CHEMOATTRACTION IN *TETRAHYMENA:* ON THE ROLE OF CHEMOKINESIS

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ABSTRACT

Chemoattraction of *Tetrahymena pyriformis,* strain GL, was measured during starvation and under different growth conditions. Log phase cells starved in buffer are attracted by certain amino acids, peptides, and proteins. Cysteine, methionine, and phenylalanine are attractants at 10^{-4} M. The peptides in proteose peptone (PP) and yeast extract (YE) are active at 10^{-6} M. Epidermal growth factor (EGF) is active at $>3 \times 10^{-5}$ M. Among the proteins, platelet derived growth factor (PDGF) is the most active (3×10^{-8} M). Cells growing in defined medium are attracted by PP, YE, and some proteins (PDGF).

Swimming speed was measured for starved cells with and without added attractants or repellents. With addition of PP the swimming speed increases from 0.42 to 0.51 mm/s., but for PDGF it is unchanged. The swimming speed of starved cells increases when the cells approach a solidified attractant (PP) as measured by the speed at a given distance. The speed of cells moving towards the attractant is higher than that of cells moving away from it.

In conclusion, certain amino acids, peptides, and proteins are chemoattractants for *Tetrahymena*. Chemokinesis likely plays a considerable role in the case of PP (and YE), since they increase swimming speeds, whereas attraction by PDGF may involve chemotaxis.

INTRODUCTION

Detailed studies on the chemosensory response of ciliated protozoa have been performed with *Paramecium* (Dryl, 1973; Van Houten *et al.,* 1982) and with *Tetrahymena* (Almagor *et al.,* 1981; Levandowsky *et al.,* 1984).

The chemosensory response can be considered as chemotaxis and/or chemokinesis. Chemotaxis refers to an oriented movement—the cell orients itself and moves towards or away from the chemical source—whereas chemokinesis denotes an unoriented change of swimming speed (orthokinesis) or turning/tumbling frequency (klinokinesis) by unoriented cells which accordingly use the gradually increasing speed to reach the attractant (Lapidus and Levandowsky, 1981).

Recently we described the effect of Platelet Derived Growth Factor (PDGF) on chemoattraction of *Tetrahymena* (Andersen *et al.,* 1984). Cells starved in buffer are also attracted by amino acids and peptides whereas cells taken directly from Holz's defined medium (Holz, 1964) as modified by Rasmussen and Modeweg-Hansen (1973)

respond only towards the peptides in Proteose Peptone (PP) and Yeast Extract (YE) and towards PDGF (Leick and Hellung-Larsen, 1985).

In the present paper we have: (1) reinvestigated the chemoattraction of a number of components including the growth factors EGF and PDGF by using four different assays for registration of the cell accumulation, (2) correlated the chemosensory response to the swimming speed and to the intracellular cAMP level, and (3) measured the cell number at certain distances from a gelified attractant and measured the swimming speed towards and away from the attractant.

We conclude that the chemosensory response of *Tetrahymena* towards peptides, at least to a large extent, can be explained by a chemokinesis, whereas the response towards PDGF, which has no effect on the swimming speed or tumbling/turning frequency, may involve a taxis mechanism.

MATERIALS AND METHODS

Isobutyl-methyl-xanthine (IBMX) was purchased from Aldrich-Chemie, W. Germany. The serum substitute Ultroser G was obtained from LKB, Bromma, Sweden. Proteose peptone (PP) and yeast extract (YE) were purchased from Difco. The binding protein for the cAMP assay was kindly supplied by Dr. P. Thams and Dr. C. J. Hedeskov, Department of Biochemistry A, University of Copenhagen. The ^{3}H-labeled cAMP was purchased from Amersham (TRK 498).

Tetrahymena pyriformis strain GL was grown at 28°C in PY-medium (0.75% PP, 0.75% YE, 1.5% glucose, 1 mM MgSO$_4$, 50 μM CaCl$_2$, and 100 μM ferric citrate) or in a defined medium (DM) containing amino acids, ribosides, vitamins, and salts (Holz, 1964). Starvation of the cells was performed by centrifugation at 500 $\times$ g, a careful wash with 10 mM Tris-HCl, pH = 7.4, and resuspension in this buffer to a cell concentration of 10^5 cells/ml. The cells were starved for 16 h at 28°C.

