THE CELLULAR BASIS OF MOVEMENT OF THE MIGRATING GREX OF THE SLIME MOULD

DICTYOSTELIUM DISCOIDEUM

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SUMMARY

Previous work has failed to suggest a mechanism for grex movement, which takes account of both the movement and contact interaction of grex cells, and properties of the whole grex. Here, some observations on the movement of grex cells under different conditions are reported. From these, it seems that the production of pseudopods by grex cells depends upon mechanical factors of their environment and that movement depends on pseudopods being able to form stable adhesion with the surfaces of other cells. Intercellular adhesions are stable to forces applied at right angles to the plane of adhesion, but a force parallel to the plane of adhesion causes the cell surfaces to slide past each other. It is suggested from observations on grex placed in aqueous media and paraffin oil that the slime sheath may play an important role in controlling the polarity of grex movement by restricting pseudopod formation in all directions except forwards. These suggestions are discussed briefly in relation to previous observations on grex movement.

INTRODUCTION

A remarkable feature of the life cycle of the slime mould is the change in the nature of cellular interaction which occurs at the aggregation stage. Prior to this, the cells behave as individual organisms and their locomotory reaction to each other tends to be one of repulsion (Samuel, 1961; Shaffer, 1962). At the onset of the aggregation stage, a precipitous reversal of this situation occurs in that the cells move together and intercellular contacts develop as the cells become incorporated into the grex. The latter shows concerted locomotory reactions to such parameters of its environment as illumination, relative humidity and ionic concentration, which demonstrate its organization and individuality (see Bonner, 1966). The mechanism of grex movement is not understood but some of the observations and suggestions which have been made concerning it will now be outlined.

Bonner (1950) has shown that all the cells in the grex produce pseudopods and thus are presumably all actively moving and contributing to the movement of the grex. This is further suggested by the work of Bonner, Koontz & Patton (1953), who showed that the speed of grex movement is directly proportional to grex volume. Shaffer (1962, 1964b, 1965a) has suggested an ingenious mechanism by which the grex, and all the cells within it, could move. Extrapolating from his observations on aggregating cells, Shaffer bases his suggested mechanism for grex movement on his theory of 'contact following'. This requires that the cells move in chains, with the anterior surface of each cell in contact with the posterior surface of the one in
front. The lateral surfaces of the cells are assumed to be stationary relative to the substratum but the cells are able to move forward by manufacturing new surface in small antero-lateral regions, 'surface sources' and absorbing it again at posterior 'surface sinks'. It seems likely that contact following may play an important part in grex movement but the mechanism for contact following proposed by Shaffer must be held in some doubt in the light of the work of Garrod & Wolpert (1968). Using fluorescent antibody staining of the cell surface, they showed that the membrane of *Dictyostelium discoideum* cells should probably be regarded as a semi-permanent structure and does not turn over completely when the cell moves through its own length.

A major difficulty in considering the movement of the grex concerns the means by which all the cells move forward at the same rate. If a cell is moving over substratum and another cell moves forward at the same rate on top of it, using the upper surface of the first cell as its substratum, the speed of the second cell relative to the true substratum will be twice that of the first. Thus, the cells in the grex cannot be crawling forwards on each other's backs, for if this were so the top layer of cells would move forward very much faster than those at the bottom (Shaffer, 1962).

In addition to the cellular aspect of grex movement, we must consider the behaviour of the grex as a whole. Grex movement is strongly polarized (Bonner, 1950). Raper (1940) found that the grex tip possessed a certain organizing property with regard to movement since, if tips were grafted into the side of a migrating grex, the cells below the level of each would follow it so that the original grex could be divided into as many smaller grex as there were tips present. Also, if the tip were cut from a migrating grex, forward movement ceased, the grex rounded up and culmination ensued without further migration. Thus, it seems that there may be some property of the grex as a whole, and of the tip in particular, which is of fundamental importance with regard to the polarity of grex movement.

