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 $\mu m^2$ (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 $2.4 \times 10^{-2}$ 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 $\mu m^3$ to 2060 $\mu m^3$, and we have taken the cell volume to be 1500 $\mu m^3$ (compared with 85 $\mu m^3$ 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 $1.18 \times 10^{-7}$ 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

Comparison of motility of *N. gruberi* amoebae on different substrata (motility expressed as μm/sec ± SD)

| Substrate used | Concentration of NaCl in medium (mM) 0 | 0.1 | 1.0 | 10 |
|---|---|---|---|---|
| Glass | 0.16±0.08 | 0.22±0.11 | 0.61±0.24 | 1.26±0.23 |
| Plastic | 0.21±0.09 | 0.32±0.20 | 0.84±0.28 | 1.25±0.29 |
| 0.5% Agar | 0.22±0.07 | 0.28±0.16 | 0.50±0.35 | 1.25±0.55 |
| Oil (FC 75) | 0.21±0.12 | 0.14±0.07 | 0.79±0.27 | 1.11±0.22 |

attention has been focussed on the wetability or non-wetability of the substrate[4]. Few reports concern the use of liquid-liquid interfaces yet recently it was found that amoebocytes from the horseshoe crab, *Limulus*, could locomote equally well on an oil-water interface as on a solid substrate although information on relative speeds was lacking[5].

In the case of the soil protozoon *Naegleria gruberi* the speed of amoeboid movement on glass can be modulated experimentally by a factor of 4 with changes in ambient electrolyte concentration[6,7]. We report here experiments that sought for the first time to compare locomotory rates of a eukaryote cell, *Naegleria*, crawling across glass, agar, plastic and fluorocarbon oil surfaces under a range of defined salt conditions.

Amoebae were deposited onto the experimental substrate by gravity and their motility measured as described in the previous paper[7]. A glass substratum was provided by circular coverslips set up conventionally in a Prior chamber[8]. The plastic substrate was prepared from untreated polystyrene petri dishes cut to the same form as the circular glass coverslips and used similarly. The agar substrate was prepared by pouring 0.5% agar in deionised water onto the bottom glass coverslip of a topless Prior chamber 24 h before use. The Prior chamber was modified to allow a reasonable volume of fluorocarbon oil FC75 (3M Company, London, England) to be accommodated in the water-oil substrate experiments. The density of the fluorocarbon liquid was stated to be 1.75 g/cm$^3$ at 25 °C. During these experiments, which were conducted at room temperature, the composition of the aqueous phase within the tissue culture chamber was changed by perfusion with deionized water or electrolyte until the specific conductivity of the eluant matched that of the solution entering the chamber.

It can be seen from the table that no significant differences were recorded in the speed of locomotion of *Naegleria* amoebae at a given concentration of NaCl regardless of which of the 4 experimental substrates were employed. These results are in marked contrast to those obtained with vertebrate fibroblasts which are able neither to locomote across moist agar[9] nor to spread on unmodified polystyrene, a substrate of low wetability[4].

Given the chemical heterogeneity of soil and the wide geographical distribution of *Naegleria* it is unlikely that this amoeba should demonstrate (restricted) specificity towards locomotory substrates. Nonetheless the interstices of soil should present (unless acid conditions prevail) one common feature to an amoeba, namely a water covered particulate surface bearing negative charge[10]. It is likely therefore that for this cell moving through its natural environment interactions with the substrate will be dominated by those nonspecific forces occurring between closely apposed plates of like charge separated by weak electrolyte. However it should be stressed that artificial substrates in the form of thin films on glass of substances which have a strong and particular affinity for the *Naegleria* cell surface can be prepared. Such experimental substrates, for example polylysine[11] and concanavalin A[12], severely impede amoeboid locomotion.

The results obtained with fluorocarbon oil are at first sight surprising but studies on vertebrate cell spreading on protein oil/water interfaces have shown that leucocytes and macrophages will spread on fluid substrata having viscosities about 10,000 times lower than those required for fibroblast spreading[13]. Thus there is probably a great difference between various cell types regarding the type of interface required for spreading and/or locomotion.

Lack of substrate specificity may not be a ubiquitous character of movement of amoebae. It has been shown that some genera e.g. *Sappinia* do not extend well on glass but do so on agar surfaces[14].

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## A bioreactor for continuous treatment of waste waters with immobilized cells of photosynthetic bacteria

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*Summary.* A bioreactor for assaying immobilized cells of photosynthetic bacteria for hydrogen production under optimal operating conditions was made. High and lasting $H_2$-photoevolutions were obtained using waste waters as substrates.

Numerous procaryotes, belonging to physiologically and taxonomically different groups, are able to produce $H_2$ (Zajic et al.). Under suitable conditions, photosynthetic bacteria of the family *Rhodospirillaceae* can photo-metabolize organic substrates forming $CO_2$ and $H_2$ (Gest et al.[2]) according to the equation: