

## Microtubules influence compaction in preimplantation mouse embryos

By BERNARD MARO<sup>1</sup> AND SUSAN J. PICKERING

*Department of Anatomy, University of Cambridge,  
Downing Street, Cambridge CB2 3DY, U.K.*

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### SUMMARY

The role of microtubules during compaction of the 8-cell-stage mouse embryo was investigated using the drugs Taxol (which leads to a non-controlled polymerization of tubulin) and Nocodazole (which causes depolymerization of microtubules). Taxol inhibits compaction in most non-compacted embryos and reverses it in already compacted embryos. These effects were observed on both cell flattening (as judged by phase-contrast microscopy) and on cell surface polarization (as judged by scanning electron microscopy and the surface binding of fluorescent concanavalin A). In contrast Nocodazole does not inhibit cell flattening, but rather accelerates its completion. Nocodazole influences the detailed organization of the surface pole and appears to reduce the incidence of surface polarization but does not reverse polarity once established to a significant extent. We conclude that microtubules exercise a constraining role during compaction, influencing cell shape, cell organization and the time at which compaction takes place.

### INTRODUCTION

The first major change in cell morphology evident in mouse development occurs during compaction at the 8-cell stage. Whereas in the early 8-cell stage individual blastomeres are rounded, during compaction cells flatten against one another to maximize cell contacts, the intercellular boundaries becoming indistinct at the level of the optical microscope (Lewis & Wright, 1935; Lehtonen, 1980). At the same time the cells develop an apical 'epithelial-like' localization of microvilli (Callarco & Epstein, 1973; Ducibella, Ukena, Karnovsky & Anderson, 1977; Reeve & Ziomek, 1981), specialized cellular junctions (Ducibella & Anderson, 1975; Magnuson, Demsey & Stackpole, 1978; Lo & Gilula, 1979; Goodall & Johnson, 1984) and show a redistribution of intracellular organelles (Ducibella, *et al.*, 1977; Reeve, 1981; Reeve & Kelly, 1983). In other cell types microtubules have been shown to be involved in the maintenance of cell shape, development of cell polarity and localization and mobility of intracellular organelles (Malech, Root & Gallin, 1977; Solomon & Magendantz, 1981; Freed & Leibowitz, 1976; Pavelka & Ellinga, 1983). Although some previous studies have reported the effects of micro-

<sup>1</sup>C.N.R.S. France

tubule depolymerizing drugs (such as colchicine or colcemid) on compaction (Ducibella & Anderson, 1975; Surani, Barton & Burling, 1980; Ducibella, 1982; Pratt, Ziomek, Reeve & Johnson, 1982; Sutherland & Calarco-Gilliam, 1983), the results were difficult to interpret because cells were allowed to pass through mitosis in the presence of the drugs, leading to a mitotic block. We have therefore evaluated the effects on compaction of two drugs that modify the organization of microtubules, applied only during the interphase of the 4th cell cycle of development (8-cell stage). The two microtubule inhibitors used in this study were Nocodazole, which induces a depolymerization of intracellular microtubules (Hoebcke, Van Nigen & De Brabander, 1976) and Taxol which leads to a non-controlled polymerization of microtubules (Schiff, Fant & Horwitz, 1979; Schiff & Horwitz, 1980).

#### MATERIALS AND METHODS

##### *Recovery and culture of embryos*

MF1 female mice (3–5 weeks; Olac) were superovulated by injections of 5 i.u. of pregnant mares serum gonadotrophin (PMS; Intervet) and human chorionic gonadotrophin (hCG; Intervet) 48 h apart. The females were paired overnight with HC-CFLP males (Hacking and Churchill) and inspected for vaginal plugs next day.

2-cell embryos were flushed from oviducts at 48 h post hCG with Medium 2 containing 4 mg/ml bovine serum albumin (M2 + BSA; Fulton and Whittingham, 1978) and cultured in Medium 16 containing 4 mg/ml BSA (M16 + BSA; Whittingham & Wales, 1969) under oil at 37 °C in 5% CO<sub>2</sub> in air.

##### *Timing of embryos*

Precisely staged embryos were obtained by selecting newly formed 8-cell embryos at hourly intervals between 65 and 70 h post hCG. The degree of compaction of the embryos was determined hourly using a Wild M5 dissecting microscope and each embryo was assigned to one of the following classes: 'non-compacted embryos' where all cell outlines were clear and separate blastomeres could be distinguished easily, 'compacting embryos' where cell outlines were less prominent though some were still visible, and 'compacted embryos' where no cell outlines were apparent. Once embryos had been assigned to the 'compacted' group, they were retained in this group even if some decompaction occurred at a later stage, when embryos reach the 8- to 16-cell-stage transition (see Fig. 2D). During further manipulations and processing of the embryos, these groups were kept strictly separate.

##### *Removal of the zona pellucida*

Removal of the zona was achieved by brief exposure of the embryos to prewarmed acid Tyrode's solution (Nicolson, Yanagimachi & Yanagimachi, 1975).

*Decompaction of the embryos*

Decompaction of compacting and compacted embryos was achieved by exposure to Ca<sup>++</sup>-free M16 + BSA for 10–15 min. at 37 °C in 5% CO<sub>2</sub> in air.

*Immunocytochemistry*

Surface polarity was assessed by incubation of zona-free embryos in 700 µg/ml FITC-labelled Concanavalin A (FITC-Con A; Polysciences) for 5 min at room temperature, followed by three washes in M2 + BSA. Labelled embryos were then placed in specially designed chambers exactly as described in Maro, Johnson, Pickering & Flach (1984a) for fixation with 3.7% formaldehyde in phosphate-buffered saline (PBS). Samples were viewed on a Leitz Ortholux microscope using the selective filter set (L2) for FITC.

For immunolabelling of intracellular microtubules, embryos were then extracted with 0.25% Triton X-100 in PBS. After three washes in PBS, embryos were labelled with affinity-purified sheep anti-tubulin antibodies (Karsenti *et al.*, 1978) and peroxidase-labelled anti-sheep immunoglobulin antibodies.

The details of this procedure are reported in Maro *et al.*, (1984a).

*Scanning Electron Microscopy*

Scanning Electron Microscopy (SEM) was used to examine the surface polarity of the specimens. The procedure used was essentially that described by Johnson & Ziomek (1982) all solutions being millipored immediately prior to use. Clean glass coverslips were coated with Poly-L-lysine (PLL, Sigma Type 1B, 1 mg/ml fresh solution) for 15 min. and washed two or three times in cacodylate buffer pH 7.4 before being placed in individual wells of a Limbro 24-well tissue culture dish containing 0.1 M-cacodylate buffer pH 7.4. Specimens were fixed in 6% glutaraldehyde (Sigma) in 0.1 M-cacodylate buffer pH 7.4 for 1 h at room temperature. They were transferred immediately to 1% glutaraldehyde in 0.1 M-cacodylate buffer pH 7.4 and then placed in the centre of freshly prepared PLL-treated coverslips. Samples were dehydrated through graded alcohols (30 min each in 20%, 40%, 60%, overnight in 70%, 30 min each in 80%, 90%, 95% and 100%) and then dried from 100% ethanol via CO<sub>2</sub> in a Polaron E3000 critical-point drying apparatus. Coverslips were mounted on stubs with silver glue and coated with a 50 nm layer of gold in a Polaron E5000 Diode sputtering system. Embryos were examined in a ISM-35CF Jeol microscope under 20kV.

*Drugs*

A stock solution of 60 µM-Taxol (LOT T-10-8 gift of Dr. J. D. Douros, N.I.H. Bethesda) in dimethylsulfoxide (DMSO) and a stock solution of 10 µM-Nocodazole (Aldrich) in DMSO were used in these experiments. The various

dilutions of these drugs made in M16 + BSA were equilibrated for 12 h in 5% CO<sub>2</sub> in air before they were used for culturing embryos. Drugs were also present during all the steps preceding fixation when the embryos were processed for immunocytochemistry, FITC-Con A labelling or SEM.

*Statistical analysis of the results*

The chi-squared test was used to assess the statistical significance of the results. Yate's continuity correction was applied in all the calculations.

Table 1. *Effect of microtubule inhibitors on the flattening of timed 8-cell embryos as judged by phase contrast microscopy.*

Percentage of embryos showing intercellular flattening at different times after division of the slowest cell (number of embryos)					
Culture in presence of	1h-2h	2h-3h	4h-5h	6h-7h	8h-9h
<b>Exp A</b>					
DMSO 0.1%	3% (129)	16% (129)	47% (129)	71% (129)	75% (77)
Control	5% (128)	20% (128)	41% (128)	78% (128)	85% (75)
<i>P</i>	NS*	NS	NS	NS	NS
<b>Exp B</b>					
DMSO 0.1%	4% (107)	16% (219)	38% (217)	60% (177)	77% (145)
Taxol 12 $\mu$ M	0 (136)	0 (302)	3% (301)	10% (239)	13% (172)
<i>P</i>	NS	$\leq 10^{-3}$	$\leq 10^{-3}$	$\leq 10^{-3}$	$\leq 10^{-3}$
<b>Exp C</b>					
DMSO 0.1%	3% (223)	15% (268)	41% (267)	67% (252)	85% (114)
Nocodazole 10 $\mu$ M	19% (226)	33% (268)	60% (266)	84% (248)	94% (110)
<i>P</i>	$\leq 10^{-3}$	$\leq 10^{-3}$	$\leq 10^{-3}$	$\leq 10^{-3}$	0.05

\*NS = non significant.

