AN EXPERIMENTAL STUDY OF THE INTERACTION BETWEEN THE SOIL AMOEBA NAEGLERIA GRUBERI AND A GLASS SUBSTRATE DURING AMOEBOID LOCOMOTION

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SUMMARY

The amoeboid locomotion of the soil protozoon Naegleria gruberi has been studied using reflexion-interference microscopy. Two types of contact are made with a planar glass substrate. One, formed at a considerable distance from the substrate in deionized water (≈ 100 nm) has been termed 'associated contact' and usually involves a considerable surface area (of the order of 100 μm²), i.e. about a third of the cell profile. From this broad platform filopodia are produced which form close contacts ('focal contacts'). In locomotion the area of associated contact is very mobile, in contrast to the focal contacts which, once established, are stable. Focal contact sites are left behind on the glass surface ('footprints') when the amoeba moves away. The cell-substrate gap in the associated contact is greatly affected by the ionic strength of the medium and particularly the valency of the cation component. This suggests that long-range forces of attraction play an important role in keeping the amoeba close to a substrate and thus allow the production of filopodia from the ventral surface to form focal contacts.

INTRODUCTION

Amoeboid locomotion involves 2 cooperative processes: the generation of the force required for movement (probably generated by an actin/myosin system) (Pollard, 1976), and the interaction between the ventral surface of the amoeba and the substrate on which it is moving, enabling the force generated to be converted into resultant locomotion. It is the second aspect which is considered in this paper. Cell adhesion has been studied for many years (reviewed by Curtis (1973)) and one aspect of it, namely, interactions between the undersurface of cells and glass substrates can be studied directly by the technique of reflexion-interference microscopy (Curtis, 1964). This method has already been used by several workers (e.g. Curtis, 1964; Izzard & Lochner, 1976; Rees, Lloyd & Thom, 1977) to examine avian and mammalian fibroblasts. Areas of close contact (focal contacts) have been described, leading to a theory of adhesion and locomotion (Rees et al. 1977). Since the Naegleria amoeba carries a net negative charge (Forrester, Gingell & Korohoda, 1967) and glass surface bears negative charges, it is to be expected that electrostatic forces of repulsion would operate between the 2 surfaces during locomotion.

The existence of long-range forces of attraction between surfaces bearing electrical
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charges of the same sign has been the subject of much discussion (Curtis (1973)). A recent paper by Gingell, Todd & Parsegian (1977) using glutaraldehyde-fixed human erythrocytes provides convincing experimental evidence for the operation of such forces. The long-range electrostatic forces of repulsion and attraction operating between 2 surfaces of like charge can be altered by changes in the concentration of electrolytes in the medium. The presence of serum in high ionic strength media used in the studies on fibroblasts makes the assessment of conditions operating in the environment of the cell and substrate difficult or impossible. However, by employing Naegleria, a soil protozoon tolerant of very low ionic-strength conditions, we have been able to look for evidence in favour of the operation of long-range forces during cell movement. A preliminary report of some of this work has been published previously (Preston & King, 1978).

METHODS

Cell culture

Naegleria gruberi (stock 1518/1c, Culture Collection of Algae and Protozoa, Cambridge) was maintained at 25 °C on monoxenic culture with Escherichia coli on 0.2 % (w/v) peptone agar plates. The protozoa were harvested in 2 mM Tris/HCl, pH 7.4 and washed several times to remove most of the bacteria. The cells were further purified by pelleting them, resuspending them in a volume of 2 ml (≈ 1 × 10⁷ - 3 × 10⁷ cells ml⁻¹) which was then applied to the top of a Ficoll/2 mM Tris/HCl gradient prepared in a 15-ml centrifuge tube. The gradient had the following constitution: 4 ml of 5 % Ficoll, 8 ml of 10 % Ficoll, 1.5 ml of 20 % Ficoll. Centrifugation was carried out at 4 °C for 10 min at 1000 rev/min in an MSE Mistral 2L centrifuge. The amoebae collected at the interface between the 10 and 20 % Ficoll. The cells were then washed free of Ficoll in 2 mM Tris/HCl. Cell number was determined with a Coulter Counter (model ZB) and amoebae were suspended in deionized water (2 μSiemens (reciprocal ohms) specific conductance) to give 10⁷ cells ml⁻¹.