The grex produces, and is surrounded by, a slime sheath. The grex cells move forward inside the slime sheath which remains stationary relative to the ground and collapses when vacated by the advancing cell mass at the posterior end. Observations on the lack of movement of particles attached to the slime sheath, which provided the above information (Bonner, 1966; Shaffer, 1965b), do not indicate where the slime sheath is synthesized. It must be made at the tip of the grex but may be produced everywhere else as well.

The above observations have been selected from published observations on the grex as being the most pertinent to an understanding of grex movement. There has hitherto been no satisfactory attempt to mould these observations into a theory of grex movement. It will be the thesis of the present paper that the polarity of grex movement must depend, in part, on two things: (i) the movement, behaviour and contact interaction of grex cells; and (ii) some property of the whole grex which organizes and coordinates the movements of the individual cells. Observations have been made on the behaviour of grex and grex cells under different conditions and from these some tentative suggestions as to the mechanism and polarity of grex movement will be made.
MATERIALS AND METHODS

Grex were grown by one of two standard methods. In the first, slime mould spores and the food organism, Escherichia coli, were spread evenly on nutrient agar containing 1 g each per l. of glucose and bacterial peptone, and buffered at pH 6·7 with 0·01 M phosphate buffer. Migrating grex appeared in such cultures after about 60 h incubation at 22 °C. Alternatively, cells were grown on rich media (Bonner, 1947; Sussman, 1966), harvested by the method of Bonner (1947) and dispensed on buffered non-nutrient agar. The non-nutrient plates were incubated either at 17 or 22 °C until grex formation occurred.

Because of the thickness of the material, it is difficult to make critical microscopic examination of the cells within an intact grex. In order to try to overcome or avoid this difficulty, three different types of preparation have been used, each of which has some advantages and some obvious defects. The first two involve the partial destruction of grex organization but provide useful information on the behaviour of grex cells in relation to one another. The preparations were as follows. (i) Grex or parts of grex were placed in Bonner's saline solution (Bonner, 1947) on a glass slide and covered with a supported coverslip. For prolonged observation, the preparation was sealed with paraffin oil. This procedure almost invariably involved the total or partial destruction of the slime sheath surround the grex. (ii) Grex were placed on a thin layer of agar and a coverslip applied so that the cells were squashed into a monolayer to facilitate observation. (iii) Grex were placed in paraffin oil on a glass slide and covered with a supported coverslip. In such preparations, the slime sheath may remain intact and slight squashing of the grex against the coverslip enables detailed observation of the cells in the surface layer. Movement of the grex accompanied by a gradual deterioration of its condition continued for up to 4 h in these preparations. Observations on grex in mineral oil were first made by Potts (1902).

Observation was carried out under phase contrast using either a Zeiss Photomicroscope or a Vicker's Patholux Microscope. Still photographs were taken using Ilford F.P.3 film. For some of the observations time-lapse cinematographic records were made using a Vinten Mk. 3 Scientific Camera and Kodak Plus-X reversal or Tri-X reversal 16-mm film. Exposures were made at a rate of one, two or four per second.

OBSERVATIONS

Preparations in Bonner's solution

Grex placed in Bonner's solution under a coverslip have never been observed to move. Moreover, the only cells which can be seen to exhibit activity under these conditions are those at the edge of the cell mass in regions where the slime sheath has been broken (Fig. 4). Those cells which are unable to make contact with a glass surface produce blebs rather than pseudopods into the surrounding solution, but elongate, flattened pseudopods are produced by edge cells in contact with slide or
coverslip. No activity can be detected in the centre of the cell mass where the cells, as far as their boundaries can be discerned, are hexagonal or cuboidal and their surfaces are in contact with other cells on all sides (Fig. 5). However, slight pressure on the coverslip can cause the mass to split, whereupon pseudopodal activity of those cells which border the split and hence have surfaces free from contact begins.

Cells migrate outwards from the edge of the mass, usually singly but sometimes in small groups, so that the mass gradually decreases in diameter and becomes ragged in appearance (Fig. 6). In many instances, as a cell moves away from the mass, its posterior end and the part of the cell with which it is in contact become attenuated into fine cytoplasmic strands which spring away from each other when contact is finally broken.

