MORPHOLOGY AND LOCOMOTION OF INDIVIDUAL EPITHELIAL CELLS IN CULTURE

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SUMMARY
The behaviour in culture of epithelial cells derived from chick embryo pigmented retina epithelium (PRE), corneal epithelium (CE) and epidermis has been studied using time-lapse cinemicrography. The analysis concentrated on the morphology and movement of individual isolated cells, lacking contacts with other cells, during a 24 h period starting 1-3 h after the cells were plated out in primary cultures.

Isolated cells from all three sources could change morphology and reversibly exhibited either a poorly spread or a well-spread morphology. While poorly spread, the different cell types all appeared similar and all blebbed vigorously. In contrast, while well spread, the cells did not bleb significantly but there were other differences between them. Well-spread CE cells were always polarized by the presence of a dominant leading lamella but well-spread PRE cells were always unpolarized. Well-spread epidermal cells exhibited both a polarized and an unpolarized morphology.

The tendency of individual isolated cells to change morphology varied with cell type. PRE cells were the most stable. Nearly 80 % of them retained the same morphology throughout the period of analysis and only 1 % of them showed three or more changes in morphology during this period. In contrast, 22 % of CE cells and 37 % of epidermal cells showed three or more changes in morphology during the period of observation. Isolated cells of all three types spent a greater proportion of the time exhibiting a poorly spread morphology than they spent exhibiting any alternative well-spread morphology.

The analysis revealed a relationship between the morphology of isolated cells and the speed of their locomotion. Only cells with a well-spread polarized morphology showed significant movement. CE and epidermal cells with this morphology moved three to four times faster than their counterparts with a poorly spread morphology or, in the case of epidermal cells, with a well-spread but unpolarized morphology. Actively moving PRE cells were not seen and this correlates with the absence of cells with a well-spread polarized morphology from cultures of this type.

These findings are discussed in the light of similar investigations of cell behaviour in other epithelial cell types and fibroblasts.

INTRODUCTION
Fibroblasts moving in culture have been studied extensively and in a number of cases the behaviour of both individual cells and populations of cells of this type have been carefully quantified (e.g. see Abercrombie & Heaysman, 1953, 1954; Abercrombie, Heaysman & Pegrum, 1970a,b; Gail & Boone, 1970; Dunn & Paddock, 1982). The movement of epithelial cells is of fundamental importance during morphogenesis, wound healing and malignant invasion but despite this the movements and interactions of epithelial cells in culture have been far less extensively documented. In particular, little is known of the behaviour of isolated cells of this type.

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Key words: cell behaviour, cell culture, epithelial cells.
type. This results, in the main, from the tendency of dissociated epithelial cells to become rapidly organized into multicellular sheets or islands of cells (e.g. see Middleton, 1973; Garrod & Steinberg, 1975; Parkinson & Edwards, 1978). Where they have been studied, isolated epithelial cells in culture have, in some cases, been reported not to show active translocation but to bleb vigorously and to remain only poorly spread upon the substratum (Trinkaus, 1963, Middleton, 1973, 1977; DiPasquale, 1975; Garrod & Steinberg, 1975; Parkinson & Edwards, 1978). In other cases, they have been seen to spread extensively and to move in an essentially fibroblast-like manner (Vaughan & Trinkaus, 1966; Garrod & Steinberg, 1975). In more detailed studies, individual isolated chick embryo pigmented retina epithelial (PRE) cells were found to be able to alternate between a poorly spread morphology showing vigorous blebbing activity and an extensively spread morphology lacking blebs (Middleton, 1977, 1982). In contrast, the majority of isolated Xenopus epidermal cells were reported to spread extensively upon the substratum and to assume a semi-circular morphology (Bereiter-Hahn et al. 1981). In an attempt to understand further the behaviour of isolated epithelial cells we have looked in detail at the morphology and movement in culture of cells derived from three different chick embryo epithelia – PRE, corneal epithelium (CE) and epidermis.

