The role of microtubules in chick blastoderm expansion—a quantitative study using colchicine

By J. R. DOWNIE

From the Department of Zoology, University of Glasgow

SUMMARY

Since their discovery, cytoplasmic microtubules have been much studied in the context of cell movement and cell shape change. Much of the work has used drugs, particularly colchicine and its relatives, which break down microtubules—the so-called anti-tubulins. Colchicine inhibits the orientated movements of many cell types in vitro, and disrupts cell shape change in several morphogenetic situations.

The investigation reported here used chick blastoderm expansion in New culture in an attempt to quantify the colchicine effect on orientated cell movement. However, although colchicine could halt blastoderm expansion entirely, a simple interpretation was not possible.

1. Colchicine at concentrations capable of blocking mitosis, and of disrupting all or most of the cytoplasmic microtubules of the cells studied, inhibited blastoderm expansion, often resulting in an overall retraction of the cell sheet.

2. Though blastoderm expansion does normally involve considerable cell proliferation, the colchicine effect could not be ascribed to a block on cell division since aminopterin, which stops cell division without affecting microtubules, did not inhibit expansion.

3. Blastoderm expansion is effected by the locomotion of a specialized band of edge cells at the blastoderm periphery. These are the only cells normally attached to the vitelline membrane—the substrate for expansion. When most of the blastoderm was excised, leaving the band of edge cells, and the cultures then treated with colchicine, expansion occurred normally. The colchicine effect on blastoderm expansion could not therefore be ascribed to a direct effect on the edge cells.

4. An alternative site of action of the drug is the remaining cells of the blastoderm. These normally become progressively flatter as expansion proceeds. If flattening in these cells is even partially dependent on their cytoplasmic microtubules, disruption of these microtubules might result in the inherent contractility of the cells resisting and eventually halting edge cell migration. That cell shape in these cells is dependent on microtubules was demonstrated by treating flat blastoderm fragments with colchicine. On incubation, the area occupied by these fragments decreased by 25–30 % more than controls.

The significance of these results in the general context of orientated cell movements and cell shape determination is discussed, with particular emphasis on the analogous system of Fundulus epiboly.

1 Author’s address: Developmental Biology Building, Department of Zoology, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.
INTRODUCTION

Colchicine has long been known for its action in blocking mitosis, and since the work of Miszurski (1949) for inhibitory effects on the movement of tissue culture cells. Only more recently have both of these effects been attributed to the action of the drug on cytoplasmic microtubules, involving specific binding to tubulin, the protein from which microtubules are built up (reviews: Margulis, 1973; Olmsted & Borisy, 1973).

Recently, a number of papers have reported different effects of colchicine (and other anti-tubulins) on the movement of a wide range of cells, both in culture and in morphogenetic situations. My reasons for believing the present results are of interest are threefold:

1. Chick blastoderm expansion offers the opportunity of a quantitative study of colchicine effects, rather than the purely qualitative ones usual in morphogenetic systems.
2. The results of the investigation are superficially at variance with those on the analogous system of teleost epiboly, as studied, using Fundulus heteroclitus, by Kessel (1960) and Trinkaus (1973). These results highlight the problems of studying the locomotion of cells in sheets, compared with single cells.
3. Vasiliev et al. (1970) and Gail & Boone (1971) have reported that colchicine (or colcemid) inhibits not so much motility in general, but rather 'orientated' motility. Both these studies were on fibroblast-like cells, not noted for strongly directional movement when isolated in culture. It seemed worthwhile to study colchicine effects on an expanding epithelium, where movement is unidirectional. Chick blastoderm expansion offers such a system, involving the consistently centrifugal and rather rapid movement over the vitelline membrane inner surface of a band of cells at the blastoderm margin (Downie & Pegrum, 1971).

Since chick blastoderm expansion normally involves a large amount of cell proliferation (Downie, 1971), it was necessary to carry out control experiments using a drug which stops cell division without affecting microtubules. Aminopterin was used for this purpose (O'Dell & McKenzie, 1963).

The present paper is part of a series on the mechanics of chick blastoderm expansion.

