more resistant hyphae, which might be a possible explanation for the recovery in growth of the pathogen after 24 h. This study suggests that volatile metabolites excluded from some of the nonparastic phylloplane microfungi may effect the growth and development of invading pathogens in an antagonistic manner. However, the present findings are true in vitro, and may not be so on leaf surfaces in natural conditions. Nevertheless, knowledge of such interactions, besides other corroboratory factors[13,14] may, in future, allow manipulation of the phylloplane microflora to maximize their potential for disease control.

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Lack of gravity effect on the speed of amoeboid locomotion in *Naegleria gruberi*[1]

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Summary. The speed of amoeboid locomotion was the same for amoebae moving on the top surface and bottom surface of a horizontal perfusion chamber i.e. cell-substrate adhesive forces must be considerably greater than the gravitational force acting on the locomoting amoeba.

In addition to force production[2] in the cytoplasm successful locomotion of amoeboid cells is dependent upon the controlled interactions between the cell surface and a substrate through which traction is mediated. Lacking a mechanism to exert traction, crawling cell locomotion would be ineffective.

In this study we have assessed the effect of gravity on cell traction by measuring the speed at which a soil amoeba *Naegleria gruberi* moved across either of 2 parallel glass coverslips held horizontally within a perfusion chamber[3]. While gravity would be expected to work against the adhesion of cells moving on the upper coverslip, it should have augmented the attachment of amoebae to the lower coverslip. Ambient electrolyte concentration has been shown previously to modulate reversibly not only the cell-substrate gap[3] but also their speed of progression e.g. these protozoons move about 4 times faster in 10 mM KCl than in deionized water[4]. Therefore by varying the ionic milieu in the perfusion chamber we were able to look for any effect of gravity on amoeboid motility over a wide range of locomotory rates.

Freshly harvested cells were introduced into a Prior chamber (W. Prior, Bishops Stortford, Herts., England) the windows of which were round coverslips separated by a lightly greased rubber seal. The medium was changed when necessary as described previously[3]. Amoebae in the chamber were selected at random and their speed of movement recorded over a period of 1 min. The paths taken by individual amoebae were traced by means of a camera lucida and the distances covered in unit time were subsequently determined with a map measuring wheel. In each medium the tracks of at least 40 amoebae were recorded; 20 on the upper coverslip (i.e. inverted) and 20 on the lower coverslip (i.e. noninverted). The experiments were carried out at room temperature (17–18 °C).

The results confirm that the presence of 10 mM KCl (or NaCl) clearly increased the speed of locomotion over that attained in deionized H_2O. Furthermore a similar increase was not observed when an osmotically equivalent (20 mM) solution of the non-electrolyte sucrose was used. However one could not show any differences between inverted and noninverted cells with regard to their speed of movement in specified media. It would therefore appear that the cell-substrate adhesive forces between *Naegleria* amoebae and glass were much greater than the force of gravity acting on the mass of the cell.

Gingell et al.[5] have studied the adhesion of fixed red blood cells to an inverted oil/H_2O interface. By reducing the ionic strength of the suspending solution, the cell-interface gap was increased[6]. When the strength of the medium was reduced to 0.3 mM NaCl about half the cells fell off. This could be viewed as a balance point between the gravitational force acting on the red cell and the adhesive force acting between the cell and the oil-water interface. The final adhesive force was considered to be produced by a balance between the long range forces of attraction (probably Van der Waals forces) and repulsive electrostatic forces set up between 2 surfaces of like charge (i.e. negatively charged cell and negatively charged interface). This situation obviously did not apply to these experiments with *Naegleria* where the ionic strength of medium can be reduced to the equivalent of 0.01 mM NaCl (= deionized water used in this study) without the cells falling off. The nature and extent of the cell-substrate interaction in *Naegleria* amoebae will be very different because of the presence of punctate focal contacts and an extensive area of 'associated contact'[3]. Although raising the ionic strength of the medium increased speed of movement there was not a similarly significant increase in the number of focal contacts produced[4]. Anyway the effect of focal contacts will be

Comparison of the speed of locomotion of *N. gruberi* amoebae in different media moving on the top coverslip (inverted) and the bottom coverslip (noninverted) of the chamber

Medium	Speed (μm/sec) ± SD Inverted	Noninverted
H_2O	0.14 ± 0.07	0.18 ± 0.09
KCl 0.1 mM	0.36 ± 0.14	0.35 ± 0.15
KCl 1 mM	0.73 ± 0.29	0.80 ± 0.28
KCl 10 mM	1.24 ± 0.21	1.14 ± 0.27
NaCl 0.1 mM	0.21 ± 0.12	0.23 ± 0.09
NaCl 1 mM	0.66 ± 0.17	0.69 ± 0.15
NaCl 10 mM	1.36 ± 0.34	1.20 ± 0.32
Sucrose 20 mM	0.31 ± 0.16	0.29 ± 0.13

ignored in the subsequent discussion, which will be concentrated on the electrodynamic forces acting between the platform of 'associated contact' and the glass substrate.

