MUTANTS OF PARAMECIUM DEFECTIVE IN CHEMOKINESIS TO FOLATE

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ABSTRACT

Ten mutant lines of *Paramecium tetraurelia* defective in attraction to folate were isolated and examined. All mutants were normal in response to other attractants and repellents tested. One mutant was able to accumulate in folate given sufficient time. All mutations were recessive and behaved as single site Mendelian lesions. Complementation tests indicate that the mutants fall into three complementation groups. Mutants of Group 2 fall into two phenotypic classes and probably represent two alleles of the mutated fol^2 gene. Possible sites of the mutants' blocks in chemoresponse are discussed.

PARAMECIA detect and respond to some soluble chemicals in their environment. These unicells swim by means of beating cilia. Changes in swimming behavior are part of their characteristic responses to the detection of chemicals. Changes in the individual cells' swimming behavior, in turn, lead to alterations in population behavior, that is, in accumulation or dispersal. Hence, chemosensory transduction can be studied on various levels in paramecia (Figure 1): the level of the population, individual motor behavior, or even membrane electrical changes because ciliary beating determines swimming behavior and ciliary beating is under membrane electrical control (ECKERT 1972; MACHEMER 1976). The study of these various levels can be facilitated by the generation of mutants to perturb one pathway component at a time. Comparison of mutant with normal individual cells and populations can lead to identification of the altered component.

In order to understand the primary steps of the chemosensory transduction pathway in Figure 1, we generated and selected mutants unable to detect or respond to folate, an attractant to wild-type paramecia. Ten mutant lines were isolated as described below and analyzed genetically and behaviorally. The ten lines fall into three complementation groups and none of these lines appears to be defective in response to any attractants or repellents other than folate. Therefore, these mutants are likely to have defects early in the chemosensory transduction response pathway, that is, in the part of the pathway specific for the detection of folate. The analyses of phenotypes and genotypes of these mutants are presented below. The possible sites of the mutant lesions discussed include the possibility of defects in a chemoreceptor for folate.

MATERIALS AND METHODS

Strains: All strains belong to *Paramecium tetraurelia* (previously called *P. aurelia* species 4; see SONNEBORN 1970) stock 51-s (endosymbiont free) or are mutants derived from stock 51-s. Mutant

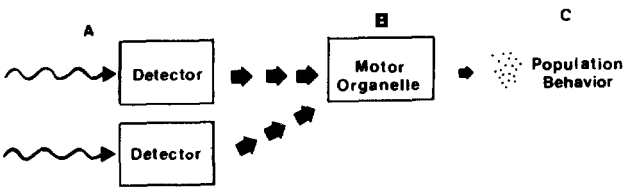

FIGURE 1.—Schematized chemosensory pathway in Paramecium. Chemical cues contact putative receptors, perhaps at the cell membrane; chemical cue is transduced into electrical cue that affects the motor organelles, cilia; altered ciliary movement of individuals indirectly causes accumulation and dispersal of a population.

lines include the ten behavioral mutants unable to normally respond to folate and strain *d4-93*, which contains a behaviorally normal, body-deformed mutation (*bdB*) used as a genetic marker here.

Culturing: Cells are routinely grown in overnight cultures of *Klebsiella pneumoniae* in Cerophyl rye grass medium.

Solutions: All solutions are buffers containing 1 mM $Ca(OH)_2$, 1.3 mM Tris (hydroxymethyl) aminomethane, 1 mM citric acid and the indicated salts at pH 7.02, with the exception of 1 mM KOH solution at pH 8.6.

Assays of chemokinesis

T-maze assay: The T-maze is a modified three-way stopcock with a two-way plug (see VAN HOUTEN *et al.* 1975). Test solution fills one arm of the T; control solution fills the other arm. The plug bore is filled with cells in control solution. The assay starts when the stopcock bore is turned to connect the test and control arms. Cells are allowed to distribute themselves between the two arms for 30 min at which time the stopcock is turned to seal the arms, the arms are emptied and the number of cells in each arm counted. The index of attraction and repulsion is I_{che} (number of cells in test arm/total cells in test and control arms). $I_{che} > 0.5$ indicates attraction; $I_{che} < 0.5$ indicates repulsion from the test solution.

