

Kinetic Analysis of Chemokinesis of *Paramecium*¹

JUDITH VAN HOUTEN,* ELAINE MARTEL,** and TONYA KASCH**

*Department of Zoology, University of Vermont, Burlington, Vermont 05405; and

**Department of Zoology, University of Iowa, Iowa City, Iowa 52242

ABSTRACT. Paramecia detect and accumulate in or disperse from some chemicals. Cells do this by changing frequency of turning and speed of swimming. There are at least two mechanisms by which cells respond: one dependent on ability to turn, one dependent on speed modulation. There are also two classes of chemicals: those that require the cells' ability to turn in order to cause accumulation and dispersal (type I), and those that apparently require only speed modulation (type II). Attractants of type I cause qualitatively similar changes in behavior to repellents of type II and the converse; therefore, assays are needed to distinguish between these two classes of chemicals, despite qualitatively similar behavior of some attractants and repellents. We examined two assays of paramecium chemoresponse, T-maze assay and well test, to understand how the T-maze distinguishes between attractants of type I and repellents of type II and why the well test does not.

PARAMECIA detect some soluble chemicals in their environment and accumulate in some and disperse from others. They accomplish this behavior not by orienting and swimming toward or away from the chemical's source, but rather by modulating frequency of turning and speed of swimming (4). Fre-

quency of avoiding reactions (abrupt turns caused by transient reversal of ciliary beating) and frequency and angle of ciliary beating, hence speed, are all under simultaneous electrical control at the cell membrane (1, 2). Nonetheless, the chemicals which cause cells to accumulate and disperse can be classified as type I, requiring the ability of cells to make turns, and type II, not requiring turns but rather speed modulation (4). In response to type I, decreased frequency of turning as cells swim up a gradient results in attraction; increased frequency of turning as cells swim up a gradient results in turning away and repulsion. In response to type II, increased speed causes dis-

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persal, and decreased net movement causes accumulation, similar in principle to a traffic jam. There are both speed and frequency of turning changes associated with response to chemicals of types I and II. However, studies of mutants unable to undergo any avoiding reactions demonstrate that speed changes are not sufficient for response to type I, and avoiding reactions are not necessary for response to type II (4).

Even though only frequency of turning or only speed may be essential for a response, the mechanism of chemokinesis becomes complicated because the qualitatively same behavior that is associated with accumulation in type I (smooth swimming due to decreased frequency of turning and slight increase in speed) causes repulsion from type II chemicals (extremely smooth swimming and fast speed due to virtually no turning) (4). Likewise, increased frequency of turning and slightly decreased speed is associated with repulsion by type I while greatly increased frequency of turning and greatly decreased speed results in almost no net movement of the cells and thereby causes accumulation by type II (4).

Since there are two mechanisms of chemokinesis for paramecia, assays for chemokinesis must be able to distinguish between attraction and repulsion despite qualitatively similar behavior of cells being characteristic of both attractants and repellents. Therefore, we have examined the previously described T-maze assay (5) and new well test that was designed to score quickly cells' first behavioral reactions to putative attractants and repellents in order to determine how normal and mutant cells distribute in these assays.

In the T-maze, test solution fills one arm and control solution fills the other arm. Cells in control solution fill the plug. Cells placed in the plug immediately begin to distribute into the two arms and will distribute uniformly throughout if the two arms contain the same solution (3). If the test arm contains an attractant of type I or II, cells will eventually accumulate in this arm; or if the test arm contains repellent I or II, cells will eventually disperse to the other arm. The assay appears to measure attractant and repellent strength whether the mechanism involved is type I or II (4).

The well test apparatus, designed to score hundreds of clones from genetic crosses, is simply three small wells interconnected by channels. Cells in this test apparatus can distinguish between wells with attractants and repellents of type I, but repellents of type II appear to be somewhat attractive in this assay. Since repellents of type II induce smooth fast swimming, cells first enter the repellent-containing arm of the T-maze or well and eventually disperse out of the arm or well, given enough time. However, the well test is a rapid assay, scored within 2 min of its start. It can only determine the first choice the cells make between wells of control and test solution. The T-maze assay, on the other hand, lasts for 30 min, enough time for cells to leave and reenter control and test solutions several times. Only early times of the T-maze may be comparable to the well test distributions.