The same population of embryos was scored at different time points in each experiment. Results are summed from 6 to 12 experimental groups. For each experimental group a control group (DMSO-treated) of the same size was usually scored (in the case of Taxol-treated embryos in which the incidence of compaction was low, experimental group size was enlarged).

## RESULTS

To avoid any interference with the mitotic process, 8-cell embryos were cultured in the presence of each drug 0–1 h after the division of the last 4-cell. First we checked that DMSO alone (in which the stock solutions of Taxol and Nocodazole were made) did not modify the kinetics of cell flattening in 8-cell embryos as judged by phase-contrast microscopy (Table 1, Exp. A).

*Effects of Taxol*

Taxol inhibited cell flattening in most 8-cell mouse embryos (Table 1, Exp. B). In contrast to the control embryos in which the cells were flattened

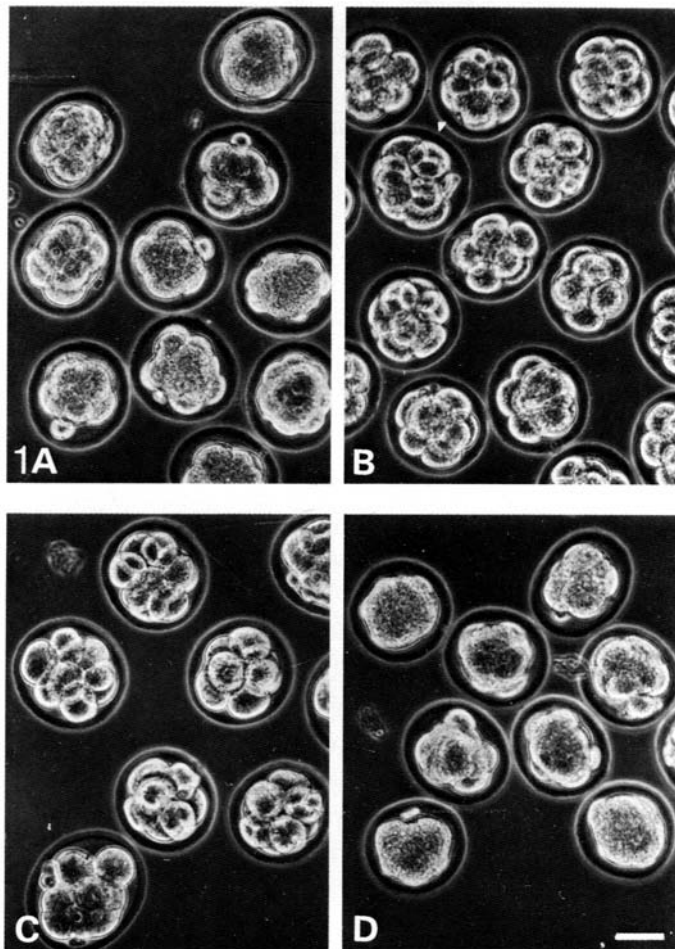


Fig. 1. Effect of Taxol on cell flattening. 8-cell embryos aged 7–8 h and cultured for 7 h in the absence (A) or in the presence (B) of 12  $\mu\text{M}$ -Taxol. Compacted 8-cell embryos cultured for 2 h in the presence (C) or in the absence (D) of 12  $\mu\text{M}$ -Taxol. Phase-contrast microscopy. Bar in (D) represents 50  $\mu\text{m}$ .