It is pertinent to mention here that the observation that aggregates formed under water do not develop polarity (Gerisch, 1960) has been repeated. Such aggregates do not appear to be surrounded by a slime sheath and cells at the edge extend pseudopods into the surrounding medium.

**Agar preparations**

Squashing a grex on agar under a coverslip results in disruption of the slime sheath and forces many of the grex cells into a monolayer. At first, vigorous pseudopodal activity is apparent in the cells at the edge of the monolayer (Fig. 7) which move outwards over the surface of the agar, thus spreading the monolayer and bringing about a more complete monolayering of the grex cells (Fig. 8). As spreading proceeds, many of the cells remote from the edge become elongated towards and move towards the points of most active spreading. This polarized movement of cells merits some description.

Whilst moving, most of these elongated cells appear to be in all-round contact with their neighbours and hyaline pseudopods are produced only at their advancing ends which are in contact with the cells in front of them. Their movement appears

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![Diagram](image.png)

Fig. 1. Diagrammatic representation of the observed relative movement of cells in contact from monolayer preparations on agar. In reality, the two cells indicated would be surrounded by and in contact with other cells, but for simplicity only two cells are shown. In (A) cell x is moving past cell y, which is stationary. The lateral surface of x slides past the surface of y as x moves forward to the position shown in (B). Cell x then changes its direction of movement to one at right angles to its previous direction (C) and cell y follows it, maintaining contact with its posterior surface (D).
similar to the ‘contact following’ described for aggregating slime mould cells by Shaffer (1962).

The pattern of contacts between the cells is constantly changing. An elongated, moving cell does not necessarily advance at the same rate as its lateral neighbours. Although the lateral surfaces of two adjacent cells are in contact, these surfaces may slide relative to each other if one cell advances faster than the other. Thus, the lateral contacts between elongated cells appear to be labile. However, if a cell moving past another (that is, having a direction of movement parallel to the surface of the other cell) changes its direction of movement to one approximately normal to the surface of the other, the other will follow and maintain contact with it (Fig. 1). This suggests a possible relationship between the stability of cell contacts and the direction of the force tending to separate cell surfaces, and is similar to the observation of Shaffer (1964) on aggregating cells.

Occasionally, another mode of following of one elongate cell by another may be observed. Here, a cell moves forward, leaving a small gap between itself and the cell immediately behind it. The following cell then extends a pseudopod to fill the gap and the sequence is repeated. This type of following is, however, very much the exception rather than the rule.

Continued spreading of the monolayer results in the appearance of large gaps between the cells comprising it. The cells bordering the gap respond by extending pseudopods into it until all the space has been filled (Figs. 9, 10), and are as vigorous in this activity as the cells at the edge of the monolayer. When an extending pseudopod meets the surface of another cell, its extension ceases if no more space is available. If, on the other hand, space is available, the direction of pseudopod formation may be redirected into that space. The pattern of gaps within the monolayer and thus the pattern of intercellular contacts is continually changing. In some cases cells extend pseudopods into a gap but do not move into it. This usually occurs when the cells involved are in contact with others which are moving in some other direction. In such situations cells adjacent to a gap may actually move away from it although actively extending pseudopods into it.

Preparations in paraffin oil

A grex placed in paraffin oil under a supported coverslip can continue movement for several hours. Slight squashing of the preparation enables the cells in the surface layer immediately beneath the coverslip to be observed under fairly critical, though by no means ideal, phase-contrast conditions. The observations which have been made on agar preparations with regard to the following of cells by others have been largely repeated on grex in oil. Apparent ‘contact following’ may occur, or very occasionally the movement of one cell may leave a small gap which its immediate neighbour will hasten to close by extending a pseudopod into it. Although it is somewhat difficult to be sure on this point, the grex in paraffin oil seems to be surrounded by a slime sheath. A slime trail is certainly left behind by the grex as it moves forward and this appears to be continuous with a thin wrinkled membrane over the grex surface. During the first 60–90 min after the grex has been placed in oil, pseudopodal
activity of the cells adjacent to the slime sheath at the tip can be detected, the direction of pseudopod formation being towards the slime sheath (Fig. 11). With the exception of the cells immediately underlying the slime sheath around the tip, very few cells appear to be elongated in the direction of grex movement and, indeed, very few cells appear to be elongated in any direction. The majority are approximately pentagonal or hexagonal and pseudopodal activity is intermittent rather than continuous and