Well test: Three small diameter wells (two 0.5-cm outer and 0.6-cm inner wells) in 0.5 cm Plexiglas are connected by short canals (Figure 2). Glass coverslips serve as bottoms of the wells. Control solution and test solution are pipetted into the two side wells and canals. At least 50 cells and control solution are pipetted into the middle well until the solutions make contact with the solutions in the canals. Cells immediately begin to distribute themselves into the side wells and the number in test and control wells are scored between 1 and 2 min from the start of the test. When at least twice as many cells are in test as control solution, the test is scored +; even distribution between wells is scored 0; at least twice as many cells in control as test well is scored −.

Mutagenesis: Cultures that had undergone about 20 fissions since the last autogamy were mutagenized by exposure to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (SONNEBORN 1970; KUNG 1971). Treated cells were distributed to flasks to grow, starve and undergo autogamy. After autogamy, cells were again fed and grown for 5–10 fissions to allow for phenomic lag before the cells were screened for folate-insensitive mutants. [SONNEBORN (1947) confirmed that *P. tetraurelia* is capable of undergoing autogamy, a process by which the micronucleus reorganizes and becomes completely homozygous.]

Screening for mutants: Two procedures were used. One mutant line (fol^{1b}) was identified using Procedure 1; nine mutant lines were identified using Procedure 2.

Procedure 1 is an enrichment process. Cells were tested in a T-maze with 1 mM K_2 folate and 2 mM KCl (control) solutions. All cells that migrated into the KCl control were grown in culture fluid and the procedure was repeated with these cells. After four repetitions to enrich for mutants, single cells were cloned and tested for the ability to be attracted to folate in the T-maze.

Procedure 2 utilized two two-way stopcocks connected to form three chambers (Figure 3). Control solution filled one chamber; an exponential gradient of 5 mM KCl control and 2.5 mM K_2 folate filled the middle chamber; 2.5 mM K_2 folate with cells filled the last chamber. The stopcocks between chambers were opened and the mutagenized cells were allowed to distribute themselves in the gradient. Cells swimming down the exponential gradient of attractant into the chamber with control solution were cloned and tested in the well test and eventually tested in the T-maze.

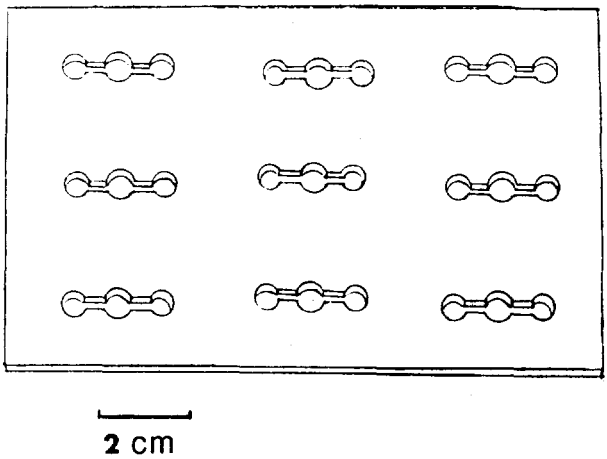

FIGURE 2.—Well test apparatus. Test and control solutions filled the side wells; cells in control solution filled the middle well. Cells distributed between the three wells. At least twice as many cells in the test as control well was scored +; an even distribution of cells was scored 0; at least twice as many cells in control as test well was scored −. The scoring was done within 2 min of the beginning of the test.

