Negative Chemotaxis of Zoospores of *Phytophthora cinnamomi*

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SUMMARY

Zoospores of *Phytophthora cinnamomi* in a concentration gradient of hydrochloric acid or its chloride salts exhibited acute, repetitive turning movements at a point which corresponded to a critical concentration of cation and not to a critical concentration gradient. The critical concentration was calculated to be $37\ \mu\text{M}$ for H^+ , $628\ \mu\text{M}$ for K^+ , $5040\ \mu\text{M}$ for NH_4^+ and $6480\ \mu\text{M}$ for Na^+ with Cl^- as the co-ionic species. The result of the negative chemotactic reaction was that zoospores swam away from the source of the chemical, regaining normal motility quickly as they returned to lower concentrations of the cation. Zoospores encysted immediately when suspensions were mixed with acids or salts having a concentration in the mixture greater than that which induced the turning reaction. They encysted swiftly at slightly lower concentrations of the cation. Negative chemotaxis appears to be largely an all-or-none response to a threshold concentration, but weaker effects caused by prolonged exposure to sub-threshold concentrations probably also occur. The response of zoospores to the various cations followed the Hoffmeister lyotropic series for cation exchange reactions, and negative chemotaxis can be interpreted as involving surface exchange phenomena.

INTRODUCTION

Zoospores of *Phytophthora cinnamomi* Rands are biflagellate unicells of a root-infecting fungus that occur naturally in the interstitial water of the larger cavities and cracks in soil. They respond to chemical, electrical and mechanical stimulation (Zentmyer, 1966; Khew & Zentmyer, 1971; Allen & Newhook, 1973) in much the same manner as zoospores of other phycomycetes (Katsura & Miyata, 1971; Khew & Zentmyer, 1971) and indeed as living cells in general (Jennings, 1906; Singer & Tasaki, 1968). One of the simplest behavioural responses in zoospores and many other organisms is that of negative chemotaxis. An 'avoiding reaction' (Jennings, 1906) occurs when an organism swims by chance to some critical point in a concentration gradient. The same type of reaction occurs when cells encounter mechanical obstructions (Jennings, 1906) and it is now known that both chemical and mechanical stimuli leading to the 'avoiding reaction' induce depolarization of the plasma-membrane prior to flagellar re-orientation (Eckert, 1972). The compounds most consistently able to induce depolarization responses and negative chemotaxis are acids and halide salts of monovalent cations (Singer & Tasaki, 1968; Eckert, 1972; Doetsch, 1972).

Despite the simplicity of the negative chemotactic reactions, there has been only one quantitative study. Smith & Doetsch (1969) examined the rate at which motile *Pseudomonas fluorescens* moved away from sources of different acids. As acid diffused from the source

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into the bacterial suspension, a band of bacterial cells developed and receded from the acid at a rate proportional to the concentration of hydrogen ion at the source. The migration rate of the response band was affected also by the degree of dissociation of the acid and the composition of the motility medium. These data were unfortunately not amenable to mathematical description.

The experimental system of Smith & Doetsch (1969) has been modified by Allen & Newhook (1973) to permit direct observation of zoospore behaviour as chemicals diffuse from an effectively infinite source into cylindrical glass capillaries containing zoospores. This method, known as the 'swim-out test', is amenable to exact mathematical description. It permits an examination of negative chemotaxis of zoospores to cations with much greater precision than in any previous study, and enables various hypotheses relating stimulus and response to be evaluated.

METHODS

Organism. *Phytophthora cinnamomi* Rands, isolate FJN1672 (Allen & Newhook, 1973), was used throughout.

Preparation of zoospores. The methods of Chee & Newhook (1966) were used to induce sporulation and obtain synchronous release of zoospores into glass-distilled water (pH 6.7 in absence of CO₂). The zoospore density was $(30 \pm 5)/\mu\text{l}$ unless otherwise stated, and the suspensions had a pH of 6.0. Disposable 5 μl 'Microcaps' (Drummond Scientific Co., U.S.A.), 32 ± 1 mm in length, were cleaned by dropping them dry into boiling 10 M-HCl and rinsing them thoroughly in glass-distilled water immediately before use. Each tube was filled by immersing one end in a bulk reservoir of zoospore suspension, and then the whole tube was submerged in this reservoir for 30 to 60 min to allow zoospore motility to stabilize.