The chemosensory response was studied at 25°C by four different methods: (1) *Capillary assay*—by use of an apparatus with horizontal capillaries for migration of the cells into a trap, which allows electronic counting of the accumulated cells (Leick and Helle, 1983). In most of the experiments reported here the outer chamber had a volume of 10 ml instead of 2 ml. The experiments were run for 2, 5, or 21 h. For further details see Leick and Helle (1983). (2) *Gel assay*—a qualitative assay for testing if substances gelified with 1% agarose (iself inert) were attractants or repellents. A small piece of gel ⅛–1 mm^3 was placed on a glass slide. Starved cells (10^4–10^5 cells/ml) were added and their distribution followed in a stereo microscope. (3) *Two-phase assay*— a semi-quantitative micro-assay with high sensitivity. Cells suspended in PY, DM, or Tris (10^5/ml) (25°C) were layered carefully in glass tubes on top of PY, DM, or Tris containing 5% of the radio opaque drug metrizamide and a defined concentration of attractant/repellent (Fig. 1). In the negative controls all cells stay in the upper phase, whereas the cells migrate into the bottom layer within 10 min if an attractant is present. If the cells are placed in the bottom layer with metrizamide they move towards the upper phase, *i.e.*, they try to avoid the metrizamide, which also slightly reduces their swimming speed. After addition of attractant the cells move downwards towards the attractant although they then have to approach the metrizamide. Gelatine, ficoll, and sucrose did not work either because they are attractants (gelatine) or because the cells get partially trapped in the bottom phase (ficoll and sucrose). (4) *Field-assay.* Analyses of cell accumulation and of swimming speed at different distances from gelified attractants/repellents: pieces of gelified attractants/repellents (⅛ mm^3) were placed at the edge of a glass slide to ensure access of oxygen to the cells when mounted with a coverslip. The object was placed on an automatic scanning stage on a Leitz
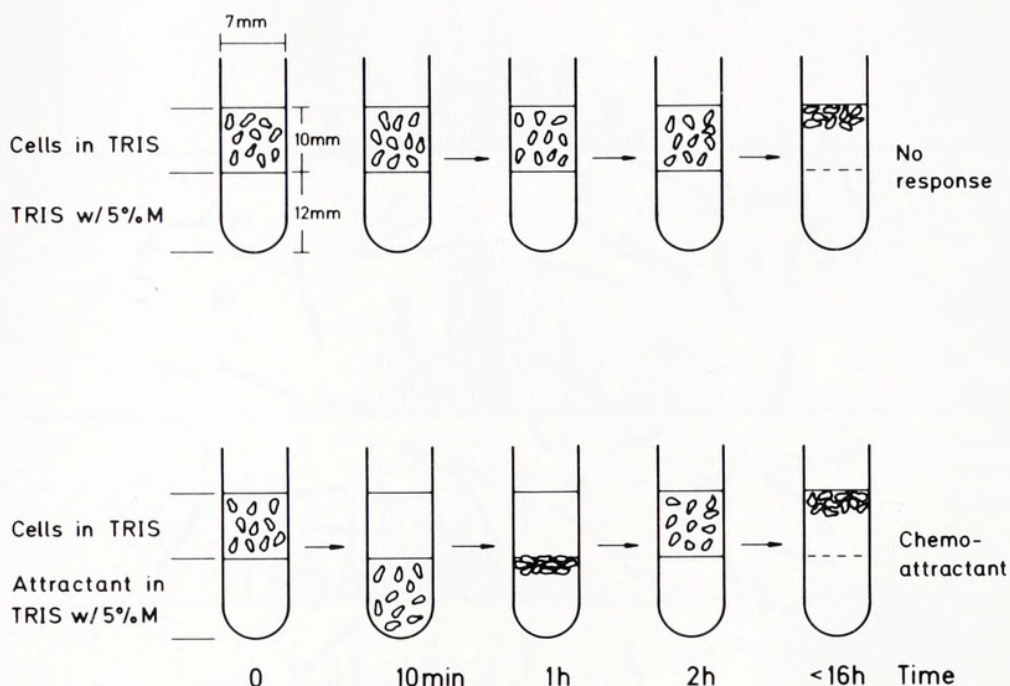
Schematic representation of the two-phase assay.

