insemination. At times, however, with *A. punctulata* eggs, the unfertilized asters persist and the sperm aster develops only slowly.

Table I describes the differences in distances, timing and rates between the events during normal fertilization and after a 30-min treatment with 10 µM taxol before insemination. It is pertinent that the rate of sperm incorporation and the formation of the fertilization cone occur at the normal speeds. However, the rate for the formation of the sperm aster is reduced from 4.9 to 1.1 µm/min, the migration of the egg nucleus from 14.6 to 3.3 µm/min, and pronuclear centration from 2.6 to 0.8 µm/min.

Antitubulin immunofluorescence microscopy of an egg treated for 15 min before insemination demonstrates that all the tubulin-containing structures in these eggs are within the sperm aster at 30 min postinsemination (Fig. 9 *a*). The adjacent, but separate, pronuclei are apparent in the accompanying phase-contrast micrograph (Fig. 9 *b*).

TEM of fertilized taxol-treated eggs at 15-min postinsemination reveals abundant microtubules and membrane vesicles within the sperm aster. In Fig. 10 *a*, the relationship between the sperm astral microtubules and the female pronucleus is depicted. Microtubule bundles run parallel with, and through, the egg nucleus. The microtubules of the sperm aster are not always aligned along a sperm astral radius (Fig. 10 *b*, *c*, and *d*) and at times appear to emanate from secondary sites within the sperm aster independent of the sperm centrioles: e.g., annulate lamellae in Fig. 10 *d*, osmiophilic center in Fig. 10 *e* and nuclear pores in Fig. 10 *f*.

Taxol added to fertilized or artificially activated eggs also induces an unusually stable monastral structure. In Fig. 11, the effects of adding 10 µM taxol at 15 min postinsemination, after the completion of the pronuclear migration, are documented. 15 min after taxol addition an arc appears near the cortex (Fig. 11 *c–e*, arrows). This crescent circumscribes the zygote nucleus (Fig. 11 *f–g*) and coalesces to a dense ring (Fig. 11 *h* and *i*). Cleavage attempts and nuclear cycles are noted (Fig. 11 *j–l*). Activation with the divalent ionophore A23187, followed by taxol treatment, results in a similar stabilized monopolar structure (22). Interestingly, activation with A23187 following taxol produces an increase in the dimensions of the taxol-induced asters, but no monopolar structure (22). 15 min appear required for the permeation of taxol into the egg cytoplasm.

Antagonistic Effect of Colcemid

The sensitivity of the nuclear migrations to a variety of microtubule assembly inhibitors, including colcemid (23), griseofulvin (24), and nocodazole (25), has been described. When the taxol-stabilized sperm aster is challenged with 10 µM colcemid, it is found that colcemid will reverse and overcome the taxol effects. Within 15 min the taxol-stabilized aster is lost and the individual pronuclei will remain at their locations until the time for mitosis when nuclear and cytokinetic cycles are observed. If unfertilized eggs are treated with both taxol (10 µM; 30 min) and colcemid (5 or 10 µM; 15 min), the taxol-induced asters are slowly lost, as judged by antitubulin immunofluorescence microscopy (Fig. 12 *a* and *b*), and the movements during fertilization resemble those of a colcemid-inhibited egg (25).

DISCUSSION

Fertilization is an ideal system in which to study the specific effects of inhibitors of cellular motility. In the sea urchin egg, microfilament-mediated motility in the fertilization cone and egg cortex appears required for sperm incorporation (26, 27). After incorporation, the formation of a microtubular array has been shown to be responsible for the intracellular translocations of the pronuclei that culminate in syngamy (12, 23–25). The proper assembly, functioning and disassembly of each motile apparatus is an absolute requisite for the proper completion of fertilization: sperm incorporation is sensitive to microfilament inhibitors and the pronuclear migrations are prevented by microtubule inhibitors (reviewed in reference 1).