Fig. 2. Tracing from a time-lapse film showing the movement of two cells through a gap between cells in a grex in paraffin oil. The advancing pseudopod of cell \(a\) makes contact respectively with the surfaces of cells \(b, c\) and \(d\). Each time a contact is made the direction of pseudopod formation by \(a\) is redirected into available space, so that this cell continues to move through the gap. The intervals between the diagrams (A–F) are 17, 16, 7½, 23½ and 28½ sec respectively.
vigorous. Single cells and short lines of three or four cells can occasionally be seen moving in almost any direction with respect to the longitudinal axis of the grex.

As the preparation ages a liquid of different refractive index from the oil begins to accumulate around the grex, most conspicuously at the tip (Fig. 12). The grex may continue moving forwards for some time after the appearance of this gap, the tip cells extending pseudopods outwards from the cell mass towards the liquid/oil interface which they are unable to penetrate. Considerable distortion of the grex shape occurs in older preparations as more liquid accumulates. Large gaps may arise within the grex. Cells then produce pseudopods into the gaps from all directions and may break contact with their neighbours and move into the gap. Again, the breaking of contact often involves the prolongation of parts of the cells involved into fine cytoplasmic strands which spring away from each other when the contact is finally broken. This situation provides the opportunity for observing the behaviour of single grex cells in relation to one another. In general, as in agar preparations, the cells seem to extend pseudopods and to move to fill the space available, i.e. until the pseudopodal surfaces of all the cells involved in filling the gap have made contact with each other. Figure 2 shows tracings from a time-lapse film following the movement of a cell through a gap in a grex in paraffin oil. It can be seen that when the advancing pseudopod of the cell makes contact with the surfaces of other cells, the direction of pseudopod formation is altered so that the cell continues moving through the gap.

**DISCUSSION**

**General hypothesis**

From the above observations it will be proposed that there are two primary factors governing the direction of movement of grex cells. First, a cell should be able to produce a pseudopod in the direction in which it is to move and, secondly, the pseudopod, once extended, should be able to form a stable adhesion in the direction of movement. These proposals are similar to those made for other systems by Gustafson & Wolpert (1963, 1967) and Weiss (1961). It seems simplest to assume that grex cells form pseudopods in a direction in which their environment provides a sufficiently low physical resistance to allow cytoplasmic outflow and that pseudopods...
form stable adhesions with the posterior surface of the cell in front. If this is so, the
slime sheath could contribute to the co-ordination of the polarity of cell movement
by providing a barrier to pseudopod extension at all points except at the tip of the
grex, where it is extended and formed. The forward movement of cells at the tip
would thus be permitted and would allow forward movement of the cells behind
them and so on, while the collapse of the slime sheath at the rear would prevent cell
movement in this direction. A diagram of this suggested mechanism for grex movement
is given in Fig. 3. These proposals with regard to cell movement and the role of the
slime sheath will now be discussed separately.

Cell movement and contact interaction

The observations which have been made on grex cells are similar to observations
on aggregating cells made by other workers (see Shaffer, 1962). However, the inter-
pretation which is placed upon them is entirely different.