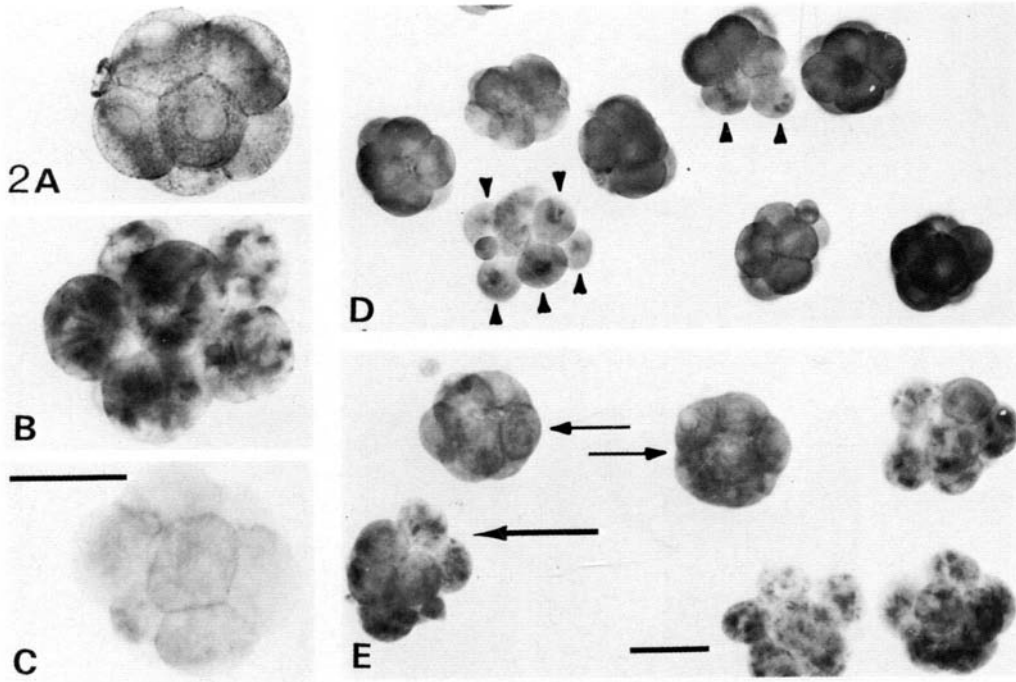


Fig. 2. Immunoperoxidase staining of tubulin in 8-cell mouse embryos. (A) control embryo; (B) embryo treated with  $12 \mu\text{M}$ -Taxol for 8 h. Note bundles of microtubules; (C) embryo treated with  $10 \mu\text{M}$ -Nocodazole for 8 h. Note loss of microtubules (as soluble tubulin is not concentrated in any particular area, the staining is weak); (D) compacted embryos after 2 h in culture; arrowheads indicate mitotic cells with tubulin concentrated in spindles; (E) compacted embryos cultured for 2 h in the presence of  $12 \mu\text{M}$ -Taxol; small arrows indicate embryos that remained flattened and with a normal tubulin staining, large arrow indicates an embryo in which two cells are decompacting and in which microtubules are reorganised. The remaining three embryos are decompacting and the cells contain bundles of microtubules. Bars in (C) and (E) represent  $50 \mu\text{m}$ .

against one another, most of the Taxol-treated embryos had clearly visible cellular outlines (Fig. 1 A, B). This effect was absent at doses below  $3 \mu\text{M}$ , detectable at  $6 \mu\text{M}$  and almost complete at  $12 \mu\text{M}$ .

The effect of Taxol on cytoplasmic microtubules was examined by immunoperoxidase staining using affinity-purified anti-tubulin antibodies (Karsenti *et al.*, 1976). Taxol induced a reorganization of cytoplasmic microtubules into randomly distributed bundles of tubules (compare Fig. 2A and B).

The effect of Taxol on cell polarization during compaction was scored in two ways: some embryos were prepared for scanning electron microscopy (SEM) and examined for poles of microvilli, whilst others were examined for a polarized surface binding of fluorescein-conjugated concanavalin A (FITC Con A). Embryos treated with Taxol had blastomeres which showed a de-

Table 2. Effect of microtubule inhibitors on the polarization of 8-cell embryos as judged by scanning electron microscopy.