FIGURE 4 TEM of taxol-treated unfertilized eggs. A survey of the taxol-treated unfertilized egg (*a*) confirms the presence of intact cortical granules and microtubule-containing zones rich in membrane vesicles and devoid of yolk. Microtubule bundles emanate in unfertilized eggs from a variety of centers including the egg nuclear envelope (*b*), along annulate lamellae (*c*), electron-transparent regions (*d*) and osmiophilic focal points (*e–f*). At times, a structure appearing partially like a centriole, with double and triple microtubule sets (*e*, arrows), is observed. *A. punctulata*. Bars; (*A*) 10 µm; (*B–D* and *F*) 1 µm; (*E*), 0.1 µm. (*A*) × 1,200. (*B* and *C*) × 13,000. (*D*) × 24,000. (*E*) × 60,000. (*F*) × 20,000.

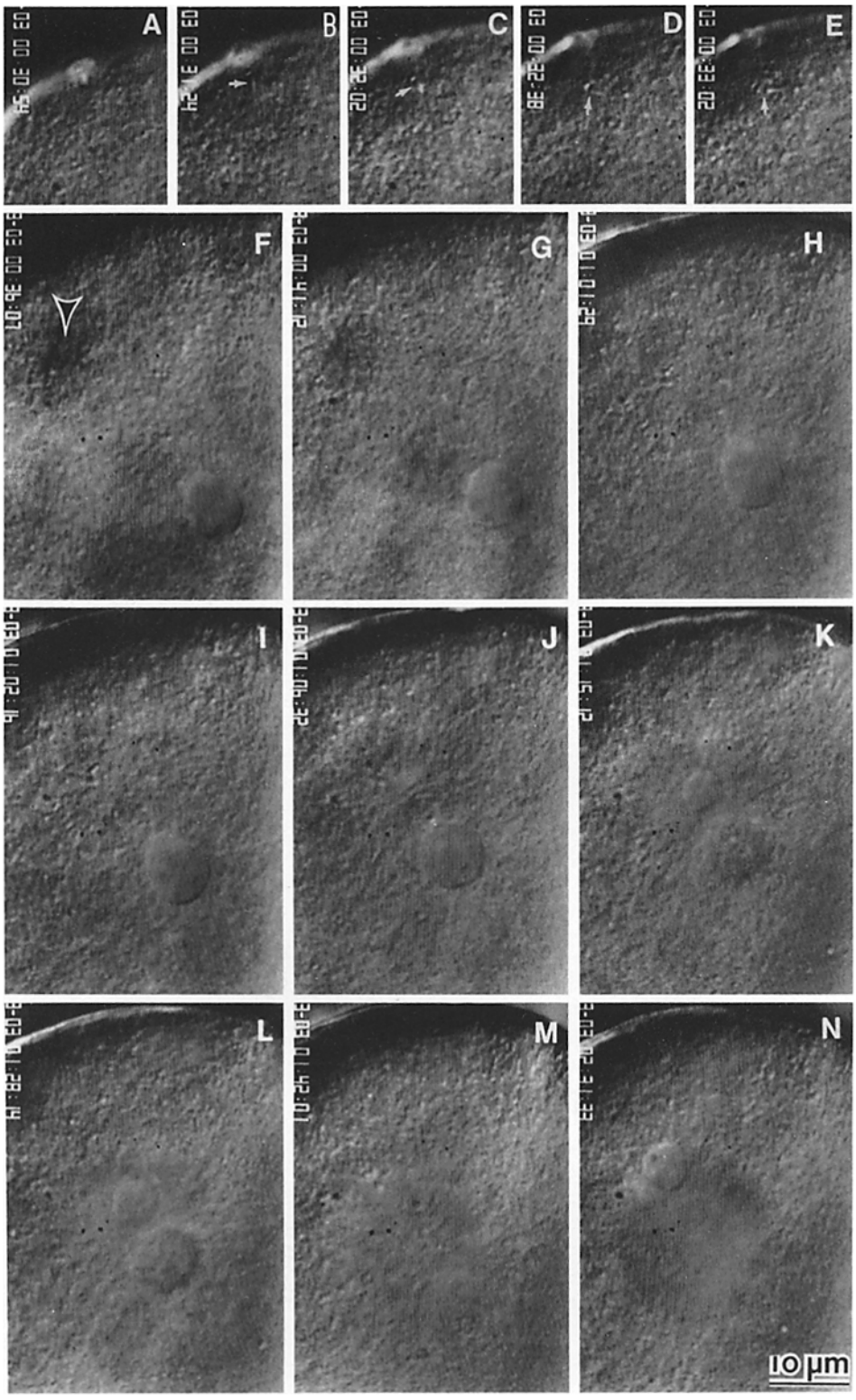


FIGURE 6 Movements during fertilization in taxol. This egg, treated with 10 µM taxol for 30 min before insemination, is able to incorporate the sperm normally. The formation of the fertilization cone (*a*), the entry of the sperm into the egg cytoplasm (*b–c*, arrows), and the rotation of the sperm at the egg cortex (*d–e*, arrows) occur on schedule. Though the sperm aster forms (*f*, arrow) and appears to contact the female pronucleus at the normal time (*g*), the normal motility and cycle of disassembly of the sperm aster are impaired in taxol. The migration of the egg nucleus, which typically requires 1 min, is not completed after 30 min (*f–j*) and the sperm aster, which typically disassembles by 15 min postinsemination, persists for at least 6 h. The sperm and egg nuclei are unable to fuse (*k–l*) though at the times for mitoses, cycles of nuclear breakdown and reformation (*m* and *n*) are noted. *L. variegatus*. Time (h:min:s) upper left. Bar, 10 µm. × 1,200.

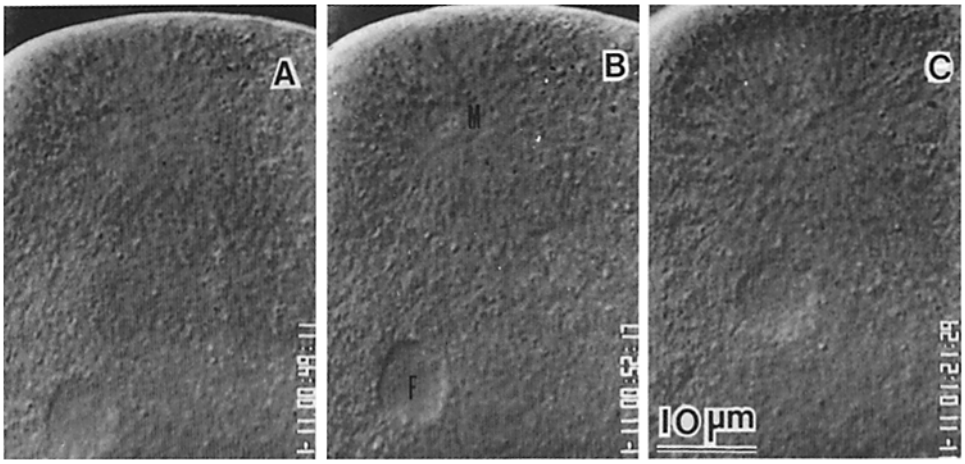


FIGURE 7 Motility of the taxol-treated sperm aster during fertilization. By 9 min postinsemination, astral fibers have contacted, and appear to distort, the female pronucleus (*a*). For the next half-hour (*b* and *c*) the female pronucleus is slowly pulled to the center of the sperm aster. *L. variegatus*. Time (h:min:s) lower right. *M*, sperm nucleus. *F*, egg nucleus. Bar, 10 μm. × 1,400.

