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Two Mechanisms of Chemotaxis in *Paramecium*

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Summary. Paramecia show chemotaxis, that is, they accumulate in or disperse from the vicinity of chemicals. This study examines both the avoiding reactions (abrupt random changes of swimming direction) and velocities of normal and mutant paramecia in attractants and repellents and shows that the animals accumulate or disperse either by changing the frequency of avoiding reactions or by changing swimming velocity. Mutations or conditions that eliminate avoiding reactions abolish the chemotaxis response to chemicals that cause accumulation or dispersal by modulation of frequency of avoiding reactions but not the response to chemicals that cause chemotaxis by modulation of velocity.

The current knowledge of the bioelectric control of the swimming behavior in *Paramecium* and observations of mutants defective in bioelectric control and in chemotaxis are used to develop a hypothesis for membrane potential control of chemotaxis: attractants that require the avoiding reaction slightly hyperpolarize the membrane; repellents that require the avoiding reaction slightly depolarize the membrane; repellents that cause chemitaxis by modulation of velocity strongly hyperpolarize the membrane.

Introduction

The behavior of *Paramecium* was carefully described by Jennings over seventy years ago (Jennings, 1906). Forward swimming and avoiding reaction (an abrupt random change of swimming direction) are the two basic components of swimming behavior. Paramecia respond to different stimuli by modulating these two components. Jennings studied the response of paramecia to stimuli such as chemicals, heat, gravity, touch, water currents, and electric fields. The responses of *Paramecium* to chemicals, termed chemotaxis, has been studied further by modern workers (Dryl, 1973; Nakatani, 1970). Since no orientation toward or away from a chemical stimulus has ever been observed, this is more accurately termed chemokinesis (Fraenkel and Gunn, 1961). This study examines the roles of velocity and the avoiding reaction and hypothesizes the physiological bases of chemokinesis by taking advantage of three modern developments. First, the bioelectric bases of ciliary motion are now known (Kinosita et al., 1965; Naitoh and Eckert, 1968). Second, two quantitative methods to assay chemokinesis have been designed (Van Houten et al., 1975). Third, mutants, each defective in one aspect of swimming behavior or in chemokinesis, are now available (Kung et al., 1975).

In *Paramecium* one cannot affect frequency of avoiding reaction without affecting velocity because these two basic components of *Paramecium* swimming behavior are both under bioelectric control at the cell membrane. The first, velocity of forward swimming determined by frequency and angle of ciliary beating, is controlled by shifts from the resting membrane potential (Machemer, 1974). The second, avoiding reaction, is a transient reversal of the cilia that corresponds to a calcium action potential (Kinosita et al., 1965; Naitoh and Eckert, 1968; Eckert, 1972). Small depolarizations of the membrane trigger action potentials and decrease frequency and angle of forward ciliary beating (Eckert, 1972; Machemer, 1974; Machemer and Eckert, 1975). Hence, small depolarizing stimuli make the cells undergo avoiding reactions more frequently and swim more slowly. Hyperpolarization has the opposite effects. Therefore, monitoring the swimming behavior of the cells is a convenient means of monitoring their electrical characteristics, for example, changes of membrane potential (E_m).

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Previously, the modulation of frequency of avoiding reactions was thought to be sufficient for accumulation in or dispersal from chemical stimuli (Jennings, 1906; Dryl, 1973). Velocity changes in the presence of attractant and repellent solutions were thought to be peripheral and were not incorporated into the understanding of a mechanism of chemokinesis (Nakatani, 1970; Kinosita et al., 1964). In this study, the frequency of avoiding reactions, velocities and chemokinesis responses of normal cells are measured in attractants and repellents and are compared with the results for behavioral mutants. These mutants are cells defective in the avoiding reaction or in chemokinesis (Kung et al., 1975). I conclude that there are two mechanisms of chemokinesis, one dependent on avoiding reaction and one on velocity modulation, and I propose that change from the resting membrane potential is the unifying parameter that controls chemoaccumulation and dispersal.

Materials and Methods

Strains and Cell Culture. The following strains of *P. tetraurelia* (previously *P. aurelia*, species 4) are used: 51-S wild type; d4-95 Pawn B; d4-94 Pawn A; d4-91 Fast-2; d4-148 Atalanta; and d4-530 chemokinesis defective (Kung et al., 1975; Kung, 1971a; Van Houten, 1977). Cells are grown in Cerophyl medium inoculated with *Aerobacter aerogenes* 24 h before use and buffered with 3.5 mM Na_2HPO_4 (Sonneborn, 1970).

