Thermosensory Behaviour in *Paramecium tetraurelia*: a Quantitative Assay and Some Factors that Influence Thermal Avoidance

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(Received 25 October 1978)

The free-swimming ciliate *Paramecium* avoids warm regions. We have developed a quantitative assay of thermal avoidance, based on the interference between thermal avoidance and the normal tendency to swim upward (negative geotaxis). *Paramecium tetraurelia* swimming in a Tris/Ca²⁺ solution avoided a region warmer than about 40 °C. Several factors influenced the strength of the response, including the concentrations of monovalent cations and Ca²⁺, the temperature of the test region, and the temperature at which the animals had been cultured. At 1 mM-Ca²⁺, increasing Na⁺ concentration enhanced avoidance of a 40 °C region, and at a constant Na⁺ concentration (0.5 mM), avoidance improved with decreased Ca²⁺. When the ratio $[Na^+]/[Ca^{2+}]^{1/2}$ was held constant, varying Na⁺ and Ca²⁺ concentrations did not affect thermal avoidance. Other monovalent cations (Cs⁺, Li⁺, Rb⁺) and hydroxylamine also enhanced thermal avoidance, and K⁺ was somewhat less effective than these. The strength of the avoidance response increased with increasing test temperature in the range of 37 to 42 °C for cells grown at 28 °C. Cells grown at 15 °C had a lower threshold for thermal avoidance, and those grown at 35 °C showed no avoidance at 40 °C and only poor avoidance at higher temperatures. Cells cultured at 15 or 35 °C and then shifted to 28 °C acquired the thermal behaviour typical of cells cultured at 28 °C. Behavioural mutants with defective Ca²⁺ channels (Pawns) are incapable of reversing their swimming direction and showed little or no thermal avoidance. We suggest that thermal avoidance is triggered by thermotropic phase transitions in the lipids of the excitable membrane of *Paramecium tetraurelia*.

INTRODUCTION

Early studies by Mendelsohn (1902*a, b, c*) and by Jennings (1904, 1906) established that the ciliate protozoan *Paramecium* avoids regions of extreme temperature and tends to aggregate in a region of intermediate temperature, which for cells grown at 24 °C is 24 to 28 °C. More recent studies (Tawada & Oosawa, 1972; Tawada & Miyamoto, 1973; Nakaoko & Oosawa, 1977) have documented the fact that *Paramecium* can respond to very small thermal gradients by altering its swimming behaviour, but the means by which such small changes are detected, and the mechanism by which the thermal stimulus is transduced into a behavioural response, remain unknown. Extensive electrophysiological and genetic studies of *Paramecium* have yielded strong evidence that a variety of chemical and physical stimuli cause depolarization of the surface membrane and the opening of a voltage-sensitive Ca²⁺ channel. The resulting influx of Ca²⁺ raises the internal Ca²⁺ concentration above a critical value, bringing about a temporary reversal in the direction of the effective stroke of the several thousand cilia and a reversal in the swimming direction, followed by forward motion in a new direction (Naitoh, 1968; Eckert, 1972; Kung *et al.*, 1975; Nelson & Kung, 1978). The result of such avoiding reactions is net motion away from the stimulus.

Fig. 1. T-tube for assaying thermal avoidance. The test arm (T) was heated by pumping hot water through tubing wrapped around it until the test temperature was reached at thermistor A; temperature in the entry arm (E) was monitored with a thermistor at B. Cells were then injected into the entry arm, as described in Methods. After 5 min, the stopcock was closed and cells were removed for counting from arms E, T and the unheated control arm (C).

Thermotaxis has been observed in several other simple organisms, including the slime mould *Physarum polycephalum* (Tso & Mansour, 1975), the nematode *Caenorhabditis elegans* (Hedgecock & Russell, 1975), the cellular slime mould *Dictyostelium discoideum* (Poff & Skokut, 1977), *Chlamydomonas* (Oosawa, 1976) and *Escherichia coli* (Maeda *et al.*, 1976). The temperature at which thermal avoidance occurs depends on the growth temperature in *Paramecium* (Jennings, 1904; Tawada & Oosawa, 1972), and in *Dictyostelium discoideum*, Poff & Skokut (1977) have suggested a possible role for membrane lipid in thermosensory transduction.