Deionized H₂O

Distilled water from a Manesty still (Model OBE, Liverpool, England) was passed through an 'Ultrapure' (mixed bed) Demineralizer Cartridge (Barnstead, Boston, Mass.). The pH of the resulting water was usually between 5.8-6.2. The specific conductance was between 0.5 and 2.0 μSiemens. For convenience when drawing the graphs the deionized water was assigned an ionic strength value of 0.01 × 10⁻⁶, since a 10 μM solution of KCl (i.e. ionic strength 0.01 × 10⁻⁶) would theoretically give a specific conductance value of 1.5 μSiemens.

Chemicals

All the chemicals used were analytical grade from BDH Chemicals, Poole, England except the sucrose which was 'Aristar' grade.

Perfusion experiments

A 100-μl sample of the purified cell suspension was inoculated into a perfusion chamber, previously filled with deionized water. The windows of the chamber were No. 2 glass coverslips (Chance, Warley, England) that had been cleaned with detergent ('Pyrene', Diversey Ltd., Barnet, Herts). After the cells had settled on the lower coverslip the chamber was inverted and perfused with deionized water to remove unattached cells and any debris. The chamber was then locked into position on the stage of the interference-reflexion microscope. Individual amoebae could be observed whilst different solutions were drawn through the chamber by a peristaltic pump. The void volume of the entire perfusion apparatus from reservoir taps to the
specimen bottle, determined by using bromophenol blue as a tracer, was ~ 2.5 ml, of which the perfusion chamber accounted for ~ 1 ml. The conductivity of experimental solutions was measured (Chandos Linear Conducting Meter, Chandos Products, New Mills, Cheshire) at 2 points: in the reservoir and in the perfusate trap (see Fig. 1). In those experiments involving an increase in ionic strength the conductivity of the perfusate matched that of the solution in the input reservoir after the passage of 10 ml of electrolyte through the chamber. When a decrease in conductivity was required, e.g., in returning to deionized water from 10 mM NaCl, it was necessary to perfuse the apparatus with larger volumes. The pH of solutions entering and leaving the apparatus was recorded using a pH meter (E.I.L. model 7010, Electronic Instruments Ltd., Chertsey, Surrey). The pH of the solutions was between 5.5 and 6.0. All experiments were carried out at an ambient temperature of 24°C.

Fig. 1. Arrangement of the microscope and perfusion system. R₁ and R₂, reservoirs; E, eluate collection vessel; *, sites where pH and conductivity were measured.

Microscopy

A Vickers M41 Photoplan stand was used with a Zeiss X100 planapochromat oil-immersion lens. Green light was selected from the output of a 200-W mercury lamp by a Balzers K4 interference filter. The immersion lens was used at an incident numerical aperture (I.N.A.) of 1.0, calculated according to the method of Izzard & Lochner (1976). A television camera (Link 156; Andover, Hampshire), connected to a videotape recorder, was positioned on the microscope to monitor the locomotory behaviour of amoebae during experiments. Figs. 3–5 (p. 149) were produced by taking 35-mm photographs of the recordings on the television monitor. When white-light interference patterns were required for estimation of the cell-substrate gap, incident illumination was provided by a 100-W quartz halogen source (Preston & King, 1978).