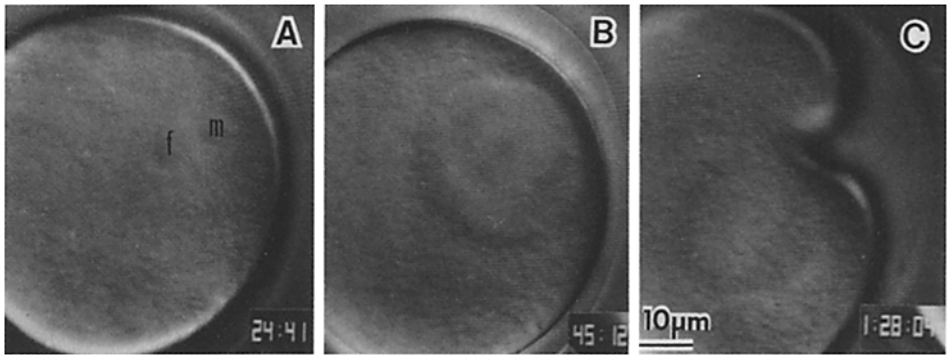


FIGURE 8 Effects of taxol when it is added 15 min before insemination. 10 μM taxol, added 15 min before insemination does not affect sperm incorporation or the formation of the sperm aster. The migration of the female pronucleus occurs on schedule, at a reduced rate, and it is moved into the sperm aster (*a*). Syngamy does not occur nor does the normal disassembly of the sperm aster (*a* and *b*). At the time for mitosis the adjacent, but separate, pronuclei underdo nuclear breakdown but the mitotic apparatus does not appear; instead the monopolar sperm aster persists (*b*). Attempts at cleavage are routinely noted with a furrow forming at the cortex adjacent to the taxol-stabilized sperm aster (*c*). These cleavage attempts are followed by cortical relaxation before the next attempt. *L. variegatus*. Time (h:min:s) lower right. *M*, sperm nucleus. *F*, egg nucleus. Bar, 10 μm. × 700.

TABLE I

Movements during Fertilization: Controls vs. Eggs Treated with 10 μM Taxol for 30 Min before Insemination

	Control	10 μM Taxol
Sperm incorporation; displacement along egg cortex		
a. distance, μm	12.4 ± 4.9	7.7 ± 5.5
b. duration, min	3.5 ± 1.1	2.8 ± 0.9
c. rate; μm/min	3.5 ± 1.3	2.4 ± 1.3
Fertilization cone		
a. distance, μm	6.7 ± 2.0	7.9 ± 3.5
b. duration, min	2.5 ± 1.3	3.3 ± 1.5
c. rate; μm/min	2.6 ± 0.9	2.6 ± 0.4
Sperm aster formation		
a. distance, μm	14.3 ± 5.5	12.9 ± 9.3
b. duration, min	2.9 ± 0.5	16.0 ± 5.6
c. rate; μm/min	4.9 ± 1.7	1.1 ± 0.9
Female pronuclear migration		
a. distance, μm	19.1 ± 7.0	19.0 ± 15.4
b. duration, min	1.0 ± 0.3	10.4 ± 8.5
c. rate; μm/min	14.6 ± 3.5	3.3 ± 3.5
Pronuclear centration		
a. distance, μm	12.3 ± 4.0	8.3 ± 8.5
b. duration, min	4.7 ± 2.3	13.3 ± 4.7
c. rate; μm/min	2.6 ± 0.9	0.8 ± 0.9

The formation of the fertilization cone and sperm incorporation, microfilament-mediated processes, are unaffected by taxol. The rates for the formation of the sperm aster, the migration of the female pronucleus and the centration of the pronuclei, microtubule-mediated events, are all drastically reduced by taxol.

Taxol has been shown to endow microtubules with an unusual stability in vitro (9) and in vivo in developing (28), differentiated (29), and cultured (10, 30, 31) cells. The effects of taxol appear related to its direct binding of microtubule-associated proteins in promoting microtubule assembly (32–34). The finding that taxol reduces the rate of microtubule "treadmilling" (35) might well have important implications in understanding its effects on cellular movements proposed to be effected by microtubule treadmilling (36, 37) such as chromosome movement during mitosis (38, 39), or in the case of fertilization, the migration of the female pronucleus (1).

In unfertilized sea urchin eggs microtubules are rarely, if ever, observed. The effects of taxol here may be indicative of an equilibrium that, in the normal unfertilized case, favors microtubule disassembly. Taxol, which seems to block the normal disassembly without affecting the rate of assembly, makes this alleged slow rate of microtubule assembly detectable. It may well be that the control of microtubule assembly during fertilization is not at the level of initiating assembly after sperm incorporation but rather at that of shifting the equilibrium from one that favors disassembly in the unfertilized egg to one that favors assembly as the sperm aster is formed. Alternatively, taxol might promote microtubule assembly, as has been reported in cultured cells (30).

