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THE MOVEMENT OF CELL CLUSTERS *IN VITRO*: MORPHOLOGY AND DIRECTIONALITY

JOHN KOLEGA
Department of Biology, Yale University, New Haven, CT 06511, *U.S.A.*

SUMMARY

The movement of cells in small groups, or clusters, was studied *in vitro* using epithelioid cells from Gordon–Kosswig melanomas (from poecelid fish) and time-lapse cinemicrography. Tumour explants cultured on glass yield cell sheets from which groups of cells separate and become independently motile clusters. These clusters typically contain 3–30 cells, but may have as many as 50. They propel themselves at speeds of 0·2–4·0 μm/min by means of broad hyaline lamellae. The distribution of lamellae around the perimeter of each cluster correlates with both direction and speed of cluster movement, i.e. a cluster moves with its most lamellar region at its leading edge, and the greater the extent of the leading lamellar region the greater the speed. Also, a cluster tends to keep moving in the same direction. This persistence is due to a relatively constant distribution of lamellae. Cells on the trailing edge usually lack lamellae and most are very elongate and oriented perpendicular to the direction of cluster movement. In general, whenever a cell elongates, there is a loss of lamellar activity along its taut edges, parallel to the axis of elongation. Thus, any region with less lamellar activity would tend to be elongated by the outward pull of the more active regions to either side and would, in consequence, suffer a further reduction in lamellar activity. In this way, the distribution of regions of lamellar activity is self-reinforcing and the result is persistence of movement in a particular direction. This phenomenon could play an important role in giving directionality to certain morphogenetic movements, such as neural crest cell migration.

INTRODUCTION

Much attention has been devoted to the movement of individual cells and the spreading of cell sheets, both *in vivo* and *in vitro*. However, little attention has been given to the intermediate form of behaviour – the movement of cells in small groups, or clusters. But, except for the work of DeHaan (1963), who observed cluster movement in the formation of chick heart primordia, and that of Trinkaus (1980), who recently observed clusters of pigment cells moving on the yolk sac of a teleost embryo, there has been no direct observation of cluster movement *in vivo*. However, circumstantial evidence for such movement abounds. For example, isolated aggregates of cells are found in fixed sections of: (1) invasive tumours (Willis, 1960; Pierce, Shikes & Fink, 1978); (2) the neural crest after its dispersive migration has begun (Weston, 1963); (3) mesenchymal cells of chick somites as they extend to form the sclerotome (Lillie, 1908); (4) chick mesoblasts as they move laterally from the primitive streak during gastrulation (Lillie, 1908); and (5) primordial germ cells during their invasion of the coelomic epithelium of the future gonadal region in chick (Meyer, 1964) and mouse (Mintz, 1957). But, in fixed sections it is impossible to tell whether these

aggregates are in fact motile clusters, or merely individually migrating cells in transient contact. In the formation of chick somite centres and progression of the chordabulb, the evidence is somewhat stronger. Spratt (1957) found that small bunches of chordabulb and somite centre cells marked with carbon or carmine particles could be found intact later on in morphogenesis, even after overtaking and passing regions marked with the other particle type. But the possibility that the marked groups of cells are somehow passing through each other as individual cells (like 2 swarms of bees) has not been excluded. In view of these various observations, it is not difficult to believe that the movement of cell clusters is reasonably commonplace during morphogenesis, and that the paucity of clear-cut demonstrations of cluster movement is due not so much to its absence as to the difficulty in observing it.

Cluster movement can be observed readily *in vitro*, however, and this fact has been exploited in the present study with a twofold purpose. First, it is hoped that, despite the stark artificiality of the *in vitro* system, the behaviour observed there will shed some light on how cells can move as groups *in vivo*. Secondly, and perhaps more importantly, clusters are a simple system in which to observe the social behaviour of potentially motile cells when their motility is constrained by attachment to other cells. How physically linked cells affect each other's movement, how directional mass motion arises from cells individually capable of motion in any direction and how the locomotively active regions of a group are determined are questions of general import to our understanding of the mechanisms of cell movement, during both morphogenesis and the spread of cancer. This study begins to address these questions in the context of cluster movement by reporting some of the characteristics and consequences of this cellular arrangement with respect to locomotor activity.

MATERIALS AND METHODS

Cells

Cells for this study were obtained from genetically induced tumours in poecelid fishes. In the Gordon–Kosswig melanoma system (Gordon, 1948; Kosswig, 1964), hybrids between *Xiphophorus maculatus* carrying the Sd^1 trait and wild-type *X. helleri* develop an invasive tumour that forms a protrusion on the side of the dorsal fin. Such fish were generously supplied by Dr Klaus Kallman of the New York Aquarium. When the hybrid fish are backcrossed to wild-type *X. helleri* the progeny also develop dorsal melanomas. For these experiments fish from the first or second backcross generation were used. The fish were anaesthetized in a solution of ethylaminobenzoate methylsulphonate (1:3000 in filtered well-water), and a lump of tissue 1–2 mm on a side was cut from the tumour. The tissue was immediately transferred to Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin and 0·25 μg/ml Fungizone. It was rinsed with 3 changes of this medium in order to reduce contamination and then plated in a 'coverglass sandwich' according to the technique of Chen *et al.* (1978). The cultures were maintained in a moist atmosphere at room temperature (22 °C). The medium was changed daily by drawing off most of the old medium with a small piece of sterile filter paper touched to the edge of the sandwich while placing a drop or two of fresh medium at the opposite edge of the coverglass. For cinemicrography, the 2 coverglasses of the sandwich were separated and mounted individually on filming chambers of the type described by Radice (1980).

Optics

Unless otherwise indicated, photomicrographs and cine films were made under phase-contrast optics. Some clusters were observed with interference reflexion microscopy, as described by Izzard & Lochner (1976).

Cinemicrography

Time-lapse films were made with a Bolex 16 mm camera and a Sage intervalometer. The interval between successive frames was 4 s with an exposure time of 0·5 s. The film (Kodak Plus-X reversal, type 7276) was processed commercially and projected for analysis on a Vanguard Motion Analyzer (Vanguard Instrument Co., Melville, N.Y.).

Film analysis

In order to examine quantitatively the role of lamellar protrusions of the cell surface in cluster movement, a method was devised for expressing the distribution of lamellae on a cluster as a vector, **L**. **L** was assigned by projecting the image of a cluster on a grid of lines oriented perpendicular and parallel to the film frame and, therefore, randomly oriented with respect to the movement of any given cluster. The spacing between grid lines was such as to give a minimum of 15–20 lines crossing the cluster image in the horizontal direction and an equal number transecting it vertically. The horizontal component of **L**, L_x, was then determined by scoring every intersection between a horizontal grid line and the outline of the cluster for the presence of a lamella. If a lamella was present with its cluster side (as opposed to the free space beyond its edge) lying to its left, it was scored as $+1$; if the cluster side was to the right, the intersection was scored as -1; and if no lamella was present, the score was 0. These scores were summed and then multiplied by the distance between the grid lines to give L_x in units that are corrected for the magnification of the particular cluster image. Similarly, the vertical component of **L**, L_y, was determined by scoring vertical grid lines for lamellae with their cluster side below $(+1)$ or above (-1) the points of intersection.