Analysis of television images

In any given ionic conditions the behaviour of the amoebae on the glass substrate was recorded for approximately 20 s. Three separate TV images were analysed for each of the experimental conditions. Five readings from the reflection-interference image of the ventral surface of the amoeba and 5 readings from background regions of the screen were obtained from each image. The measurement of the light intensity from the screen was carried out using a highsensitivity photometer (model J37, Vickers Instruments, York, England). The aperture was reduced to a small hole of approximately 4 mm², which was held against the TV screen and the reading recorded. Under our standard operating conditions the area of 4 mm² was equivalent to a surface area on the amoeba of about 0.5 µm². Most of the results have been expressed as 'image ratio', i.e. the ratio of the light intensity from the ventral surface of the amoeba divided by the
light intensity of the background. Therefore, (a) when background is equal to the light intensity from the amoeba, image ratio = 1.0; (b) when destructive interference occurs, image ratio < 1.0; (c) when constructive interference occurs, image ratio > 1.0.

The image ratio recorded under any given experimental condition was the ratio of the average of 15 readings (from 3 separate TV images) from the amoeba surface and from the background.

The position of focal contacts and associated contacts was drawn directly onto plastic sheets placed over the T.V. monitor screen. From these traces the pictures shown in Fig. 6 and values in Table 1, p. 151 were obtained.

RESULTS

Behaviour of living cells in deionized water

Phase-contrast microscopy. Naegleria gruberi, a limax amoeba, is roughly cylindrical and displays a predominantly monopodial style of movement. Locomotion is achieved by the production, often eruptive, of smooth, hyaline bulges anteriorly, accompanied by forward flow of endoplasm. The surface of an amoeba may be considerably folded in the tail region (uroid), from which thin trailing filopodia emanate. These cytoplasmic extensions may reach several micrometres in length before detaching from the substrate and recoiling forwards into the uroid (Fig. 2).

Reflexion-interference microscopy (RIM). Previous attempts to obtain information on the morphology of an amoeba's ventral surface and its dynamic behaviour with respect to a glass substrate by viewing cells from the side (Dellinger, 1906; Bell & Jeon, 1963) have been severely limited by diffraction at intervening gap distances of less than half the wavelength of the illuminating source (= 270 nm in the case of green light). However, the nature of incident light RIM makes possible an examination of both these features of amoeboid movement.

The interference image produced from the ventral surface of the amoeba consists of 2 distinct components. Firstly, there is a broad area of contact showing a constructive interference pattern which we term 'associated contact'; secondly small dark areas of close contact (= 0.4 μm in diameter) which, by analogy with fibroblasts, we term 'focal contacts' (Fig. 3). In white light the interference image from the associated contact was white to whitish grey and no interference colours were observed, showing that the associated contact was present as a broad platform held at a fairly constant...
Amoeboid motion of *Naegleria gruberi*
distance from the glass substrate. By reference to the Newtonian interference colour
series we estimate the gap distance to be in the order of 100 nm. The platform may
fluctuate between existing as one large area or as separated parts over short time
periods (see Fig. 6, 10-s interval traces), and represents the closest approach made to
the substrate by the bulk of the cell. Furthermore the entire associated contact
behaves uniformly even when partitioned into several islands.

![Fig. 6](image)

**Fig. 6.** Sequence of line drawings obtained from the RIM/Video recorder showing
the behaviour of a single amoeba moving on a glass coverslip over a 70-s period. The
top left-hand picture represents the situation at time 0 followed by a sequence of
frames at 10-s intervals. The profile line represents the boundary of the 'associated
contact'. ●, focal contact; ×, new focal contact formed in previous 10 s; +, one
particular focal contact marked distinctively to provide a point of reference. × 2200,
scale bar represents 10 μm.

Within areas of associated contact underlying the anterior two thirds of a cell,
microextensions may appear which form adhesions to the substrate, thus bridging the
intervening 100-nm gap. We have termed these microextensions 'filopodia' to denote
their foot-like character and small diameter. However, it must be remembered that
these filopodia are usually very short (i.e. ≈ 100 nm in deionized water) when pro-
duced from the ventral surface.