FIGURE 1. Schematic representation of the principle in the two-phase assay for chemoattraction. 400 μl *Tetrahymena pyriformis* log cells grown in PY, DM, or starved in Tris were adjusted to 10^5/ml at 25°C and carefully layered in glass tubes (inner diameter: 7 mm) on top of 400 μl PY, DM, or Tris containing 5% w/v metrizamide. The bottom phase was added attractant and/or repellent in the experimental tubes. Visual readings were performed at different times after set-up. In some cases the assay was performed in polyvinyl micro-cuvettes and the optical density at 450 nm of the bottom phase was registered after 10 minutes.

Periplan microscope coupled to a Leitz Tass Image Analyzer (objective 2½×, final magnification 7×). A program was developed to automatically count the cell density per field in 10 adjacent fields each measuring 2.4 × 2.1 mm. Whenever the last field had been analyzed the scanning stage automatically relocated the first field in focus and the analyses were repeated. Another program, which could be activated whenever desired, was developed to measure swimming speed and turning/tumbling frequency. Upon activation six successive images of the same field, each detected with a 200 ms delay, were fused within an image memory and shown on the video screen. Thus the track of each cell in an one-second period was directly visible and the swimming velocities were automatically calculated from the length of each track. Furthermore, turning/tumbling of a cell was directly visualized by deviation from a linear track (Fig. 2).

Intracellular levels of cyclic AMP were determined by a protein binding assay essentially as described by Geisler *et al.* (1977). Labeled cAMP and unlabeled cAMP (to be measured) compete for binding to a binding protein isolated from rabbit muscle. 2,8-^{3}H-Adenosine-3',5'-cyclic phosphate ammonium salt (30–50 Ci/mmol) was diluted to 1 pmol/50 μl with assay buffer before use. Standards containing 0.25–12 pmol/50 μl of unlabeled cAMP were prepared from a stock solution.

RESULTS

The swimming behavior (swimming speed and turning/tumbling frequency) was studied in cells grown in PY-medium and starved in Tris-buffer and with starved cells

Swimming tracks of T. pyriformis in PY-medium

FIGURE 2.   Swimming tracks. *Tetrahymena pyriformis* cells (4×10^4 cells/ml) logarithmically growing in PY-medium were placed under a coverslip supported by small coverslips to give a chamber with a depth of 300 μm. The cells (25°C) were examined with a Leitz Tass Image Analyzer at 7× magnification and swimming tracks were generated electronically (see Materials and Methods). The Figure shows a representative collection of tracks. For reasons of clarity only 63 tracks are shown here. Each track corresponds to the movement of a single cell within 1 s and serves as a basis for calculation of swimming velocities and for estimation of per cent cells showing 0, 1, 2 etc. turns within 1 s.

after addition of various compounds. Figure 2 shows a representative number of swimming tracks from cells growing logarithmically in PY-medium at 25°C. On the basis of the tracks in Figure 2 and analogous pictures for other conditions it was found that ~50% of the cells are "runners," *i.e.,* show no turns whereas ~25% show one turn within 1 s. The percentages for starved cells were 40% and 35%, respectively, in agreement with the results of Almagor *et al.* (1981). Additions to starved cells of amino acids or various proteins (*e.g.,* PDGF) were without effect on the percentage of runners.