A region of a cluster was identified as being part of a lamella if (1) the cytoplasm was clear and agranular and thereby distinct from the main body of the cell, and (2) the region had a convex outline with no sharp discontinuities. The latter criterion distinguished between spread lamellae and lamellae that were undergoing detachment from the substratum and retraction into the cell body. The film footage preceding and following the cluster image under examination was viewed frequently to check for spreading and ruffling activity of the cluster margin and thereby confirm the still-image identification of lamellae.

The cluster velocity, **V**, was determined as follows. The direction of **V** was defined by a line drawn between the centre of the cluster at the time of interest and the centre of the cluster 10 min later. The rightward direction was taken as the positive x axis and the upward direction as the positive y axis so that the direction of **V** could be directly compared with the direction of **L**. The cluster's speed was defined by the straight-line distance between the centre of the cluster before and after the same 10-min interval. The centre of the cluster was taken as the centre of a rectangle whose sides were constrained to lie parallel to the frames of the film and whose dimensions were just large enough to contain the outline of the cluster.

RESULTS

Formation of clusters

Culture of Gordon–Kosswig melanomas on glass yields a large population of non-pigmented epithelioid cells. These cells initially migrate from the explant as a confluent monolayer. But, because different regions of the monolayer advance at slightly different rates and in slightly different directions, within 24 h the sheet begins to pull apart. Tongues of cells extend radially from the edge of the sheet,