In order to understand how the longer T-maze test distinguishes between type I and II attractants and repellents and why the well assay does not, we studied time courses of accumulation and dispersal in these assays. We also tested the prediction that cells will at first be attracted and then be repelled by repellents II in the T-maze. An understanding of assays of chemoaccumulation is necessary for an accurate description of the mechanisms of chemokinesis.

MATERIALS AND METHODS

Strains. *Paramecium tetraurelia* stock 51-S, and mutants derived from this stock, were used throughout. Cells were grown

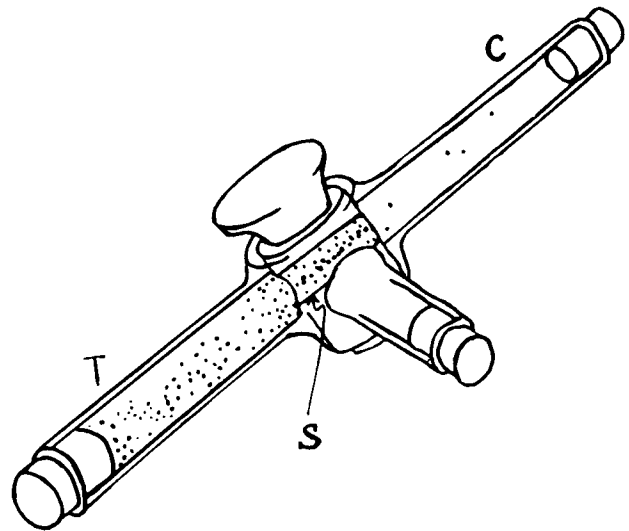


Fig. 1. T-maze assay. Cells in control solution fill the stopcock plug (S); control solution fills C arm; test solution fills T arm. Assay begins when plug is turned to connect the two arms. Cells distribute for 30 min at which time the plug is turned, arms emptied and cells counted. Index of chemokinesis is calculated as explained in Materials and Methods.

in overnight cultures of *Aerobacter aerogenes* in Cerophyl medium.

Solutions. All solutions were buffers containing 1 mM Ca, 1 mM Tris (hydroxymethyl) diaminomethane, 1 mM citric acid, and indicated salts at pH 7.02 (except 1 mM KOH solution at pH 8.6).

T-maze assay. This assay is a modification of the three-way stopcock with a two-way straight bore plug (3, 5) (Fig. 1). Test and control solutions fill the two arms of the T; cells in control solution fill the plug. The assay begins with turning the plug to interface with the two arms. After 30 min or other specified times, the plug is turned, arms emptied, and cells counted. Index of chemokinesis (I_{che}) is defined as number of cells in test arm/number of cells in both test and control arms. $I_{che} > 0.5$ indicates attraction; $I_{che} < 0.5$ indicates repulsion.

Well test. Well test apparatus consists of three wells of 0.5 cm diameter with connecting canals 0.25 cm long in 0.5 cm thick plexiglass (Fig. 2). A glass coverslip serves as the bottom of the wells and canals. Control and test solutions fill the side wells and canals; cells in control solution are pipetted into the middle well until contact is made with the solutions in the canals. At this point the cells (at least 50) begin to distribute into the side wells. The distribution is scored between one and two minutes. (Diffusion rapidly breaks down the step gradient of test and control solutions making later scoring inaccurate.) At least twice as many cells in test well as control well is scored as +; even distribution of cells is scored 0; at least twice as many cells in control well is scored as -.