The extremely stable, small points of closest approach designated 'focal contacts',
arise only beneath associated contacts. Areas of associated contact may, however, form
and disappear without focal contacts developing from them. When a focal contact
does arise it serves to consolidate that part of the associated contact from which the
bridging filopod subtends. The focal contacts remain fixed in position as an amoeba
moves forward (Fig. 6 and Table 1). Eventually the bridging filopodia become visible
at the uroid. It is frequently found that filopodia extend back from the rear of the cell
to contact points on the glass. As the cell moves forward these filopodia are put under
Table 1. Analysis of focal contacts formed over a 70-s period

<table>
<thead>
<tr>
<th>Time, s</th>
<th>Total no. of focal contacts</th>
<th>New focal contacts formed in previous 10-s period</th>
<th>Focal contacts inside area of associated contact</th>
<th>Focal contacts outside area of associated contact</th>
<th>Area of associated contact, ( \mu m^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>96</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>101</td>
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<tr>
<td>20</td>
<td>11</td>
<td>7</td>
<td>11</td>
<td>0</td>
<td>99</td>
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<td>12</td>
<td>3</td>
<td>12</td>
<td>0</td>
<td>115</td>
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<td>40</td>
<td>16</td>
<td>4</td>
<td>14</td>
<td>2</td>
<td>132</td>
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<tr>
<td>50</td>
<td>21</td>
<td>5</td>
<td>18</td>
<td>3</td>
<td>147</td>
</tr>
<tr>
<td>60</td>
<td>22</td>
<td>1</td>
<td>17</td>
<td>5</td>
<td>121</td>
</tr>
<tr>
<td>70</td>
<td>23</td>
<td>1</td>
<td>9</td>
<td>14</td>
<td>72</td>
</tr>
</tbody>
</table>

At time 0 only the focal contacts within the associated contact are shown. Using phase contrast microscopy the whole cell profile was about 300 \( \mu m \).

Fig. 7. Effect of different ionic strength solutions of KCl on the reflexion-interference image obtained from the ventral surface of *Naegleria* amoebae. Ordinate, photometer reading (arbitrary units); abscissa, ionic strength of KCl (\( \mu \)) in the medium (log scale); \( \circ \), photometer readings obtained from the surface of the amoeba; \( \bullet \), photometer readings obtained from the background. Each point represents the average of 5 readings obtained from a single television image and the standard deviation is shown by the bars. The dotted line represents the average value of the background (i.e. obtained from 40 results).
stress and eventually break. However, it is not the glass-filopodium contact which is broken but the actual filopodium itself. Thus cellular material is left behind at the focal contact point (footprint).

**Influence of electrolyte composition of medium on associated contact**

**Monovalent ions.** Fig. 7 showed the effect of KCl concentration on the reflection-interference image obtained from the ventral surface of an amoeba. At low concentrations of electrolyte (less than 0.3 mM) the interference pattern obtained was of a constructive nature and had about the same relative intensity as that obtained in deionized water. A rapid change occurred between 0.2 and 2.0 mM as the constructive interference pattern changed to a destructive interference pattern (Figs. 3-5). At higher electrolyte concentrations the image obtained was not affected, i.e. remained dark. On returning the cells to deionized water the constructive interference pattern originally present was restored (i.e. the electrolyte effect was reversible). A given concentration of electrolyte would give a reflection image which had the same light intensity as the background, i.e., an image ratio of 1.0. This value was obtained from the relevant graph of image ratio against electrolyte concentrations (e.g. Figs. 7, 8). It was assumed that when the image ratio was 1.0, no matter what ionic conditions produced it, the gap distance has a fixed value. Therefore the effectiveness of ions in bringing the substrate surface and cell surface closer together could be judged by the reciprocal of the concentration of ion used to obtain an image ratio of 1.0 (see Table 2). Essentially similar results were obtained using NaCl.
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Divalent anions. Similar results to those described above were obtained using (NH₄)₂SO₄ and Na₂SO₄. Consideration of the contribution of the cation and anion to the ionic strength of the solution showed that at the cross-over point (i.e. image ratio = 1.0), whereas the cation component was about the same as the value obtained for NaCl and KCl, the anion component was twice as great.