MATERIALS AND METHODS

(i) Quantitative drug effects on blastoderm expansion

Fertile hens' eggs were incubated for 20–24 h to reach Hamburger & Hamilton (1951) stages 3–4 and then set up as New (1955) cultures. Embryos on their culture rings were transferred to fresh thin albumen medium, containing 10% medium '199' (Biocult labs.) or 10% medium '199' + drug. To ensure immediate effects, the drugs were also added to the ventral (uppermost) surface of embryos in 2–3 drops of '199', controls receiving '199' alone. Embryos were then staged,
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and outline drawings made, using a Wild M 5 microscope and drawing tube. The culture rings carried fixed orientation marks to allow exact superimposition of succeeding drawings. Further drawings were made after 4½, and 16–20 h incubation at 38 °C. Expansion distances for each embryo were calculated as the mean of expansion at ten arbitrarily chosen points round its circumference. In the same general way, measurements were made on the expansion of blastoderm edges, with the centre of the embryo (about four-fifths of the total blastoderm tissue) excised.

Finally, measurements were made on the size of fragments of 1- and 3-day yolk-sac tissue, lying on vitelline membrane as set up for New cultures, and incubated for 4 h in control or colchicine-containing medium. To give as accurate a measure as possible, tracings were made of the outline records, and these tracings weighed. Colchicine and aminopterin solutions were made up in medium ‘199’ and either used immediately or stored for a maximum of 2 weeks at 4 °C.

(ii) Morphological drug effects on blastoderm cells

(a) To check the effects of the drugs on cell division, control and experimental cultures from each batch of experiments, and from each time period were fixed in Bouin’s fluid. As a preliminary, part of the hypoblast and adhering yolk was removed using a jet of chick Ringer from a fine pipette. This exposes the epiblast to view in whole mount preparations, making it unnecessary to cut sections. Embryos were stained in Ehrlich’s haematoxylin and mounted flat in Canada balsam.

(b) To check the effects on cell ultrastructure, control and experimental embryos were prepared for electron microscopy by fixation in 2 % glutaraldehyde with post-fixation in 1 % osmium tetroxide. Staining of thin sections was with uranyl acetate and lead citrate.

(iii) Filming of colchicine-affected blastoderm expansion

Blastoderm edge cells are unfortunately rather difficult to film (Bellairs, Boyde & Heaysman, 1969; Downie, 1971) because of the thickness and high refractility of the underlying vitelline membrane, and because of the yolk obscuring them. In this work time-lapse films were made of the edges of blastoderms set up as New cultures in Vaseline-sealed plastic petri dishes, using a Wild time-lapse apparatus and inverted microscope with phase-contrast optics. To obtain a short enough working distance, the floor of the dish was replaced by a glass coverslip, and a minimum of albumen left between coverslip and vitelline membrane.

RESULTS

(i) Effects of colchicine on blastoderm expansion

Table 1 shows the effect of colchicine on blastoderm expansion in the first 4½ h incubation, and Fig. 1 some representative drawings. Preliminary
Table 1. Expansion distances after 4½ h colchicine treatment

<table>
<thead>
<tr>
<th>Treatment (colchicine in μg/ml)</th>
<th>No. of cultures</th>
<th>Expansion distance (mm), mean ± s.d.</th>
<th>Significance of difference from control (Student's t test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16</td>
<td>0·81 ± 0·20</td>
<td></td>
</tr>
<tr>
<td>1·0</td>
<td>16</td>
<td>−0·29 ± 0·74</td>
<td>$P &lt; 0·001$</td>
</tr>
<tr>
<td>0·2</td>
<td>22</td>
<td>0·61 ± 0·19</td>
<td>0·01 &gt; $P &gt; 0·002$</td>
</tr>
<tr>
<td>0·1</td>
<td>19</td>
<td>0·77 ± 0·23</td>
<td>$P &gt; 0·1$</td>
</tr>
</tbody>
</table>

Fig. 1. Colchicine effects on the expansion of whole blastoderms. Outline drawings at 0, 4·5 and 20 h of cultures with expansion distances close to the mean for each treatment. (a) 0·1 μg/ml colchicine, (b) 0·2 μg/ml colchicine, (c) 1·0 μg/ml colchicine, (d) control.