To determine to what extent the chemosensory effect of PP is mediated by an increase in the swimming speed, this parameter was measured for starved cells (Tris control), for cells growing in PY-medium, and for various conditions where different compounds were added to starved cells (Fig. 3). The rationale for adding imidazole and IBMX was to decrease and increase, respectively, the intracellular concentration of cAMP (Wells *et al.,* 1975). The means of the distributions $\pm$ standard deviations (mm/s) were 0.40 $\pm$ 0.10 (+ imidazole), 0.42 $\pm$ 0.08 (control), 0.51 $\pm$ 0.11 (+PP), 0.52 $\pm$ 0.08 (PY-medium), 0.52 $\pm$ 0.11 (+dbcAMP), 0.63 $\pm$ 0.10 (+IBMX), and 0.63 $\pm$ 0.12 (+IBMX + dbcAMP). By applying *t*-tests on the random distributions we found significant differences ($P < 0.001$) for comparisons between the control value and all other values except that of imidazole (.10 $< P <$ .20). Thus, the cells swim faster in PY than when starved and the addition of PP increases the swimming speed to the level of that of cells in PY. Interestingly, this speed value is also obtained by
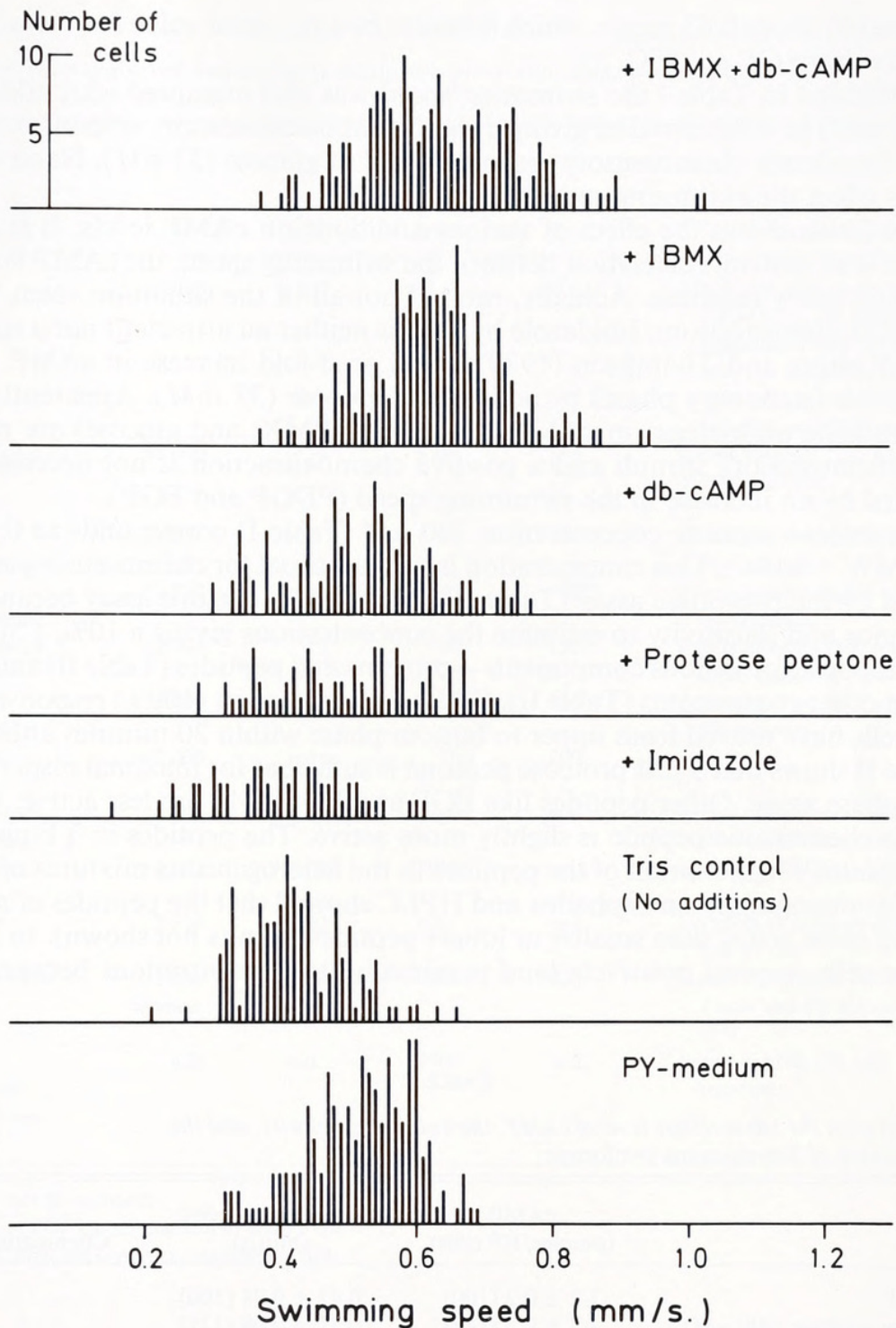
FIGURE 3. Distribution of swimming speed under different conditions. On the basis of the swimming tracks showing 0 turns (Fig. 2) the swimming speed was calculated for *T. pyriformis* cells grown in PY-medium (bottom profile) and for cells starved for 16 hours in 10 mM Tris-buffer, pH = 7.4 (Tris control). The other profiles show the effect (after 20 minutes) of addition of imidazole (10 mM), proteose peptone (0.1% $\sim$ 300 μM), db-cAMP (1 μM), IBMX (1 mM), and IBMX + db-cAMP. The number of tracks analyzed was (from top to bottom) 170, 200, 130, 80, 60, 130, and 170.