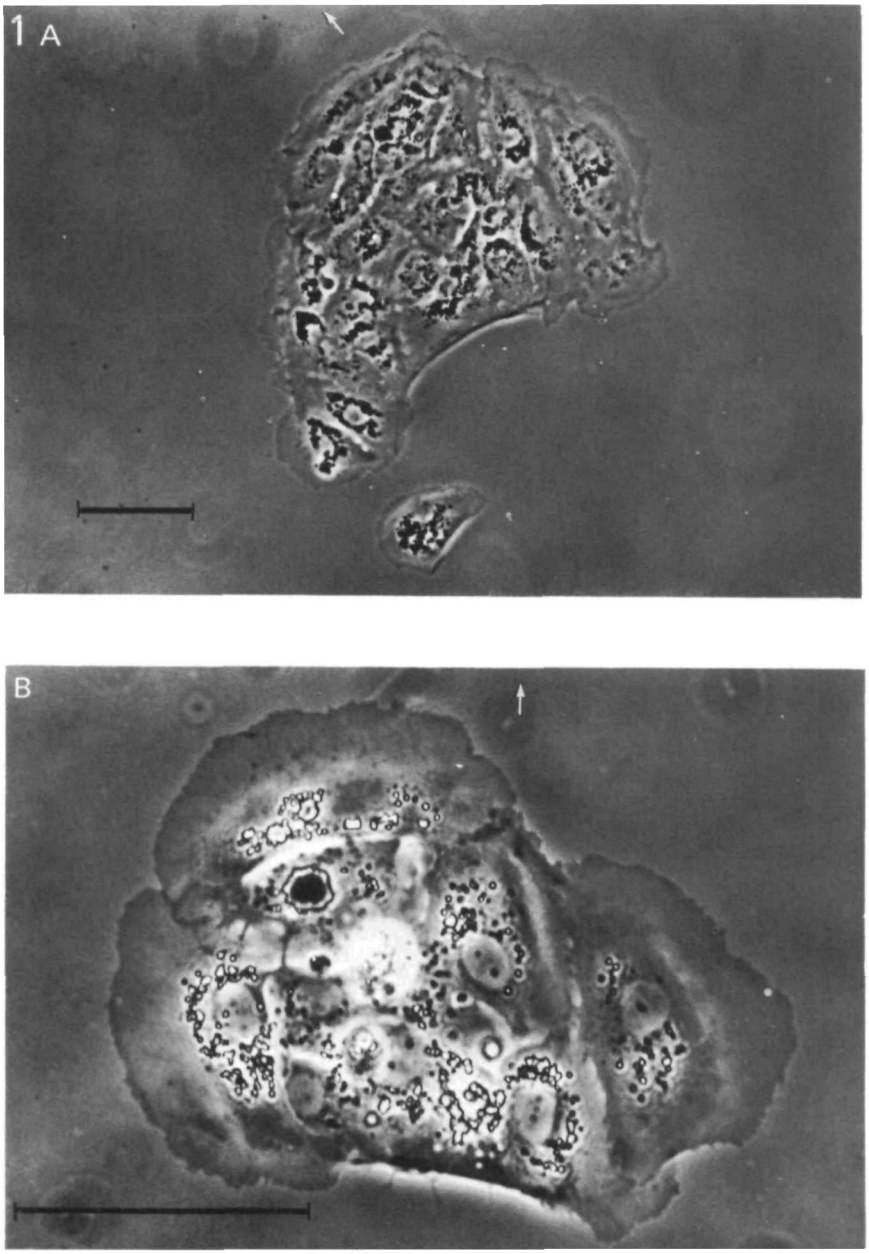


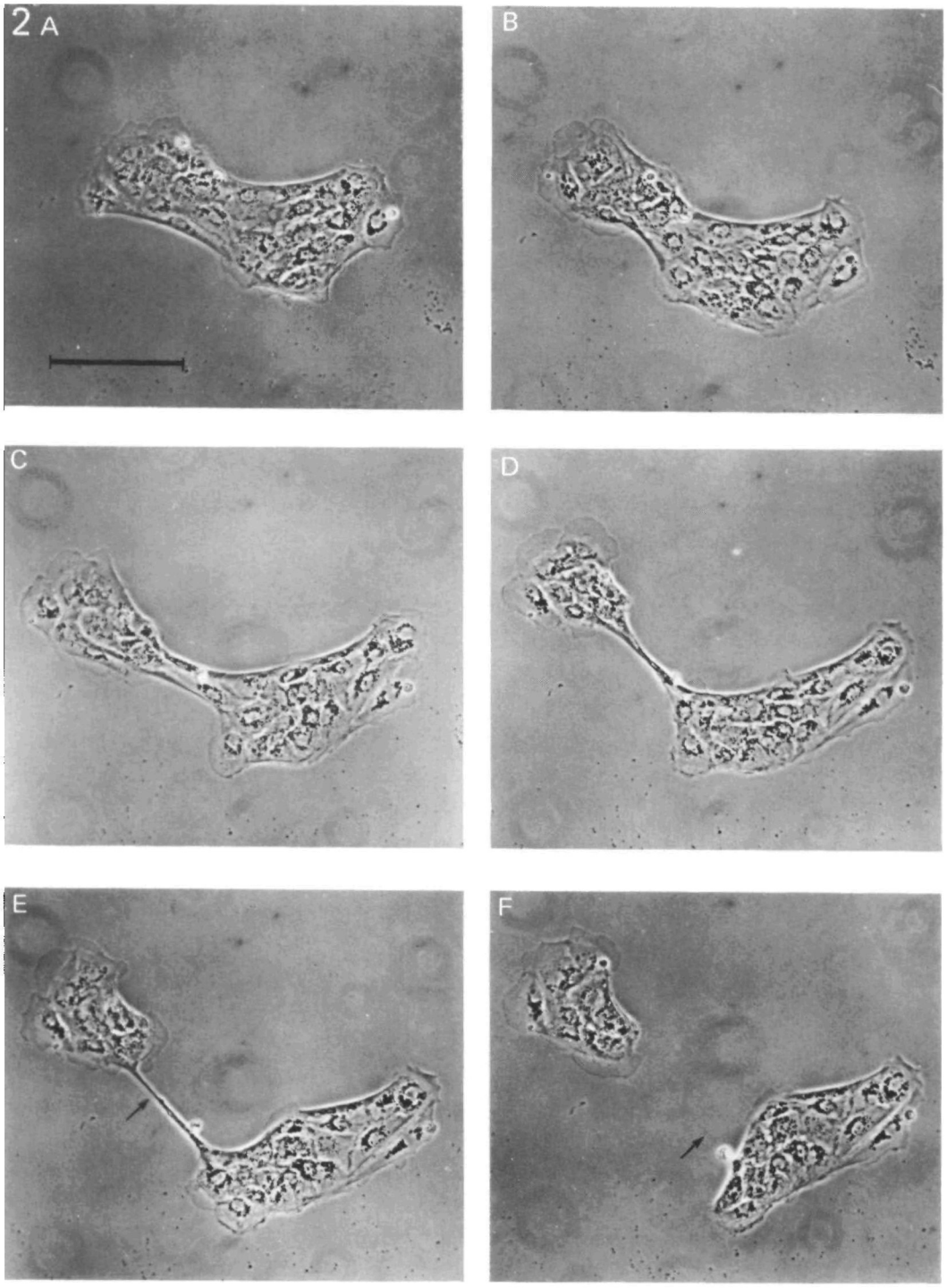
Fig. 1. Two typical clusters (A and B). In A, the cluster contains approximately 20 cells. An isolated single cell, moving from right to left, is present directly below the cluster. The cluster in B contains 7 cells. In both cases, an arrow indicates the direction of movement of the cluster. Observe the large and numerous lamellae around most of the perimeter of each cluster as well as the conspicuously alamellar region at the trailing edge. The alamellar regions have smooth, catenary contours, suggesting that the cluster is a tense sheet spread between lamellar attachments. Also note that the clusters are slightly longer along an axis perpendicular to the direction of movement. Interestingly, there is a striking similarity between the single cell in A and the whole clusters both in shape and in location of lamellar regions. Bars, 50 μm.

separate from it in a manner identical to the fission of an isolated cluster (see below), and become independently motile groups of cells. These groups, or clusters, like the sheet whence they came, are monolayers of closely apposed, predominantly polygonal cells. There may be as few as 3 or as many as 50 cells in a cluster, arranged in no distinguishable pattern (Fig. 1). There is usually a slight elongation of the cluster along an axis perpendicular to the direction of movement, but there are generally no gross asymmetries in the cluster geometry. The one exception to this occurs when a cluster is splitting into 2 smaller clusters: just as sheets of cells will fragment into clusters, clusters of cells can fragment into smaller clusters and even single cells. During this process, 2 groups of cells of the cluster move in different directions. These regions essentially retain the normal cluster morphology, but the cells in between become extremely elongate, until rupture either of the elongate cells or their intercellular attachments occurs (Fig. 2).

Clusters are not always coming apart. An individual cluster can maintain its integrity for hours at a time, and intact clusters can be found after 3–4 days of culture. Since the average cluster speed is of the order of 2 μm/min (30 observations), there is considerable *en masse* movement of clusters between fissions.

Movement of clusters

One of the outstanding features of the cell clusters in this system is the abundance of lamellae around the cluster perimeter. Because the lamella, or, more precisely, the lamellipodium at its edge (Abercrombie, Heaysman & Pegrum, 1970), is considered the locomotive organelle of primary importance in a variety of cell movements on plane substrata (fibroblast and leukocyte locomotion, epithelial spreading, epidermal wound closure), it seemed appropriate to ask if the distribution of lamellae on a cluster is in any way related to the cluster's locomotive behaviour. To do this, the distribution of lamellae was converted to a vector, **L**, as described in Materials and methods. **L** is a measure of how much of the cluster's edge exists as lamellae unbalanced by lamellae protruding in the opposite direction. The direction of **L** is that in which the 'net lamella' faces. When this direction is compared with the direction of cluster movement (Fig. 3), a strong tendency towards coincidence is found: the mean difference between the directions is $-8{\cdot}8°$, which is not significantly different from 0° ($R = 12{\cdot}7$ with $R_{\text{critical}} = 14{\cdot}5$ at 99% confidence levels; test of Stephens, 1962). In other words, lamellae are so distributed around a cluster's perimeter that their net active frontage points in the direction of movement. Furthermore, the magnitude of **L**, $|\mathbf{L}|$, shows a positive correlation with cluster speed (Fig. 4). However it is also clear that this is not a strict one-to-one relationship: a large lamellar imbalance can sometimes be found on a slow-moving cluster, and a speedy cluster may, on occasion, have a relatively small $|\mathbf{L}|$. This may be due, in part, to the way in which $|\mathbf{L}|$ and speed are measured. $|\mathbf{L}|$ can be determined from a single image of a cluster and, therefore, is essentially an instantaneous measurement. Speed, on the other hand, must be determined over a time-interval long enough to allow accurate measurement of both the interval and the cluster's displacement. Consequently, it is really an average of the speed during that interval. This average may not accurately reflect the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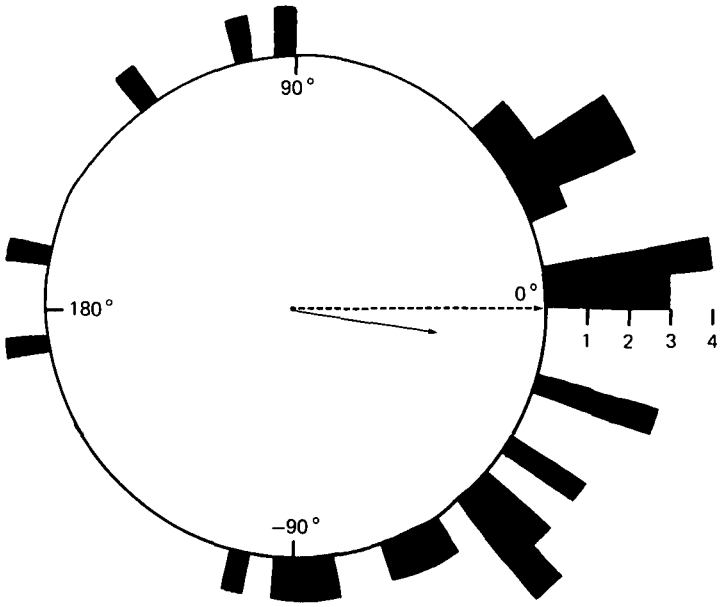