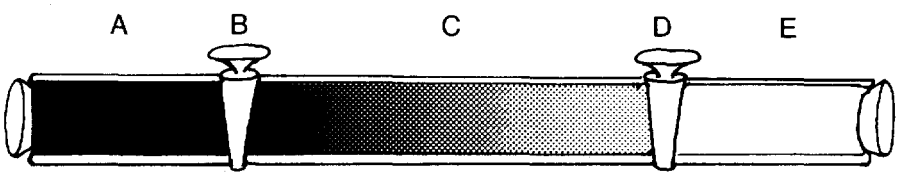

FIGURE 3.—Apparatus for screening for chemokinesis-defective mutants. An exponential gradient of attractant to relative repellent in chamber C was separated from uniform concentrations of attractant (2.5 mM K_2 folate) in chamber A and relative repellent 5 mM KCl in chamber E by stopcocks B and D. Cells from a mutagenized population started in chamber A and all cells swimming down the gradient into relative repellent were collected from chamber E.

Genetic analyses

Crosses: Cells were crossed to d4-93, a recessive body-deformed mutant of 51-s, as described by SONNEBORN (1970) and KUNG (1971). Clones of the F_2 generation were isolated after the F_1 was induced to undergo autogamy. The F_1 and F_2 were scored for body morphology and behavior in well tests or T-mazes. The presence of the body deformation did not impair the cells from accumulating in attractant (data not shown; see VAN HOUTEN 1976).

Complementation tests: Folate mutants with body deformation markers were crossed with a second mutant with normal body morphology. The F_1 generation was scored for body and behavior. Some F_2 generations were isolated as a check on body deformation segregation.

RESULTS

Phenotypes

Mutants were tested for attraction to and dispersal from a series of normal attractants and repellents. The four representative mutants described (fol^{1a}, fol^{1b}, fol^{2b} and fol^{3}), although abnormal in their population response to folate, were within the wild-type range in response to all other attractants and repellents tested in the T-maze (Figure 4). Normal cells are attracted to folate in the T-maze and well test assays whereas the mutants fol^{1a}, fol^{1b} and fol^{3} do not

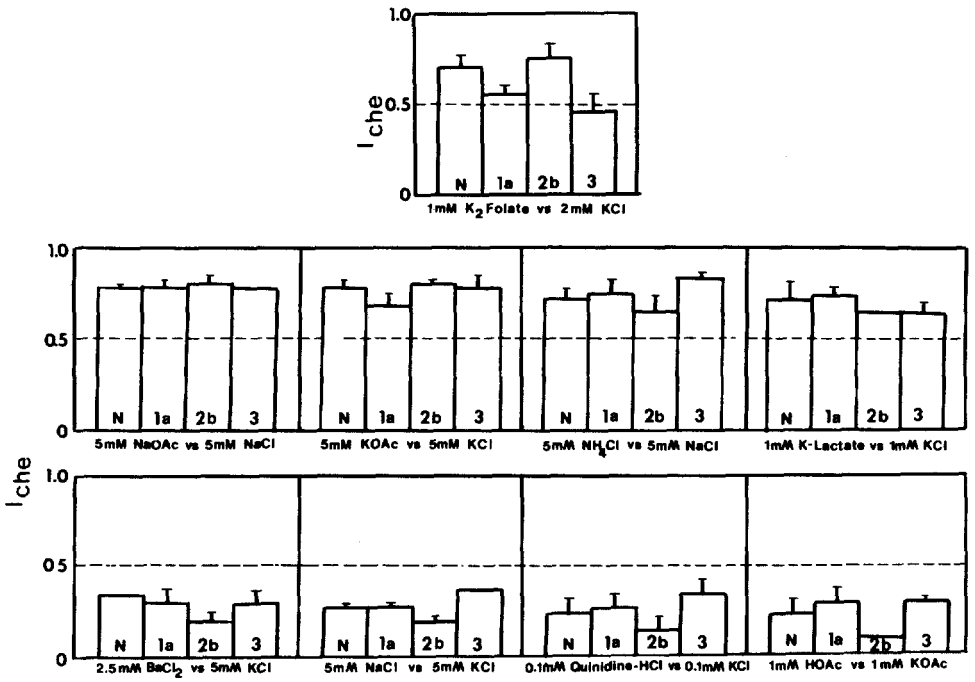

FIGURE 4.—T-maze assays of normal and folate mutant paramecia. a) Assays in attractants 5 mM NH_4^+, 5 mM OAc^-, 5 mM lactate ion, and 1 mM folate ion; b) assays in repellents 5 mM NaCl, 2.5 mM $BaCl_2$, 2.5 μM H^+ (1 mM HOAc, pH 5.6) and 0.1 mM quinidine-HCl. N = normal cells; 1a = *fol*^{1a}; 1b = *fol*^{1b}; 2a = *fol*^{2a}; 3 = *fol*^{3}. $I_{che} > 0.5$ indicates attraction; $I_{che} < 0.5$ indicates repulsion. Solutions were buffers described in MATERIALS AND METHODS and indicated salts at pH 7.02, except HOAc at pH 5.6. Error bars indicate one standard deviation. $n = 2$ (with no error bars) – 61.

accumulate in folate relative to chloride in either test (Figures 4 and 5). Mutant *fol*^{2b} is not initially attracted to folate, as indicated in the rapid well test (Figure 5), but is eventually attracted in the T-maze assay (Figure 4). Time is apparently not limiting for responses of the other mutant lines.

Genetic analysis of mutants

All mutant lines were crossed to *d4-93*, a behaviorally normal line with a recessive body deformation marker. The F_1 generation of each cross was scored in well tests or T-mazes to determine if the mutations were dominant or recessive. As indicated in Table 1, all F_1s were wild type for behavior and morphology, indicating that all mutations were recessive. F_2 clones of each cross were scored for attraction to folate relative to chloride and for body shape. As indicated in Table 1, all F_2 generations segregate approximately 1:1:1:1 for normal body and behavior:normal body with fol$^-$ behavior:body deformed with normal behavior:body deformed with fol$^-$ behavior, as judged by a chi-square test at the 0.2 level of significance. These ratios indicate that each mutant harbored a genic, single site lesion that segregated in a Mendelian fashion, unlinked to the body-deformed locus.

Since all mutants were recessive, complementation tests could be performed to determine the number of loci represented by the mutants. Double fol$^-$ behavior and body deformed mutants of each mating type were generated in