The configuration of microtubules in taxol-treated unfertilized eggs is quite different from that observed during fertilization when the sperm centrioles are the only assembly sites during artificial activation, when a single radial monaster forms

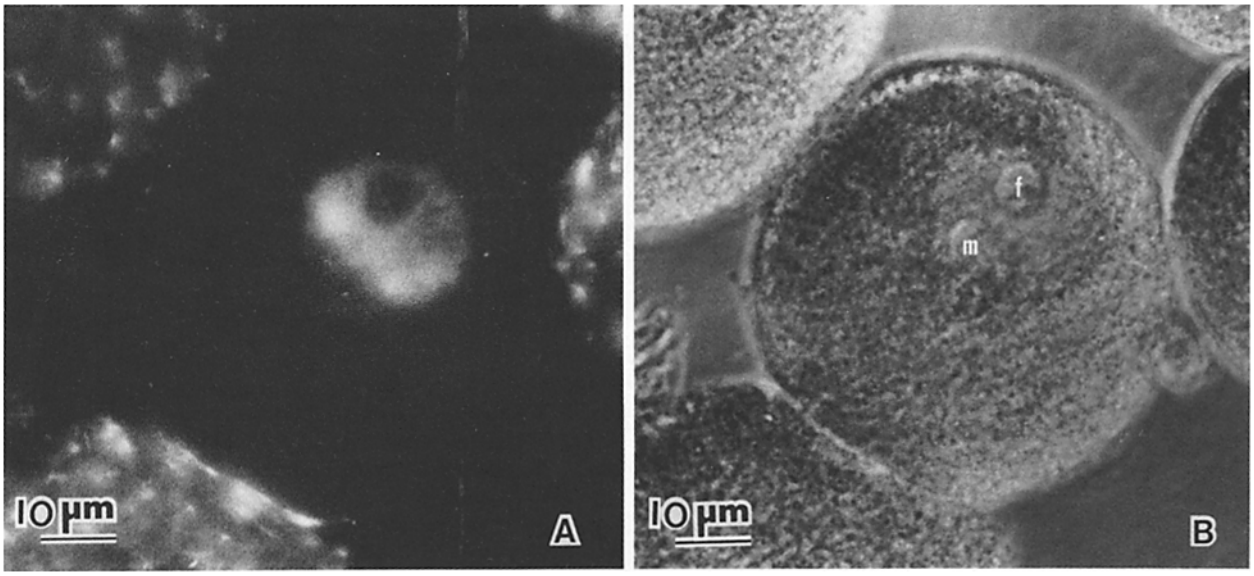


FIGURE 9 Antitubulin immunofluorescence microscopy of taxol-treated fertilized egg. When eggs treated with 10 μM taxol for 15 min before insemination are processed for immunofluorescence staining at 30 min postinsemination, the only tubulin-containing structure is the stabilized sperm aster (*a*). The unstained darker circular regions are the male and female pronuclei (*b*); phase-contrast microscopy. *A. punctulata*. *m*, sperm nucleus. *f*, egg nucleus. Bar, 10 μm. × 1,000.

around the centering egg nucleus (7, 20–22), or after parthenogenetic activation with heavy water (5, 6), when only a few (less than a dozen) larger asters form around the egg nucleus and at the subcortical regions (unpublished results). Within fertilized eggs, the only microtubule structure stabilized by taxol is the sperm aster, precluding the possibility, at least in these sea urchin species, of anastral cortical microtubules (15) active during the pronuclear migrations at fertilization. Interestingly, fertilized eggs display a single, albeit huge, aster, in contrast to results reported in mitotic cultured cells (30) in which supernumerary microtubule organizing centers were found. The disappearance of the unfertilized asters after insemination could be caused either by their disassembly as the sperm aster grows or by their aggregation into the forming sperm asters; video, TEM, and immunofluorescence microscopy support the latter alternative.

At fertilization the formation of the fertilization cone and the lateral displacement of the sperm along the egg cortex appear unaffected; this finding is consistent with the model in which the surface events are mediated by egg cortical microfilaments (11). Sperm tail motility is also unaffected, underscoring the effect of taxol on assembling, not assembled, microtubules. However, the intracellular movements of the sperm and egg nuclei are dramatically altered by taxol. Though the initial formation of the sperm aster appears largely unaffected, all of the later movements mediated by the sperm aster are altered. However, the sperm centriole is the dominant organizing center in fertilized eggs. The taxol-stabilized sperm aster, though immotile, does not interfere with the nuclear and cleavage attempt cycles.