Fig. 3. Comparison of the distribution of lamellae with direction of cluster movement. **L**, the vector representing the lamellar distribution (see Materials and methods), was determined for 6 different clusters at a total of 38 different time points. The actual direction of cluster movement was determined at the same time, and the angular difference between the 2 vectors was measured in each case. The histogram represents the circular distribution of these angular differences. Broken arrow, vector representation of the mean angular difference in the ideal case, where the directions of **L** and movement coincide exactly (0°, r (radius) = 1·0). Solid arrow, actual mean angular difference (−8·8°, r = 0·6).

Fig. 2. Cluster fission. A cluster of 20–25 cells was photographed at intervals as it split into 2 smaller clusters. (A) The cluster begins to split. The distribution of lamellae is distinctly bipolar. As in ordinary clusters, the alamellar regions have smooth, catenary contours. (B) Same cluster, 5 min after (A); the cluster is slightly more elongate than in (A); the elongation is due primarily to the movement of the highly lamellar cells at the upper left (note their displacement relative to the immobile debris on the coverslip). (C) 5 min after (B); the elongation of the cluster is now very distinct. The cluster is polarized into 2 segments moving in opposite directions and connected only by 2 or 3 cells. (D) 5 min after (C); the connection between the 2 segments has been reduced to a narrow strand much like the extended tail of a chick heart fibroblast. (E) 5 min after (D); the connection between the 2 daughter clusters is just about to break. An arrow marks the point of rupture, which occurs within 20 s after the photomicrograph was taken. (F) 2 min after (E); the ruptured connection retracts rapidly into the cluster. There is already no trace of an extension from the cluster at the upper left, and only the very end of the retracting strand is visible on the other cluster (arrow). Bar, 100 μm.

instantaneous speed at the moment |**L**| is determined. This could severely reduce the correlation between |**L**| and speed, particularly if sharp changes occur in either variable. In fact, rapid retractions of lamellae do occur quite frequently along the trailing edges of clusters, causing sudden increases in the lamellar imbalance.

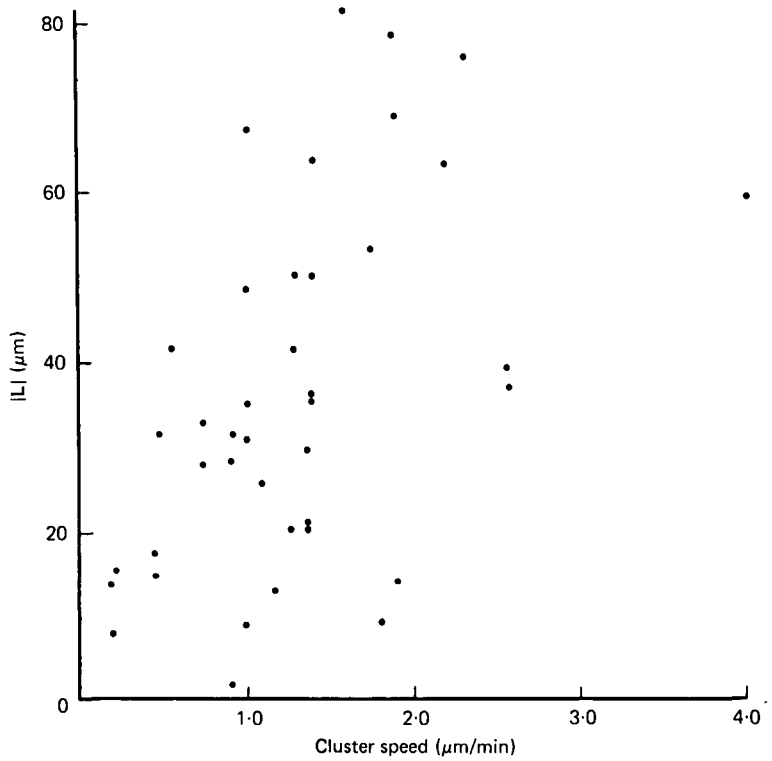


Fig. 4. Comparison of the magnitude of **L** with cluster speed. For 6 different clusters at a total of 40 different time points, the magnitude of **L** and the speed of movement were determined as described in Materials and methods. There is a definite positive correlation between the 2 variables ($r = 0{\cdot}5$ with 95 % confidence limits of $0{\cdot}2 \leqslant r \leqslant 0{\cdot}75$). The fact that the correlation is positive even at the extreme limits of confidence indicates that increased lamellar imbalance is somehow related to faster cluster movement.

Another possible explanation for the inconsistent correlation between speed and |**L**| is that other factors are involved in determining the rate of movement. For example, speed may be influenced by the degree of adhesion to the substratum (Couchman & Rees, 1979; Shure, Young, Kolega & Chen, 1979; Keller, Barandun, Kistler & Ploem, 1979), the distribution of points of adhesion, the rate of spreading at the leading edge, and so on. There are also more subtle variations in lamellar distribution, such as differences in shape and width of lamellae, that are not taken into account when calculating |**L**|.