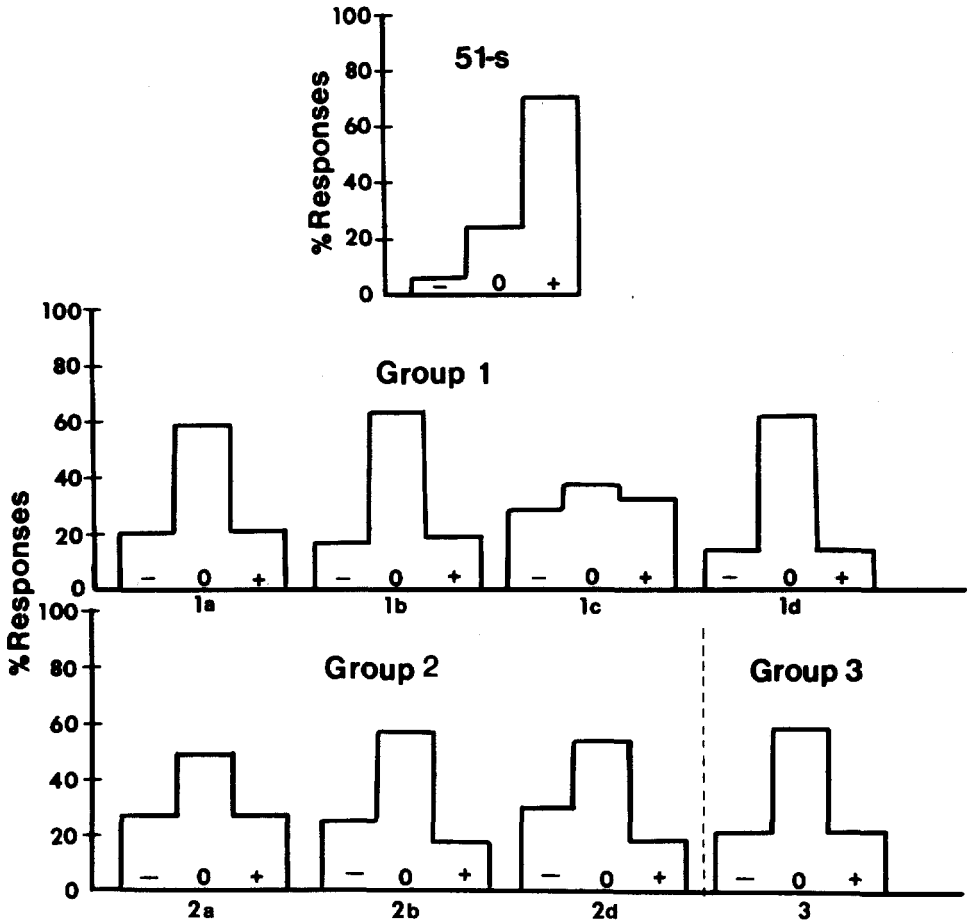

FIGURE 5.—Well test assays of normal and folate mutant responses to folate. 1 mM K_2 folate solution filled test well; 2 mM KCl filled control well. + indicates at least twice as many cells moved into folate as control well; 0 indicates an even distribution of cells; – indicates at least twice as many cells were in control as folate well at 2 min. Chi-square test indicates that the mutants' distributions are significantly different from the normal distribution. $n = 33 - 107$.

the F_2 of crosses to *d4-93*. These double mutants were crossed to other fol$^-$ mutants with normal body morphology and scored in the F_1 for body and behavior for complementation of the two fol$^-$ mutations (Table 2). Some double body-deformed fol$^-$ mutants were crossed to their parental strains as complementation controls. Some complementation crosses were carried out to the F_2 generation in order to detect linkage or suppression in double fol$^-$ mutants (Table 3). The results in Tables 2 and 3 indicate that there are three complementation groups. Each group is discussed in turn below.

fol^{1a} and *fol*^{1b} are in complementation Group 1 (Table 2) and were selected by two different procedures from separate mutagenized populations. There are three other members of this group and none can be distinguished from other group members by obvious differences in phenotype (Figures 4 and 5). No wild-type recombinants are recovered from crosses of mutants within Group 1 (Table 3). However, sample sizes are relatively small and rare recombinants would be

TABLE 1

F_1 phenotypes and autogamous F_2 segregations between the fol⁻ mutants and d4-93

Lines	F_1[a]	F_2[b]			fol⁻ and deformed
		Normal	fol⁻	Deformed	
fol-1a	Normal	11	11	11	14
fol-1b	Normal	22	21	24	30
fol-1c	Normal	25	21	20	23
fol-1d	Normal	11	11	9	9
fol-1e	Normal	8	13	5	9
fol-2a	Normal	11	13	10	14
fol-2b	Normal	14	11	6	17
fol-2c	Normal	4	9	5	11
fol-2d[c]	Normal	—	—	—	—
fol-3	Normal	12	12	8	15

[a] F_1 phenotype scored in well test assay.
[b] Pass chi-square test at the 0.2 level of significance for expected 1:1:1:1 ratio of phenotypes for single-site mutations.
[c] F_2 generations were not isolated. Only F_1 phenotypes for dominance tests were scored.