Solutions. All solutions contain 1.3 mM Tris (Tris(hydroxy-methyl)aminomethane), 1 mM citric acid, 1 mM $Ca(OH)_2$, pH 7 adjusted with Tris. Test and control solutions contain salts added before adjustment of pH. In the case of the KOH solution, the control, 1 mM KCl solution, is prepared first and the pH is adjusted to 7 with Tris. The 1 mM KOH solution is made with carbonate-free KOH (Fisher Dilutit) with the same addition of Tris and the pH is not further adjusted. The final pH is measured consistently at 8.65.

In each set of experiments, there is only one ion species that differs between control and test solution. For example, 5 mM KCl in buffer pH 7 is control for 5 mM potassium acetate (KOAc) in buffer pH 7. Acetate is an attractant relative to chloride, and the counter ion in both solutions, potassium, cannot be responsible for accumulation of animals in KOAc.

T-maze Assay. The T-maze is an assay for chemokinesis designed to present a test and a control solution to a population of animals (Van Houten et al., 1975). Before the tests, the cells are centrifuged at 350 × g for 2 min in an oil testing centrifuge and are resuspended in the control solution to be used. The cells are diluted again in control solution to a density of about 500 to 1000 cells per ml. Cells in control solution are placed in the shortened stem of a 3-way stopcock. One arm of the T and the plug are filled with control solution; a test solution fills the other arm. The stopcock is opened and the cells are allowed to migrate for 30 min. An index of chemokinesis is defined as I_{che} = animals in the test solution arm/animals in test and control solution arms at the end of the test. An I_{che} around 0.5 indicates indifference to the test solution; $I_{che} > 0.5$ indicates attraction; $I_{che} < 0.5$ indicates repulsion. I_{che} data are presented as averages ± one standard deviation. I_{che}'s are summarized in Table 2 for easy reference.

The modified T-maze has a two-way plug in a three-way stopcock. The test solution fills one arm of the T; the control solution fills the other. Animals in control solution fill the plug. As soon as the plug is opened the animals interface with the control or test solution, creating a step gradient of test solution. I_{che} is calculated in the same manner.

The terms attractants and repellents used in this paper are operationally defined. Both metal ions and organic molecules are tested for their chemokinetic potency. Metal ions can act directly by fluxes through ion channels or gates. However, either inorganic or organic attractants and repellents may first be bound by receptors and the reception transduced to signals in the form of ion fluxes.

Measurement of Frequency of Avoiding Reaction (F_{AR}) and Velocity (V). A few cells are transferred with a minimum amount of culture fluid to control solution. After 30 min, a single cell is transferred into a small pool of control or test solution. The frequency of avoiding reaction (F_{AR}) in min^{-1} is measured by monitoring the locomotion of paramecia, one at a time, under a stereomicroscope while recording each avoiding reaction on an event recorder. The small and uniform delay (~0.5 s) in registering the events has little consequence in the experiments since the number of avoiding reactions is integrated through 1 min intervals before further analyses. The effective swimming speed (V) is determined by measuring the lengths of tracks made by swimming paramecia in dark-field long-exposure macrophotographs (Kinosita et al., 1964; Chang and Kung, 1973). Cells are centrifuged, resuspended in control solution for 30 min and then $^1/_2$ Pasteur pipet of cells is injected into a pool of test solution. Long exposure photographs are taken at about 5 s, 15 min, and 30 min after injection. Only the tracks clearly in focus are measured. No attempts are made to choose the straight paths. End-to-end distances of tracks that contain bends (avoiding reactions) are also included, although such tracks tend to go out of focus and are often excluded for this reason. Straight paths with no ARs are given more weight. Therefore, the true average distances and, hence true Vs in KCl, are smaller than those measured when AR is not present. The measurements most likely underestimate the difference between the V in KCl and the V in KOH or KOAc.

Note that the F_{AR} and V measured should not be taken to mean the F_{AR} and V in the T-maze. They are measured after a sudden transfer into a uniform concentration of test solution and not in a spatial diffusion gradient of the test substance. The sudden transfer should, however, simulate a sharp step gradient to which the animals respond in the modified T-maze (Van Houten, 1976). For a test of KCl versus KOAc, I_{che} is maximal within 40 s, while the gradient is still very steep and little diffusion has occurred (Van Houten, 1976). Upon swimming into the test arm, the cells should be experiencing a temporal gradient. Therefore, the F_{AR} and V experiments that utilize a sudden transfer of cells (a temporal gradient) should measure behavioral changes that have relevance for chemokinesis.