Swim-out test. Solutions of known molarity were prepared from A.R. grade HCl, KCl, NaCl, or NH₄Cl with glass-distilled water. The hydrogen ion activity of all solutions was determined by using glass-calomel electrodes. The solutions were dispensed separately into 90 mm diam polystyrene Petri-dish bases to a depth of 5 mm. Glass capillaries, filled with zoospore suspension as above, were submerged in these dishes and zoospore behaviour was observed microscopically by means of dark-field illumination. Some swim-out tests were terminated after 30 min by transferring tubes to water at 60 °C. This immediately immobilized the zoospores without altering their distribution. Counts were then made at successive 2 mm intervals, and both the total (residual) count and a mean count for corresponding positions at each end of the tube were calculated (Allen, 1973). The distribution of zoospores in tubes placed in hot water without exposure to test solutions was used for controlled comparisons.

Band migration. The formation of response bands was observed and their positions relative to the open ends were recorded (± 0.05 mm) with a travelling microscope at eight different times between 0.5 and 64.0 min (± 5 s) after submerging each tube in the test solution (Allen, 1973). The measurements at each end of the tube were averaged for each time. Four tubes (two on each of two different occasions) were observed for each test solution; the standard error was approximately 5 % of the mean. All tests were carried out on a laboratory bench at ambient temperature (22 to 24 °C).

Indicator test. Crystals of bromocresol green (Gurr, 1960) were dissolved in glass-distilled water (0.01 %, w/v) and the solutions were adjusted to pH 6.0 using 0.1 M-NaOH. Approximately 25 ml of this solution was retained; and the remainder was titrated to pH 2.15 with 1.0 M-HCl and then dispensed into Petri dishes as above. During titration the colour of the indicator solution was blue at H⁺ concentrations less than 25 μM and changed

Table 1. *Diffusion coefficients of the compounds used for swim-out tests*

Compound	Diffusion coefficient (mm ² /s)	Reference
HCl	0.0033	Longworth (1954)
KCl	0.0019	Weast (1971)
NaCl	0.0015	Weast (1971)
NH ₄ Cl	0.0019	Weast (1971) from limiting conductance data

to yellow at concentrations of H⁺ greater than 200 μ M. The concentration of H⁺ increased in direct proportion to the amount of titrant added. Clean glass capillaries, 800 μ m internal diam, were sealed at one end in a Bunsen-burner flame so that the lumen was 16.0 ± 0.5 mm in length. The lumen of each tube was filled by injection with indicator solution (pH 6.0) and the tubes were submerged in test solution as in the zoospore experiments. The colour of the indicator solution changed as acid diffused into the tubes and the position of the boundary (blue to pale green, corresponding to 25 to 80 μ M-H⁺) was recorded (± 0.5 mm) at eight different times as in the zoospore experiments. These measurements were compared with those of response-band positions at the same intervals of time as in zoospore experiments conducted with the same concentration of HCl at the source.

Mathematical description. The concentration (C) of the diffusing chemical at any position in the tube was calculated for any time after the start of the experiment ($t = 0$) by using Fick's second law of diffusion (Crank, 1956)

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \quad (1)$$

with boundary conditions

$$C(0,t) = C(b,t) = C_0; \quad C(x,0) = 0 \quad (2)$$

and defining b as the length of the glass capillary and x as the distance from one end. The solution may be written

$$C(x,t) = C_0 \left\{ 1 - \sum_{n=1,3}^{\infty} \left[\frac{4}{n\pi} \sin \left(\frac{n\pi x}{b} \right) \times \exp(-n^2\pi^2 Dt/b^2) \right] \right\}. \quad (3)$$

The diffusion coefficient (D) was assumed to be constant for any given chemical (Table 1). Numerical solutions of eqn 3 yield concentration curves at time t (e.g. Fig. 1) which are symmetrical about the centre of the tube ($x = b/2 = 16$ mm). They are valid for diffusion into tubes with one end sealed (as in the indicator test) if b is taken as twice the length of the tube.

RESULTS

Zoospore behaviour in isotropic solutions

Zoospores swimming freely in distilled water had normal motility as described by Allen & Newhook (1973). Their flagella extended fore and aft of the rigid zoospore body giving each cell an effective length (L) of 30 μ m or more and a capacity for locomotion at speeds exceeding 5 L/s. Motility was maintained for more than 8 h. However, when suspensions of zoospores in distilled water were mixed in equal proportions with 10 mM solutions of HCl or its salts, zoospore motility ceased immediately and the cells encysted or, in some instances, ruptured before encystment was completed. Zoospores also lost motility quickly and encysted when mixed with HCl and KCl of greater dilution; suspensions mixed in equal proportions with 20 μ M-HCl or 1 mM-KCl lost motility within 30 s.