Cells at the edge of a grex mass or adjacent to a gap always seem to be more
pseudopodally active than cells which are in all-round contact with others. Also,
when a cell is moving through a gap, pseudopodal extension and movement cease
or are redirected when the anterior surface of the pseudopod makes contact with
the surface of another cell. Strictly speaking, there appear to be three situations in
which this can occur. Pseudopodal extension ceases or is redirected: (i) when the
surface of an extending pseudopod comes into contact with the surface of a stationary
cell; (ii) when the surface of an extending pseudopod comes into contact with the
surface of a cell moving in the same direction as, but more slowly than, the cell
producing the pseudopod; and (iii) when the surface of an extending pseudopod
comes into contact with the surface of a pseudopod being extended by another cell
in a direction opposite to its own direction of extension (Fig. 2). This behaviour is
strikingly similar to that shown by cells in other systems where cells adjacent to
a gap (Gustafson & Wolpert, 1962, 1963, 1967) or to a free edge (DuPraw, 1965;
Vaughan & Trinkaus, 1966; Trinkaus & Lentz, 1967) produce pseudopods into
the available space. The term 'contact paralysis' has been coined to describe this
behaviour (Gustafson & Wolpert, 1967) and has been discussed by Wolpert & Gingell
(1968). (Contact paralysis is distinct from 'contact inhibition' (Abercrombie &
Heaysman, 1954), which describes the situation in which cells cannot move over
each other. Contact inhibition is not a property of slime mould cells, from late
aggregation onwards at least, since the cells move on top of each other during grex
formation and movement.) It must be stressed, however, that contact in itself does
not inhibit pseudopod formation by grex cells, since 'contact following' occurs.
Contact following seems to involve pseudopodal activity of the following cells which
maintains contact with the posterior surface of the leading cell. In view of this, it
would seem simplest to account for the observed contact paralysis of pseudopodal
activity in grex cells by assuming that, in circumstances such as those enumerated
above, the contact imposes a mechanical restriction to pseudopod formation by the
cells involved. A cell which is in contact with other cells on all sides would then be
unable to form a pseudopod because the surrounding cells represent a mechanical
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barrier to such activity. If one of the surrounding cells were to move away, the physical resistance to pseudopod formation would be reduced on that side, whether or not contact with the moving cell were maintained. The cell would, therefore, tend to form a pseudopod, and to move, in that direction.

In order that a cell may move, the extended pseudopod must be able to form a stable adhesion with the substratum in the direction in which it is to move (Gustafson & Wolpert, 1963). Thus, if a cell can form a more stable adhesion in one direction than in another it will tend to move in the former direction. From the present observations, there is considerable evidence that the surface of a cell moving perpendicularly away from that of another provides a stable adhesion for the latter and that adhesions are stable when the forces tending to break them are applied perpendicularly to the plane of adhesion. The evidence is as follows: (i) a cell extending pseudopods into a gap may move away from a gap when contact following another cell; (ii) the breaking of adhesions in a direction perpendicular to the plane of adhesion involves ‘stretching’ of the cell surface involved; (iii) ‘contact following’ occurs much more frequently than the alternative form of following where the advancing cell leaves a gap into which the following cell extends a pseudopod. On the basis of this evidence, it seems reasonable to propose that cells within the grex make stable adhesions with and obtain traction on the posterior surfaces of the cells in front of them.

Of particular interest is the observation that unlike anterior-posterior adhesion, lateral adhesion between cells seems to be unstable in that when cell movement tends to apply a shearing force parallel to the plane of adhesion, the cell surfaces slide past each other. Bonner (1952) and Bonner & Adams (1958) have shown that cells are able to move through the grex mass, and cells must change their relative positions during the transition from aggregate to migrating grex and from migrating to culminating stages. The ability of cells to slide past each other would facilitate these activities. It also seems likely that cells within the grex may be able to slide past those above and below them, so that the rate of movement of any particular cell will be governed by the rate of movement of the cell in front of it, and the rate of movement of all cells by the rate of movement of those at the extreme tip.

An important theoretical point is raised by the relative stability of intercellular adhesion to tensile and shearing forces. Since Garrod & Wolpert (1968) have shown that the surface of a slime mould cell is a relatively permanent structure, this behaviour is unlikely to be explicable by assuming turnover of the cell surface. On the other hand, this type of behaviour can be predicted from the colloid-electrostatic double-layer theory of cell adhesion (Curtis, 1962), as will be shown in a later paper. Briefly the London/van der Waal’s forces, which, on this theory, are supposed to hold the cell surfaces together, act perpendicularly to those surfaces and provide no resistance to a force applied at right angles to their direction of action; that is, to a shearing force, parallel to the plane of adhesion.
The slime sheath as an organizer of grex polarity