We have devised a quantitative assay for thermal avoidance in *Paramecium* and have used it to study some of the factors, including growth temperature, which govern this behaviour. We have also begun to study thermal avoidance in mutants originally selected for defective swimming behaviour in response to other types of stimuli, as well as mutants selected for defective thermal avoidance.

METHODS

Cells and culture media. *Paramecium tetraurelia* (formerly *P. aurelia*, species 4), stock 51s and mutants Pawn A (d4-94) and Pawn B (d4-95) were gifts from C. Kung. They were grown at 28 °C in Cerophyl medium (Sonneborn, 1950) with *Enterobacter cloacae*.

Solutions. The control solution contained 1 mM-Ca(OH)₂, 1 mM-citric acid and 1 mM-Tris in deionized, distilled water. This solution was chosen to allow direct comparisons of our experiments with electrophysiological studies in which the same solution was used. All solutions were adjusted to pH 7.0 with Tris or HCl, except in the experiment with 3-(*N*-morpholino)propanesulphonic acid (MOPS) buffer, which was adjusted to pH 7.0 with citric acid. Cations were added as chloride salts.

T-tube assay apparatus. The test apparatus was similar to that used by Van Houten *et al.* (1975) to study chemotaxis in *Paramecium*. A T-shaped tube was fashioned from a two-way stopcock shell (Pyrex S8702) by attaching an extra arm (C) at the stopcock (see Fig. 1). This added arm was 5.9 cm long, 0.5 cm in diameter, and held a volume of 1.2 ml. The other two arms were 11 cm long, 0.65 cm in diameter, and held 3.7 ml each. A Teflon three-way stopcock plug with an inner diameter of 1 mm was used with this T-tube shell. To eliminate thermal convection, the T-tube was used in a vertical position so that the uppermost arm was the test arm (T) (i.e. the heated arm) and the lower arm was the entry arm (E). The small arm perpendicular to these two opposing arms was the control arm (C) (Fig. 1). Two thermistors (Fenwal Electronics

GB32J2) were embedded in epoxy near the three-way stopcock (A, B) to monitor the temperatures on both the T and E sides. The temperature was measured with a digital voltmeter accurate to within $\pm 0.05^\circ\text{C}$.

The thermistor at T was used routinely to monitor temperature in the heated arm, but to avoid obstructing the path of cells entering the T arm from the stopcock, the thermistor was placed several millimetres above and to the side of the stopcock opening. To determine the actual temperature at the stopcock opening, in a separate experiment we positioned a thermistor at the opening and recorded its output and that of the permanently mounted thermistor as the temperature in T was varied. From the calibration curve thus obtained, it was possible to approximate ($\pm 0.2^\circ\text{C}$) the temperature at the stopcock opening using a thermistor several millimetres away, out of the swimming path. The temperatures reported here are the temperatures at the stopcock opening, where the avoidance presumably occurs.

T-tube assay of thermotaxis. Cells were harvested in the exponential phase of growth (1500 to 2000 cells ml^{-1}) by centrifugation in a 100 ml pear-shaped vessel in an oil-testing centrifuge (International HN-S) for 2 min at 500 rev. min^{-1} , washed in control solution, then suspended in control solution at a density of about 20000 cells ml^{-1} . In most experiments, cells were held in this control solution at room temperature (25 to 28°C) for 60 min before thermotaxis assays. For the last 30 min of this pre-incubation, air was bubbled into the suspension with a fine capillary to ensure uniform suspension.