Cell–cell interactions

If the presence of lamellae is indeed evidence of locomotive force, then unbalanced lamellae on one side of a cluster will necessarily pull it along in that direction. But with lamellae located around most of the cluster perimeter, as is generally observed, there should be a considerable amount of tension within the cluster due to lamellae pulling on it in several different directions. There are a number of observations that

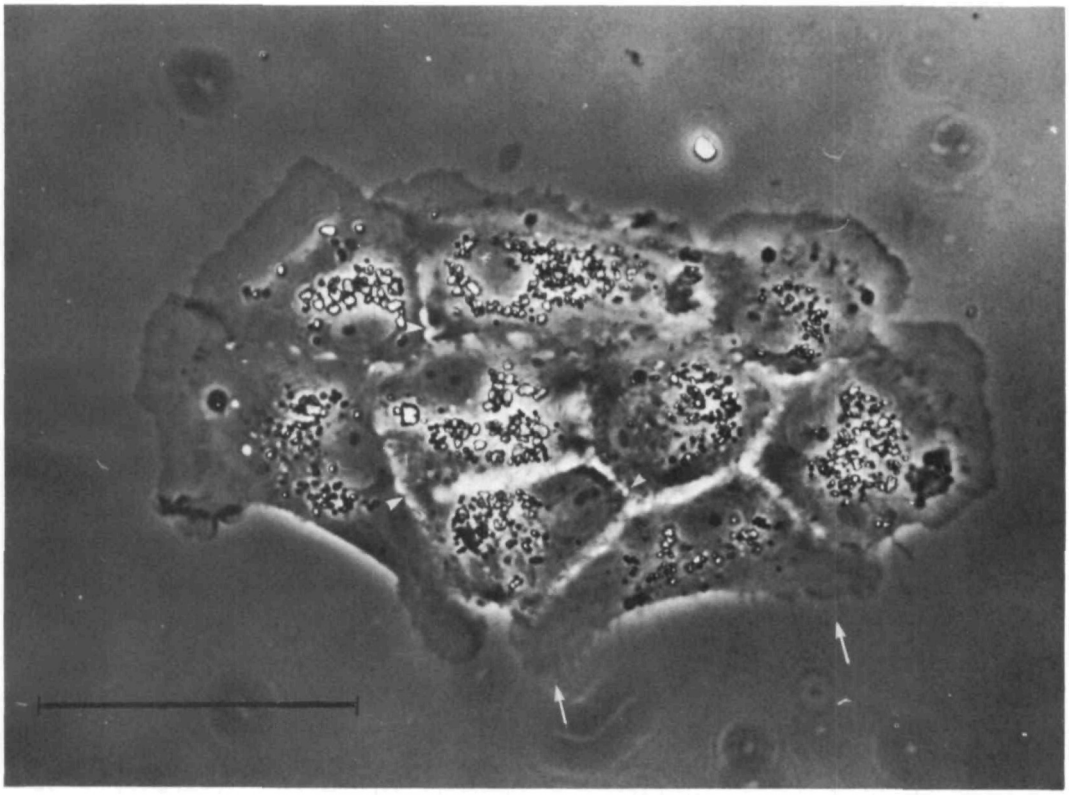
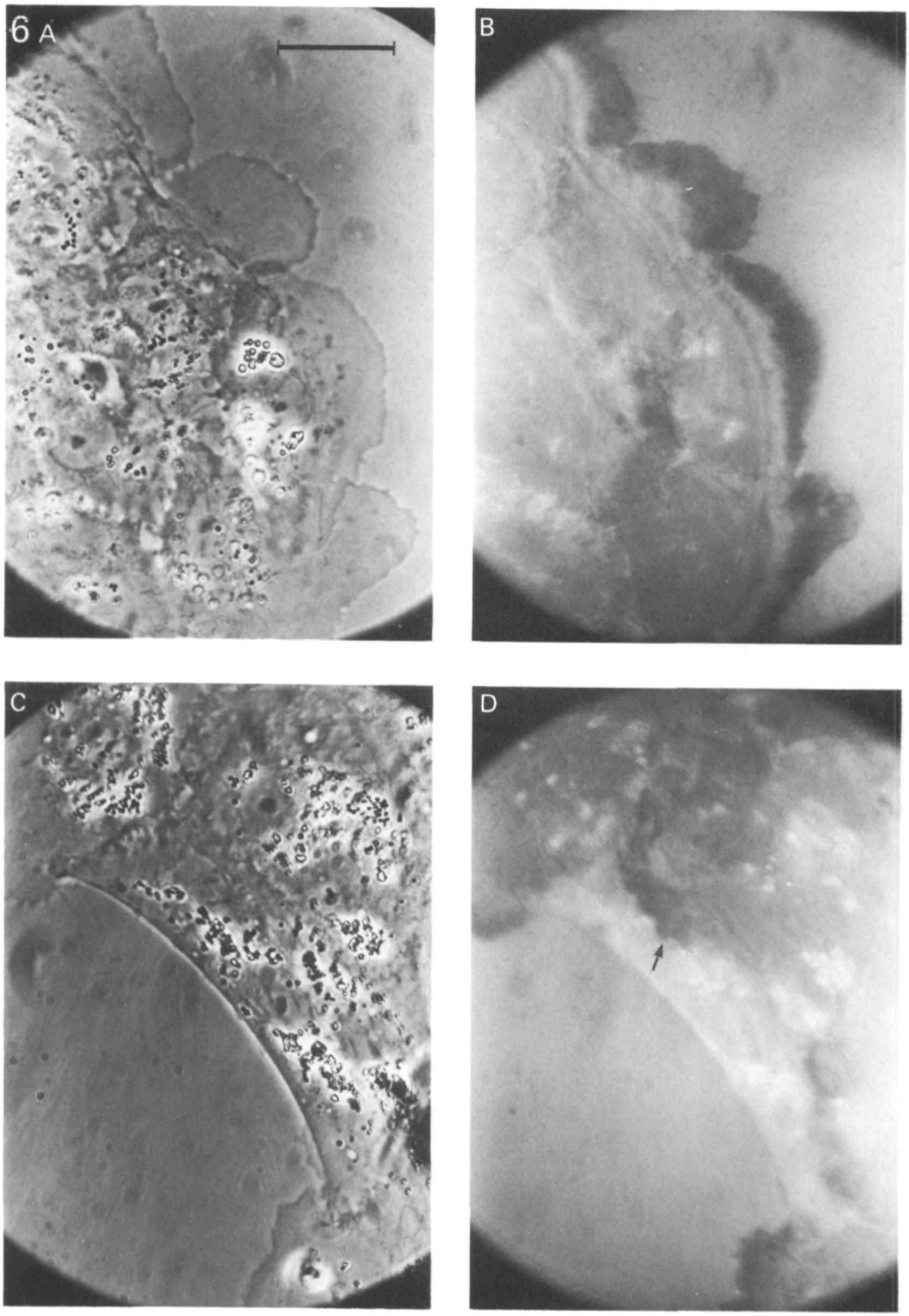