Experiments showed no effect at 0·01 μg/ml colchicine.¹ The full results show no inhibition at 0·1 μg/ml, but a significant reduction of expansion at 0·2 μg/ml; while at 1·0 μg/ml, hardly any expansion occurs and 9 out of 16 cultures show an overall retraction.

In the overnight incubation period (4½ h to about 20 h) this difference was intensified. Control and 0·1 μg/ml cultures continued to expand until baulked by the culture ring. In 1·0 μg/ml cultures, retraction continued, with occasional small areas of expansion. In 0·2 μg/ml cultures, results were variable. There was either some irregular expansion, or an overall retraction.

Reversibility of these effects was not good. Blastoderms exposed for 4½ h to 1·0 or 0·2 μg/ml colchicine and then incubated in control medium did not expand normally. However, colchicine effects are often not reversible without prolonged washing out, which was not done here. The concentration range used here is similar to that used by several other authors for locomotion inhibition studies on tissue culture cells.

¹ 1·0 μg/ml = 0·25 × 10⁻⁵ M colchicine. There is some argument over effective concentration. Some use mitotic arrest as the criterion of minimum effective concentration. Others, notably Goldman (1971), prefer to ensure that all cytoplasmic microtubules have disappeared. This generally requires a much higher concentration, and may produce toxic effects (Ramsey & Harris, 1973).
Table 2. Expansion and retraction rates in filmed colchicine-treated blastoderms

<table>
<thead>
<tr>
<th>Culture no.</th>
<th>Expansion rate (µm/h) before addition of colchicine (range)</th>
<th>Duration of expansion after colchicine addition (min)</th>
<th>Rate of expansion after addition (µm/h)</th>
<th>Retraction rate after cessation of expansion (µm/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200-315</td>
<td>26</td>
<td>140</td>
<td>57</td>
</tr>
<tr>
<td>2</td>
<td>155-215</td>
<td>39</td>
<td>65</td>
<td>195</td>
</tr>
<tr>
<td>3</td>
<td>560-670</td>
<td>80</td>
<td>350</td>
<td>95</td>
</tr>
</tbody>
</table>

Time-lapse films of the addition of 1·0 µg/ml colchicine to expanding blastoderms show first a slowing of the rate of expansion; then, within \( \frac{1}{2} \)–1 \( \frac{1}{2} \) h, expansion stops and the edge starts to retract. The flattened leading lamellae of the edge cells can be seen retracting and loosening their grip on the vitelline membrane. This leads to a rapid retraction, until the edge cells attach again. Table 2 gives details of films of three such cultures.

(ii) Effects of aminopterin on blastoderm expansion

Since blastoderm expansion normally involves considerable cell proliferation, inhibition by colchicine might be the result of the block in cell division, rather than interference with edge cell locomotion. This possibility is made unlikely by the work of Bellairs (1954) who, using an in ovo injection method, found aminopterin had no significant effect on the total area covered by blastoderm expansion, though the yolk-sac was sometimes an open meshwork, presumably due to the lack of cells. It seemed useful to repeat this work using the rather more certain and quantifiable New culture method.

Embryos were treated with 300 or 33 µg aminopterin in '199' (the higher concentrations used by O’Dell & McKenzie, 1963), added ventrally to New cultures. As with the colchicine experiments, expansion distances after 4 \( \frac{1}{2} \) h and after overnight incubation were recorded. There was no significant difference between controls and experimentals in the 4 \( \frac{1}{2} \) h period with either concentration (for 300 µg: controls (8) 1·24 ± 0·30 mm; experimentals (11) 1·11 ± 0·24 mm, \( P > 0·10 \); for 30 µg: controls (16) 0·81 ± 0·20 mm; experimentals (11) 0·71 ± 0·11 mm, \( P > 0·10 \) ) and expansion continued apparently normally in overnight incubation, not accurately measurable because of contact with the culture ring. Morphogenesis within the area pellucida was almost completely inhibited in all experimental cultures.