TABLE 2

Complementation tests of fol⁻ mutants

Line	Crossed to representatives of:				
	Group 1		Group 2		Group 3
	fol-1b	*fol-1d*	*fol-2a*	*fol-2b*	*fol-3*
Group 1					
fol-1a	−			+	+
fol-1b	−	−	+	+	+
fol-1c	−	−			
fol-1d	−			+	
fol-1e	−			+	
Group 2					
fol-2a	+		−	−	+[a]
fol-2b	+		−		+
fol-2c				−	
fol-2d	+			−	
Group 3					
fol-3	(+)		(+[a])	(+)	

[a] F_1 is different from parents, but clearly not wild type. F_2 segregation ratios of this cross and *fol-3* × *fol-2b* (Table 3) indicate that both *fol-2a* and *fol-2b* have mutations in genes separate from and unlinked to *fol-3*. Parentheses indicate that same cross appears elsewhere in table.

missed. Crosses of mutants of Group 1 to mutants of other groups segregate in approximately 1:3 ratios (normal:fol⁻ phenotypes), as expected for unlinked mutants in different genes, with the exceptions of *fol-1b* and *fol-1c* crossed to *fol-2d* (Table 3). In these crosses, more wild-type progeny appear in the F_2 generation than expected. The progeny fall into a 1:3 ratio that barely passes a chi-square test for a 1:3 ratio at the 0.2 level of significance. Since the preponderance of

TABLE 3

Autogamous F_2 segregations of crosses between fol⁻ mutants

Cross				F_2			
Line with body-deformed marker	Group	×	Line	Group	Normal	fol⁻	Deformed	fol⁻ and deformed
fol^{1b}	1		*fol*^{1a}	1	0	73	0	64
			fol^{1d}	1	0	28	0	19
			fol^{2a}	2	7	12	4	17
			fol^{2d}	2	21	58	22	41
			fol^{3}	3	17	37	6	34
fol^{1c}	1		*fol*^{1d}	1	0	25	0	23
			fol^{2d}	2	14	25	19	31
fol^{2a}	2		*fol*^{2b}	2	0	42	1[a]	39
fol^{2b}	2		*fol*^{1a}	1	15	34	9	34
			fol^{1e}	1	8	19	4	12
			fol^{2a}	2	0	12	0	8
			fol^{3}	3	10	17	2	13
fol^{3}	3		*fol*^{2a}	2	5	16	5	11

All crosses pass chi-square test at 0.2–0.05 level of significance for 1:3:1:3 and 0:1:0:1 ratios expected for intergenic and intragenic crosses, respectively.

[a] See text for explanation.

wild-type phenotypes appears in crosses of two separate *fol*^{1} lines, it is possible that there is some suppression or other interaction between *fol*^{2d} and *fol*^{1} gene products. However, the intergenic suppression is not complete because the crosses of *fol*^{2d} with *fol*^{1b} and *fol*^{1c} fail to fit a 1:1 ratio at the level of 0.05 and 0.01, respectively. It is interesting to note, however, that cross of *fol*^{1c} and *fol*^{2d} does fit an hypothesis of a third gene as suppressor of *fol*^{1c} gene, a 3:5 (fol⁻ to normal) ratio. The F_2 passes a test of this ratio at the 0.99 level of significance. This suppressor would not explain the excess wild-type progeny in the cross of *fol*^{1b} with *fol*^{2d} as the F_2 progeny of this cross fail to fit a 3:5 ratio at the level of 0.1–0.5 significance. This will be investigated further.

Group 2 is presently comprised of four lines of mutants (Table 2) that all originate from the same mutated population of cells and were isolated by the same Procedure 2 (see METHODS). Phenotypes within Group 2 are not identical, however. *fol*^{2a} and *fol*^{2d} never show attraction to folate in well tests or T-mazes (Figures 4 and 5; I_{che} for *fol*^{2d} = 0.46 ± 0.02). *fol*^{2b} and *fol*^{2c} are not attracted to folate in the well tests but, given sufficient time to respond, are eventually attracted in the T-maze (Figures 4 and 5; I_{che} for *fol*^{2c} = 0.64 ± 0.05). It is likely that *fol*^{2a} and *fol*^{2b} are different alleles of the same gene *fol*^{2} and that *fol*^{2d} and *fol*^{2c} are copies of *fol*^{2a} and *fol*^{2b}, respectively. Members of Group 2 show no intergenic interaction when crossed with mutants of other groups, except for *fol*^{2d} (discussed above) (Table 3). Members of Group 2 crossed with each other are expected to show no wild-type progeny. However, *fol*^{2a} and *fol*^{2b} did yield 1 clone out of 82 that scored consistently as normal in the well test (Table 3). When the reciprocal cross was performed (Table 3) no wild-type progeny were detected, but the sample size was smaller than in the previous cross and rare