Paramecia adapt and F_{AR} and V return to basal levels when solutions are not changing (Dryl, 1959a; Van Houten, 1976, 1977; Machemer, 1976; Kung and Gee, unpublished observations). Animals in the T-maze are presumably adapting to uniform concentrations of attractant or control chemical in the arms of the maze and reacting to the gradient around the stopcock plug. The use of 30 min incubation before measurement of V and F_{AR} necessarily means some adaptation to the incubation solution. However, the incubation is used because i) cells have to be washed free of undefined culture fluid, which is a strong attractant (Van Houten, 1976); ii) cells show negative geotaxis in buffer and can be concentrated at the top of a cylinder of buffer; iii) variable incubations up to 30 min prior to chemokinesis assays show no significant change in I_{che} (unpublished observations); iv) there is little difference in

the initial F_{AR} in transfer of cells to 5 mM KCl directly from culture fluid or after the short incubation in 5 mM KCl (Van Houten, unpublished observations).

Results

Classical Mechanisms of Chemokinesis

Fraenkel and Gunn (1961) show that accumulation and dispersal of animals can be due to different mechanisms (Table 1). For example, for some animals, decreasing the rate of random turns (klinokinesis) causes accumulation. For others, decreasing the velocity of locomotion (orthokinesis) is the cause. The findings for paramecia are summarized under two mechanisms of attraction and repulsion below.

Table 1. Classical mechanisms of chemokinesis

Mechanism	Behavior required	changes required for	
		Attraction	Repulsion
Klinokinesis	Avoiding reaction	Decreased F_{AR}[a]	Increased F_{AR}
Orthokinesis	Velocity modulation	Decreased V[b]	Increased V

[a] F_{AR} Frequency of avoiding, or rate of change of direction; [b] Average velocity of swimming: based on Fraenkel and Gunn (1961)

Attraction with Decreasing F_{AR}: Mechanism I

Acetate was found to be an attractant relative to chloride, whether potassium was used as counter ion, that is, with 5 mM KCl and 5 mM KOAc filling the control and test arms of the T-maze ($I_{che} = 0.84 \pm 0.07$, $n = 17$), or sodium was used as counter ion ($I_{che} = 0.82 \pm 0.11$, $n = 7$). The frequency of avoiding reactions was measured upon transfer of cells to 5 mM KCl or attractant 5 mM KOAc (Table 3). The cells consistently show fewer ARs in attractant. This is true of the F_{AR} in attractant NaOAc relative to NaCl as well (Van Houten, 1977).

Solutions that abolish AR prohibit attraction. Paramecia do not show AR in solutions where $(Ca^{++})/(M^{+})$ is high (M^{+} = concentration of other cations) (Naitoh and Eckert, 1968). In such solutions, the threshold of action potentials is high. Little or no avoiding reaction is observed in 2.5 mM Ca^{++} solutions. The attraction to 5 mM acetate from chloride is extinguished with 2.5 mM Ca^{++} as counter ion ($I_{che} = 0.46 \pm 0.21$, $n = 4$).

Mutants without AR cannot be attracted. Pawn mutants have lost their ability to generate action

Table 2. Indices of chemokinesis in *P. tetraurelia*

C arm[a]	T arm[a]	I_{che}[b]		
		Wild type	Pawn	Other mutants
Attraction I				Fast-2
5 mM KCl	KOAc	0.84 ± 0.07	0.55 ± 0.05	0.87 ± 0.09
5 mM NaCl	NaOAc	0.82 ± 0.11	0.41 ± 0.11	0.51 ± 0.08
5 mM KCl	K lactate	0.83 ± 0.06	0.46 ± 0.13	$0.79'' \pm 0.11$
2.5 mM $CaCl_2$	$Ca(OAc)_2$	0.46 ± 0.21	–	–
Repulsion I				Atalanta
0.1 mM KCl	quinidine-HCl	0.08 ± 0.04	$0.44' \pm 0.03$	0.51 ± 0.13
5 mM KCl	NaCl	0.27 ± 0.13	0.60 ± 0.11	–
5 mM KCl	2.5 mM $BaCl_2$	0.21 ± 0.11	0.46 ± 0.14	–
5.5 mM NaCl	5 mM TEA^{+} 0.5 mM NaCl	0.08 ± 0.11	0.48 ± 0.07	–
Repulsion II				$Acetate^{-}$
1 mM KCl	KOH	0.38 ± 0.05	0.34 ± 0.08	–
5 mM NaCl	2.5 mM $CaCl_2$	0.24 ± 0.07	–	–
5 mM NaCl	NaOAc	(0.82)	(0.41)	0.14 ± 0.05
5 mM KCl	NaCl	(0.27)	(0.60)	Fast-2 0.36 ± 0.05