RESULTS

Early events in T-maze comparable to those in well test. Repellent II KOH induces fast smooth swimming in normal cells (4). In the T-maze at 30 min I_{che} is 0.38 ± 0.05 (standard deviation) indicating repulsion from KOH while the well tests indicate attraction to KOH during the first minute of the assay (Table I). The time course studies of the distribution of normal cells in the T-maze also show slight attraction at very early times (1–5 min) with repulsion by 20 min (Fig. 3). The cells

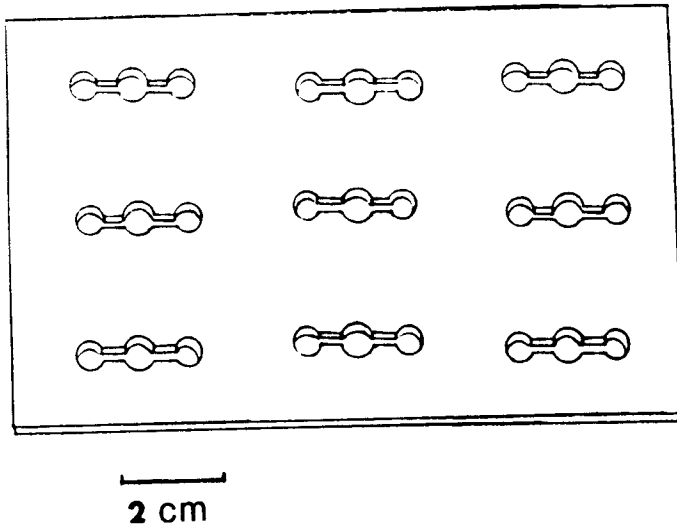


Fig. 2. Well test apparatus. Test and control solutions fill the side wells, cells in control solution fill the middle well, and cells then distribute among the three wells. At least twice as many cells in the test as control well is scored +; an even distribution of cells is scored 0; at least twice as many cells in control as test well is scored -. The scoring is done within 2 min of the beginning of the test.

appear to swim fast and smoothly into the KOH solution at first in both assays. With time in the T-maze, cells disperse from the KOH. If the time of the T-maze assay were not normally 30 min, the repulsion by KOH would be missed. Likewise, if cells were trapped in the first arm they entered, for example, by using a capillary sized stopcock plug, KOH would appear to be an attractant similar to acetate (OAc^-) or ammonium (NH_4^+) ions (3, 4). Time course of normal cells in the type I attractants OAc^- and NH_4^+ indicates that cells swim fast and smoothly into these solutions and remain in them (Figs. 4a, d), unlike KOH.

Differentiation of mutant phenotypes. Both mutants d4-539 and d4-538 do not accumulate in NH_4^+ at 30 min in the T-maze ($I_{che} = 0.48$ and 0.44 , respectively). However, while d4-538 is not attracted to NH_4^+ in either the T-maze or well test, d4-539 is attracted in a well test assay (Table I). Time courses of d4-539 and d4-538 in the T-maze demonstrate that d4-538 appears not to respond to the presence of NH_4^+ at any time during the assay (Fig. 4b) while d4-539 detects NH_4^+ and is attracted at first but does not maintain this accumulation (Fig. 4c). Time

TABLE I. Well test assays of responses to NH_4Cl and KOH.

Line	% Tests scored ^a					
	NH_4Cl vs. NaCl			KOH vs. KCl		
	+	0	-	+	0	-
51-S (normal)	72	28	0	64	7	29
d4-539	77	19	3	—	—	—
d4-538 ^b	34	38	28	—	—	—

^a + = at least twice as many cells in test as in control well; 0 = same number of cells in test and control wells; - = at least twice as many cells in control as test well. 5 mM NH_4Cl buffer and 1 mM KOH buffer are test solutions; 5 mM NaCl and 1 mM KCl are respective control solutions. Data are expressed as % of total tests scored as +, 0 or -. Number of tests ranged from 12 to 28 for each series.

^b Distribution significantly different from normal by chi-square test.