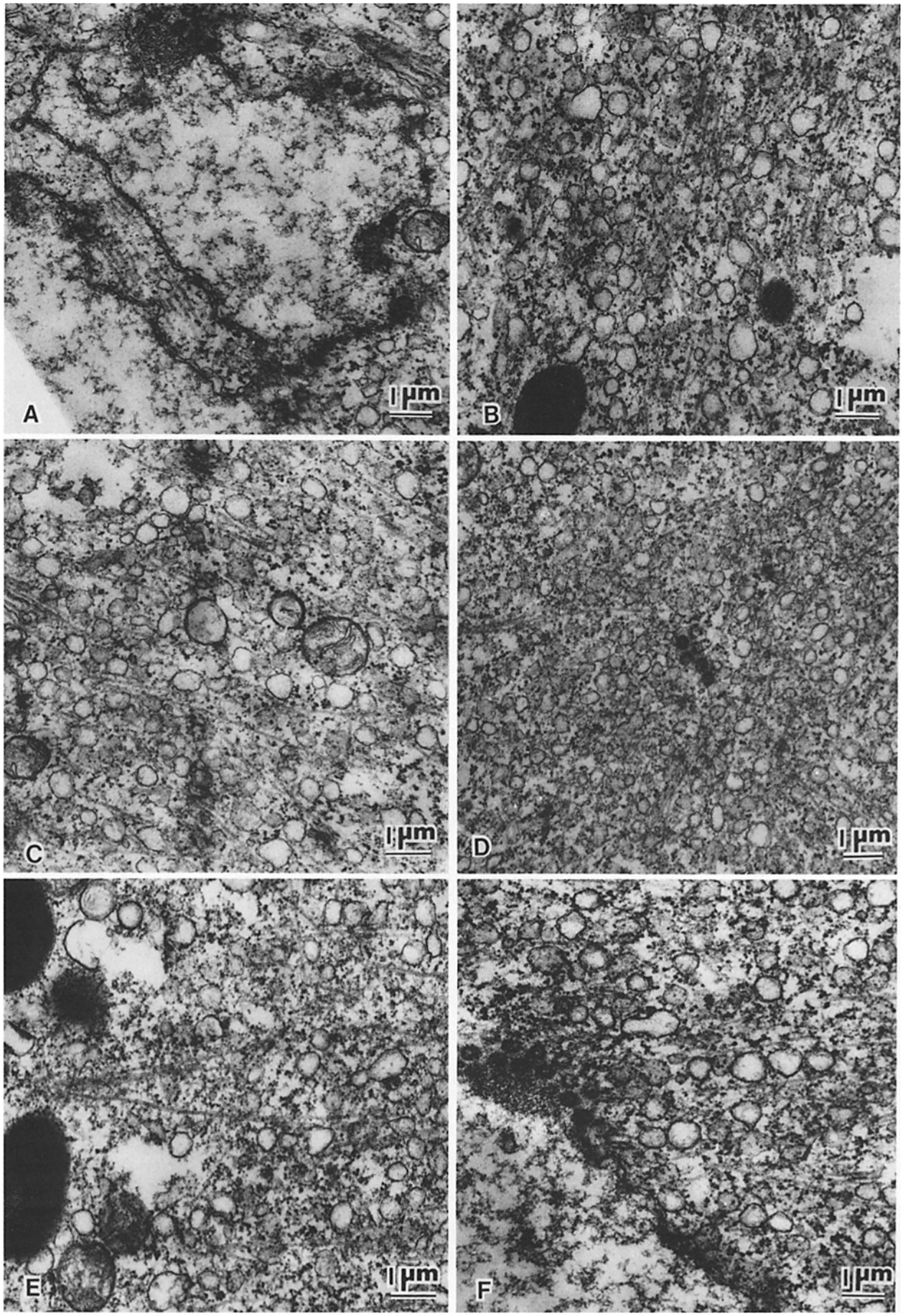
In conclusion, taxol induces the appearance of microtubules in unfertilized eggs, which is consistent with a model in which a normally undetectable amount of microtubule assembly occurs in the unfertilized egg but is outweighed by microtubule disassembly. Taxol then, by blocking disassembly in the unfertilized eggs, permits the detection of this alleged slow microtubule assembly rate. Alternatively, taxol might well promote microtubule assembly. It does not appear to affect the functioning of the stable microtubules of the sperm axoneme. During fertilization, taxol does not influence the rate of sperm incorporation or the formation of the fertilization cone, supporting the ideas regarding the role of egg cortical microfilaments during sperm incorporation. The formation of the sperm aster, the centripetal migration of the male pronucleus, the migration of the female pronucleus and pronuclear centration are all affected by taxol and syngamy is precluded in the presence of this drug; these findings demonstrate a requirement for microtubule disassembly during the nuclear movements at fertilization and lead to speculations concerning the importance of microtubule disassembly during motility generally.