Fig. 1. Distribution of zoospores in glass capillaries before (◇) and after (◆) submergence in 5 mM-HCl for 30 min. Each point (◇) is the mean of 24 observations and its height represents more than twice the standard error. Each point (◆) is the mean of six observations and the standard error is indicated as a vertical line where appropriate. The theoretical concentration distribution of HCl at 30 min is represented by the thick line.

The motility of zoospores in capillary tubes was disoriented by collision with the walls as described by Allen & Newhook (1973). However, zoospores swam actively in these tubes for more than 8 h, and were uniformly distributed at the commencement of swim-out tests as indicated by the distribution counts in control tubes (Fig. 1).

Effect of hydrochloric acid

When tubes with zoospores were submerged in test solutions containing $1 \mu\text{M-H}^+$ or less, zoospores swam out into the surrounding solution. However, when tubes with zoospores were submerged in $10 \mu\text{M-HCl}$, zoospores swam at first to the entrance of the tube where they turned and swam back into the tube. The turning involved a series of re-orientation movements (Allen & Newhook, 1973) as zoospores attempted to swim away from the mouth. After 30 min, zoospores began to leave the tube but motility in the surrounding medium was abnormally slow ($< 1 \text{ L/s}$) and encystment generally occurred within 1 mm of the end of the tube. After approximately 60 min, encystment progressed into the tube from the ends so that at 8 h no motile cells remained. No cells ruptured during encystment under these conditions.

Zoospores not only failed to swim out of tubes submerged in HCl solutions of 0.1 mM or more, but a response band developed at each end of the tube and this receded continually from the source of acid (Fig. 2). Bands receded from 100 mM-HCl at a faster rate than from 0.1 mM-HCl . The response bands were initially less than 0.1 mm wide and consisted of only a few cells, but their width and density increased with time. At 30 min the bands in tubes submerged in 5 mM-HCl were approximately 4 mm wide and the proximal edge had migrated approximately 8 mm (Fig. 1). This edge corresponded closely with the apparent 'edge' of the diffusion zone (Fig. 1) as calculated from eqn 3.

In tubes submerged in HCl solutions of 1 to 100 mM , zoospores turned at the proximal edge of the response band as abruptly as if they had encountered a mechanical obstruction (Allen & Newhook, 1973), particularly in the early stages of band development. These cells

Fig. 2. Migration of response bands of zoospores swimming in tubes submerged in various concentrations of hydrochloric acid. All test solutions and zoospore suspensions were prepared using glass-distilled water. The radius of each symbol represents the standard error of a mean based on eight observations. The lines are theoretical predictions of band position calculated for two different hypotheses. The thin lines represent predictions of position based on a best estimate (Table 2) of a critical concentration *gradient* at the response band region. The thick lines represent predictions based on a critical *concentration* ($37 \mu\text{M}$). The broken line gives the predicted position based on an estimate of critical concentration of $27 \mu\text{M}$.

stopped, pivoted, moved forward in a new direction, and repeated this procedure until oriented away from the source of acid. In so doing they moved transversely to the gradient and collided frequently with the walls of the tube. Zoospores that remained on the acid side of the response band were always non-motile.

Zoospores regained normal motion quickly as they swam away from the region where 'avoiding reactions' (*sensu* Jennings, 1906) were occurring. Thus the distribution of zoospores at 30 min (Fig. 1) was characterized by a cleared zone near the end where zoospores had successfully 'avoided' the diffusing acid, a region where the zoospores had concentrated during the re-orientation process, and a region in which the frequency of zoospores decreased with increasing distance to a value equal to or above that of the frequency in tubes that had been immobilized immediately before exposure to chemical solutions (i.e. controls). Zoospores always ultimately encysted in tubes submerged in HCl solutions, beginning 50 min after tubes were submerged in 100 mM-HCl and 8 h after tubes were submerged in 0.1 mM-HCl.

Response bands did not develop in tubes in which zoospores had encysted or had been immobilized with heat before testing.