For the purposes of this investigation the continuation of blastoderm expansion on overnight incubation is the more significant observation since examination of yolk-sac epiblast cells (in fixed and stained whole mounts) reveals that cell division does not cease in the first 4 \( \frac{1}{2} \) h incubation period (mitotic index of three experimental embryos, all mitotic stages represented – 2·8, 2·2, 2·2 %, compared with between 2·0 and 4·0 % for five control embryos). It does stop
with overnight incubation (16–21 h): experimental embryos show no mitotic figures at all; controls have an index of 2–3. This is perhaps unsurprising if the aminopterin effect is only on DNA synthesis, and the cell cycle time is about 8–10 h (Wyllie, 1972) since cells entering mitosis in the first 4–5 h incubation period may have escaped the block. These results do, however, contrast with those of O'Dell & McKenzie (1963), who found aminopterin caused metaphase clumping.

It is clear, then, that in the total absence of cell division, blastoderm expansion can continue, at least for a time. The epiblast cells adopt an alternative strategy, that of increasing the area occupied by each cell. Such areas can be calculated from the number of nuclei within a fixed counting area. The area occupied by a single epiblast cell (calculated from the number of cells occupying a fixed area of epiblast sheet) in three 16–21 h incubated control embryos was 350, 450 and 370 $\mu\text{m}^2$ respectively; and in four aminopterin-treated embryos 1160, 1160, 1010 and 675 $\mu\text{m}^2$. The finding of Bellairs (1954), that with aminopterin treatment the yolk-sac became an open meshwork, was not seen in New cultures, perhaps because the incubation period was not long enough. Bellairs incubated embryos for up to 48 h after addition of the drug.

(iii) Effects of colchicine on blastoderm edges alone

Though partly discounting the importance of cell division as such, the aminopterin results highlight the role of cell flattening in blastoderm expansion. During the early stages of expansion, epiblast cells change from a close-packed columnar arrangement to form a thin sheet of very flattened cells, the area occupied by individual cells increasing from 142 $\mu\text{m}^2$ (cells near edge of blastoderm) at the start of incubation to 243 $\mu\text{m}^2$ (cells midway between area pellucida and blastoderm edge) after 24 h (Downie, in preparation). Shape changes of this kind are often accomplished by the redeployment of cytoplasmic microtubules (Tilney, 1971; Burnside, 1971). Could the colchicine inhibition of expansion be due to an inhibition of this shape change, rather than, or as well as, an effect on the locomotion of the edge cells? The most probable mechanism for such an effect is as follows: the contractility of cells lacking the ability to maintain a flattened shape (owing to the breakdown of their microtubules) exerts a tension against the centrifugal movements of the edge cells, capable of halting, or even reversing, that movement.

This postulated mechanism suggests a test. What happens to colchicine-treated blastoderms if the bulk of the blastoderm is removed, leaving the edge cells essentially alone?

Blastoderm centres were removed, leaving edges intact, plus a narrow ring of tissue representing in all about 20% of the total blastoderm area, and expansion in New culture followed, as before, for 4½ h. The results were surprisingly clear-cut (see Fig. 2 for representative drawings).

Isolated edges treated with 1·0 $\mu\text{g}/\text{ml}$ colchicine expanded at an almost
Fig. 2. Colchicine effects on isolated edges compared to whole blastoderms. Outline drawings at 0 and 4.5 h of cultures with expansion distances close to the mean for each treatment. (a) Edge alone, 1.0 μg/ml colchicine. The solid lines represent the positions of the outer edge; the dotted lines the cut margin, (b) whole blastoderm, 1.0 μg/ml colchicine, (c) whole blastoderm, control.

Fig. 2. Colchicine effects on isolated edges compared to whole blastoderms. Outline drawings at 0 and 4.5 h of cultures with expansion distances close to the mean for each treatment. (a) Edge alone, 1.0 μg/ml colchicine. The solid lines represent the positions of the outer edge; the dotted lines the cut margin, (b) whole blastoderm, 1.0 μg/ml colchicine, (c) whole blastoderm, control.

identical rate to whole control embryos (colchicine-treated embryos (13) 1.21 ± 0.33 mm in 4.5 h; controls (8) 1.24 ± 0.32 mm P > 0.1). Other results (in preparation) have shown that isolated edges under control conditions expand at a rate indistinguishable from whole embryos.