Fig. 5. Photomicrograph showing the apparent strain within the cluster sheet. Direction of motion is toward the top of the picture. There are many gaps between cells. Cell–cell contacts are generally punctate (arrowheads) with regions of separation between attachment points. Retraction fibres are visible along parts of the trailing edge recently occupied by a large lamella, of which portions still remain (arrows). The remaining alamellar region has the smooth catenary contour characteristic of the loose non-adherent edge of a sheet under tension (see Fig. 6). Bar, 50 μm.

suggest that this is, in fact, the case. First, cell–cell contacts are frequently punctate, with a wide separation of the regions between the attachment points, as if the cells involved were being pulled apart (Fig. 5). Second, when cell–cell attachments break, the cells retract sharply from the point of rupture (Fig. 2). Likewise, the loss of lamellae from a region of the cluster's edge is accompanied by a sharp retraction away from that region and towards other, still active lamellae. This is presumably due to the loss of cell–substratum adhesions. In both cases, the recoil of cells from the broken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attachment (cell–cell or cell–substratum) is not restricted to one or two cells, but involves large regions of the cluster, only excepting those cells that are firmly anchored to the substratum by their lamellae. That lamellae serve as cluster–substratum attachments is confirmed by interference–reflexion microscopy. With interference–reflexion optics it is possible to visualize regions of close contact between the cell and the substratum that presumably coincide with cell–substratum adhesions (Curtis, 1964; Izzard & Lochner, 1976, 1980). In these clusters, lamellae invariably coincide with areas of close contact (Fig. 6), in contrast to alamellar regions. Finally, alamellar regions of a cluster have catenary contours (Figs. 1, 2, 5), as if an entire cluster were a rubber sheet stretched out and then pinned down at its lamellae. Where no lamellae are maintaining tension on the sheet, it tends to retract centripetally.

Yet, despite this tension – this appearance of force being exerted in several directions at once – clusters do not jerk randomly about or shuffle back and forth in their paths. Rather, they move with considerable persistence. That is, a cluster tends to continue moving in the direction in which it is already headed. This is visually evident in the polarity of the paths of cluster movement (Fig. 7). The forward progress of each cluster is much greater than its lateral or retrograde wanderings. Furthermore, most, if not all, of the lateral or retrograde deviations can be attributed to transient changes in the cluster shape (e.g. the extension of an unsuccessful lamella), which change the calculated centre of the cluster (see Materials and methods) without necessarily representing a translocation of the group. Thus, for periods of at least as long as 80–90 min, the paths appear to approximate straight lines. This impression can be given statistical tangibility by the method of Gail & Boone (1970). In their analysis, the angle between the direction of movement at consecutive time points is measured. If motion is random, this angle will vary randomly over the entire range from $+180°$ to $-180°$; but if motion is persistent, the angle will be $0°$. In the paths of 4 different clusters using time-intervals of 30 min, the interstitial angles were highly non-random ($Z = 6{\cdot}16$ with $Z_{\text{critical}} = 4{\cdot}2$ at 99% confidence levels) and, according to the test of Stephens (1962), fell within a circular normal distribution whose mean was not significantly different from $0°$ ($R = 6{\cdot}57$ with $R_{\text{critical}} = 6{\cdot}8$ at 99% confidence levels).

According to the results presented in previous sections, if a cluster is to move with persistence, the direction of **L** must be relatively constant for long periods. This is accomplished simply by maintaining lamellae in the same location around the cluster.

Fig. 6. Coincidence of lamellae and cell-substratum contacts. (A) Phase contrast image of a portion of the leading edge of a cluster. (B) Interference–reflexion image of the same region as in (A). Lamellar regions are consistently darker than the rest of the cluster, indicating that cell–substratum separation is much smaller under the lamellae. These regions of close contact between cell and substratum presumably correspond to cell–substratum adhesions. (C) Phase contrast image of a portion of the cluster's trailing edge. (D) Interference–reflexion image of the same region as in (C). Again, the darkest areas are associated with lamellae. Note in particular the very wide separation (light grey to white) under the long alamellar portion of the edge. The dark region under the interior of the cluster (arrow) may be a lamella extended by a submarginal cell into the gap under the tautly suspended marginal cell. Bar, 20 μm.

Specifically, cells in the leading rank always have lamellae along their entire free edges, cells on the lateral margins almost always have lamellae, and the trailing cells are largely alamellar. There is very little fluctuation in the lamellar distribution along the leading and lateral margins. But along the trailing edge, lamellae appear and disappear quite frequently, particularly near the lateral margins. It is these lateral transient appearances of lamellae that account for the vast majority of changes in lamellar distribution of a cluster. Note that, since these trailing lamellae face directly backward and, therefore, directly along the axis of motion, their appearance and disappearance affect only the magnitude of **L**, not its direction.

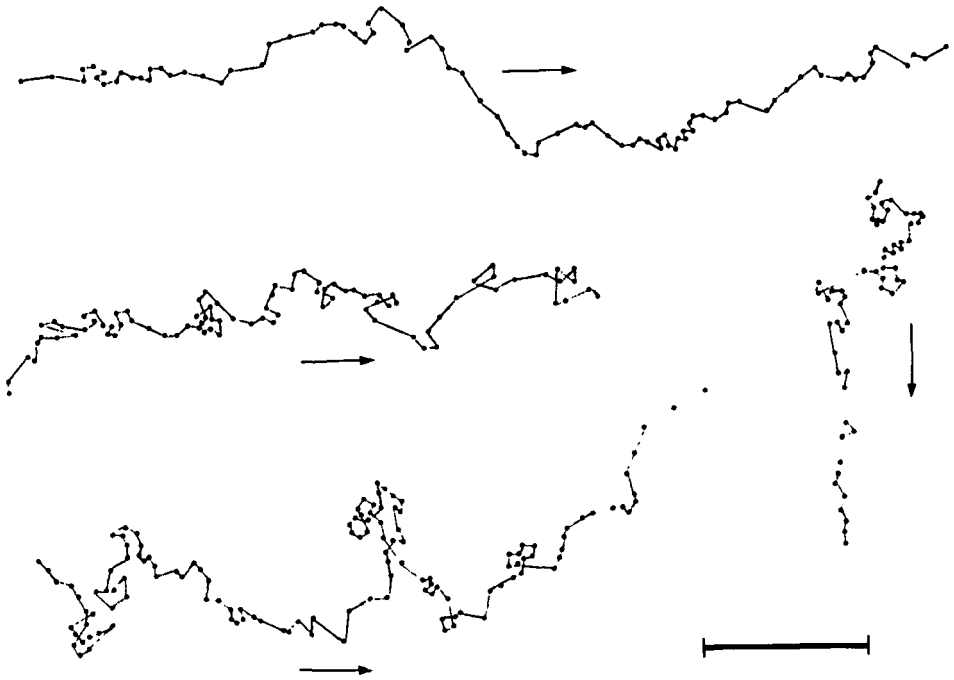

Fig. 7. The paths of 4 typical clusters. The points mark the location of the centre of the cluster (see Materials and methods) at 1 min intervals. Note that the net displacement of each cluster in the direction of the arrows is much larger than the lateral and retrograde motions. Most of the small deviations in the path may be attributed to changes in cluster shape (e.g. the transient extension of an unsuccessful lamella), which cause slight shifts in the calculated cluster centre. Bar, 50 μm.