Divalent cations. From Fig. 8 and Table 2 it can be seen that divalent cations were very effective in bringing the cell and substrate closer together.

<table>
<thead>
<tr>
<th>Electrolyte used</th>
<th>Molarity of solution</th>
<th>Relative* effectiveness of electrolyte</th>
<th>Ionic strength† of solution</th>
<th>Anion component (0.5 C₁Z₁)</th>
<th>Cation component (0.5 C₂Z₂)</th>
<th>Relative‡ effectiveness of cation component</th>
</tr>
</thead>
<tbody>
<tr>
<td>LaCl₃</td>
<td>3.5 x 10⁻⁶</td>
<td>215</td>
<td>21 x 10⁻⁸</td>
<td>5 x 10⁻⁸</td>
<td>16 x 10⁻⁸</td>
<td>24</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>3.7 x 10⁻⁶</td>
<td>20.7</td>
<td>110 x 10⁻⁶</td>
<td>37 x 10⁻⁸</td>
<td>73 x 10⁻⁸</td>
<td>5.3</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>3.2 x 10⁻⁶</td>
<td>23.8</td>
<td>92 x 10⁻⁶</td>
<td>32 x 10⁻⁸</td>
<td>63 x 10⁻⁸</td>
<td>6.1</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>3.0 x 10⁻⁶</td>
<td>2.4</td>
<td>90 x 10⁻⁶</td>
<td>640 x 10⁻⁶</td>
<td>320 x 10⁻⁶</td>
<td>1.2</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>3.0 x 10⁻⁶</td>
<td>2.6</td>
<td>900 x 10⁻⁶</td>
<td>600 x 10⁻⁶</td>
<td>300 x 10⁻⁶</td>
<td>1.3</td>
</tr>
<tr>
<td>NaCl</td>
<td>6.4 x 10⁻⁶</td>
<td>1.2</td>
<td>640 x 10⁻⁶</td>
<td>320 x 10⁻⁶</td>
<td>320 x 10⁻⁶</td>
<td>1.2</td>
</tr>
<tr>
<td>KCl</td>
<td>7.8 x 10⁻⁶</td>
<td>1.0</td>
<td>780 x 10⁻⁶</td>
<td>390 x 10⁻⁶</td>
<td>390 x 10⁻⁶</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* The relative effectiveness of the electrolyte was obtained by taking reciprocals of the values obtained in column 2, i.e. the molarity of the electrolyte solution producing an image ratio of 1.0, and adjusting the values so that KCl had an 'effectiveness' of 1.0.
† The ionic strength of the solution (μ) was calculated using the formula: \( \mu = 0.5(C_1Z_1 + C_2Z_2) \) where \( C_1 = \) concentration of anion (g ions/l), \( Z_1 = \) valency of anion, \( C_2 = \) concentration of cation (g ions/l), \( Z_2 = \) valency of cation.
‡ The relative effectiveness of the cation component was obtained by taking reciprocals of the values obtained in column 6, i.e. cation component of the ionic strength of the solution producing an image ratio of 1.0, and adjusting the values so that the K⁺ component in the KCl solution had an 'effectiveness' of 1.0.

Trivalent cations. Lanthanum chloride was even more effective than CaCl₂ or MgCl₂ in bringing the cell closer to the substrate, producing an image ratio of 1.0 at 3.5 µM.

Presence of a non-electrolyte (sucrose). The use of sucrose solutions at concentrations up to 200 mM failed to produce a change from constructive to destructive interference. In fact at high sucrose concentrations the interference image became slightly lighter.