The test arm was filled with the solution to be tested, then sealed with a Parafilm-covered cork. The rest of the T-tube apparatus was filled with the same solution, the entry arm was closed with a serum stopper, and the control arm was left open. Tygon tubing ($\frac{1}{16}$ in diam., $\frac{1}{32}$ in wall) was wrapped around the test arm near the stopcock and distilled water at 80 to 90°C was circulated through the tubing with a peristaltic pump to heat the arm. The temperature at the thermistor in T was monitored and adjusted by altering the pump speed. During this heating the T-tube was mounted in a vertical position with the stopcock open and the test arm uppermost.

When the required temperature was reached at the test arm thermistor, the cells were injected slowly through the side of the serum stopper at the bottom of the entry arm (at room temperature) from a 1 ml syringe with a 20 gauge needle. Fluid displaced by the injection flowed out the control arm and the temperature of the test arm did not change. The temperatures at both thermistors were logged every minute and 5 min after injection of the cells the stopcock was closed to terminate the assay. The thermistor near the top of the entry arm (B) read between 28 and 31°C in all experiments. Cells were removed from each of the three arms and counted after dilution and immobilization in 1.0 mM-NiCl₂.

From the numbers of cells in each arm, we calculated an 'index of thermotaxis', I_T :

$$I_T = \frac{\text{cells in T}}{\text{cells in C} + \text{cells in T}}$$

When I_T is near unity, little thermal avoidance has occurred; with effective thermal avoidance, the value of I_T approaches zero. In control experiments with all three arms at the same temperature (room temperature), I_T was approximately 0.93. To control for variations in the rate of migration upward, the fraction of cells that escaped from the entry arm was measured, and expressed as I_M :

$$I_M = \frac{\text{cells in T} + \text{cells in C}}{\text{total cells (T+C+E)}}$$

I_M is obviously a function of the length of the assay, as well as the swimming velocity and the frequency of direction changes. In control solution, I_M was 0.1 to 0.2 for most of our experiments lasting 5 min. *Paramecium* showed very strong negative geotaxis under these conditions; with all arms at the same temperature, virtually all cells injected into arm E had entered T or C within 30 min. When the T arm was heated, the natural tendency to swim upward was counterbalanced by the tendency to avoid the warm region, and I_T decreased.

'Hot wire' assay of thermotaxis. The procedure for dark-field photography was adapted from that of Chang & Kung (1973). A shallow glass-bottomed trough 6.3 cm long, 0.8 cm wide and 2 mm deep was mounted on a tripod beneath a Polaroid MP-4 Land camera with a 35 mm lens. The solutions in the trough were the same as previously described except that 0.02 % methocel was added to reduce convection currents. A Nichrome wire bisecting the trough in the viewing area of the camera was heated electrically (2 to 3 V) until the temperature near the wire, measured with a thermistor embedded in the trough wall, was 40°C . Cells were suspended in control solution as previously described and 2 drops of this suspension were added to one side of the trough. Photographs were taken by exposing no. 42 Polaroid film (ASA200) for 4 to 5 s.

Growth at high and low temperatures. An exponential phase culture at 28°C was used to inoculate Cerophyl medium previously inoculated with bacteria, and these cultures were then incubated at 15 or 35°C . This procedure was repeated for each series of experiments to avoid enrichment for thermotolerant mutants.

Fig. 2. Thermal avoidance in the T-tube. The wild-type (51s) and Pawn A and B mutants (d4-94 and d4-95, respectively) were tested for thermal avoidance at four temperatures. The wild-type was assayed without added Na^+ (○) and with 0.5 mM- Na^+ (▲). Since Pawn mutants showed very poor avoidance at 0 or 0.5 mM- Na^+ , 1 mM- Na^+ was added in the experiments with Pawn A (△) and Pawn B (●) mutants, to obtain a maximum estimate of thermotactic ability. Mean values $\pm$ standard deviations are shown; the number of experiments, n , was 4, except for the wild-type at 40 °C ($n = 8$) and for Pawn B for all temperatures except 42 °C ($n = 1$). With no heat applied to the test arm: $I_T = 0.92 \pm 0.03$, $I_M = 0.16 \pm 0.06$ for the wild-type in 0.5 mM- Na^+ ; $I_T = 0.95 \pm 0.01$, $I_M = 0.21 \pm 0.03$ for the wild-type in control solution; $I_T = 0.93 \pm 0.02$, $I_M = 0.17 \pm 0.01$ for Pawn A; $I_T = 0.95 \pm 0.02$, $I_M = 0.19 \pm 0.03$ for Pawn B.