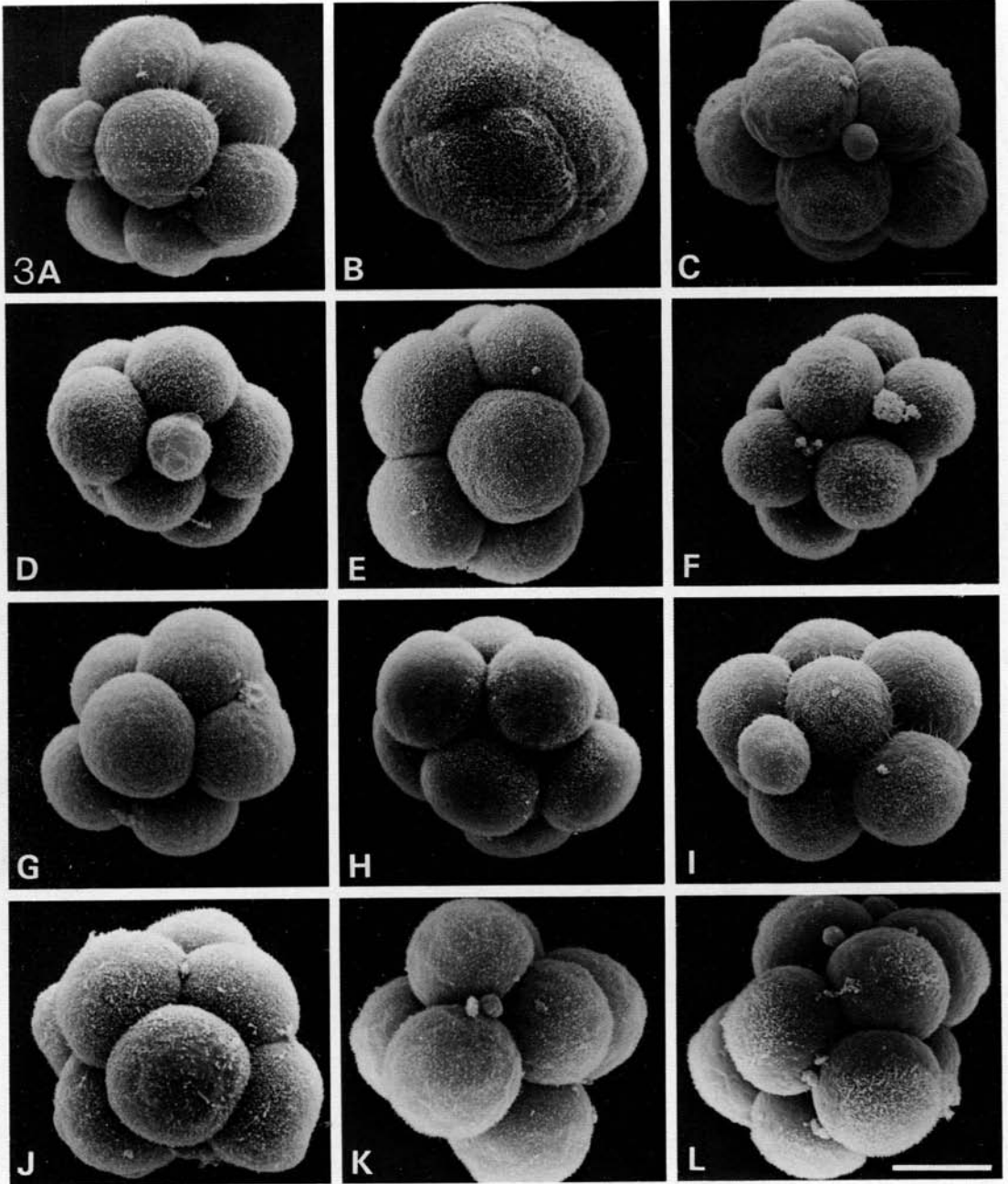
Embryo cultured for 8 h in presence of	DMSO 0.1%		Taxol 12 $\mu\text{M}$		Nocodazole 10 $\mu\text{M}$	
	n	non-polarized polarized	n	non-polarized polarized	n	non-polarized polarized
non-flattened	4	4 (100%)	33	24 (76%) 9 (24%)	10	9 (90%) 1 (10%)
flattened	35	0 (100%)	11	3 (27%) 8 (73%)	54	38* (70%) 16 (30%)

\*'bald poles' were observed on nine of these embryos (see Fig. 3h for example).

Table 3. Effect of microtubule inhibitors on the polarization of 8-cell embryos as judged by the FITC-Con A binding pattern.

8 h culture in presence of	Number of embryos	Number of embryos with a given percentage of polarized cells*			Number of polarized cells	Number of polarized cells (compared to DMSO)
		0-25%	26-50%	51-75%		
DMSO 0.1%	15	0	0	5 (33%) 10 (67%)	114	87 (76%) -
Taxol 12 $\mu\text{M}$	16	9 (56%)	2 (13%)	4 (25%) 1 (6%)	128	44 (34%) $\leq 10^{-3}$
Nocodazole 10 $\mu\text{M}$	21	7 (33%)	4 (19%)	4 (19%) 6 (29%)	143	74 (52%) $\leq 10^{-3}$

\*in some embryos less than eight cells could be scored.





creased incidence of polarized microvillous distribution (Table 2 and Figure 3 A–C and D–F) and polar FITC-Con A binding patterns (Table 3 and Figure 4 A, B) when compared to controls. Those few embryos which appeared to flatten in the presence of Taxol also showed a higher incidence of blastomeres with a microvillous pole than those which did not flatten (Table 2).