Behaviour of fixed cells

Fixed cells were allowed to settle on the lower coverslip in the perfusion chamber when the ambient solution was 0.2 M NaCl. After 15 min the chamber was inverted. The cells remaining on the inverted coverslip were counted and deionized water was run in until the effluent had the same conductivity as the deionized water. The cells were again counted and it was noted that very few cells had fallen off. When individual cells were observed using reflexion-interference microscopy small areas of dark contact could be seen. In marked contrast to the situation observed in focal contacts with live cells, Newton's rings were associated with each of these contact areas.

Fixed cells were allowed to settle on the lower coverslip in the perfusion chamber when the ambient solution was deionized water. After 15 min the chamber was
inverted and all the cells fell off. Thus a high-ionic-strength solution appeared to be required in the first instance to allow the cells to come close to the glass and make contact.

DISCUSSION

From Figs. 7, 8 and Table 2 showing the effect of electrolyte on the gap distance of the 'associated contact' it can be seen that: (1) Increase in electrolyte concentration decreases the gap distance between amoeba and glass substrate. (2) The valency of the cation has a very marked effect. (3) The valency of the anion component has little effect on the gap distance (compare the results using (NH₄)₂SO₄ and Na₂SO₄ with those using NaCl or CaCl₂). (4) There is a general lack of specificity when divalent cations were compared (CaCl₂ and MgCl₂) and when monovalent cations were compared (NaCl and KCl). This contrasts with the frequent biological discrimination between Mg²⁺ and Ca²⁺ (and K⁺ and Na⁺).

These results could be explained in 2 ways. Firstly, the alteration of ionic strength might have a direct effect on the length of the filopodium, i.e. as the electrolyte concentration increases, the filopodia from the ventral surface might shorten, thereby reducing the separation gap of the associated contact. We consider this explanation unlikely for 2 reasons. Filopodia emanating from the uroid do not alter in length when the electrolyte concentration is suddenly changed and islands of associated contact (Fig. 6, 20-s frame) can exist without focal contacts being present. Furthermore, the gap distance found in these isolated areas is the same as that found in associated contacts present on the same cell at the same time but possessing focal contacts. Secondly, the change in electrolyte concentration might have a direct effect on the long-range forces of attraction and repulsion. It is this concept we wish to develop in the ensuing discussion.

Positively charged, i.e. counter ions, present in the aqueous medium can react with the surface negative charges present on the cells and glass substrate. This results in the formation of an electrical double layer, consisting of 2 parts: an inner region which may contain adsorbed ions and an outer diffuse layer (see Shaw, 1966). The 'thickness' of the outer diffuse layer can be represented by the Debye length (1/K) and can be calculated from the following formula:

$$\frac{1}{K} = \frac{1}{3.27 \times Z \sqrt{C}} \text{nm},$$

where Z = valency of counter ion and C = molecular concentration of counter ion. The simplest way of viewing this physicochemical phenomenon is to consider the repulsive forces extending a considerable distance into the medium when very little electrolyte is present (e.g. in deionized water). However, when a reasonable amount of electrolyte is present (e.g. 10 mM KCl), the repulsive forces extend only a short distance from the charged surface. Using the formula given above when the KCl concentration is 10 μM (this value would be approximately the same as our deionized water),
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The Debye length would be approximately 100 nm. When the NaCl concentration is 10 mM, the Debye length would be approximately 3 nm.

Thus in general terms the diminution of the size of the outer diffuse layer as the electrolyte concentration is raised could account in part for the results obtained. It would be expected from the formula used in calculating the Debye length that the efficiency of monovalent: divalent: trivalent cations in reducing the Debye length would be proportional to the square of the valency, i.e. a ratio of 1:4:9 for equimolecular solutions and thus directly related to the ionic strength of the cation component. From Table 2 it can be seen that the multivalent cations are more effective than would be suggested from considerations of Debye length alone. This anomaly is probably due to interactions between counter ions and the charged surface occurring in the inner part of the double layer. Interactions of this type could probably be detected by cell electrophoresis, which has not been carried out in this study.