Exponential growth rates at 15 °C were about three times lower than at 28 °C, and twice as high at 35 °C relative to 28 °C. Cultures were harvested and washed as described above, but were pre-incubated at room temperature in control solution for only 5 min before assays, to avoid adaptation to room temperature.

RESULTS

Thermal avoidance in the T-tube apparatus

When all three arms of the test apparatus contained the same solution (control solution plus 0.5 mM- Na^+) at the same temperature (room temperature), the observed I_T was 0.92 ± 0.03 and I_M was 0.16 ± 0.06 . When the test arm was heated at 40 °C, there was no significant change in I_M , but I_T decreased to 0.22 ± 0.04 , indicating effective thermal avoidance. In control solution (no added Na^+), measurable thermal avoidance occurred at 39 °C, and avoidance increased at higher temperatures (Fig. 2). The addition of 0.5 mM- Na^+ to the test solution had no effect at 39 °C but significantly enhanced thermal avoidance at 40 and 41 °C (Fig. 2); at 42 °C avoidance was virtually complete with or without added Na^+ . The index of motility (I_M) was not a function of temperature and did not change significantly on addition of 0.5 mM- Na^+ . The mean I_M was 0.13 ± 0.04 in control solution and 0.09 ± 0.04 in 0.5 mM- Na^+ .

Effect of cations and hydroxylamine on thermal avoidance

With the test arm at 40 °C, thermal avoidance increased on addition of Na^+ to the test solution at concentrations above 0.125 mM (Table 1). In controls with no heat applied to the test arm, I_T was about 0.93 at all Na^+ concentrations. I_M was the same whether the test arm was heated or not, but decreased at Na^+ concentrations above 1 mM, presumably as a result of more frequent direction changes (ciliary reversals).

Table 1. *Effect of monovalent cations and hydroxylamine on thermal avoidance in Paramecium tetraurelia*

The index of thermotaxis (I_T) and the index of motility (I_M) were determined as described in Methods. The test arm (T) was either heated to 40 °C or unheated. Cations were added to the control solution as indicated. All values given are means $\pm$ standard deviations; n is the number of independent trials.