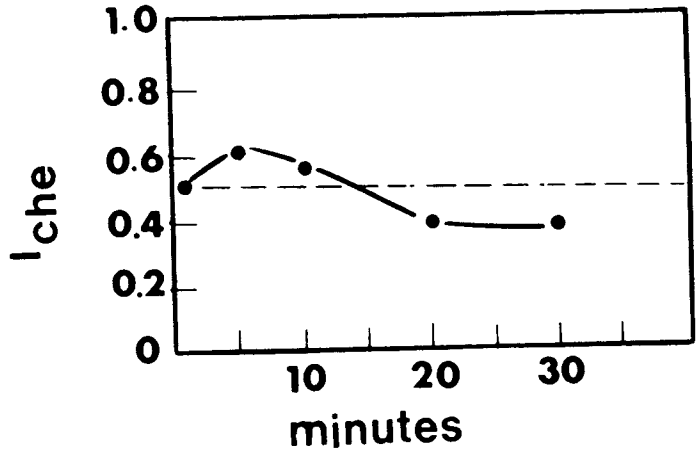


Fig. 3. Time course of normal cells distributing between KOH and KCl in T-maze. T-mazes with 1 mM KOH in test arm and 1 mM KCl in control arm were tested with normal cells for various times. The resulting indices of chemokinesis (I_{che} 's) are plotted against time in min. Data points are average I_{che} 's from two T-mazes. Graph is a representative example of three replicate time courses.

courses of the mutants in T-mazes with OAc^- as attractant indicate that d4-538 can accumulate normally and that d4-539 accumulates, but with kinetics that consistently appear to be different from normal (Figs. 4e, f).

A closer examination of T-maze distributions. Not all cells of a normal population accumulate in NH_4^+ in the T-maze. Rather, only approximately 85% are found in the test arm. In order to determine whether a subset of the population does not detect NH_4^+ , at various times we stopped a T-maze assay, replaced the solution in the control arm and plug, and reopened the stopcock bore to let the cells in attractant in the test arm continue to distribute in the T-maze for the remainder of the usual 30-min assay. If the cells in the attractant arm were a special subset of the population, they would not move out of the attractant arm into control solution. If, however, the cells in the attractant arm were not a special subset, they would redistribute through the T-maze with approximately 85% in attractant and 15% in control solutions. A different T-maze was used for each time point. I_{che} 's were calculated for distributions of cells at the time the T-maze assay was first interrupted and at the end of the 30-min assay. I_{che} 's for the intermediate times and final distributions at 30 min are given in Table II. Ninety-three percent of the cells that move out of the stopcock plug move into the test arm by 5 min ($I_{che} = 0.93$) and 14% of these cells eventually move out of the test arm into the control arm

TABLE II. Time course of normal attraction to NH_4^+ in the T-maze.^a

Time C arm refilled (min)	I_{che} at time of refilling	I_{che} at 30 min
5	0.93 ± 0.04	0.86 ± 0.05
10	0.91 ± 0.02	0.87 ± 0.04
15	0.89 ± 0.03	0.86 ± 0.06
20	0.85 ± 0.05	0.83 ± 0.03
25	0.81 ± 0.08	0.93 ± 0.01
30		0.79

^a Data are averages of three T-maze tests ± 1 standard deviation, except 30 min I_{che} , which is from one T-maze test.