Why does the trailing edge produce fewer effective lamellae? The shape of the cells on the trailing edge may be an important factor. These cells are not stretched out in the direction of movement as one might expect if they were simply being dragged along. Rather, their long axes tend to lie at right angles to the direction of movement (Fig. 8). Thus, the long side of the cell is a free edge, and yet it has no lamellae. Looking at other cells on a cluster's perimeter, one finds that this unusual absence of lamellae from a free edge is actually characteristic of the long side of elongate cells (Fig. 9). That is, highly elongate cells rarely have lamellae on those edges lying parallel to the cell's long axis. Furthermore, when individual cells are followed

through the film record, it is found that many of the elongate, alamellar cells were at one time shorter and had broad lamellae on their free edges. The lamellae disappeared only as the cells became more extended in form, and the loss of lamellae was usually a rapid, all-at-once phenomenon, as if the cell reaches some critical degree of extension beyond which it can no longer maintain lamellae on its lateral margins. In the rare instances where a highly elongate cell does have lamellar protrusions on its long side, it is always the case that the cell is in the *process* of elongation, having been caught just before the lamellae disappear. It should be noted that lamellae are very stable on cells that are not highly extended, i.e. they were never observed to disappear suddenly, except from elongating cells.

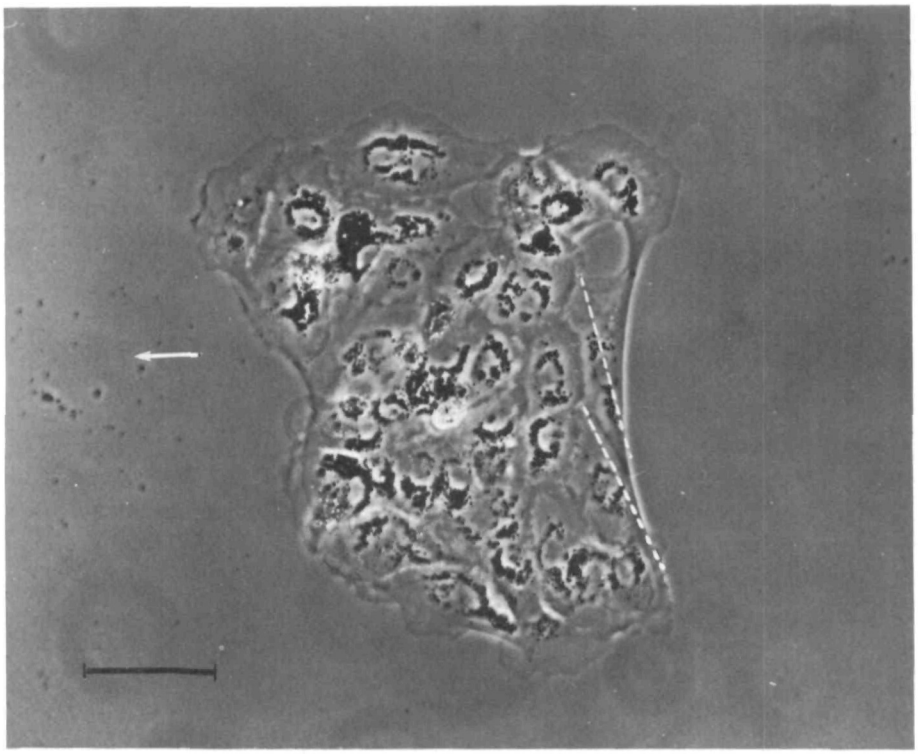

Fig. 8. Elongation of the trailing edge. Arrow points in the direction of motion of the cluster. Broken lines mark the long axes of 2 cells on the trailing edge. These 2 cells are elongate in an orientation nearly perpendicular to the direction of movement. They also lack lamellae, despite the fact that in both cases the long side of the cell has a free edge. Bar, 50 μm.

There are, nonetheless, some cells that lack lamellae along their long edges, but are not highly elongate. These can be placed in one of 3 categories. (1) Cells that were previously very oblong, but, due to changes in cluster shape, now have a less asymmetric geometry. Within the next 10 min of observation, these cells either produce new lamellae or are stretched out again. (2) Cells that have just retracted from a more extended form, following breakage of cell–cell or cell–substratum adhesions. Again,

these cells soon produce new lamellae or are extended again. (3) Cells in which only a very small region of the cell has access to the free space at the cluster's edge. Such regions often fail to produce a lamella even if the body of the cell is not noticeably extended. Perhaps this failure arises from spatial limitations, e.g. because so little of the cellular machinery has access to the free edge. Alternatively, this particular part of the cell may be extended relative to its normal shape without the rest of the cell being distorted. This possibility is suggested by the very large lamellae found flanking such regions, and the very taut contours of the regions themselves.