(iv) Effects of colchicine on isolated yolk-sac fragments

These results suggest that a microtubule-dependent flattening of those yolk-sac cells not at the edge may be necessary to maintain in the blastoderm sheet a tension low enough for the edge cells to overcome. Two deductions can be made from this idea (a) tension is important in blastoderm expansion. New (1959) first showed this to be true, and further work is in progress to analyse the role of tension in blastoderm expansion; (b) the flattened shape of yolk-sac cells is at least partly maintained by microtubules. The following observations show that this is so.

Polygonal pieces of yolk-sac, with as straight edges as possible, and in a size range 2–8 mm² were cut from both 24 h embryos (from between the edge and the area pellucida) and 72 h embryos (from the area vitellina, near the distal margin of the area vasculosa) and placed flat, epiblast down on vitelline membrane set up as for New cultures. The objective was to find how the size of these fragments (reflecting the area occupied by their individual cells) altered on incubation with control medium or with colchicine. Vitelline membrane provided a surface on which shape change occurred with apparent freedom. On agar or glass, the tissue fragments failed to flatten out well, and often stuck irregularly. Interpretation is somewhat complicated by using whole yolk-sac fragments, rather than epiblast alone, since this involves an interaction between epiblast, hypoblast and any intervening material. This, however, is the real situation: in how far do epiblast microtubules maintain the shape of the whole yolk-sac? Furthermore, isolated epiblast is difficult to handle, being
Table 3. Size changes in yolk-sac fragments after 4 h incubation

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Treatment</th>
<th>No. of fragments</th>
<th>% retraction from original (mean ± s.d.)</th>
<th>Significance of difference from control (Student's t test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-day yolk sac</td>
<td>Control</td>
<td>20</td>
<td>10.5 ± 12.9</td>
<td>---</td>
</tr>
<tr>
<td>3-day yolk sac</td>
<td>Control</td>
<td>21</td>
<td>0.8 ± 9.3</td>
<td>---</td>
</tr>
<tr>
<td>1-day yolk sac</td>
<td>10 μg/ml colchicine</td>
<td>22</td>
<td>38.5 ± 8.0</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>1-day yolk sac</td>
<td>1 μg/ml colchicine</td>
<td>21</td>
<td>37.0 ± 14.0</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>3-day yolk sac</td>
<td>10 μg/ml colchicine</td>
<td>21</td>
<td>30.5 ± 19.3</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>3-day yolk sac</td>
<td>1 μg/ml colchicine</td>
<td>19</td>
<td>27.5 ± 13.6</td>
<td>( P &lt; 0.001 )</td>
</tr>
</tbody>
</table>

Fig. 3. Colchicine effects on isolated fragments of 1-day incubated yolk sac: three representative fragments with each treatment. Outer lines: outlines of fragments at the start. Inner lines: after 4 h incubation. (a) Control, (b) 1-0 μg/ml colchicine, (c) 10-0 μg/ml colchicine.

rather sticky tissue which tends to curl up. A more serious complication is that on incubation, the edges of such fragments eventually attach to the vitelline membrane and start to spread the fragment. This, however, seems to happen equally to control and colchicine-treated fragments, between 4 and 6 h after explantation: experimental incubations were therefore limited to 4 h.

Table 3 gives the results of these experiments (and Fig. 3 some representative drawings), with size changes given as a percentage retraction from the original size.

Colchicine incubation, whether at high or lower concentration, whether on 1-day or 3-day tissue results in a size retraction, compared with controls, of 25–30%. This confirms the idea that the yolk-sac area is at least partially maintained by microtubules.

(v) Cytological effects of colchicine on epiblast cells

There is little point in postulating a major role for cytoplasmic microtubules in any cell type, on the basis of colchicine experiments, without evidence that colchicine does affect microtubules in these particular cells.
(a) Light-microscope observations on colchicine-treated embryos

As well as inhibiting blastoderm expansion, 1·0 and 0·2 μg/ml colchicine completely inhibited morphogenesis within the area pellucida, whereas control embryos, and embryos treated with 0·1 μg/ml colchicine proceeded on overnight incubation to stage 10 or so, after explantation at stage 4. After 4½ h incubation with 1·0 or 0·2 μg/ml colchicine, epiblast cell mitosis was completely blocked with accumulation of cells in metaphase (up to 34%) and no later mitotic phases visible. Mitotic index of 0·1 μg/ml colchicine-treated embryos was indistinguishable at 4½ h from controls (3–4%), but on overnight incubation, some highly abnormal mitotic figures appeared, suggesting some small effect at this concentration.