It is a pleasure to acknowledge Dr. John D. Douros, Natural Products Branch, National Cancer Institute, for the generous gifts of taxol.

G. Schatten is a recipient of a Research Career Development Award of the National Institutes of Health (NIH) (HD 00363). The support of this research by the NIH (research grant HD 12913; microscope grant RR 1466) is gratefully acknowledged.

Portions of this study were presented at the Twenty-first Meeting of the American Society for Cell Biology (40).

FIGURE 10 TEM of fertilized taxol-treated eggs at 15 min postinsemination demonstrates the formation of numerous microtubule bundles in association with, and into, female pronuclear envelope (*a*), oriented primarily, though not exclusively, radial to the sperm aster (*b*, *c*, and *d*) and, at times, emanating within the sperm aster from microtubule-organizing centers other than the sperm centrioles (*d–f*). The abundance of membranes, and the lack of yolk platelets, within the taxol-stabilized sperm aster is noteworthy (*d*). *A. punctulata*. Bars, 1 μm. (*A–C* and *E*) × 9,000. (*D* and *F*) × 7,500.


A
1 µm
B
1 µm
C
1 µm
D
1 µm
E
1 µm
F
1 µm

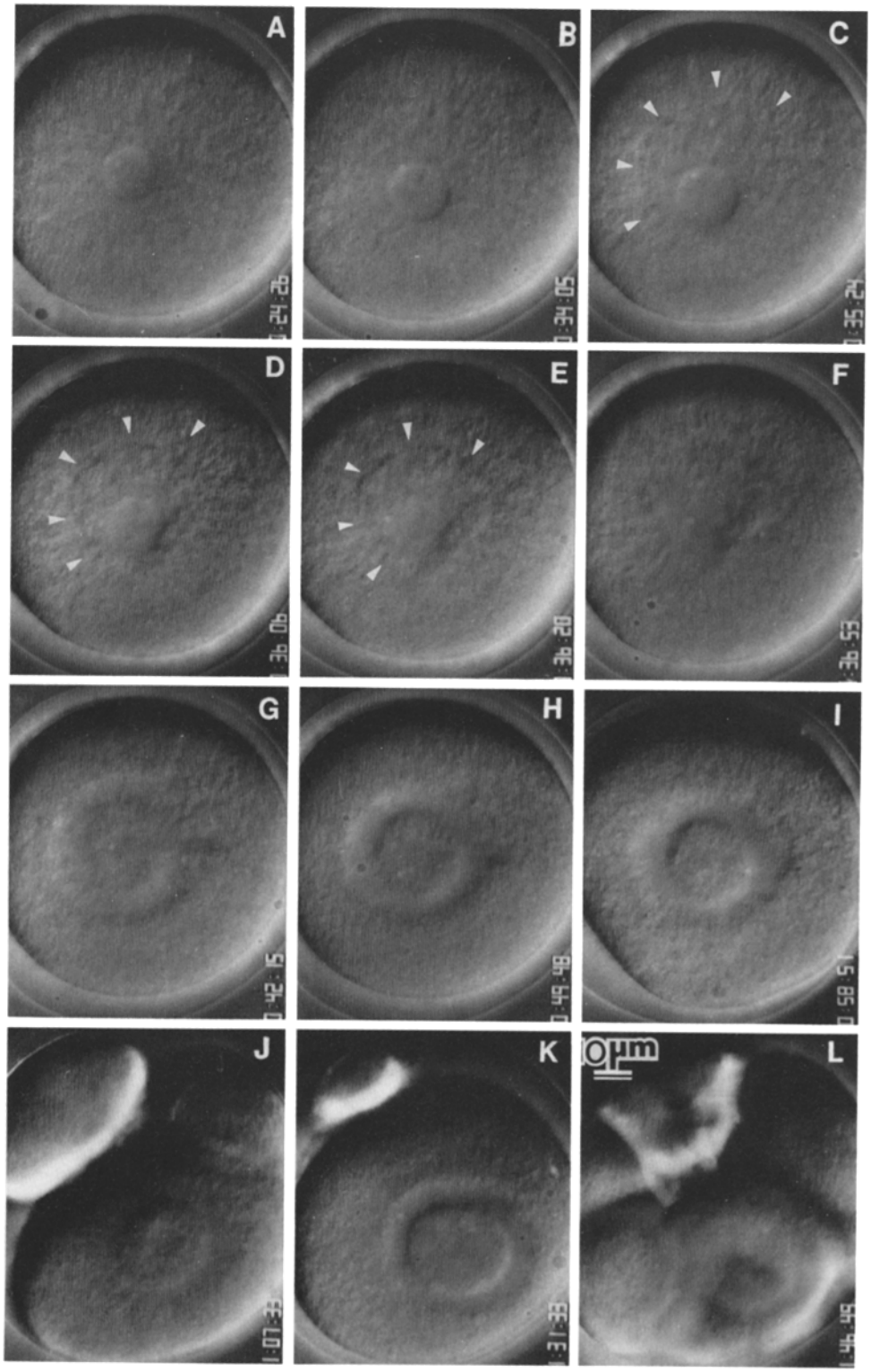


FIGURE 11 Effect of taxol added postsyngamy. 10 μM taxol, added 15 min post-insemination, does not affect the pronuclear migrations or centration (*a*). Fifteen min. after its addition (*b*), the monaster develops irregularities near the egg surface (*b* and *c*; arrowheads), which move centrad (*d–f*; arrowheads). By 50 min, a stable unusual ring surrounds the zygote nucleus (*g*). The bipolar mitotic apparatus does not form. However cycles of nuclear breakdown and reformation, chromosome condensation and decondensation, and cleavage attempts are observed (*h–l*). *L. variegatus*. Time (min:s) lower right. Bar, 10 μm. × 600.

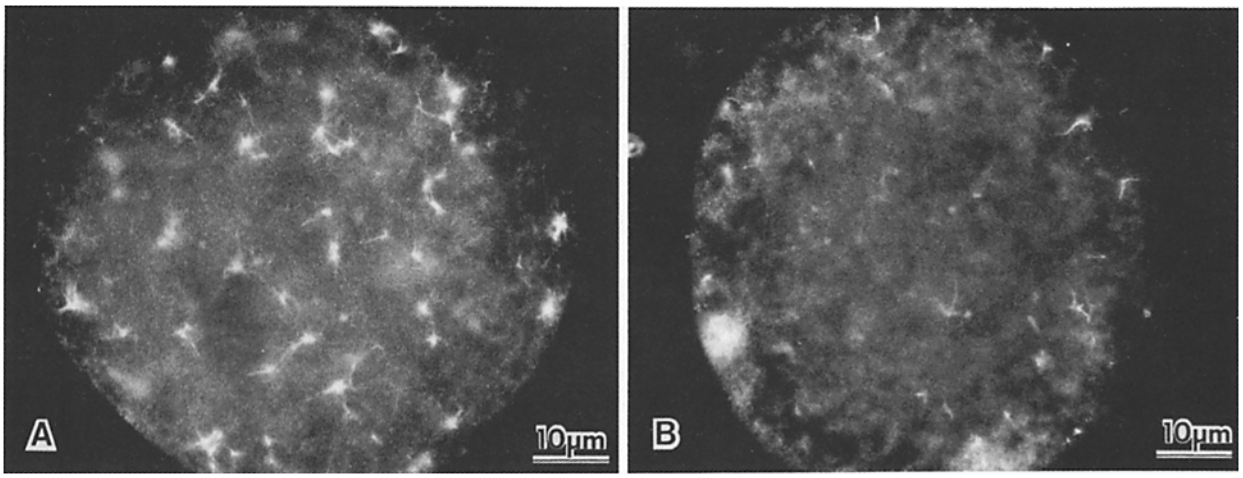


FIGURE 12 Colcemid reversal of taxol-induced asters in unfertilized eggs. (*A*). 10 μM taxol, 30 min, then 10 μM colcemid added for additional 15 min. (*B*). As *A*, but fixed after 30 min. *A. punctulata*. Bars, 10 μm. × 1,000.

Received for publication 28 December 1981, and in revised form 4 May 1982.

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