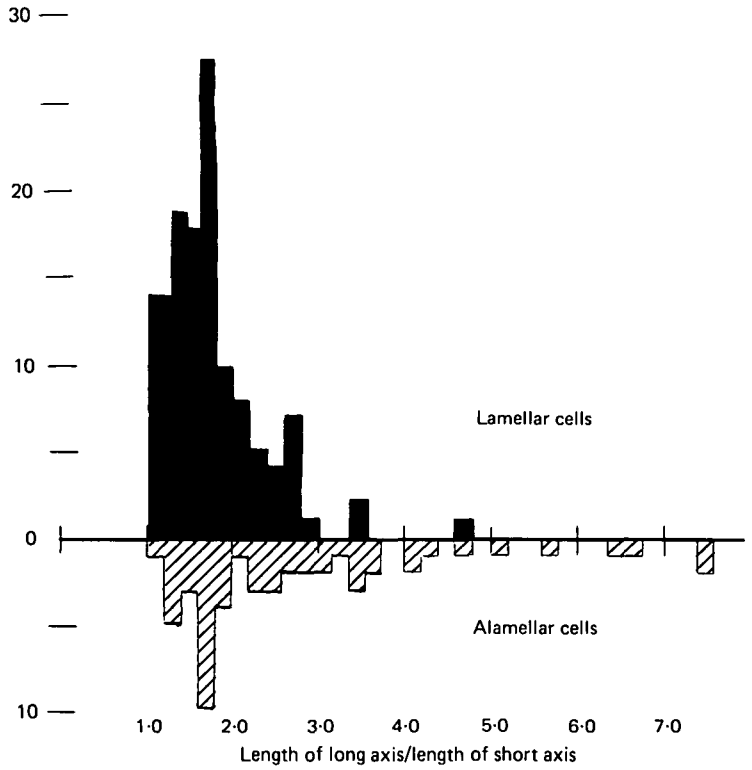

Fig. 9. The presence or absence of lamellae as a function of cell shape. For 7 different clusters, all cells located on the perimeter were assigned a long and short axis. The long axis was defined by a straight line drawn between the 2 most widely separated points within the cell body, exclusive of lamellae. The short axis was defined by a straight line drawn perpendicular to the long axis at the region of the cell having the greatest width (again exclusive of lamellae) in this direction. Each cell was then classified according to its degree of elongation (the ratio of the lengths of the long and short axes) and whether or not there was a lamella present anywhere along the edge lying parallel (+ or $-45°$) to the long axis. For each long:short axis ratio, the number of cells having such lamellae is represented by a solid bar, and the number of alamellar cells is represented by a shaded bar.

The vast majority of lamellar cells have low long:short axis ratios; almost all of the highly elongate cells lack lamellae.

DISCUSSION

Non-pigmented epithelioid cells from Gordon–Kosswig melanomas translocate readily *in vitro* as small groups of cells. In terms of the fundamental locomotor machinery, there is no evidence that this movement involves any novel devices. The motive force appears to be provided by the same broad hyaline lamellae found on the leading edges of migrating epithelia both *in vitro* (e.g. see Harrison, 1910; Matsumoto, 1918; Vaughan & Trinkaus, 1966; Radice, 1980*a*) and *in situ* (Trinkaus & Lentz, 1967; Bellairs, Boyde & Heaysman, 1969; Radice, 1980*b*) and on fibroblasts moving on planar substrata (e.g. see Goodrich, 1924; Abercrombie & Ambrose, 1958). What makes cluster movement unusual in this system is that collective movement must arise from cells oriented so that they should move in opposing directions. Consider that, under most circumstances, cluster cells will form active lamellae when given a free edge: as single cells, they invariably display enormous leading lamellae and migrate very rapidly (often exceeding speeds of 10 μm/min); in sheets, such as those that grow out from the tumour explants, the advancing margins are occupied entirely by lamellae; even the locomotively inactive cells lying in the internal regions of a sheet or cluster quickly produce lamellae when given a free edge, as cells change positions or split off from the cluster. In short, these cells always try to move when given a space into which to go. But a cluster has empty space on all sides, so one would expect locomotor activity in all directions. In fact, lamellae are present around most of the cluster perimeter. Also, cells within the cluster appear to be under considerable tension, suggesting that the peripheral, lamellar cells are indeed attempting to migrate outward, but are restrained by their attachments to other cells.

How then can there be translocation of the whole cluster? It occurs only because there are never lamellae around the entire perimeter. Clusters move with great fidelity in the direction in which any imbalance of lamellae exists, with the most lamellar region leading, and the speed of movement correlates with the extent of that imbalance. This behaviour is explained simply, if each lamella, or, more precisely, the intracellular locomotory machinery using the lamella as an attachment to the substratum, pulls in whichever direction the lamella is extended, and the cluster is displaced towards the strongest pull; i.e. towards the side having the largest lamellar edge.

However, as noted by Albrecht-Buehler (1979), studying clusters of PTK-1 cells moving *in vitro*, cluster movement is more complex than a simple tug-of-war among cells. He found cluster speed to be independent of the number of cells in a cluster, indicating that cluster motion is not the summation of random cell motion within the cluster. Nor could the motion be the net result of randomly distributed locomotor activity on the cluster perimeter. In the system I have studied, the ability of more than 20 cells to move as a unit at speeds as high as 4 μm/min, and to do so with persistence, again indicates that some locomotor interaction is necessary among the individual cells of a cluster.

The observation that highly elongate cells fail to maintain lamellae along their long axes suggests a relatively simple mechanism by which cluster cells might 'coordinate'

their locomotor activity. In a cluster, most, if not all, of the cluster–substratum adhesions are associated with the lamellar regions of the cluster's edges. If some portion of the perimeter lacks lamellae because it has been pulled off the substratum by the collective forces exerted by other lamellae, or because it never had any lamellae to begin with (e.g. the new edge that is produced when a cluster splits in two), then it will lack attachments to the substratum (Fig. 6). This region, having no anchorage at which to resist the tensile forces within the cluster, will be stretched out by the cells on either side. It also fails to exert any radial force on the cluster, allowing the cluster to translocate in the opposite direction (i.e. with the unattached side becoming the trailing edge). This would account for the slight elongation of clusters perpendicular to the direction of movement, and would also explain the shape and orientation of cells on the trailing edge. In addition, because the elongated cells are apparently less capable of forming new lamellar attachments, they cannot regain their less oblong form unless given some slack by the other marginal cells, which continue to pull radially on the cluster. Thus, any alamellar region would tend to remain as such. This would lead to the preservation of the lamellar distribution around the cluster and, therefore, to a constant direction of movement, i.e. persistence. It is interesting to note that the absence of lamellae from elongate surfaces also occurs in isolated single cells (note the single cell in Fig. 1 A). Also, the elongate tail of an individual fibroblast has no lamellae on its taut surfaces, nor does the rear margin of a *Fundulus* 'fan cell', which moves at right angles to its elongate axis (Goodrich, 1924). Not surprisingly, both of these cell types exhibit highly persistent motion.

In vivo, cluster movement could exist as a mechanism for changing the location of an entire population of cells without disturbing the spatial relationships among other cells. In contrast to the mass movement of individual cells, it might serve to minimize the loss of cells from the initial population and the possibility of cells wandering off to develop in inappropriate places. Alternatively, clusters may simply be intermediates in dispersive migrations such as that of neural crest cells. Here the advantage of cluster movement is its strong polarity (persistence), which may be important in getting cells rapidly away from the origin of migration.

The last proposition must be viewed with the cautionary note that it is not yet possible to translate directly the observations made in 2-dimensional systems into 3 dimensions. Because there is only a single interface between cell and substratum, a monolayer of cells moving on a glass plane has a relatively limited number of sites for functional locomotive activity. But in a ball of cells surrounded by extracellular matrix, the potential cell–substratum interactions are considerably more complex. Nonetheless, locomotive activity at the cluster boundaries will still create tension and shape changes within the cluster. The current study on planar clusters tells us that such mechanical events can influence local locomotive activity and thereby profoundly affect the locomotive behaviour of the cluster as a whole.

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