addition of db-cAMP. IBMX gives a further increase in swimming speed, but this value cannot be further increased by addition of db-cAMP. Simultaneous addition of imidazole and IBMX (with 10-fold molar excess of imidazole) gives no effect. When IBMX is added to cells in PY or to starved cells with PP added, the swimming speed

is increased to about 0.63 mm/s, which seems to be a maximal value for *Tetrahymena pyriformis* strain GL at 25°C.

As indicated in Table I the swimming speed was also measured after addition of PDGF (9 n*M*) (a concentration giving a significant chemosensory response), of EGF (20 μ*M*) (moderate chemosensory response), and of glucose (37 n*M*). None of these additions affect the swimming speed.

Table I also shows the effect of various additions on cAMP levels. It is evident that there is no general correlation between the swimming speed, the cAMP level, and the chemosensory response. Actually, most if not all of the situations seem to have their specific characteristics. Imidazole by itself is neither an attractant nor a repellent. Nandini-Kishore and Thompson (1979) found an 8-fold increase in cAMP level of *T. pyriformis* (stationary phase) by addition of glucose (37 m*M*). Apparently, compounds causing an increase in cAMP (db-cAMP, IBMX, and glucose) are not necessarily chemosensory stimuli and a positive chemoattraction is not necessarily accompanied by an increase in the swimming speed (PDGF and EGF).

The proteose peptone concentration 300 μ*M* (Table I) corresponds to 0.1% (assuming MW = 3.000). This concentration is supraoptimal for chemosensory response measured by the two-phase assay (Table II). We chose to use this assay because of its convenience and sensitivity to estimate the concentrations giving a 10%, a 50%, and a 100% response for various components—proteins and peptides (Table II) and amino acids and other components (Table II). One hundred percent (100%) response means that all cells have moved from upper to bottom phase within 20 minutes after set-up.

Table II shows that 9 μ*M* proteose peptone is sufficient for maximal response with the two-phase assay. Other peptides like EGF and EGF 3–14 are less active, whereas leukocyte chemotactic peptide is slightly more active. The peptides in YE give comparable results. Fractionation of the peptides in the heterogeneous mixtures of PP and YE by chromatography on Sephadex and HPLC showed that the peptides of medium length are more active than smaller or longer peptides (results not shown). In the case of PP the cells respond positively (and maximally) to concentrations between 9 μ*M*

TABLE I

Relation between the intracellular level of cAMP, the swimming velocity, and the chemoattraction of Tetrahymena pyriformis

	cAMP (pmoles/10^6 cells)	Swimming speed (mm/s)	Chemoattraction
Control	1.2 ± 0.2 (100)	0.42 ± 0.08 (100)	
Proteose peptone (300 μ*M*)	1.7 ± 0.3 (142)	0.51 ± 0.08 (121)	+
db-cAMP (1 μ*M*)	4.1 ± 0.3 (342)	0.52 ± 0.11 (124)	0
IBMX (1 m*M*)	2.8 ± 0.4 (233)	0.63 ± 0.10 (150)	−
Imidazole (10 m*M*)	1.6 ± 0.3 (133)	0.40 ± 0.10 (95)	0*
PDGF (9 n*M*)	n.d.	0.41 ± 0.09 (98)	+
EGF (20 μ*M*)	n.d.	0.41 ± 0.09 (98)	(+)
Glucose (37 m*M*)	n.d.	0.43 ± 0.08 (102)	0

Various compounds were added to cells starved in Tris-buffer for 16 h (control) (25°C). After 30 minutes aliquots were analyzed for level of cAMP (average of 4 separate experiments), swimming speed was measured at the same time (average of at least three separate experiments yielding 60–200 tracks). The results are given as mean (±S.D.). Chemoattraction was measured with the capillary assay, the gel assay, and the two-phase assay, which in all cases gave the same results. Numbers in parenthesis show percent compared to control. +: Attractant, −: Repellent.
* Imidazole abolishes the effect of IBMX when present in 10-fold molar excess.