recombinants would have been missed. The wild-type F_2 progeny was not likely to result from an error in scoring and, therefore, may represent a recombinant between *fol*^{2a} and *fol*^{2b}.

One mutant, *fol*^{3}, comprises Group 3 (Table 2). *fol*^{3} was isolated from the same population that included mutants of Groups 1 and 2. *fol*^{3} shows no strong intergenic interaction when crossed with members of Groups 1 and 2 (Table 3).

DISCUSSION AND CONCLUSIONS

We have isolated ten lines of mutants that are not normal in response to folate but are normal in all other chemokinesis responses tested. The time and concentration of the tests are apparently not limiting except for *fol*^{2b} and *fol*^{2c}, which will eventually accumulate in folate, given sufficient time in the T-maze. These mutants are of the same complementation group as *fol*^{2a} and *fol*^{2d} that are never attracted to folate.

The ten lines of mutants all harbor recessive, single site, genic mutations that fall into three complementation groups. Crosses between either of two members of complementation Group 1 and *fol*^{2d} of Group 2 produce more wild-type F_2 progeny than expected, but less than expected for full suppression interaction between the genes *fol*^{1} and *fol*^{2}. The crosses will be repeated and some of the wild-type phenotype progeny analyzed to determine if they are double mutants, true wild type, or heterozygotes because of unusual but possible macronuclear regeneration after autogamy.

Members of Group 2 fall into two phenotype classes: a) never attracted to folate (characteristic of *fol*^{2a} and *fol*^{2d}) and b) attracted in the T-maze at 30 min (characteristic of *fol*^{2b} and *fol*^{2c}). Time course studies of responses in the T-maze have confirmed that normal cells are immediately attracted to folate in the T-maze; *fol*^{2a} is never attracted; and *fol*^{2b} begins to show slight attraction only after 5 min (DINALLO, unpublished results). Crosses between *fol*^{2a} and *fol*^{2b} have produced 1 clone of wild-type-phenotype out of 82 total progeny. The existence of a wild-type recombinant would be consistent with the mutants *fol*^{2a} and *fol*^{2b} being different alleles and producing different phenotypes. The percent recombination (approximately 2.4%), however, would be higher than would be expected for intragenic recombination unless the gene were of considerable size. More attempts will be made to isolate and analyze such rare recombinants.

The two methods of selection for fol^{-} mutants were very different. Procedure 1 involved a slow enrichment procedure with retesting in the T-maze whereas method 2 involved cloning of all cells swimming down a gradient of folate attractant, similar to the method used to select *d4-530*, a mutant repelled by the attractant sodium acetate (VAN HOUTEN 1976, 1977). All clones were retested in Procedure 2, using the well test, a fast test of attraction or repulsion. The well test measures the initial responses of cells to attractants and control solutions and is comparable to tests run for only 2–5 min in the T-maze (VAN HOUTEN, MARTEL and KASCH, unpublished results). Cells could conceivably be unable to adapt and sustain population responses for 30 min in the T-maze assay and yet respond normally and quickly in the well test. Such abnormal cells would be detected by Procedure 1 but overlooked by Procedure 2. [Such lines unable to respond normally to ammonium have been isolated by Procedure 1 (VAN HOUTEN, MARTEL and KASCH, unpublished results).]