Effect of other cations

Response bands were formed when tubes were submerged in 10 mM solutions of HCl, KCl, NaCl and NH_4Cl having pH values of 2.15, 5.90, 5.50 and 5.60 respectively. The rate at which the response bands receded from the ends of the tubes after submergence varied with the different chlorides (Fig. 3) but was not consistently related to pH at the source or to the diffusion coefficient. For example, response bands receded from KCl at a faster rate than from NH_4Cl despite the similarity of the diffusion coefficients (Table 1) and pH. In addition, the position of the response band for KCl at 32 min (Fig. 3) was 5.0 mm from the

Fig. 3. Migration of response bands of zoospores swimming in tubes submerged in 10 mM solutions of HCl ($\diamond$), KCl ($\blacklozenge$), NH_4Cl ($\square$) and NaCl ($\blacksquare$). The vertical height of each symbol (sometimes extended) represents twice the standard error of a mean based on eight observations. The lines were predicted from eqn 3, based on the best estimates of constant concentration at the response band region for each compound (Table 3).

end, which is less than the distance measured (> 7.0 mm) at an equivalent time ($32 D_{\text{KCl}}/D_{\text{HCl}} = 18.4$ min) for hydrochloric acid. Nevertheless, the method of response-band formation and the pattern of encystment was consistent in each case with observations made in previous experiments involving HCl at various concentrations.

Evaluation of hypotheses relating stimulus and response

Two classes of response have been considered in attempting to explain the data obtained here. The first class is that of a continuous response to some function of the concentration or concentration gradient; such a response is one in which over a wide range, a greater intensity of stimulus leads to a greater response. In the second class, the response is a threshold effect. In this case the organism exhibits either a total response or no response at all, according to whether some threshold in concentration or concentration gradient (or some function of these variables) is exceeded. In a threshold response, once the response has been initiated a greater stimulus will produce no greater response.

Continuous response to concentration or gradient. Recent studies on positive chemotaxis (Dahlquist, Lovely & Koshland, 1970) have yielded evidence for a continuous response to the gradient of the logarithmic concentration (i.e. to $1/C \times \partial C/\partial x$). A continuous response could lead to band formation in the capillaries if the speed of migration (v) were proportional to the concentration or concentration gradient. That is, a continuous response to some function of the concentration [$v = f(C)$] could lead to band formation provided that $f(C)$ decreased with increasing distance from the ends of the tube at any fixed time. If for example the speed were proportional to the concentration gradient ($\partial C/\partial x$), then $1/v \times \partial C/\partial x$ would be constant at the band position as the band moved in from the ends of the tubes.

Calculations based on eqn 3 indicate that the concentration at the band position remains

Table 2. *Best fit values for critical chemotactic concentration (α) and concentration gradient (β) for experiments with different hydrochloric acid concentrations*

Test solution		Best estimate of α (μM)	Best estimate of β ($\mu\text{M}/\text{mm}$)
Nominal concentration (mm)	pH		
0.1	4.10	27	N.C.
1	3.10	35	47
10	2.15	37	60
100	1.20	38	79

N.C., Not calculated.

All estimates have been corrected for hydrogen ion activity as measured by glass-calomel electrodes.

roughly constant with time while the concentration gradient does not. The possibility of a continuous response to the concentration can thus be eliminated, since the speed of movement of the band varies greatly as the band migrates inwards (Fig. 2) although the concentration at the band position remains the same. The speed of movement of the band is roughly proportional to the concentration gradient (and hence to $1/C \times \partial C/\partial x$) at the band position. Comparison of the experimentally observed band position and the concentration gradient in the tubes for differing initial concentrations, however, shows that the proportionality constant varies systematically with the starting concentration and hence the possibility of a continuous response to concentration gradient or to $1/C \times \partial C/\partial x$ can also be eliminated. Indeed a continuous response to $1/C \times \partial C/\partial x$ would not lead to band formation since this ratio increases with increasing distance from the ends of the tube at any fixed time.

Threshold response to concentration or gradient. Microscopic observations of the response of individual zoospores provides strong evidence for a threshold effect above which an avoiding reaction is initiated. Such a response would lead to a correlation of band position with the position in the tube where the threshold is traversed.