MATERIALS AND METHODS

Cell culture

The medium throughout consisted of Ham's F-10 tissue culture medium (Flow Ltd) supplemented with 10% foetal calf serum (Grand Island Biological Co) and containing 50 units/ml penicillin and 50 µg/ml streptomycin.

Single-cell suspensions of PRE cells from 10-day-old chick embryos were prepared as described by Middleton (1977) and single-cell suspensions of CE cells from 12-day-old chick embryos were prepared as described by Brown & Middleton (1981). Epidermal cells were prepared from skin dissected from the backs of 8-day-old chick embryos, pooled in Earle's saline and cut into pieces approximately 5 mm². After two washes in calcium- and magnesium-free (CMF) Earle's saline, the tissue was incubated in 0·5% trypsin in CMF at room temperature for 10 min. The fragments of skin were then washed in CMF and transferred to medium containing 1·5 mg/ml deoxyribonuclease (Sigma). The epidermis was separated from the underlying dermis and the resultant sheets were dissociated by incubation in 0·1% papain, using the formulation described by Steinberg (1962), for 5 min at 37°C. After dissociation the cells were washed in three changes of medium.

Samples (0·2 ml) of suspensions of PRE, CE and epidermal cells, containing 2·4 × 10⁵ cells/ml, 6·4 × 10⁵ cells/ml and 4·0 × 10⁵ cells/ml, respectively, were plated out into 9 mm diameter glass rings attached to collagen-coated coverslips (see Middleton & Pegrum, 1976) with silicone grease. Cultures were maintained at 37°C in an atmosphere of 5% CO₂ in air.

Filming and film analysis

Cultures were incubated for 1–3 h and then transferred to filming slides. To maintain the pH of the cultures during filming the medium was supplemented with 20 mM-HEPES. Cine films (16 mm) were obtained using Bolex-Wild time-lapse filming equipment in conjunction with a Reichert Biovert inverted microscope equipped with phase-contrast optics and a heated stage. The films were exposed at 1 frame/25 s.

Analysis of the films started 3–5 h after the cells were plated out and continued for the subsequent 24 h. The morphology exhibited by 75 isolated cells of each type was monitored continuously throughout this period. To obtain this number of cells three films each of CE and epidermal
cultures, and six films of PRE cultures, were analysed and the data pooled appropriately. The speed of locomotion of a total of 18 isolated cells of each type was recorded throughout the period of analysis. Six cells were selected from each of three films of the various cell types to give this total. The films were projected onto paper so as to give a total magnification (microscope plus projector) of ×1600. The morphology and position of each of the selected cells was recorded at the start of analysis and subsequently at intervals equivalent to 30 min of real time. The centre of the nucleus was used to define the position of the cells at each time point. The projected distance that this moved in each 30 min period was measured and converted to true distance.

The data that we derived in different parts of this investigation were not always normally distributed and therefore statistical comparisons were made using the distribution-free Kolmogorov-Smirnov 2-sample test (Siegel, 1956).

RESULTS

Analysis of the films began 3.5 h after the cells were plated out. At this stage cultures of all three cell types contained numerous isolated cells (Fig. 1A,B,C) but, in cultures of PRE cells in particular, some small islands of cells were also present (Fig. 1A). The analysis continued for an additional 24 h by which time virtually all the cells of each type had become incorporated into coherent sheets of cells and few if any isolated cells remained in the cultures.

It was clear from our films that, irrespective of their source, individual isolated cells could undergo striking alterations in morphology and that these alterations often appeared to be associated with changes in their speed of movement. We have analysed these observations in more detail.