[a] C and T arms refer to the arms of the T-maze filled with control and test solutions respectively. [b] I_{che} (Index of chemokinesis) is defined as the number of animals in the T arm/total animals in T and C arms. Data are averages ± one standard deviation. $I_{che} > 0.5$ indicates attraction; $I_{che} < 0.5$ indicates repulsion; $I_{che} \sim 0.5$ indicates indifference. 'Modified T'maze was used. See text for description. I_{che} is calculated in the same way. ''90 min instead of 30 min for test duration. Parentheses indicate numbers given more than once in the table

Table 3. Frequency of avoiding reactions

Min after transfer	Attractant I		Repellent I		Repellent II	
	F_{AR} in control solution 5 mM KCl	F_{AR} in attractant solution 5 mM KOAc	F_{AR} in control solution 0.1 mM KCl	F_{AR} in repellent solution 0.1 mM quinidine-HCl	F_{AR} in control solution 1 mM KCl	F_{AR} in repellent solution 1 mM KOH
1	1.8±1.6	0.8±1.0′	2.2±4.0	13.9± 7.6*	5.1±8.9	0±0*
2			2.6±5.4	12.7± 8.8*	4.6±8.2	0±0
3			1.3±1.9	37.8±33.9*	5.7±7.0	0±0*
4			0.5±1.1	41.3±34.5*	2.6±3.1	0.1±0.3*
5	1.4±1.4	0.9±1.3′	1.2±1.9	22.4±19.5*	3.6±4.9	0±0*
6			1.4±2.5	18.8±28.8*		
10	2.1±1.8	0.8±0.9′				
15	2.8±2.0	0.2±0.4′				
n	20	20	10	6–10″	10	10

Data are the average number of avoiding reactions in minute intervals (F_{AR}) of n cells after transfer to control or test solution ± one standard deviation

* Averages of test and control F_{AR} are significantly different by the Mann-Whitney U test. ′Averages of test and control F_{AR} are significantly different by comparison of means of Poisson distributions of F_{AR}. All solutions contain salts indicated in buffer pH 7, except KOH solution pH 8.6 (see Methods). ″When cells are transferred to quinidine-HCl after 4 min some undergo a prolonged backward swimming component of the AR that can last for more than 1 min. Although the cells are undergoing an AR, the F_{AR} is not meaningful. Therefore, such cells are not included in the calculation of F_{AR} and n for the quinidine experiment is reduced from 10 to 6 by 6 min. The small sample size magnifies the variations in these measurements

potentials, and hence, have no avoiding reactions (Kung, 1971b; Kung and Eckert, 1972; Satow and Kung, 1976a). Pawn B (d4-95) shows no attraction to 5 mM acetate with K^+ or Na^+ as counter ion ($I_{che}=0.55\pm0.05$, $n=3$; $I_{che}=0.41\pm0.11$, $n=4$, respectively). It also shows no attraction to 5 mM lactate ($I_{che}=0.46\pm0.13$, $n=4$), although wild type is attracted ($I_{che}=0.83\pm0.06$, $n=8$).

Fast-2 is a mutant that is insensitive to sodium solutions (Satow and Kung, 1976b). Unlike wild type that show frequent AR in sodium, Fast-2 shows *no* AR and swims fast forward. The mutant is normal in other solutions. As expected, Fast-2 shows normal attraction to 5 mM acetate with K^+ as counter ion ($I_{che}=0.79\pm0.11$, $n=4$) and not with Na^+ as counter ion ($I_{che}=0.51\pm0.08$, $n=12$).

Repulsion with Increasing F_{AR}: Mechanism I

Attraction to a test solution by a decrease in F_{AR} entails repulsion from a control solution by a relative increase in F_{AR}. Thus, the results given in the previous section show that, relative to attractants (acetate or lactate), a neutral agent (chloride) repels paramecia by causing a relative increase in their F_{AR} and that solutions or mutations which abolish AR prohibit repulsion by this mechanism.