Taxol was also able to reverse cell flattening when already compacted embryos were cultured in the presence of the drug (Figure 1 C, D and Table 4). Cells not only became more rounded, but the proportion with surface poles was also lower (Figure 3 J, Figure 4 D and Table 5). A reorganization of microtubules was also observed in those embryos decompacted by Taxol (Figure 2 E), whereas a normal tubular pattern was present in those embryos that remained compact in the presence of Taxol (Figure 2 E, arrows). The analysis of these data is complicated by the fact that cells undergoing mitosis at the 8- to 16-cell stage rounded up transiently (Figure 2 D, arrowheads). We have checked that the decompacting effect of Taxol was not due to an underlying mitotic event. Thus only about 10% of cells were found to be mitotic in both control and Taxol-treated embryos after a 2 h incubation as assessed by phase-contrast microscopy (Table 5) and by examination of toluidine-blue-stained serial thick sections.

#### *Effects of Nocodazole*

In contrast to Taxol, Nocodazole blocks tubulin polymerization and, at the dose used here (10  $\mu\text{M}$ ), led to a disruption of cytoplasmic microtubules (compare Figure 2 A, and C). Nocodazole did not inhibit cell flattening but reduced the time interval between the last mitotic division from 4 to 8 cells and compaction (Table 1, Exp. C). This effect of Nocodazole in promoting cell flattening was not observed on populations of timed 4-cell embryos. Noco-

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Fig. 3. Scanning electron microscopy of 8-cell mouse embryos. Embryos in C, F, G, H, I, K and L were exposed to  $\text{Ca}^{++}$ -free medium prior to fixation to facilitate the observation of the microvillous poles.

(A–C) 8–9 h old control embryos, treated with DMSO for 8 h. (A) non-flattened, non-polarized; (B) compacted; (C) compacted treated with  $\text{Ca}^{++}$ -free medium, polarized.

(D–F) 8–9 h old embryos treated with 12  $\mu\text{M}$ -Taxol for 8 h. (D) non-flattened, non-polarized; (E) non-flattened, semi-polarized; (F) compacted treated with  $\text{Ca}^{++}$ -free medium, polarized.

(G–I) 8–9 h old embryos treated with 10  $\mu\text{M}$ -Nocodazole for 8 h. (G) compacted treated with  $\text{Ca}^{++}$ -free medium, non-polarized; (H) compacted treated with  $\text{Ca}^{++}$ -free medium, 'bald poles'; (I) compacted treated with  $\text{Ca}^{++}$ -free medium, polarized.

(J) compacted embryo treated for 2 h with 12  $\mu\text{M}$ -Taxol. Non-flattened, non-polarized.

(K–L) compacted embryos treated for 2 h with 10  $\mu\text{M}$ -Nocodazole. (K) compacted treated with  $\text{Ca}^{++}$ -free medium, non-polarized; (L) compacted treated with  $\text{Ca}^{++}$ -free medium, polarized. Bar in (L) represents 20  $\mu\text{m}$ .

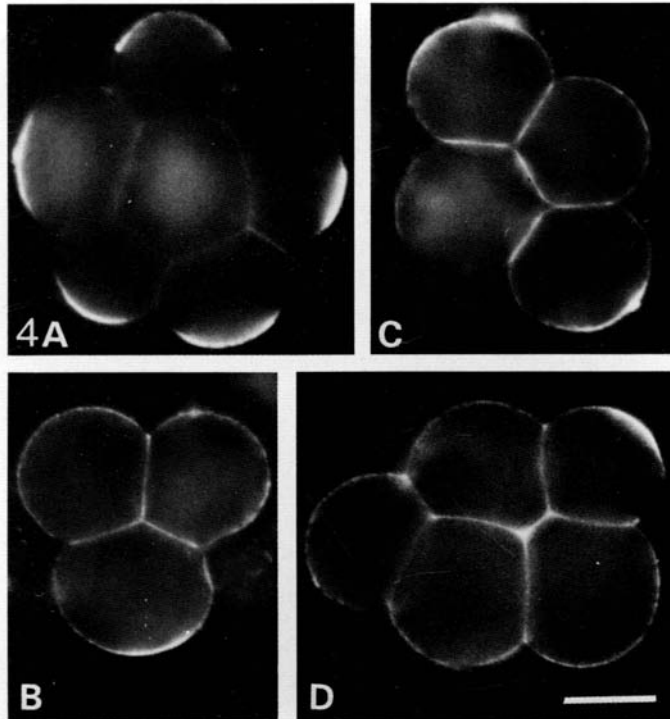


Fig. 4. FITC-Con A staining of 8-cell mouse embryos. (A) DMSO-treated control embryo, 8-9 h old; all cells polar. (B) 8-9 h old embryo treated with 12  $\mu\text{M}$ -Taxol for 8 h; 1 cell polar, 2 cells non polar. (C) 8-9 h old embryo treated with 10  $\mu\text{M}$ -Nocodazole for 8 h; 2 cells polar, 2 cells non-polar. (D) compacted embryo treated with 12  $\mu\text{M}$ -Taxol for 2 h; 1 cell polar, 4 cells non polar. Bar in (D) represents 20  $\mu\text{m}$ .