Fig. 1. Appearance of the cultures 3.5 h after plating out. A, PRE cells; B, CE cells; C, epidermal cells. Phase-contrast. Bar, 100 μm.
Cell morphology

Our films showed that individual isolated cells of all three types could adopt, reversibly, either a poorly spread or a well-spread morphology. While they were poorly spread the different types of cell all looked similar and they all blebbed vigorously (Fig. 2A,C,E). In contrast, while well spread, the cells from the various sources did not bleb significantly but there were other differences between them. Well-spread CE cells adopted a fibroblast-like morphology usually polarized by the presence of a dominant leading lamella (Fig. 2D). Well-spread PRE cells, on the other hand, were unpolarized; they lacked a dominant leading lamella but lamellar cytoplasm was usually present around much of their periphery (Fig. 2A). Well-spread epidermal cells could adopt either a polarized or an unpolarized morphology. When polarized (Fig. 2F) they resembled well-spread CE cells. When unpolarized they sometimes resembled well-spread PRE cells but more commonly they adopted a more polygonal form with concave margins (Fig. 2G).

Our films allowed us to monitor the behaviour of the cells continuously over a period of 24 h (see Materials and Methods) and 75 of the filmed cells of each type were selected for further study. We chose cells that initially were isolated, and although many of them subsequently made and broke contacts with other cells, we restricted our analysis to those periods when the cells were isolated. For the purposes of analysis we recognised three categories of cell morphology: (1) poorly spread, shown by all three cell types; (2) well-spread but unpolarized, shown by PRE and epidermal cells; and (3) well-spread and polarized shown by CE and epidermal cells. Inevitably some cells had morphologies that were intermediate between these categories and such cells were allocated to the category that they most closely resembled. We recorded any changes in the morphology of the selected cells and the length of time that each cell spent exhibiting a particular morphology. Where necessary, the data obtained in this way were pooled to provide average values for each cell type.

Table 1 shows that individual isolated cells of all three types exhibited changes in morphology during the period of analysis but that their tendency to do this varied considerably with cell type. Nearly 80 % of PRE cells retained the same morphology throughout, while in contrast the majority of both CE and epidermal cells showed at least one change in morphology during this period. Only 1 % of PRE cells showed three or more changes in morphology during the period of analysis while the corresponding values for CE and epidermal cells were 22 % and 37 %, respectively.

It is clear from Table 2 that isolated cells of all three types, on average, spent more time exhibiting a poorly spread morphology than they spent exhibiting any alternative category of morphology. In the case of both PRE and CE cells the proportion of time spent poorly spread was significantly greater than the proportion of time spent

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Fig. 2. Morphology adopted by isolated cells. A, B. PRE cells; A, poorly spread; B, well-spread, unpolarized. C, D. CE cells; C, poorly spread; D, well-spread, polarized. E-G. Epidermal cells; E, poorly spread; F, well-spread, polarized; G, well-spread, unpolarized. Phase-contrast. Bar, 10 μm.
Table 1. Number of changes in morphology shown by the cells during the 24 h period of observation

<table>
<thead>
<tr>
<th>No of changes in morphology</th>
<th>% of cells(^\dagger)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE cells</td>
</tr>
<tr>
<td>0</td>
<td>79</td>
</tr>
<tr>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>3 or more</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^\dagger\)n = 75 for each cell type.

Table 2. Analysis of cell morphology

<table>
<thead>
<tr>
<th></th>
<th>Average total time spent as isolated cells ± S.E.M. (h)(^\dagger)</th>
<th>Average % of total time ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Poorly spread</td>
<td>Well-spread</td>
</tr>
<tr>
<td>PRE cells</td>
<td>6.55 ± 0.58</td>
<td>79.5 ± 7.6</td>
</tr>
<tr>
<td>CE cells</td>
<td>3.78 ± 0.38</td>
<td>74.1 ± 8.8</td>
</tr>
<tr>
<td>Epidermal</td>
<td>3.45 ± 0.37</td>
<td>41.2 ± 6.4</td>
</tr>
</tbody>
</table>

\(^\dagger\)n = 75 for each cell type; n.a., not applicable.

displaying their respective well-spread morphologies (PRE cells \(\chi^2 = 32.7, P < 0.001\); CE cells \(\chi^2 = 12.6, 0.01 > P > 0.001\)). However, in the case of the epidermal cells there was no significant difference between the proportion of time spent poorly spread and the total proportion of time spent exhibiting one or other well-spread morphology \(\chi^2 = 2.53, 0.3 > P > 0.2\). There was no significant difference between the proportion of time that well-spread cells of this type spent polarized and the proportion that they spent unpolarized \(\chi^2 = 0.43, 0.9 > P > 0.8\).