The data for migration from various concentrations of HCl were examined for two threshold hypotheses: either the band was always at some point of critical absolute concentration (α), that is

$$C(x,t) = \alpha \quad (4)$$

or the band position corresponded to some point of critical concentration gradient (β), that is

$$\partial C/\partial x (x,t) = \beta. \quad (5)$$

Solutions of eqns 4 and 5 at various times corresponding to the times of observation of the band positions were obtained with $C(x,t)$ given by eqn 3 and using a least-squares fitting procedure to optimize the constants α and β for each experiment. The estimates of α were similar for all experiments, whereas those of β were not (Table 2). Predictions of band position based on the best estimate of β for any one experiment were not related to the experimental observations (Fig. 2), whereas those based on α provided a remarkably good fit to the experimental data. Further, when a single value of $\alpha = 37 \mu\text{M-HCl}$ was used to predict the band position in all of the experiments, the predictions were within the limits of experimental error for all of the 23 observations involving concentrations of HCl of 1 mm or greater. The fit to the 0.1 mm data is very sensitive to small changes in the threshold parameter α , and in Fig. 2 the fits for $\alpha = 27$ and $\alpha = 37 \mu\text{M}$ are both shown. The poorer fit in this case may be due to inaccuracies in the measured pH (a change of 0.1 in pH leads

Table 3. *The critical and relative concentrations of different chlorides of monovalent cations inducing the 'avoiding reaction' in zoospores of P. cinnamomi swimming in distilled water of pH 6.0*

Compound at source	Critical concentration, α (μM)	Value relative to KCl
HCl	37	$\times 0.06$
KCl	628	$\times 1.00$
NH_4Cl	5040	$\times 8.03$
NaCl	6480	$\times 10.3$

to a 20 % change in α), to the larger experimental errors and wider bands in this experiment, to a possible effect on the pH by the organisms themselves, or to a combination of such effects. All of the data are consistent with the hypothesis that the band position is at a position of critical hydrogen ion concentration of $37 \pm 10 \mu\text{M}$.

The hypothesis of response at a critical concentration gradient is therefore untenable. It is also clear from the fits to α and β that a threshold response to $1/C \times \partial C / \partial x$ would not provide a satisfactory fit to the experimental data. The hypothesis of Jennings (1906), however, which related response to a critical absolute concentration, provides a very satisfactory explanation of the experimental observations.

Indicator test on hydrochloric acid diffusion

The diffusion of hydrogen ions into glass capillaries previously filled with indicator solution (pH 6.0) can be detected by observing the migration of the colour boundary which corresponds to a region of known hydrogen ion concentration. The point of colour change in the indicator solution receded inwards as diffusion from 10 mM-HCl progressed into the tube. The rate of migration of this colour boundary was almost identical to that of response bands in two separate zoospore tests which had 10 mM-HCl at the source (Figs. 2 and 3). There was a linear relationship between response-band position (Y_t , mm) and colour-boundary position (X_t , mm) at the various times of measurement, the regression equations being:

$$\hat{Y}_{t,A} = 1.09 X_t - 0.23; r = 0.9923, \quad (6)$$

$$\hat{Y}_{t,B} = 1.13 X_t - 0.16; r = 0.9980, \quad (7)$$

where A and B were data for 10 mM-HCl illustrated in Figs. 2 and 3 respectively. The critical correlation coefficient for $P = 0.001$ was $r = 0.9249$ in both instances. It can be seen that the regression coefficients were approximately unity, and the expressions (6) and (7) extrapolate close to the origin at the beginning of the experiment (i.e. when $X_t = 0$; $Y_t = 0$).

The colour change in bromocresol green involves a simple chemical reaction dependent on the concentration of H^+ (Gurr, 1960). Since the colour boundary corresponded to a concentration of 25 to 80 μM H^+ , and this encompassed the predicted concentration at the response-band region (37 μM), the indicator test corroborates the theoretical considerations.

Effect of other cations

The best estimates of critical chemotactic concentrations (α) for various chlorides of monovalent cations were calculated (eqn 4) and are presented in Table 3. The resultant theoretical predictions are illustrated in Fig. 3. The same agreement between observed and predicted values occurs for the salts as for hydrochloric acid, but the concentration at which 'avoiding reaction' occurred in zoospores varied more than 100-fold for the different chlorides (Table 3).