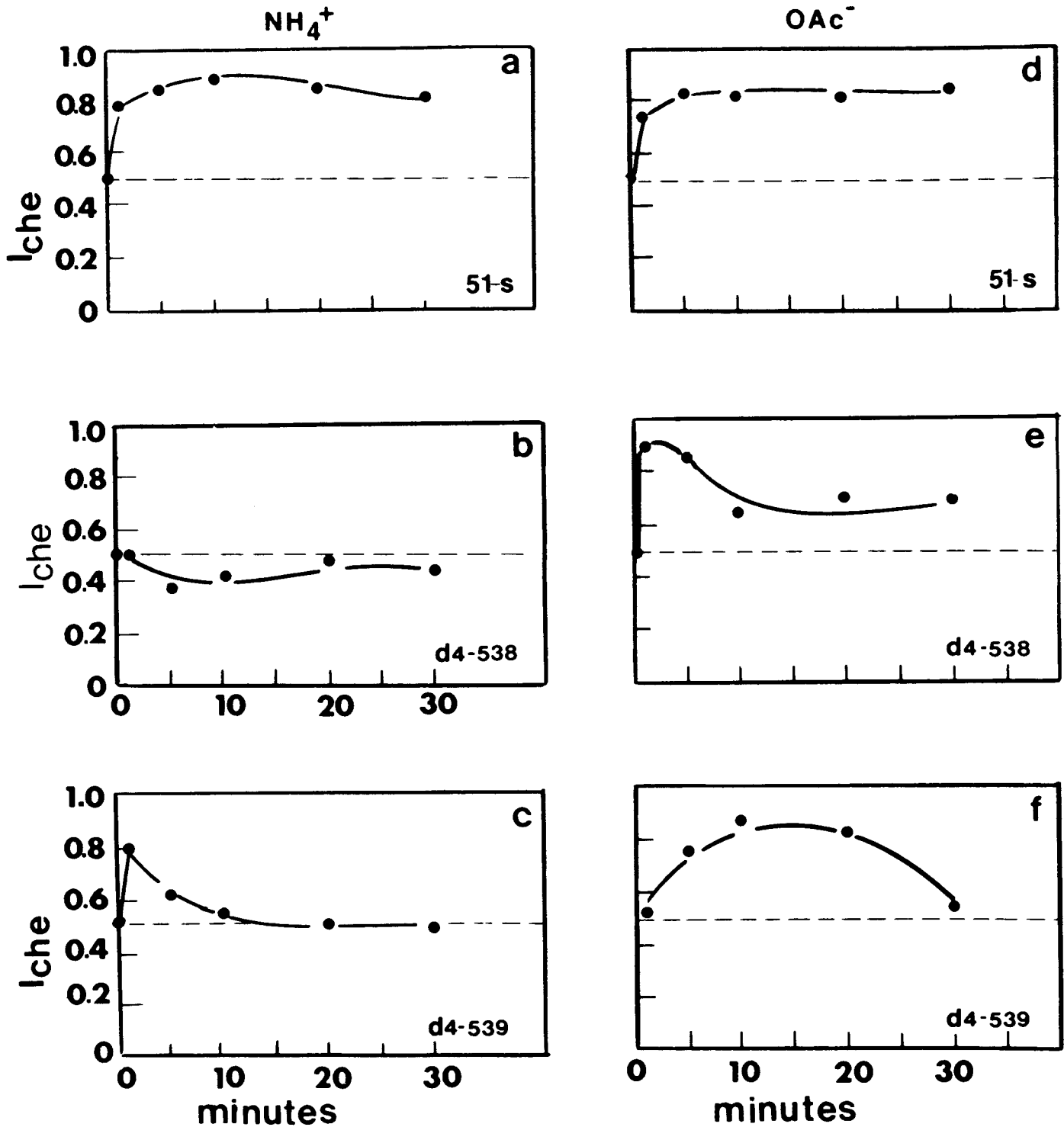


Fig. 4. Time courses of normal and mutant cells in T-mazes. Five mM NH_4Cl filled test arms and 5 mM NaCl filled control arms of tests in panels a, b, and c. Five mM NaOAc filled test arms and 5 mM NaCl filled control arms of tests in panels d, e, and f. I_{che} 's of normal (51-S) cells are in panels a and d; d4-538 in panels b and e; d4-539 in panels c and f. Data points are average I_{che} 's from two T-mazes. Graphs are representative examples of time courses repeated two to three times each.

(I_{che} at 30 min = 0.86). Similarly, at each time examined, the cells that accumulated in the test arm redistribute, and some of these cells that once were in the test arm were found in the control arm at 30 min. These results indicate that the population that accumulates is not a special subset of the starting popu-

lation, but instead accumulation is the result of each cell having an increased probability of distribution into the test arm (due to a biased random walk) (4). They also show that cells actually do leave arms of the T and redistribute during the 30-min assay.

The I_{che} 's at intermediate times provide a time course similar

to Fig. 4a. Note that in both time courses, the initial I_{che} 's are higher than the final I_{che} at 30 min from an undisturbed T-maze. Also, the intermediate I_{che} 's are slightly higher than the 30-min I_{che} 's for the same T-maze, except for the 25-min interruption point (Table II). (We believe that the 5 min between the refilling of the control arm and plug and the end of the test at 30 min were not sufficient time for redistribution of cells between test and control arms and, therefore, the high I_{che} for the T-maze with control arm refilled at 25 min is an artifact of the test design. We observed this result consistently.) The higher I_{che} 's for intermediate times in this test and for similar tests with OAc^- as attractant (data not shown) and for early time points in conventional time courses (Fig. 4) probably are results of the initial smooth, fast swimming response of cells into the attractant arm. After cells redistribute with time and adapt to the solutions, the I_{che} approaches an equilibrium value (although a true equilibrium is impossible due to diffusion of the step gradient).

DISCUSSION

Time courses of the I_{che} 's in the T-maze assay with KOH as repellent II, show that cells first accumulate in KOH and then disperse. This early accumulation is similar to the accumulation of cells in KOH in the short-term well test. The time courses in T-mazes with OAc^- or NH_4^+ as attractants also show high initial accumulation in the attractant arm, but show little subsequent dispersal. Cells accumulate in OAc^- and NH_4^+ in the well test assay as well. Therefore, it is important to allow time for cells to redistribute in the T-maze assay and not inadvertently to trap cells in the arm in which they first enter, such as by using a capillary size stopcock plug, in order to distinguish repellents II from attractants I. Well tests or short times in the T-maze cannot make this distinction. Short times in the T-maze seem to be useful in measuring increased motility as opposed to accumulation.

Time for redistribution is also important in order to understand better the phenotypes of mutants such as d4-539 and d4-538 that do not respond normally to NH_4^+ . While neither show accumulation after 30 min in the T-maze, d4-539 does accumulate at early times (less than 5 min) in the T-maze (Fig. 4c) and in the well test. The early transient accumulation of d4-539 may be due to failure of this mutant to adapt in NH_4Cl or $NaCl$ (6). This possibility is being tested. The other abnormal time courses in Figs. 4e and f as yet have no explanation. This also points out the advantage of using several assays in characterizing mutants to distinguish between strains that never respond to an attractant from those that detect but cannot maintain the accumulation response.

At various times, T-maze control arms and plug were emptied, refilled with control solution, and the test resumed. I_{che} 's at the intermediate times and at the final 30-min distributions indicate that a higher percentage of cells initially swim into the

test NH_4^+ arm than is found there at 30 min. Again cells initially enter solutions that induce fast, smooth swimming and then redistribute between test and control arms. These tests also confirm that cells do leave arms to cross the stopcock plug and enter another arm and that the cells in the two arms do not represent separate subpopulations, one able to detect or respond to attractant, the other not able to do so.

The well test was recently developed to facilitate scoring hundreds of clones of cells during a screening for chemoreception mutants or for segregation of chemoreception phenotypes in F2 generations of crosses. It is important for us to be cognizant of the types of responses this assay measures in order to use the well test effectively and appropriately. For example, use of the well test in screening for mutants should eliminate the possibility of finding mutants like d4-539 that fail to maintain an accumulation response. Also, the well test will not discriminate between mutants that never detect an attractant from those that detect but are slow to show accumulation, perhaps due to defects in the steps of the chemosensory pathway downstream from the receptor binding functions.

The results of this paper warn against confusing assays of *Paramecium* motility with assays of accumulation and dispersal (6). Similar problems exist in the assays of leukocyte chemotaxis inadvertently measuring motility (7, 8). It will be especially important to understand the distribution of cells in an assay of chemokinesis before using the data in a description of a mechanism of accumulation and dispersal and before designing a computer simulation of the population behavior.

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