**Speed of locomotion**

Preliminary observations of our films suggested that there was a relationship between the morphology of the isolated cells and their speed of locomotion. In general, the only cells to show significant translocation were those with a well-spread polarized morphology. Poorly spread and well-spread unpolarized cells seemed to do little more than oscillate over relatively limited areas of the substratum. We analysed this in more detail. A total of 18 isolated cells of each type were selected and their morphology and speed of locomotion were recorded at 30 min intervals throughout the 24 h period of analysis (see Materials and Methods). We recognised the same three categories of cell morphology as used in the previous part of this investigation and we again restricted our analysis to those periods during which the cells were isolated.
**Table 3. Speed of locomotion of cells with different morphologies**

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Mean speed of locomotion ± S.E.M. (µm/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE cells</td>
<td></td>
</tr>
<tr>
<td>Poorly spread</td>
<td>2.3 ± 0.2 (n = 202)</td>
</tr>
<tr>
<td>Well-spread unpolarized</td>
<td>2.8 ± 0.3 (n = 109)</td>
</tr>
<tr>
<td>Well-spread polarized</td>
<td></td>
</tr>
<tr>
<td>CE cells</td>
<td></td>
</tr>
<tr>
<td>Poorly spread</td>
<td>2.5 ± 0.3 (n = 168)</td>
</tr>
<tr>
<td>Well-spread polarized</td>
<td>10.1 ± 1.0 (n = 53)</td>
</tr>
<tr>
<td>Well-spread unpolarized</td>
<td></td>
</tr>
<tr>
<td>Epidermal cells</td>
<td></td>
</tr>
<tr>
<td>Poorly spread</td>
<td>5.0 ± 0.8 (n = 93)</td>
</tr>
<tr>
<td>Well-spread unpolarized</td>
<td>3.7 ± 0.6 (n = 76)</td>
</tr>
<tr>
<td>Well-spread polarized</td>
<td>15.4 ± 1.0 (n = 89)</td>
</tr>
</tbody>
</table>

**Table 4. Comparisons between the stated distributions of speeds of cell locomotion**

<table>
<thead>
<tr>
<th>Line</th>
<th>Cell type</th>
<th>Comparison made</th>
<th>( \chi^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CE cells</td>
<td>Well-spread polarized versus poorly spread</td>
<td>44.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>Epidermal cells</td>
<td>Well-spread polarized versus poorly spread</td>
<td>49.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Well-spread polarized versus well-spread unpolarized</td>
<td>49.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Well-spread unpolarized versus poorly spread</td>
<td>1.44</td>
<td>0.5 &gt; P &gt; 0.1</td>
</tr>
<tr>
<td>5</td>
<td>PRE cells</td>
<td>Well-spread unpolarized versus poorly spread</td>
<td>2.45</td>
<td>0.5 &gt; P &gt; 0.1</td>
</tr>
<tr>
<td>6</td>
<td>CE/epidermal cells</td>
<td>Well-spread polarized CE versus well-spread polarized epidermal cells</td>
<td>11.13</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The data in Table 3 show that the greatest speeds of locomotion were achieved by cells exhibiting a well-spread polarized morphology. CE and epidermal cells with this morphology moved significantly faster than when exhibiting any alternative morphology (Table 4, lines 1, 2, 3). On average, well-spread polarized cells of both these types moved three to four times faster than when poorly spread or, in the case of epidermal
cells, when well-spread but unpolarized. The highest speeds of locomotion were shown by well-spread polarized epidermal cells, which on average moved significantly faster than CE cells with the same morphology (Table 4, line 6). There was no significant difference between the speed of movement of epidermal cells when poorly spread and when well-spread but unpolarized (Table 4, line 4), and the same was true of PRE cells (Table 4, line 5).

**DISCUSSION**

Isolated epithelial cells of the three types studied in this investigation showed less tendency to spread extensively on the substratum and to show polarized locomotion than would be expected of many types of isolated fibroblastic cells cultured under similar conditions. Since it has been suggested in previous reports that similar behaviour is shown by cultured epithelial cells derived from *Fundulus gastrulae* (Trinkaus, 1963), and chick embryo liver (Garrod & Steinberg, 1975) and amnion (Vaughan & Trinkhaus, 1966), it may well be characteristic of many epithelial cell types.

Although their behaviour differed in detail, isolated cells of the three types studied here spent a substantial proportion of the time poorly spread and blebbing vigorously (Table 2). In general, normal fibroblasts would only be expected to display such behaviour after partial detachment from the substratum or during mitosis. The reason for this difference in behaviour between epithelial cells and fibroblasts is unknown. One possible explanation may be that in general epithelial cells do not develop sufficient cell-substratum adhesion to enable them to spread as extensively as do fibroblasts. Such an explanation would not, however, account for the observation made here and elsewhere (Middleton, 1977; Brown & Middleton, 1981) that isolated epithelial cells do spread extensively upon the substratum for a significant proportion of the time (Table 2). Neither can it account for the fact that poorly spread isolated epithelial cells, of a number of types, spread extensively on the substratum shortly after making contact with another cell of the same type (see below). A possible alternative explanation may be that isolated epithelial cells bleb and fail to spread because, unlike fibroblasts, they do not contain sufficient microtubules to localize their protrusive activity and to stabilize their spreading lamellae (DiPasquale, 1975). Again this suggestion is difficult to reconcile with the finding that for a proportion of the time isolated epithelial cells lack blebs and spread extensively on the substratum. Indeed ultrastructural and immunofluorescence observations have now revealed the presence of abundant microtubules in cultured epithelial cells from a number of sources (Lonchampt *et al.* 1976; Bershadsky *et al.* 1978; Asch, Medina & Brinkley, 1979), and indirect immunofluorescence studies have shown that they are also present in the three cell types studied here (Middleton, unpublished observations). However, there is some evidence to suggest that the distribution of microtubules within the cytoplasm differs between fibroblasts and epithelial cells (Bershadsky *et al.* 1978), but it remains to be shown that this is responsible for the differing behaviour of these cell types.
While they were poorly spread, isolated cells of all three types had a very similar appearance and they all blebbed vigorously. However, there was considerable variation both in the average proportion of the time that each cell type spent displaying this morphology (Table 2) and in the frequency with which isolated cells of each type adopted an alternative morphology (Table 1). Poorly spread isolated cells of the three types used here are known to spread extensively on the substratum shortly after making contact with another cell or island of cells of the same type (Middleton, 1977; Brown & Middleton, 1981; Brown, 1983) and this response has been called contact-induced spreading (Middleton, 1977). However, it is clear both from our data (Table 2), and from that obtained in previous investigations (Middleton, 1977; Brown & Middleton, 1981) that contact with other cells is not an absolute prerequisite for the spreading of epithelial cells and such spreading, in the absence of cell contact, has been termed spontaneous spreading (Middleton, 1982). Isolated epithelial cells of all three types displayed spontaneous spreading and as they did so their blebbing ceased. Since this is also a characteristic of contact-induced spreading, it seems likely that in these cells blebbing and extensive spreading are mutually incompatible. It has been suggested that in some fibroblasts blebs may provide a reservoir of cell surface that can be used during extensive spreading (Erickson & Trinkaus, 1976) and it may well be that the same is true for these epithelial cells.