TABLE II

Chemoattraction of starved Tetrahymena pyriformis *towards proteins and peptides.*
The two-phase assay was used

Compound	MW	Concentration giving a certain response			Comments
		10%	50%	100%	
PDGF	30.000	1 nM	6 nM	30 nM	PDGF was kept as stock solution in 0.5 M HAc. An aliquot was neutralized with NaOH before use. The acetate by itself showed 10% attraction at 3 nM which is the conc. in PDGF at 30 nM.
Serum albumin fat-free (bovine)	68.000	4	n.d.	65	Excess albumin (265 nM) showed 90% response.
Histone 3 (calf thymus)	15.324	n.d.	65	110**	Excess histone 3 (600 nM) showed 0% response.
Histone 3 (calf thymus)	11.282	n.d.	45	90**	Excess histone 4 (800 nM) showed 0% response.
Serum albumin (human)	68.000	15	70	135	Excess albumin (300 nM) showed 100% response.
Lysozyme (egg white)	14.400	7	70	150	Excess lysozyme (1.2 μM) showed 0% response.
Hexokinase (yeast)	102.000	n.d.	50	200	
Cytochrome C (horse heart)	12.500	n.d.	160	400	Excess cytochrome C (1.6 μM) showed 80% response.
EGF (mouse)	6.045	3300	32000	n.d.	No effect of EGF at 330 nM and 33 nM.
EGF 3–14* (mouse)	1.310	4200	n.d.	n.d.	No effect at concentrations 420 nM and 42 nM.
Proteose peptone	3.000 average	1000	4500	9000	Excess PP showed 100% response at 1 mM and 0% response at 10 mM.
Leucocyte chemotactic peptide (f-met-leu-phe)	438	n.d.	2500	n.d.	Excess peptide (50 μM) showed 0% response.

n.d. = not determined.
* Part of EGF containing amino acids 3–14.
** Maximal response was consistently 90%.

and 1 mM, but they are not attracted by 10 mM (Table II). Such an optimal plateau of concentration is seen for the majority of attractants.

A number of proteins were tested and it is evident that all are considerably more active on a molar basis than the peptides EGF and PP (Table II). This is especially true for PDGF, but a number of other proteins are also active in the nanomolar range. We carefully checked the effect of salts and have found an attractant effect of acetate (10^{-4}–10^{-3} M). Therefore the effect of PDGF (stock in strong HAc) was corrected for the effect of acetate. A number of other anions (bicarbonate, sulphate, phosphate) showed no effect in concentrations 10^{-9}–10^{-3} M. No effect was observed with various lithium salts (10^{-5}–10^{-3} M) or with sodium vanadate (10^{-5}–10^{-3} M). It is noticeable that 4-fold excess of BSA and cytochrome C compared to their optimal concentration

show 80–90% chemoattraction, whereas 5-fold and 8-fold excess of histone 3 and lysozyme, respectively, show 0% response.

Some of the amino acids (three of the nine tested) namely cysteine, methionine, and phenylalanine show a positive though not complete response at concentration of about 10^{-4} M (Table III). A mixture of 10 amino acids (10^{-4} M) consistently gave 100% response, but dilution to 10^{-5} M reduced the response to about 25%. A number of other low molecular weight substances were tested including carbohydrates and cyclic nucleotides. All of them showed no or only a slight response.