The suggestion that the slime sheath which surrounds the grex may be instrumental in controlling the polarity of grex movement was put forward by Francis, whose work has been discussed by Shaffer (1964a, 1965b), though his results have never been published. In the present study two pieces of evidence seem to suggest the importance of the slime sheath in this respect. First, plunging the grex into aqueous solution seems to disperse the slime sheath, and no sheath is formed by under-water aggregates. Previously, it has been shown that when a *D. discoideum* grex is placed in water it loses polarity and rounds up (Shaffer, 1962) and, when aggregates are allowed to form under water, polarity is not developed unless the aggregate contacts the air/water interface (Gerisch, 1960). It seems possible that the absence of polarity in under-water grex may be connected with the absence of slime sheath. Secondly, the grex continues polarized movement for some time in paraffin oil and in these conditions the slime sheath seems to persist.

It has been proposed above that grex cells form pseudopods and hence move when they are not physically restricted from doing so by their environment. If this is true, polarity of grex movement could be produced if the slime sheath restricted the pseudopodal activity of all cells except those at the tip. These cells would then be able to move forward and would allow those behind to move forward and so on.

In order to substantiate this hypothesis it is first necessary to show that the slime sheath does restrict cell movement. The fact that cells within the grex are not elongated in the direction of movement (Bonner, 1950) suggests that this is so. Here it has been shown that cells in the grex in oil preparations, other than those at the tip, are rounded and that their pseudopodal activity is intermittent. When a grex is squashed on agar the cells are also rounded initially. Later, however, they become elongated as they move outwards from the cell mass. They then have a similar appearance to cells in aggregation streams. This transition seems to suggest that, in squashing the grex, some restriction to their movement has been removed. When the slime sheath is dispersed by placing the grex in an aqueous medium, the cells all round the edge of the mass are able to form pseudopods into the surrounding medium. It is perhaps not surprising that such a cell mass cannot develop polarity under these conditions, since cell movement can occur with equal facility in all directions.

It seems quite probable, then, that the slime sheath restricts cell movement. It also seems likely, as Shaffer (1964a) has pointed out, that the slime sheath at the tip of the grex to some extent restricts cell movement in a forward direction. For the slime sheath to be effective in controlling grex polarity it is only necessary that at the tip the slime sheath should restrict cell movement less than it does elsewhere. Although critical microscopy is impossible on oil preparations, it does seem that the cells at the tip of the grex are able to extend pseudopods in a forward direction, which may indicate that there is a lack of restriction to their pseudopodal activity in this region.

The question now arises as to how a reduction in the resistance of the slime sheath to cell locomotion could be brought about at the tip of the normal grex. There
have been two suggestions in the literature as to how this might occur. Francis (see Shaffer, 1964a; Bonner, 1966), apparently in contradiction to his own results, suggested that the slime sheath at the tip might be a liquid of low surface tension. The tip cells would be able to move forward because they would be able to deform the sheath at the tip more easily than elsewhere. Shaffer (1964a), criticizing Francis' view, suggested that there is actually a membranous slime sheath at the tip of the grex and that the tip cells are able to move forward because they synthesize the slime sheath. Tacit in both of these suggestions is the assumption that slime sheath is synthesized only at the front of the grex.

Either of these possibilities could provide a basis for grex polarity which would seem to fit in with the above suggestion concerning factors which control cell movement. However, a little further evidence relating to slime sheath synthesis emerges from the present study. When a grex has been in paraffin oil for some time, liquid begins to accumulate around the cell mass, particularly at the tip. This may well be slime which is produced by the grex cells but which does not condense to form a slime sheath over the grex surface, or at least does not do so as rapidly as it would in air. Further, D. R. Garrod, J. F. Palmer and L. Wolpert (unpublished results), in an investigation of the electrophysiological properties of the grex, have found a graded anterio-posterior difference in properties of the slime sheath which may indicate that it increases in rigidity toward the rear end of the grex. Both these facts would appear to support the suggestion of Francis. However, I have also found that when grex are fixed in a cationic fixative the cells may be dissected out from the inside leaving a bag of slime sheath which entirely retains the shape of the grex, even at the extreme tip. This may indicate that the slime sheath forms an actual membrane at the tip surface and would tend to support Shaffer. In the light of these observations it seems most reasonable to suggest the slime is produced in liquid form by the grex cells, and that this liquid is liberated on to the surface at the grex tip, where it condenses to form a membranous slime sheath.