To make repulsion more than a trivial case of the inverse of attraction, I have chosen to study chemicals relative to an arbitrary solution, KCl. I found both attractants and repellents relative to this arbitrary neutral solution. The strongest organic repellents known to date are quinine (Dryl, 1973) and its isomer, quinidine. T-maze tests with wild type paramecia, starting with the control arm of 0.1 mM KCl and the test arm of 0.1 mM quinidine-HCl, give an I_{che} of 0.08 ± 0.04, $n=7$. Direct measurement of the F_{AR} shows that paramecia in 0.1 mM quinidine-HCl give avoiding reactions up to about 40/min soon after transfer from 0.1 mM KCl (Table 3). The behavior in quinidine-HCl is complex, and the F_{AR} shows an eventual decay by 15 min. The control transfer from KCl to KCl shows low F_{AR} that decreases in frequency with time (Table 3). The repulsion from quinidine- and quinine-HCl is not due merely to trapping of the cells by frequent AR at the boundary of the entry arm and plug of the T-maze. Conditions were used under which hundreds of animals migrated out of the stem and then distrib-

uted between the control and test arm (Van Houten et al., 1975; Van Houten, 1976).

Ions known to increase F_{AR} repel. Na^+ causes frequent and repeated AR in paramecia (Satow and Kung, 1976a; Satow and Kung, 1974). K^+ elicits many fewer AR at concentrations tested. Relative to 5 mM KCl, 5 mM NaCl is repulsive yielding an I_{che} of 0.27 ± 0.13 ($n=4$). Barium (Ba^{++}) and tetraethyl ammonium (TEA^+) cause action potentials and, therefore, AR (Satow and Kung, 1974; Satow and Kung, 1976c). Solutions containing Ba^{++} or TEA^+ are repulsive to paramecia relative to K^+ and Na^+. Standard T-maze tests of 5 mM KCl versus 2.5 mM $BaCl_2$ give $I_{che} = 0.21 \pm 0.11$, $n=7$, away from $BaCl_2$. Tests of 5.5 mM NaCl versus 5 mM TEA-Cl and 0.5 mM NaCl give $I_{che} = 0.08 \pm 0.11$, $n=4$, away from TEA-Cl.

Mutants lacking AR are not repelled by this mechanism. Pawn B gives I_{che}'s of the following values: $I_{che} = 0.44 \pm 0.03$, $n=2$, in the modified T-maze tests of 0.1 mM KCl versus 0.1 mM quinidine-HCl; $I_{che} = 0.46 \pm 0.14$, $n=8$, in the Ba^{++} experiment and $I_{che} = 0.40 \pm 0.07$, $n=7$, in the TEA^+ experiment described above. Atalanta, a mutant most likely defective in its cilia, is capable of generating action potentials. However, these action potentials cause transient halts instead of avoiding reactions (Kung et al., 1975). Atalanta is not repelled by 0.1 mM quinidine-HCl ($I_{che} = 0.51 \pm 0.13$, $n=4$).

Repulsion with Increasing V When F_{AR} is Near Zero: Mechanism II

Hydroxide ion (OH^-) repels by increasing V. Wild type give an I_{che} of 0.38 ± 0.05, $n=12$, in tests of 1 mM Cl^- against 1 mM OH^- (pH 7.05 versus 8.65) with K^+ as counter ion. After transfer from KCl to KOH, paramecia show *no avoiding reaction*, while the control transfer from KCl to KCl shows the usual frequent ARs and the usual decrease with time (Table 3). This is surprising since reduction of F_{AR} in KOH is expected to cause attraction instead of repulsion. Part of the repulsion mechanism is the modulation of speed, V. A small but significant increase in V is noticed when tracks of paramecia transferred from KCl to KOH are compared to those from KCl to KCl. After a few minutes, the tracks of paramecia in KOH become slightly longer than those in KCl. The speeds of movement calculated from the end-to-end distances of the tracks are 0.91 ± 0.03 (standard error of the mean) mm/s ($n=47$) in KCl and 1.33 ± 0.03 (s.e.m.) mm/s ($n=52$) in KOH, 30 min after transfer ($t=2.13$, $0.02 > P > 0.01$).

Mutants defective in avoiding reaction are still repelled by velocity modulation. Pawn B is repelled by OH^- to the same extent as wild type. In the 1 mM OH^- against Cl^- test, Pawn shows an $I_{che} = 0.34 \pm 0.08$, $n=7$. This observation provides further evidence that AR is not a factor in this form of repulsion. The speed measurements of Pawn show that the mutants make the same velocity changes as wild type in response to KOH. Pawns swim at 1.07 ± 0.07 (s.e.m.) mm/s in KCl and 1.33 ± 0.06 (s.e.m.) mm/s in KOH ($n=11$ and 19) 30 min after the transfers ($t=2.5$, $0.02 > P > 0.01$).