Measurements with the capillary assay have shown that proteose peptone (0.1%) attracts all of the cells (10^4/ml) in a 2 ml outer chamber (Leick and Hellung-Larsen, 1985). With the purpose of getting information about the mechanism behind this response four different experiments were performed (results not shown): (1) If the size of the outer chamber is increased to 15 ml, 40 ml, or 80 ml, and the cell concentration is kept constant at 10^4 cells/ml, the *absolute* amounts of cells trapped were 60, 70, and 80×10^3 cells, respectively. (2) If IBMX (1 mM) is added to the outer chamber the cells accumulate in the trap since IBMX is a repellent. (3) If IBMX (1 mM) is added to inner and the outer chamber with PP present in the inner chamber the time necessary for complete accumulation of cells is decreased. (4) Only 7% of color dye (bromophenol blue) diffuses out of the inner chamber during 24 hours (spectrophotometric measurement). These capillary apparatus results seem to indicate that (a) short-ranged concentration gradients are formed close to the outlets of the capillaries and (b) the accumulation of cells is strongly dependent on the swimming speed of the cells in the outer chamber, because the gradient is short compared to the dimensions of the outer chamber.

TABLE III

Chemoattraction by starved Tetrahymena pyriformis *towards amino acids and other low molecular weight substances. The two-phase assay was used*

Compound	Optimal concentration range (M)	Response
Asp	10^{-4}–5×10^{-4}	Weak*
Citrullin	2×10^{-4}– 10^{-3}	Negative
Cys	2×10^{-4}–2×10^{-3}	Positive
His	2×10^{-4}–2×10^{-3}	Weak
Ile	2×10^{-4}– 10^{-3}	Negative
Leu	2×10^{-4}– 10^{-3}	Negative
Met	2×10^{-4}– 10^{-3}	Positive
Phe	2×10^{-4}–2×10^{-3}	Positive
Thr	2×10^{-4}–5×10^{-3}	Weak
Trp	2×10^{-4}– 10^{-3}	Negative
Mixture of amino acids**	10^{-4} of each	Positive
Betaine	2×10^{-4}– 10^{-3}	Weak
Cadaverin	2×10^{-4}– 10^{-2}	Weak
Dibuturyl-cAMP	10^{-4}– 10^{-3}	Negative
Dibuturyl-cGMP	10^{-4}– 10^{-3}	Negative
Glucose	10^{-4}– 10^{-2}	Negative
Glutathione	2×10^{-4}–2×10^{-3}	Weak
Glycerol	10^{-3}– 10^{-2}	Negative
Glycerophosphate sodium salt	10^{-3}– 10^{-2}	Negative
Sucrose	10^{-3}– 10^{-2}	Negative
Thymine	10^{-3}– 10^{-2}	Negative

* Weak: accumulation after 10–20 min of 5–20% of the cells initially present in the upper phase.
** Contains ala, arg, asn, glu, gln, gly, his, ile, leu, lys, met, phe, pro, ser, thr, trp, and val.

With the field assay (Table IV) it was possible to answer some important questions: how rapidly do the cells respond to various chemical stimuli? Do they accelerate on their way towards the attractant? Is the swimming speed greater towards than away from the attractant? The results are shown in Table IV. At time 0 there is the same number of cells in all fields but after only 1 min the cells accumulate in field 1. Apparently some cells are drained from fields 2 and 3, others enter from areas outside the scanned area, which had a width of 2.1 mm.

As indicated in the lower part of Table IV the cells in field 10 swim 0.38 mm/s [comparable to the control value for starved cells (0.42) in Figure 2]. In field 6 where accumulation of cells is about to increase (720 after 16 min) the swimming speed is 0.44 mm/s. Thus, the speed increases with decreasing distance to the attractant. After 12 minutes the swimming speed was measured in field 2. The cells moving towards the attractant have a higher speed than those swimming away from it. The values differed significantly ($P < 0.001$). In some experiments we used Ultroser G (a mixture of proteins such as adhesion and growth factors) as an attractant instead of PP. The results became more reproducible with Ultroser G probably due to the fact that the proteins in Ultroser G diffuse more slowly out of the gel than the small peptides in PP.