This suggestion has the attraction that it may provide an explanation of a puzzling observation on grex movement. Bonner et al. (1953) have shown that the speed of grex movement is directly proportional to grex volume. The above suggestions as to the control of grex cell movement would mean that the speed of the whole grex would be determined by the rate at which the cells at the anterior end could advance. A membranous slime sheath over the grex tip would restrict the forward movement of tip cells, so that the speed of grex movement would depend on the rate at which this restricting barrier could be advanced. Therefore, if tip slime sheath is formed from liquid slime produced internally by the grex cells, the speed of grex movement will depend on the rate at which slime sheath can be produced from liquid slime. Suppose that liquid slime is produced either by all the grex cells or by a constant proportion of them at the front end, and that each cell produces slime at a constant rate regardless of the size of the grex. As the size of the grex increases, the amount of slime produced per unit time will increase as the cube of the linear dimension. However, as grex size increases the area of the slime sheath at the tip will increase only as the square of the linear dimension, so that in a large grex there will be
proportionately more liquid slime to form a slime sheath proportionately smaller
in area. Thus in a large grex the rate of formation of slime sheath will be greater
than in a small grex, so that the cells of a large grex will be permitted to move
forward faster than those of a small one.

Although it is suggested here that slime sheath synthesis is the main factor con-
trolling polarity of grex movement, there are situations in which the deformability
of the slime sheath could also play a part. When the tip of a grex is amputated the
remaining rear portion ceases to move forward (Raper, 1940) but the cells at the
back continue to move forward, so that the cell mass becomes rounded. A particle
placed on the slime sheath of such a rear portion remains stationary until the culmi-
nation process begins, which indicates that during rounding up the slime sheath
is being deformed. The continued polarized movement of the cells in this situation
could possibly be accounted for if there were an anterior-posterior gradient in slime
sheath rigidity. That this cannot be the whole explanation is, however, shown by
the fact that grafting a tip on to the posterior end of a decapitated grex does not
reverse its polarity (Raper, 1940). This is probably the most serious difficulty in
supposing that the polarity of grex movement is entirely controlled by mechanical
factors. It does seem, however, that mechanical factors could play an important
part and the behaviour of grex cells appears to support the contentions of previous
workers in this respect.

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Fig. 4. The edge of the tip of a grex a few minutes after being placed in Bonner's solution, showing the pseudopodal activity of cells at the edge.

Fig. 5. Cells in the centre of a grex (slightly squashed) shortly after being placed in Bonner's solution, showing that each cell is in contact with others on all sides.

Fig. 6. Edge of a grex about 90 min after being placed in Bonner's solution, showing cells moving away from the edge singly and in small groups.
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Fig. 7. Photograph taken 5 min after squashing a grex on agar, showing the vigorous pseudopodal activity of cells at the edge of the monolayer.

Fig. 8. Photograph of the same preparation as in Fig. 7 but taken 90 min later. The cells are actively moving outwards over the surface of the agar, bringing about a more complete monolayering of the cell mass. Cells at the edge are showing pseudopodal activity while those away from the edge are elongated in the direction of spreading and moving in that direction.

Fig. 9. Same preparation as in Figs. 7 and 8 but taken 3 h after Fig. 7. A gap has appeared in the monolayer (arrow) into which the surrounding cells are producing pseudopods.

Fig. 10. Taken 3½ min after Fig. 9. The original gap (arrow) has been almost closed by the activity of the surrounding cells, while other gaps have appeared.
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Fig. 11. Photograph of the tip of a grex 1½ h after being placed in paraffin oil. A small amount of liquid is beginning to accumulate around the grex (lower left of photograph) and pseudopodal activity of cells around the edge of the grex can just be discerned.

Fig. 12. Photograph of the tip of a grex 3½ h after being placed in paraffin oil, showing the accumulation of a considerable amount of liquid in front of the most anterior cells.