Parsegian and Gingell[7] have calculated that the attractive force between a cell and quartz substrate at a separation distance of 5 nm to be $= 2.4 \times 10^4$ dynes cm^{-2}.

Using this figure and making the following assumptions regarding *Naegleria* amoebae in 10 mM KCl on glass:

a) In 10 mM KCl the area of closest apposition of *Naegleria* is about 5 nm from the substrate (consistent with an upper limit of 20 nm)[3].

b) *Naegleria* amoeba had similar properties to the cell considered by Parsegian and Gingell[7].

c) Force of attraction between cell and glass is the same as that between cell and quartz.

d) Total area of close apposition (= 'associated contact') is about 100 μm^2 (Preston and King[3]).

The force of attraction $= 2.4 \times 10^{-4}$ dynes $\mu m^{-2} \times$ area of contact $= 2.4 \times 10^{-2}$ dynes.

For the situation in deionized water, applying the same assumption as above except that the cell-substrate gap is now about 100 nm^3 and using the force/distance proportionality equation of Israelachvili[8], i.e. force is proportional to the reciprocal of the gap distance[2]. Then if a value of 2.4×10^{-2} dynes is obtained at a gap distance of 5 nm, the force of attraction in deionized water $= 6.0 \times 10^{-5}$ dynes. Thus according to this estimate the attractive forces between *Naegleria* and glass would be 400 times greater in 10 mM KCl than in deionized water. We have previously shown that *Naegleria* amoebae are more strongly adherent to glass in 10 mM KCl than in deionized H_2O[9].

It is relatively easy to calculate the gravitational force acting on an 'inverted' *Naegleria* amoeba and compare the value to that obtained for the attractive forces.

Weik and John[10] found the cell volume of *N. gruberi* amoebae to be in the range of 1140 μm^3 to 2060 μm^3, and we have taken the cell volume to be 1500 μm^3 (compared with 85 μm^3 for the human erythrocyte). Assuming the density of the amoeba is about the same as a human granulocyte[11], i.e. 1.080 g/cm^3, then the mass of an individual cell $= 1.62 \times 10^{-3}$ μg.

Since the density of the cell was taken to be 1.080 g/cm^3 the relative density in water will be 0.080 g/cm^3 and the effective weight of a single cell $= 0.12 \times 10^{-3}$ μg. As the acceleration due to gravity is 981 cms^{-2}, the effective gravitational force acting on the amoeba will be 1.18×10^{-7} dynes.

Using the theoretically derived figures for attractive forces then the gravitational force represents about 0.3% of the value of the attractive forces when the amoebae are moving on glass in deionized water; and only about 0.0008% of the attractive forces when the amoebae are moving on glass in 10 mM KCl. It is not therefore surprising that gravity played no observable effect in the studies reported here.

However it should be remembered that not all amoebae adhere well to glass. In such cases, for example, *Thecamoeba, Sappinia, Vannella simplex* which cover a wide range of cell size, classical hanging drop preparations are not usually successful[12]. Thus it is possible that in these amoebae gravity might well be effective in determining the efficiency of locomotion.

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Lack of substrate specificity on the speed of amoeboid locomotion in *Naegleria gruberi*[1]

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Summary. Comparison of locomotory rates of *Naegleria* on glass, agar, plastic and fluorocarbon oil under a range of defined electrolyte concentrations showed the speed of amoeboid movement to be independent of the substrate's nature.

Experiments performed 50 years ago by Mast[2] and Hopkins[3] showed that amoeboid locomotion of *Amoeba* could proceed on various types of glass and paraffin. However there was some doubt about the significance of the quantitative differences that were observed[3] in view of the possibility of chemicals (particularly ions) being transferred from the substrate to the medium. In the analysis of vertebrate cell, particularly fibroblast, locomotion much