When Na^+ and K^+ are compared, Na^+ is repulsive and K^+ attractive to normal paramecia (see previous section on repulsion by increasing F_{AR}). The repulsion from sodium is clearly due to its ability to cause repeated action potentials (Satow and Kung, 1974) and therefore, avoiding reactions (Kung, 1971b). As described above, the Fast-2 gives no ARs in a sodium solution. Yet, Fast-2 is not attracted or indifferent to sodium as predicted by the mechanisms involving F_{AR}. Instead, it is repelled by sodium. It gives an I_{che} of 0.36 ± 0.05 ($n=4$) away from 5 mM NaCl to 5 mM KCl, in which it shows AR. Again, this repulsion cannot be explained by changes in F_{AR}. In sodium solution it swims fast, up to 2 to 3 times faster than wild type (Kung, 1971b). This fast swimming in sodium is, therefore, responsible for its repulsion from sodium.

In solutions where calcium is the only inorganic cation, paramecia speed up considerably and give few AR (Satow and Kung, 1976b). In contrast, paramecia bathed in solutions containing both calcium and sodium, such as Dryl's solution (Dryl, 1959b) and the culture medium (Sonneborn, 1970) always show a few AR and do not swim fast. The T-maze experiments show that 2.5 mM Ca^{++} is a repellent compared to 5.0 mM Na^+ ($I_{che} = 0.24 \pm 0.07$, $n=4$), even though the ionic strengths are similar (7.5×10^{-3} and 5×10^{-3} M) and both $CaCl_2$ and NaCl are dissociated.

Attraction with Increasing F_{AR} and Decreasing V: Mechanism II

One would think that there ought to be an attraction type II symmetric to repulsion type II. In attraction II, F_{AR} should be very high, translational speed should be very low (due to both the slow ciliary beating frequency and the large amount of time spent in performing the frequent ARs) and the paramecia should oscillate in place. The lack of significant translation would tend to confine the cells, hence they would accumulate. This is the principle used in screening for behavioral mutants (Kung, 1971b) and can be

easily demonstrated by dropping animals into a pool of high concentrations of Ba^{++} or Na^{+} solutions (Kung et al., 1975). Preliminary T-maze experiments demonstrate this kind of accumulation with 1 mM $BaCl_2$ relative to 2 mM NaCl ($I_{che} = 0.77 \pm 0.22$, $n = 4$).

Discussion

Two Mechanisms of Chemokinesis

In *Paramecium*, probably as in other microorganisms, F_{AR} and V seem to be at odds in their contributions to accumulation and dispersal. In attractants, such as acetate, where decreasing F_{AR} is expected, paramecia speed up, which tends to disperse them (Table 1). In relative repellents, such as quinidine, where increasing F_{AR} is expected, paramecia slow down and should, thus, accumulate (Table 1). However, Rohlf and Davenport (1969) have simulated accumulation predictable by klinokinesis using decreased frequency of turning (with adaptation) alone and with an increased velocity. This combination of increased V and decreased F_{AR} was more efficient at accumulating simulated swimming animals than decreased F_{AR} (klinokinesis) alone. Therefore, modulation of F_{AR}, possibly in combination with V, must be responsible for attraction in solutions of acetate and dispersal from quinidine. However, there are experimental conditions under which paramecia do show atttraction and repulsion by velocity modulation alone, as the data on Mechanism II demonstrate. By elimination of the AR while keeping most V modulations intact, as in Pawn, it was possible to determine that ARs are not essential for repulsion by Mechanism II. However, for normal paramecia with ARs, F_{AR} apparently must be close to zero in repellent, such as KOH, to be dispersed by this mechanism.

This study may have limitations in focussing on only two components of behavior, the AR and forward swimming velocity. Local changes in ciliary activity on the cell are possible in response to chemical stimuli. However, Rohlf and Davenport (1969) have shown by computer simulation that F_{AR} and V are sufficient, in at least some situations, for accumulation. It remains to be shown why one or the other components, F_{AR} or V, dominates giving opposite chemokinesis results. The nature of the interactions of F_{AR} and V requires a mathematical description. Such a description is now being developed in collaboration with M. Levandowsky.