Table 4. *Effect of Taxol on compacted 8-cell mouse embryos as judged by phase contrast microscopy*

Treatment	Percentage of embryos in which cell flattening was retained at different times after drug addition (number of embryos)				
	0h	1h	2h	3h	4h
DMSO 0.1%	100% (106)	92% (106)	78% (106)	64% (75)	33% (51)
Taxol 12 $\mu\text{M}$	100% (137)	42% (137)	26% (137)	22% (37)	5% (43)
<i>P</i>	-	$\leq 10^{-3}$	$\leq 10^{-3}$	$\leq 10^{-3}$	$\leq 10^{-2}$

Once some blastomeres in an embryo rounded, this embryo was removed from the culture. In the case of the DMSO-treated embryo, this corresponds to cell entering mitosis.

Table 5. Effect on incidence of polarity of addition of Taxol to compacted 8-cell embryos as judged by FITC-Con A binding and scanning electron microscopy (SEM).

2h culture in presence of	FITC-Con A Analysis						SEM Analysis						
	Number of embryos	Number of embryos with a given percentage of polarized cells			Number of cells scored	Number of polarized cells scored	Number of mitotic cells scored	Number of embryos	Number of embryos		non flattened with polar cells		
		0-25%	26-50%	51-75%					76-100%	flattened with polar cells		non polar cells	
DMSO 0.1%	24	2 (8%)	5 (21%)	10 (42%)	7 (29%)	192	134 (70%)	20 (10%)	24	19* (79%)	0	3 (13%)	2 (8%)
Taxol 12 μM	18	10 (56%)	7 (39%)	1 (6%)	0	142	40 (28%)	16 (11%)	17	2 (12%)	0	2 (12%)	13 (76%)
P							≤10 <sup>-3</sup>	NS					

\*In two of these embryos, one cell was not flattened on the others and was also not polar.

dazole also reduced the incidence of cells scored as polarized as judged by the examination of both the surface distribution of microvilli (Table 2 and Figure 3 G-I) and the FITC-Con A binding patterns (Table 3 and Figure 4, A, C). However, of the poles detected, many tended to be more extensive and less sharply defined than in control (compare Fig. 3 C and I, and Fig. 4 A and C), and it is therefore possible that these values are underestimated since in the intact embryo the complete cell surface cannot be scored and thus very diffuse poles might be missed. 'Bald poles', corresponding to a decreased incidence of microvilli in the apical region of the cell surface were also observed (Fig. 3 H).

Nocodazole by itself does not reverse compaction, unless cells enter mitosis and become blocked. 34 compacted embryos were cultured for 2 h in the presence of 10  $\mu$ M-Nocodazole and processed for SEM. Surface poles of microvilli were observed in 76% of these embryos, while in 24% polarized cells were not detected (Figure 3 K, L). In 40 control compacted embryos the equivalent values were 93% and 7%. The difference observed between these two groups is not significant statistically ( $P \approx 0.07$ ).

#### DISCUSSION

The experiments reported here were designed to investigate the role of microtubules during the process of compaction. In order to simplify the interpretation of the effects of drugs that affect microtubules, examination of embryos and exposure to drugs were restricted to the non-mitotic phase of the 8-cell stage. Taxol, a drug which induces a non-controlled polymerisation of microtubules in cells (Schiff *et al.*, 1979; Schiff & Horwitz, 1980) blocked compaction in a high proportion of 8-cell mouse embryos. The two features of compaction examined, cell flattening and polarization, were affected coordinately. This drug was also able to reverse both these features of compaction in already compacted embryos. A small population of Taxol-resistant embryos was observed both in prevention and reversal of compaction, and resistance was correlated with the absence of reorganization of the microtubule network into bundles of microtubules as assessed by immunocytochemistry. To our knowledge Taxol is the first agent described that is able to block and reverse both cell flattening and cell polarization.