(b) Electron-microscope observations on colchicine-treated embryos

Epiblast cells, both at the edge and back from the edge of the blastoderm were examined for the presence of cytoplasmic microtubules. When present, they were generally near the cortex of the cells at the vitelline membrane side, and aligned roughly parallel to the cell surface. It is difficult to make precisely quantitative statements on microtubule numbers when these are not in obvious patterns. It will take further work to provide an accurate description of how the microtubules are distributed.

Control embryos: after 4½ or 19 h in culture, the cells maintained a normal healthy appearance, and showed abundant cortical microtubules. Cells at the edge, attached to the vitelline membrane, seemed to have fewer microtubules than cells not at the edge. This may relate to the apparent unresponsiveness of edge cells alone to colchicine:

(1) 0·1 μg/ml colchicine: microtubules remained abundant after 4½ or 19 h culture.

(2) 0·2 μg/ml colchicine: the cells were generally healthy after 4½ or 19 h culture, with prominent cell junctions. Microtubules were still quite abundant after 4½ h, but much reduced after 19 h (though not totally absent). This relates well to the time course of expansion inhibition at this concentration.

(3) 1·0 μg/ml colchicine: the cells were healthy in appearance after 4½ h, but very vacuolated and rounded, with dense granular cytoplasm after 19 h. There were few if any microtubules after 4½ or 19 h.

Aminopterin treatment had no noticeable effect on microtubules.

Treatment with 0·2 and 1·0 μg/ml colchicine certainly reduced numbers of cortical cytoplasmic microtubules during the course of the experiments, but may not have totally removed them. This is perhaps not surprising. Tubulin is regarded as sensitive to colchicine when in the unpolymerized state. Newly forming microtubule systems, such as mitotic spindle fibres, are therefore likely to be much more quickly sensitive to colchicine than established cortical microtubules (shown here by the rapid and complete arrest of mitosis).
DISCUSSION

The results may be summarized as follows. (1) Colchicine, at concentrations capable of blocking mitosis, and of breaking down all or most cytoplasmic microtubules, inhibits chick blastoderm expansion, often causing an overall retraction of the cell sheet (though the edge cells still adhere to the vitelline membrane). (2) This effect is not due simply to the block on cell division, nor does it appear due to a block on the orientated migration of the blastoderm edge cells. (3) The site of action may be the microtubules of those yolk-sac cells not at the edge. It is suggested that in these cells, microtubules maintain the flattened cell shape, and that flattening is essential for the massive area increase involved in blastoderm expansion. Breakdown of the microtubules results in the inherent contractility of the cells resisting and eventually halting edge cell migration. (4) A simple experimental demonstration of flattened-shape maintenance in yolk-sac cells is given.

There is little point in reviewing here the vast number of colchicine effects on cells, and, derived from them, the many roles postulated for microtubules. Two general aspects are relevant: microtubules and cell motility; and microtubules and cell shape maintenance.

(a) Microtubules and cell motility

Though this relationship has now been widely studied using various anti-tubulins, there are still no completely consistent conclusions. This is perhaps unsurprising in view of the rather different systems and techniques used. Colchicine or its relations may stop cell translocation entirely (Goldman, 1971), reduce it (Gail & Boone, 1971), have little effect (Ramsey & Harris, 1973; Bandmann, Rydgren & Norberg, 1974) or have effects varying from cell to cell (Spooner, Yamada & Wessells, 1971). There is more general agreement that ‘directional’ or ‘orientated’ movement is much reduced: colchicine-treated cells lose their polarity, ruffle, and extend leading lamellae all around the periphery rather than at restricted points (Vasiliev et al. 1970; Goldman, 1971; Gail & Boone, 1971; Bandmann et al. 1974).

In the context of chick blastoderm spreading, all this work suffers from a serious weakness – it deals with cells which normally move as individuals (fibroblasts, glial cells, leucocytes) rather than part of a cell sheet. Cells that are part of a sheet cannot so easily lose their orientation, since their centripetal and lateral margins are firmly bound to other cells, rather than free to spread on the substratum.