DISCUSSION

The main purpose of the present paper was to elucidate the relative role of chemokinesis in chemoattraction of *Tetrahymena pyriformis*, Strain GL. We have confirmed that the turning/tumbling frequency may be modulated by the presence of attractants and repellents (Almagor et al., 1981) and that the swimming speed may be increased when the cells approach the attractant (Levandowsky et al., 1984). How-

TABLE IV

Analyses of cell accumulation and swimming speed of Tetrahymena pyriformis *as a function of distance from attractant*

Field number		1	2	3	6	10
Mean distance from attractant (mm)		1.2	3.6	6.0	13.2	22.8
Cell number per field at various times after addition of cells	0 min	600	600	510	600	600
	1 min	1350	580	510	n.d.	n.d.
	2 min	1920	550	380	n.d.	n.d.
	12 min	≫1920	1730	450	n.d.	n.d.
	16 min	≫1920	≫1920	1720	720	610
Swimming speed (mm/s.) 12 min after addition of cells		0.55	0.56 ± 0.07 (towards attractant) 0.49 ± 0.06 (away from attractant)		0.44	0.38

A chamber was made on top of a glass slide by means of piece of agarose gel (1% HSA agarose in 10 m*M* Tris, pH = 7.4) and a coverslip to give a depth of 600 μm. One piece of gel containing attractant (0.5% PP in Tris-buffer) was placed close to the edge of the coverslip thereby allowing sufficient access to oxygen. Cells starved in Tris buffer overnight (2×10^5 cells/ml) were added under the coverslip (25°C). The cell number and the swimming speed were registered in fields (2.4 mm × 2.1 mm) with a certain distance from the gel with attractant. The swimming speed was based on the length of tracks during a period of 1 s. A number of 50 tracks was analyzed per calculation. The results are given as mean (±S.D.).

ever, these observations have been extended in the following way: PP influences the swimming speed—probably due to cAMP concentration—whereas other attractants do not (Table I). These data are in reasonable agreement with those of Voichick *et al.* (1973) and Nandini-Kishore and Thompson (1979). We have been aware of the fact that the swimming speed is strongly dependent on the culture temperature (Hill, 1983). The chemosensory attraction of *T. pyriformis* is not only seen with starved cells. Actually cells in DM respond towards PP, YE, and PDGF, and cells in PY-medium respond towards PDGF.

Interestingly, the optimal concentration of PP for growth is not the same as that for chemoattraction. The same holds true for the amino acids of DM. In both cases the optimal chemoattraction is seen at a 10–50 fold lower concentration than that in the growth media.

The role of chemokinesis is obvious from the measurements of swimming speed after various additions (Table I) and in different distances from an attractant (Table IV). Values for swimming speed at 25°C (0.40–0.63 mm/s.) are comparable to those reported by Hill (1983) and Levandowsky *et al.* (1984) for *T. thermophila.* The correlation of increase in swimming speed to increase in cAMP level seems to be positive (Table I).

By use of the capillary assay, we found earlier with *T. thermophila,* B7, that a mixture of amino acids show chemoattraction (Leick and Hellung-Larsen, 1985). The two-phase assay used in the present study allows us to conclude that for *T. pyriformis* cysteine, methionine, and phenylalanine are attractants, whereas histidine, isoleucine, leucine, and threonine, and tryptophan are not. Levandowsky *et al.* (1984), using *T. thermophila,* CU strain 307, found cysteine, methionine, histidine, and leucine to be positive. Almagor *et al.* (1981), using *T. thermophila,* strain WH 52, scored leucine and methionine as positive. We have no explanation for the differences, but we have at least confirmed that an interesting specificity exists for some of the amino acids. Furthermore, the present data indicate that the amino acids (on a molar basis) are less active than peptides and proteins. The peptides in PP and YE are good attractants in micromolar concentration. Apparently those of middle-length are most active, but all peptides tested so far show some chemoattraction. Proteins are on a molar basis the most efficient chemoattractants and considerable specificity exists (Table II). Thus, 6 nM PDGF gives 50% response whereas about a 10-fold higher concentration is needed for histones 3 and 4.

Attempts to demonstrate receptors in *Tetrahymena* for PDGF by use of ^{125}I-PDGF (Heldin *et al.,* 1981), to induce tyrosine-specific phosphorylation by PDGF (Ek and Heldin, 1982), and to measure PDGF and EGF in extracts or exudates of cells by a radioreceptor assay thus far have been unsuccessful (Heldin and Hellung-Larsen, unpub.).

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