In a study of adhesion of the alga Chlorella to glass, Nordin, Tsuchiya & Fredrickson (1967) showed that increasing the concentration of NaCl decreased the electrophoretic mobility of the negatively charged algal cells and the negative charge on the glass, and this was associated with increased adhesion of the cells to glass. FeCl₃ at very low concentrations (20 μM) was effective in producing strong cell-substrate adhesion. When the concentration of FeCl₃ was raised to 100 μM, positively charged cells (as judged by cell electrophoresis) and positively charged glass were obtained. The cell-substrate adhesion was reduced but it was still greater than the adhesion of cells to glass in distilled water. We consider that the observations made on the change of gap distance on the associated contact between amoebae and glass surfaces using varying concentrations of electrolyte provide convincing evidence for the operation of repulsive forces. In deionized water these repulsive forces extend over considerable distances (> 50 nm) from each surface. Whether long-range forces of attraction play a part in the amoeba-substrate association is a more difficult problem. Two pieces of evidence suggest that forces of attraction operate here. Firstly, a separated area of associated contact can be formed (e.g. the 20-s frame from Fig. 6), and may exist for some seconds without the formation of a focal contact, but the reflexion-interference images from these areas are similar to those obtained from areas with focal contacts. It must be remembered that the force of gravity is operating against any forces of attraction. Secondly, in distilled water when a single large area of associated contact is established with focal contacts (e.g. 10-s frame from Fig. 6), adjacent focal contacts may be widely separated (e.g. 5 μm), but the associated contact presents an even reflexion-interference image with the amoeba surface held about 100 nm off the glass substrate. Without long-range forces of attraction one would expect the ventral surface to move further away from the substrate in areas not in close proximity to a focal contact, thereby producing higher-order interference colours. These were never found.

The large area of membrane associated with the ventral surface which can respond to change in ionic strength of the medium suggests that very little resistance is offered by the membrane in adopting a conformation which allows the long-range electrostatic forces to hold the membrane at a defined distance from the substrate. This is surprising as it is to be expected that gel-like ectoplasm would be present as a tube around the
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amoeba (Allen, 1961), offering resistance to the formation of a large area of associated contact. If rigid cytoskeletal elements were abundant in the cell and anchored to the plasma membrane (Rees et al. 1977) it is unlikely that this large area of contact could be produced. The plastic nature of the *Naegleria* membrane as revealed by reflexion-interference microscopy and the very rapid locomotion of this amoeba relative to mammalian fibroblasts, suggest a very different relationship between the cytoskeletal elements and the cell membrane in these two widely different cell types. The reflexion-interference images of rat fibroblasts obtained by Rees et al. (1977) are complex compared with the results we have obtained. Preliminary experiments with avian fibroblasts have shown that the ventral surface of this cell is less responsive to changes in ionic strength of the medium (King, Heaysman & Preston - unpublished results) than *Naegleria* amoebae.

We have had considerable difficulty in finding a suitable fixative for *Naegleria* amoeba, presumably due to the lack of exposed protein in the cell membrane (King & Preston, 1977). Glutaraldehyde-fixed amoebae have many surface protuberances. We assume that the dark areas of contact are due to these protuberances making contact with the glass surface. The presence of Newton’s rings indicates that the protuberances have a conical profile near the point of contact, rather than the cylindrical profile suggested for the filopodium associated with the focal contact in live cells. The failure to detach the cells from the glass substrate using very low ionic strength solution is in contrast to the results of Gingell et al. (1977), who found that half the attached glutaraldehyde-fixed erythrocytes fell off when the NaCl concentration was reduced to about 300 μM. This difference is probably due to a lack of protuberances from the erythrocyte surface.