DISCUSSION

Validity and limitations of the all-or-none hypothesis. The results confirm and extend the earlier studies by Jennings (1906) and Smith & Doetsch (1969) on negative chemotaxis in unicellular organisms. The observation by Smith & Doetsch (1969) that the migration of the response band is proportional to the concentration of chemical external to the capillary can be adequately explained in terms of diffusion theory (eqn 1). The microscopic observations and mathematical calculation provide strong evidence for a threshold effect in which individual cells undergo acute, repetitive orientation reactions at a concentration of ions inside the capillary which is constant for each salt, but which varies from salt to salt, showing specificity for the cation. However, there are two observations which are not explained by the all-or-none hypothesis. The first is the inability of zoospores to swim out of tubes submerged in $10\ \mu\text{M}$ -HCl. This external concentration is considerably below the estimated threshold value ($37\ \mu\text{M}$) for an acute 'avoiding reaction'. The second observation is that the sharpness of the response band diminished with time, a phenomenon already noted by Smith & Doetsch (1969). This effect would be expected if the critical concentration varied somewhat between individual zoospores. The band position would then be a measure only of the mean threshold and the width a measure of the spread in threshold amongst individual cells. As the experiment progresses the gradient at the point of critical concentration decreases and the variation in threshold response would be reflected in a band extending over a greater region of the tube. These observations suggest a limited response below the 'threshold' value, and a total response above this value.

The extent to which zoospores respond to subthreshold concentrations may be related to the rate at which they encounter increasing concentrations of stimulant. In the early stages of an experiment, particularly when there is a high concentration at the source, there will be a steep concentration gradient at the point where zoospores respond. Zoospores swimming into this region will encounter this point suddenly. As the experiment progresses, the concentration gradient at the point of critical concentration will gradually decrease, so that zoospores swimming toward the source of chemical will encounter concentrations approaching the threshold for response over an increasingly extended period of time. The observation that zoospores encyst within 30 s when exposed to isotropic solutions of $10\ \mu\text{M}$ -HCl suggests that motility may be affected by prolonged exposure to a concentration of stimulant below the threshold for an acute response. An additional factor may be localized turbulence caused by the swimming activity of zoospores themselves. This would alter the concentration gradient, and hence the rate of diffusion (eqn 1), and help to explain the increasing error of the observed values in the later stages of the experiment (Fig. 2). The errors at the beginning of the experiment were probably related to errors in making the observations at the given times.

Mechanism of chemoreception. The relative magnitudes of the observed threshold concentrations of the different cations (Table 3) follow the classic Hoffmeister lyotropic series for cation exchange reactions. The position of H^+ in this series indicates that the surface properties of the zoospore flagella are consistent with those displayed by exchange resins having fixed anionic groups of low acid strength (Helfferich, 1962). Meves & Chandler (1965) have observed a similar selectivity in their studies of excitability in squid axons, the relative selectivity of $\text{Na}^+:\text{K}^+$ (8–12:1) being similar to that reported in Table 3. Tasaki, Singer & Takenaka (1965) consider the external surface of squid axon to have a net negative charge derived from dissociation of carboxylate radicals. Since zoospores also have a net surface charge which is negative (Khew & Zentmyer, 1971), it appears that the same type of

exchange reaction is involved in signal reception in zoospores as in depolarizing events in squid axon membranes.

Mechanism of response. The effect of cations on membrane-mediated reactions may be related directly to their effects on zoospore conformation. Allen & Newhook (1973) have observed that in normal motility the flagella are extended fore and aft of the zoospore body and exhibit high activity. This extended conformation requires the existence of a strong force opposing the natural tendency for all proteins to adopt a random coil configuration of high entropy. The most probable force is one of electrostatic repulsion between adjacent negative charges along the surface of the flagellum. If this force is strong enough the rod-like configuration can be maintained, but diminution of this force would result in collapse of the flagellum and therefore impairment of the normal swimming motion. In the presence of distilled water only, the force between these negative charges would be maximal because overall electro-neutrality could be maintained by surface orientation of polar water molecules. This would not effectively shield adjacent fixed charges at the membrane surface. However, as the concentration of ions increased in the bulk solution, the fixed negative charges would be surrounded by a cloud of small ions (both positive and negative). This would effectively shield the fixed surface charges and the electrostatic force would decrease. When the concentration of ions at the surface of the flagellum reached a critical value the shielding of fixed charges by small ions would be great enough to allow the flagellum to relax from its extended form. An unstable state of motility would ensue, with the aft flagellum continuing to function normally at first and the forward flagellum becoming ineffectual or exhibiting asymmetric beating.

Evidence for such a relaxation effect can be deduced from the published photographs of turning activity in monoflagellate gametes of *Blastocladiella emersonii* (Miles & Holwill, 1969) and *Turbularia crocea* (Miller & Brokaw, 1970). Either asymmetric beating or differential activity of zoospore flagella could lead to the 'avoiding reaction', but direct photographic evidence has yet to be obtained.

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