We now have two studies of colchicine effects on epibolic spreading, representing one kind of cell-sheet movement – that of Kessel (1960) on Fundulus epiboly (recently confirmed but not formally written-up by Trinkaus, 1973) and the present work on chick blastoderm expansion. Kessel and Trinkaus found that Fundulus epiboly occurs normally in the presence of enough colchicine to
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block mitosis. Trinkaus reports that the rate of spreading is normal for the first few hours, 'then it slows down a little'. As we have seen, colchicine in adequate concentration first slows down, then stops, and then may reverse chick blastoderm expansion: but that this effect is not on the migration of the edge cells. The difference between Fundulus and chick may lie in the amount of surface to be covered by epibolic spreading, and the rate of covering. Since the area is greater and rate more rapid in chick, the contribution from cell flattening may be correspondingly greater, and therefore more sensitive to microtubule breakdown.

Why then should the highly orientated movement of the edge cells of these two kinds of cell sheet be insensitive to colchicine treatment? One or two speculations are possible. If colchicine really does inhibit, not the mechanism of movement of cultured cells but their orientation system, then chick blastoderm spreading and Fundulus epiboly may be protected by the rather specialized arrangement of their edge cells (Downie & Pegrum, 1971) which may confer orientation even in the absence of microtubules.

In some preliminary experiments (Downie, 1971) I have found that the behaviour of cells at the periphery of yolk-sac epiblast explants (which do not have the specialized blastoderm edge) becomes rather abnormal after colcemid treatment: spreading is inhibited, and the cells show random to and fro movements. It is difficult, however, to be sure how much the tension/cell flattening effect, which confuses the issue in whole blastoderms, is also important in explants.

(b) Colchicine and cell shape maintenance

Though microtubules are often instrumental in the development of cell shape (review, Tilney, 1971) by as yet uncertain means (Burnside, 1971), their role in the maintenance of shape is more variable. This seems to depend on whether or not other stabilizing factors are present, such as other kinds of intracellular fibril, intercellular contacts, or attachment to a stable substratum. In the single-celled heliozoans, axopodial shape is maintained by microtubules (Tilney, 1968). In chick lens development, cell elongation is colchicine-sensitive, but already elongated cells are not (Pearce & Zwann, 1970; Piatigorsky, Webster & Wollberg, 1972); presumably, intercellular contacts, the basal lamina and internal fibrillar material all contribute to stabilization. However, Handel & Roth (1971) concluded that microtubules were at least partly responsible for shape maintenance in the highly asymmetrical cells of the chick neural tube.

In chick yolk sac, shape maintenance is mainly by (a) the tension in the sheet produced by the edge cells (loosening of the edge of a 24 h blastoderm produces an area reduction of around 25% (Downie, in preparation)) and (b) microtubules in the epiblast cells (as we have seen, isolated yolk-sac fragments retract a further 25–30% after colchicine treatment). The contribution of any other intracellular factors, and the details of microtubule arrangement must await further study.
Intercellular factors are probably unimportant since the very flattened epiblast cells have a low proportion of their surface area shared with their neighbours, reducing the stabilizing role of cell contacts; and their basal contacts are with the equally unstable hypoblast.

The final point for discussion is the suggestion that, in the absence of microtubules, the epiblast cells may exert a contractile force acting against, and eventually reversing, the centrifugal migration of the edge cells. Bhisey & Freed (1971) have noted an analogous situation in mouse macrophages. Normal macrophages have a polygonal shape and move in the ‘gliding’ way of fibroblasts. After colchicine treatment, the leading lamellae transform into narrow amoeboid pseudopods with obvious cytoplasmic flow. Bhisey and Freed suggest that microtubules normally maintain the shape of the cell against the contractile tendencies of ‘cortical’ microfilaments. In the absence of the microtubules, the cortex contracts producing cytoplasmic flow and shape transformation. Such an effect in the cells of the epiblast could provide the force necessary to halt blastoderm expansion.

I should like to thank Professor D. R. Newth for reading the manuscript and suggesting several improvements. This work was started while I was in receipt of an S.R.C. studentship and under the guidance of Professor M. Abercrombie at University College London, and continued in the Department of Zoology, University of Glasgow.

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(Received 14 February 1975)