A focal contact is interpreted as the interaction site between a filopodium produced from the ventral surface of the amoebae and the glass substrate. Filopodia can be found on the dorsal surface (Preston & King, 1978) but only those produced on the ventral surface can be effective for the process of forward movement on a planar substratum to occur. In deionized water when the gap distance between the cell body and the glass is 100 nm or more then the filopodium would be 100 nm or more long and have a maximum diameter of 0.4 μm. The problem of the repulsive forces between the glass and the cell surface associated with filopodium could be overcome if the force generated were large enough and the amount of cell pushed through the gap was small enough. This has been proposed on theoretical grounds by Pethica (1961), who pointed out that a cell could overcome repulsive forces and make close approaches to other cells provided the cell first made contact at portions of membrane having a radius of curvature not greater than about 0.1 μm. We have suggested that filopodia produced from the ventral surface of the *Naegleria* amoeba are responsible for the formation of the focal contacts. These filopodia would have a diameter not exceeding 0.4 μm. If one considers the advancing end of a filopodium (prior to making contact with the substrate) as pointed or rounded, it would be of the right order of magnitude to fit into line with the considerations discussed above. Since anionic sites on the surface of *Naegleria* amoebae are mobile (i.e. can be capped off using cationized ferritin (King & Preston, 1977)), it is possible that the mobile negative charges in the membrane on
Amoeboid motion of *Naegleria gruberi*

the advancing filopodium might be repelled by the repulsive charges set up due to the negative charges on the glass. Thus problems associated with the formation of close contact between 2 similarly charged surfaces would not arise.

One would not expect the long-range forces between cell and substrate to offer resistance to shearing forces acting parallel to the planar surface of the substrate (Gingell, 1971). Therefore we consider that the function of the focal contact filopodia in *Naegleria* is to anchor amoebae to the substratum in order that cytoplasmic streaming and pseudopodial production can be converted into translational cell movement. Hence, these filopodia must be capable of bracing an amoeba against those forces generated by the interaction of the erupting pseudopodia with the viscous forces of the environment. One would not look to the cell membrane itself to provide this mechanical strength (Evans & Hochmuth, 1976), but rather to the cytoplasmic contents of the filopodia.

In locomotion focal contacts are formed at the anterior end and broken at the posterior end of the cell. Unless the focal contact filopodia were broken at the uroid, they would antagonize the progression of the amoeba. It is tempting to speculate that this process of formation and destruction may be controlled by the cyclic conversion of consistency states (i.e. sol ⇔ gel) within the cytoplasm. These changes have previously been implicated in general theories of amoeboid locomotion (Mast, 1926; Allen, 1961).

Since focal contacts may be left behind on a glass coverslip it is clear that there must be a depletion of the cell membrane as a result of locomotion. Assuming a focal contact to be spherical and 0.4 μm in diameter and assuming an amoeba to be a sphere of 16 μm diameter then each focal contact would represent 0.06% of available cell surface area. The number of focal contacts produced varies from amoeba to amoeba and even from time to time for a given cell. Therefore we cannot put an accurate figure on the rate of focal contact production and the consequent rate of loss of cell surface area. There is evidence that amoeba are capable of replacing surface membrane depleted by endocytosis very rapidly (Weismann & Korn, 1967) so one would expect *Naegleria* to be capable of compensating for loss of cell surface in discarded focal contacts.

Considering the variety of locomotory patterns encompassed within the Sarcodina it is likely that the problem of making and breaking adhesions to the substratum during cell movement has been solved in a variety of ways. *Amoeba proteus* for example does not leave permanent focal contacts behind as judged by reflexion-interference microscopy (Habery, 1971). This cell like many other freshwater amoebae bears a prominent extracellular coat. It may be that these polysaccharide coats might play a part in securing adhesion to an inert substratum in similar fashion to that employed by bacteria. It is becoming clear that extracellular coats on bacteria are involved in cell adhesion (Costerton, Geesey & Cheng, 1978). For example, *Klebsiella pneumoniae* has capsular polymer fibrils composed of mannuronic acid, galactose and mannose, radiating out farther than 600 nm (Cagle, 1975).

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REFERENCES


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