The average proportion of the time for which the cells were spontaneously spread and the morphology that they adopted when spread in this way again varied with cell type. Isolated CE and PRE cells were spontaneously spread for a similar proportion of the time (Table 2), but while such CE cells were almost invariably polarized by the presence of a dominant leading lamella the equivalent PRE cells were always unpolarized. Epidermal cells were spontaneously spread for a much greater proportion of the time and differed from the other cell types in that they could be either polarized or unpolarized. We were surprised not to record the presence of any well-spread polarized PRE cells since they have been previously described (Middleton, 1977). We can only assume that this failure is due to minor differences in culture techniques or to sampling error. Nevertheless, it is clear that under identical culture conditions isolated epithelial cells of different types can vary both in the proportion of the time for which they are spontaneously spread and in the extent to which they become polarized. The reasons for these differences between the cells are unknown but they are clearly of importance in determining the behaviour of the cells since only those cells with a polarized morphology show significant translocation.

It is clear from the data in Table 3 that there is a close relationship between the morphology adopted by these epithelial cells and their speed of locomotion. Poorly spread and well-spread but unpolarized cells of whatever type moved only very slowly and on average did not achieve speeds greater than 5 μm/h. Cells with these morphologies remained, for the most part, essentially stationary and such movements as they did show were limited to minor oscillations induced by their vigorous blebbing or by protrusions of transient lamellae rather than by persistent locomotion in a given direction. Surprisingly, DiPasquale (1975) has described isolated vigorously blebbing epithelial cells moving at speeds varying between 30 and 240 μm/h. However,
we found that only cells with a well-spread polarized morphology showed significant locomotion. CE and epidermal cells with this morphology moved significantly faster than when displaying any other morphology (Table 4). PRE cells with this morphology were not seen and this correlated with the absence of actively moving cells from these cultures. On average, well-spread polarized cells moved between three and four times faster than their counterparts with an alternative morphology (Table 3).

A strict comparison between the speeds of locomotion of isolated cells observed here and those previously recorded for other types of isolated cells is difficult, since the way in which the position of the cells is defined and the time intervals over which cell movements are measured vary in different investigations. Nevertheless, the speeds attained by polarized CE and epidermal cells (approximately 10 μm/h and 15 μm/h, respectively) were substantially lower than those reported for some other isolated cells. The average speed of locomotion of embryonic chick heart fibroblasts has been variously recorded as approximately 82 μm/h (Abercrombie & Heaysman, 1953) and 36 μm/h (Abercrombie et al., 1970a) with a maximum speed equivalent to 250 μm/h (Abercrombie & Heaysman, 1953). Embryonic mouse muscle fibroblasts have been described moving with an average speed of about 41 μm/h (Abercrombie et al., 1970a) and isolated Xenopus epidermal cells are reported to move with an average speed of 480 μm/h (Bereiter-Hahn et al., 1981), and with a maximum speed equivalent to 1200 μm/h (Bereiter-Hahn, 1967).

We noted that, even when well-spread and polarized, both CE and epidermal cells were stationary for long periods, apparently being prevented from moving forwards by the strength of cell substratum adhesions at the rear of the cell. These stationary periods inevitably reduced the average speed of locomotion that we recorded, but cannot alone account for the low values that we obtained since even when fully motile we never observed cells moving faster than a speed equivalent to approximately 44 μm/h (Brown, 1983).

Although varying with cell type, the behaviour of isolated cells of all three types of epithelium in this investigation differed significantly from that of many types of isolated fibroblasts cultured under similar conditions. In general, the isolated epithelial cells showed less tendency to spread extensively on the substratum, less tendency to assume a polarized morphology and less effective translocation than do most fibroblasts. The significance of these differences and the extent to which they are more general requires further study.

REFERENCES
Behaviour of epithelial cells in culture


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