A Hypothesis for Membrane Potential Control of Chemokinesis

When change in velocity from control is plotted on the ordinate and change in F_{AR} from control is plotted on the abscissa, all of *Paramecium* normal behavior falls into two quadrants (Fig. 1): Increasing velocity and decreasing F_{AR} in the upper left quadrant, decreasing velocity and increasing F_{AR} in the bottom right quadrant. Behavior within these quadrants is further restricted to a shaded sector that is determined or reflected by change in membrane potential from control.

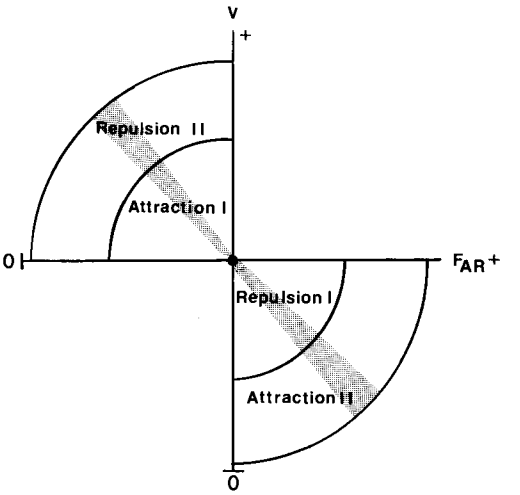

Fig. 1. A graphical description of *Paramecium* behavior. Behavior of cells in control solution is at the origin. An increase or decrease in velocity (V) from control is plotted on the y axis. An increase or decrease in the frequency of avoiding reactions (F_{AR}) is plotted on the x axis. Behavior of normal animals falls in the upper left or lower right quadrants. Behavior is restricted to an area represented by a shaded sector that is determined or reflected by the E_m

Behaviors of cells in attractants I and repellents II are qualitatively similar. Both decrease F_{AR} and increase V and both fall within the upper left quadrant of Figure 1. Since F_{AR} in repellent II is close to zero, the behavior in repellent II falls farther from the origin than behavior in attractant I. Likewise, behaviors of cells in repellent I and attractant II are similar. Since both types of stimuli elicit frequent AR and slow V, behaviors in both repellent I and attractant II fall into the lower right quadrant.

Applying current concepts of the bioelectric control of ciliary motion, a hyperpolarization from resting E_m will temporarily reduce the triggering of action potentials by bringing the potential away from threshold, thus lowering F_{AR}. A hyperpolarization will also increase frequency of ciliary beating and the cells will swim slightly faster. These are the behaviors of the upper left quadrant of Figure 1. A depolarization will temporarily elicit frequent action potentials because the potential is closer to threshold, thus increasing F_{AR}. The frequency of ciliary beating will also decrease temporarily and the cells will slow down. These are the behaviors of the lower right quadrant.

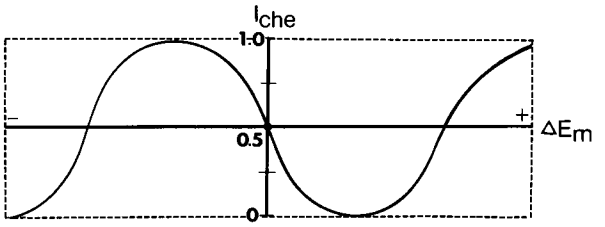

Fig. 2. A summary of a hypothesis for membrane potential control of chemokinesis. E_m is plotted on the x axis; I_{che} on the y axis. E_m in control is at the origin. $I_{che} > 0.5$ indicates attraction; $I_{che} < 0.5$ indicates repulsion. The graph is meant to be a qualitative description at present

These changes in behavior should last until a new resting membrane potential and threshold are established (Machemer, 1976; Eckert and Machemer, 1975). It seems apparent that direction and magnitude of membrane potential changes away from resting E_m will account for all the behaviors described for chemokinesis. The predictions of a membrane potential control hypothesis are described below.

The predictions of the hypothesis are illustrated in Figure 2. Change from control resting E_m is plotted against I_{che}. Small hyperpolarizations result in attraction ($I_{che} > 0.5$) due to decreased F_{AR}, perhaps in combination with increased V; larger hyperpolarizations result in repulsion ($I_{che} < 0.5$) due to fast swimming that is not interrupted by AR. Small depolarizations cause repulsion by increasing F_{AR}; larger depolarizations cause attraction by the greatly increased F_{AR} and decreased V that cause the animals to turn in place and make very little translational progress.