Nocodazole, a microtubule-disrupting drug (Hoebeker *et al.*, 1976), had more subtle effects on compaction reducing the interval between the last mitotic division (at the 4- to 8-cell-stage transition) and compaction, and reducing the incidence of cells scored as clearly polarized. Of the many poles that did form most were larger and less clearly defined than those in controls, an observation also suggested from a previous study (Pratt *et al.*, 1982) in which colcemid was used. The drug did not reverse compaction unless the cells were going into the 8- to 16-cell-stage division, when cells undergoing mitosis rounded up. The decompacting effect reported for other microtubule-

disrupting drugs such as colchicine or colcemid (Surani *et al.*, 1980; Pratt *et al.*, 1982; Sutherland & Calarco-Gilliam, 1983) was in fact due to rounding up of mitotic cells blocked by the drug rather than reversal of compaction as such. Ducibella did report a lack of effect of these drugs on cell flattening during interphase but the absence of kinetic studies meant that the accelerated compaction was not observed (Ducibella & Anderson, 1975; Ducibella, 1982).

In many systems, the use of drugs or the injection of antibodies reacting with tubulin (Wehland & Willingham, 1983) has demonstrated that microtubules control cell shape (Wells & Mallucci, 1978; Solomon, 1980; Solomon & Magendanz, 1981) and the intracellular organization of various organelles (Freed & Leibowitz, 1976; Toyoma, Forry-Schaudies, Hoffman & Holtzer, 1982; Summerhayes, Wong & Chen, 1983; Pavelka & Ellinga, 1983; Maro, Sauron, Paulin & Bornens, 1983). Microtubules have also been shown to be important in the control of oriented migration in neutrophils (Malech *et al.*, 1977) limiting the ability of the cell to move and change direction (Rich & Hoffstein, 1981). In lymphocytes, the use of anti-microtubule drugs led to a disruption of the normal relationship between the position of the centrosome and the position of the cap of surface immunoglobulin induced by anti-immunoglobulin antibodies, and to the release of the inhibition of surface immunoglobulin capping caused by high concentrations of Concanavalin A (Yahara & Edelman, 1973; Rogers, Khoshbaf & Brown, 1981; Paatero & Brown, 1982). Finally, the inhibition of myocyte contraction by dibutyryl cyclic AMP can be reversed by the addition of colcemid to the culture medium (Bollon, Porterfield, Fuseler & Shay, 1982). Nocodazole disrupts microtubules and destroys their interactions with other cellular components. In contrast, Taxol induces the formation of microtubule bundles destroying the spatial control of the microtubule network and stabilizing the interactions between microtubules and some associated cell components via microtubule-associated protein (MAPs) (Albertini, Herman & Sherline, 1984; Maro, *et al.*, 1983, 1984b; Johnson & Maro, in preparation).

The effects of anti-microtubule drugs on compaction of the 8-cell mouse embryo can be related to these results. The observation that disruption of microtubules accelerates intercellular flattening, whilst uncontrolled assembly and stabilization of microtubules prevents and reverse it, argues that microtubules have a constraining effect on cell shape at the 8-cell stage. The process of intercellular flattening itself appears remarkably independent of microtubules once this restraining effect is removed, and presumably is dependent simply upon an interaction between the microfilament system and the cell surface (Johnson & Maro, 1984b; Sutherland & Callarco-Gillam, 1983).

Both drugs inhibit or reduce the incidence of cell surface polarization which occurs at compaction, but only Taxol was able to disperse surface poles once they had developed. It seems clear that the development of a surface pole is not consistent with an uncontrolled proliferation of microtubules. Moreover,

the Taxol-induced rearrangement of the microtubule network leads to an active dispersion of the surface microvilli, resulting in the disappearance of surface poles. In contrast, although the incidence of poles appear to be reduced in the absence of microtubules (Nocodazole present), some poles do develop and, once poles have developed, they are relatively resistant to subsequent loss of microtubules. It is possible that the incidence of poles in the presence of Nocodazole was in fact higher than reported here, since the poles that were scored tended to occupy a larger part of the exposed apical surface of the blastomeres and had less sharply defined boundaries than on control blastomeres. With such poles, using the techniques applied here, it could be possible to miscore some 'broadly' polarized cells as non polar. Evidence consistent with this interpretation comes from examination of sectioned material by transmission electron microscopy, in which poles of microvilli are found to develop in the presence of colcemid (Ducibella, 1982). It seems that, in contrast with control poles, poles formed in the absence of microtubule passively disperse over the newly exposed apical surface when embryos are decompacted by exposure to  $\text{Ca}^{++}$ -free medium. Thus, Nocodazole may well have had its primary effect not on whether surface poles had developed but on their detailed organization and their stabilization once formed. From our results, it appears that the primary role of microtubules during compaction of the 8-cell mouse embryo, is to play a constraining rôle, being involved in the regulation of change in cell shape and organization and in their timing, rather than being actively involved in the actual mechanisms of cell flattening and polarization.

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