TABLE 4

Stocks, genotypes, phenotypes and genic symbols of fol⁻ mutants of P. tetraurelia

Stock	Genotype	Complementation group	Notes
d4-534	fol^{1a}/fol^{1a}	1	Note attracted to folate; otherwise appears normal
d4-535	fol^{1b}/fol^{1b}	1	Only mutant isolated by Procedure 1; similar to d4-534
d4-536	fol^{2a}/fol^{2a}	2	Not attracted to folate; otherwise appears normal
d4-537	fol^{2b}/fol^{2b}	2	Attracted to folate only if allowed to distribute more than 5 min in T-maze assay
d4-538	fol^{3}/fol^{3}	3	Only member of complementation Group 3; not attracted to folate; otherwise appears normal

Rescreening with the well test in Procedure 2 insured the isolation of mutants that were not capable of an initial response to attractant. The development of Procedure 2 stems from our interest in chemoreceptors. Mutants with defective receptors should be among those not able to respond even initially to folate. Mutants that require more time to accumulate in folate, although probably not having defective receptor function, would also be detected by Procedure 2 but overlooked by Procedure 1. Although all members of the complementation Group 2 have been isolated by the same Procedure 2, mutants fol^{2a} and fol^{2d} never respond to folate in wells or T-mazes, whereas fol^{2b} and fol^{2c} do not initially, but eventually, accumulate in the T-maze. These results demonstrate that Procedure 2 is successful at isolating mutants of either type of phenotype described above.

Two mutants of the same complementation Group 1 (fol^{1a} and fol^{1b}) were isolated by Procedures 1 and 2, respectively. Neither mutant is ever attracted to folate. Therefore, both procedures can be used to detect mutants of such phenotypes. Procedure 1 is less effective than Procedure 2, however, since fol^{1b} is the only mutant fol⁻ line isolated by this method.

It appears that eight of these mutant lines described possibly never detect the presence of folate in the external medium since they do not respond in the well test assay or in T-maze. If putative receptors for folate are defective in these mutants, the chemical signal will not be successfully transduced into the electrical signal in folate (VAN HOUTEN 1979) that in turn alters swimming behavior. The eight mutant cell lines successfully accumulate in or disperse from other compounds and give normal avoiding reactions in response to cations K, Ba and Na. Therefore, it is likely that the ciliary apparatus is normal and that some step in the chemosensory transduction pathway before the electrical signal arriving at the cilia is affected. We are presently examining binding and uptake of folate to determine if among these complementation groups are mutants defective in putative membrane chemoreceptors for folate.

Five lines of mutants are now assigned the standard *d4* designation (derived stocks of *P. tetraurelia*). Table 4 summarizes these designations and mutant symbols.

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LITERATURE CITED

ECKERT, R., 1972 Bioelectric control of cilia. Science **176:** 473–481.

KUNG, C., 1971 Genic mutations with altered system of excitation in *Paramecium aurelia*. II. Mutageneses, screening and genetic analysis of mutants. Genetics **69:** 29–45.

MACHEMER, H., 1976 Interactions of membrane potential and cations in regulation of ciliary activity in *Paramecium*. J. Exp. Biol. **65:** 427–448.

SONNEBORN, T. M., 1970 Methods in *Paramecium* research. pp. 243–339. In: *Methods of Cell Physiology*, Vol. 4. Edited by D. M. PRESCOTT. Academic Press, New York.

VAN HOUTEN, J., 1976 Ph.D. Dissertation. University of California, Santa Barbara.

VAN HOUTEN, J., 1977 A mutant of *Paramecium* defective in chemotaxis. Science **198:** 746–749.

VAN HOUTEN, J., 1979 Membrane potential changes during chemotaxis in *Paramecium*. Science **204:** 1100–1103.

VAN HOUTEN, J., H. HANSMA and C. KUNG, 1975 Two quantitative assays for chemotaxis in *Paramecium*. J. Comp. Physiol. **104:** 211–223.

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