Some of the predictions have been verified or tested. 1) The cation Repellents I Ba^{++}, Na^+, and TEA^+ are known to depolarize the membrane relative to KCl and cause frequent action potentials (Satow and Kung, 1974; 1976c). 2) The mutant Fast-2 hyperpolarizes in NaCl solutions and, hence gives no action potentials and swims fast in sodium (Satow and Kung, 1976b). NaCl is a type II repellent to Fast-2 and the strong hyperpolarization of the mutant in NaCl is expected. 3) Anion Attractants I KOAc, K-lactate and K_2-folate slightly hyperpolarize the membrane relative to controls (6–12 mV) (Van Houten, 1978). 4) The acetate chemokinetic mutant, d4-530, is repelled by Mechanism II from NaOAc into NaCl, while the normal cells are attracted to NaOAc by Mechanism I (Van Houten, 1977). Both cell types hyperpolarize in NaOAc as the hypothesis predicts (Van Houten, unpublished observations).

Extremely large depolarizing stimuli are not accounted for. The present hypothesis predicts an increased F_{AR} and decreased V resulting in attraction type II. However, *very* large depolarizations can decrease the frequency of action potentials by reducing the electromotive force for calcium influx as the E_m approaches the calcium equilibrium potential (Eckert, 1972). If attraction to a very large depolarizing stimulus were observed, it probably would be due to a decreased F_{AR} and not due to a large increase in F_{AR}.

Adaptation

After change of solution, the cells change membrane potential and change frequencies of ciliary beating and action potentials. With time in the new solution, a new resting potential is established, a new threshold for action potentials is established relative to this new resting E_m, and ciliary beating frequency and angle are changed back to a resting level (Machemer, 1976; Eckert and Machemer, 1975). This accommodation of E_m and adjustment of the threshold corresponds to the observed adaptation of the F_{AR} and V with time (Van Houten, 1976). Again, changes in electrical properties are reflected in the behavior of the cells. This adaptation of behavior is necessary in the classical mechanisms of accumulation (Table 1) (Fraenkel and Gunn, 1961) and in computer simulations of accumulation that more closely approximate the mechanisms in *Paramecium* by varying both F_{AR} and V (Rohlf and Davenport, 1969).

Jennings observed that cells immediately perform ARs upon swimming out of an area of attractant (1906). The immediate response with AR is beneficial in turning the cell until it is once again headed toward the attractant. Accommodations of the resting membrane potential may be responsible for the immediacy of the response. For example, if a cell were to swim into an area of attractant, the E_m would hyperpolarize and the cell would temporarily swim faster and more smoothly. Eventually a new resting E_m would be established and the threshold for APs would shift in the negative direction. Upon swimming out of the area of attractant, the cell would depolarize and action potentials would easily be triggered due to the new more negative threshold. If the threshold had not been adjusted, a large depolarization would have been required to trigger an immediate AR in response to the new environment.

A consequence of membrane and behavior adaptation is that not absolute E_m but the magnitude of change of E_m from control will be important in determining the extent of accumulation or dispersal. Despite having different E_ms, cells adapted in different ionic environments show similar resting ciliary frequency and angle and similar *changes* of frequency and angle with changes of potential away from resting E_m (Machemer, 1976).

Role of Membrane Potential in Other Chemokinesis Systems

Membrane potential changes during chemokinesis have been found in bacteria (Szmelcman and Adler, 1976), slime molds (Hato et al., 1976) and macrophages (Gallin and Gallin, 1977). The relationships of these E_m changes to the behavior of the cells are not as well understood as in *Paramecium*. A mechanism of transducing chemical sensing by changes in membrane potential, conductance, or ion gradients may be common to cells from procaryotes to the sensory organelles of the metazoan systems. These sensory organelles almost invariably include modified cilia (Barber, 1974) reminiscent of the ciliated chemosensitive *Paramecium*.

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Note Added in Proof: Pawns accumulate in attractant II 1 mM $BaCl_2$ relative to 2 mM KCl ($I_{che} = 0.77 \pm 0.09$, $n = 4$). The relatively slow swimming of Pawn in $BaCl_2$ must be sufficient to accumulate animals without the need for AR. 2.5 mM $BaCl_2$ relative to 5 mM KCl is repellent I to wild type and is "neutral" to Pawn (Table 2). Therefore, the absolute concentration is not important in determining chemokinesis response but rather the change in potential from that in control to the potential in test solution.

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