Addition	n	T arm at 40 °C		T arm unheated	
		I_T	I_M	I_T	I_M
None (control)	8	0.41 $\pm$ 0.04	0.17 $\pm$ 0.03	0.95 $\pm$ 0.01	0.21 $\pm$ 0.03
0.063 mM-Na ⁺	8	0.37 $\pm$ 0.10	0.15 $\pm$ 0.03	0.93 $\pm$ 0.02	0.19 $\pm$ 0.04
0.125 mM-Na ⁺	8	0.34 $\pm$ 0.09	0.15 $\pm$ 0.03	0.94 $\pm$ 0.01	0.20 $\pm$ 0.02
0.25 mM-Na ⁺	8	0.29 $\pm$ 0.07	0.14 $\pm$ 0.04	0.95 $\pm$ 0.02	0.17 $\pm$ 0.06
0.5 mM-Na ⁺	8	0.22 $\pm$ 0.04	0.12 $\pm$ 0.05	0.92 $\pm$ 0.03	0.16 $\pm$ 0.06
1.0 mM-Na ⁺	8	0.12 $\pm$ 0.07	0.04 $\pm$ 0.02	0.95 $\pm$ 0.01	0.08 $\pm$ 0.03
2.0 mM-Na ⁺	8	0.06 $\pm$ 0.03	0.03 $\pm$ 0.03	0.89 $\pm$ 0.03	0.05 $\pm$ 0.03
0.5 mM-Cs ⁺	4	0.11 $\pm$ 0.07	0.06 $\pm$ 0.02	0.93 $\pm$ 0.02	0.10 $\pm$ 0.03
0.5 mM-Li ⁺	4	0.14 $\pm$ 0.04	0.02 $\pm$ 0.01	0.94 $\pm$ 0.02	0.06 $\pm$ 0.01
0.5 mM-Rb ⁺	4	0.18 $\pm$ 0.09	0.11 $\pm$ 0.03	0.90 $\pm$ 0.02	0.13 $\pm$ 0.04
0.5 mM-NH ₂ OH	4	0.11 $\pm$ 0.03	0.11 $\pm$ 0.02	0.92 $\pm$ 0.01	0.14 $\pm$ 0.05
0.5 mM-K ⁺	4	0.37 $\pm$ 0.04	0.14 $\pm$ 0.02	0.93 $\pm$ 0.01	0.25 $\pm$ 0.04
1.0 mM-K ⁺	4	0.19 $\pm$ 0.08	0.10 $\pm$ 0.02	0.93 $\pm$ 0.01	0.24 $\pm$ 0.06

Table 2. *Effect on thermal avoidance of different Na⁺ and Ca²⁺ concentrations*

For details, see legend to Table 1, except that the Ca²⁺ and Na⁺ concentrations in the control solution were varied as indicated. For all experiments there were four independent trials.

Additions	[Na ⁺]/[Ca ²⁺] [‡]	T arm at 40 °C		T arm unheated	
		I_T	I_M	I_T	I_M
0.5 mM-Na ⁺ , 2.0 mM-Ca ²⁺	0.354	0.36 $\pm$ 0.06	0.18 $\pm$ 0.03	0.93 $\pm$ 0.01	0.18 $\pm$ 0.03
0.707 mM-Na ⁺ , 2.0 mM-Ca ²⁺	0.500	0.28 $\pm$ 0.07	0.16 $\pm$ 0.05	0.93 $\pm$ 0.01	0.19 $\pm$ 0.04
0.5 mM-Na ⁺ , 1.0 mM-Ca ²⁺	0.500	0.22 $\pm$ 0.04	0.12 $\pm$ 0.05	0.92 $\pm$ 0.03	0.16 $\pm$ 0.06
0.356 mM-Na ⁺ , 0.5 mM-Ca ²⁺	0.500	0.26 $\pm$ 0.06	0.08 $\pm$ 0.04	0.94 $\pm$ 0.02	0.17 $\pm$ 0.01
0.5 mM-Na ⁺ , 0.5 mM-Ca ²⁺	0.707	0.10 $\pm$ 0.05	0.06 $\pm$ 0.02	0.90 $\pm$ 0.03	0.07 $\pm$ 0.03

Several other monovalent cations enhanced thermal avoidance when present at 0.5 mM (Table 1). With the test arm at 40 °C, Li⁺, Cs⁺ or hydroxylamine at 0.5 mM allowed better thermal avoidance than 0.5 mM-Na⁺, and Rb⁺ was as effective as Na⁺ at the same concentration. K⁺ enhanced thermotaxis significantly at 1 mM, but not at 0.5 mM.

When the Na⁺ concentration was held constant, raising the concentration of Ca²⁺ resulted in less thermal avoidance (Table 2) and greater migration upward, measured by I_M . When [Na⁺] and [Ca²⁺] were varied together so as to maintain a constant ratio [Na⁺]/[Ca²⁺][‡], I_T was essentially constant (Table 2), suggesting that it is this ratio, and not the absolute concentration of the ions, which determines thermosensitivity. Migration upward (I_M) was also unaffected as long as the ratio was constant.

Effect of growth temperature on thermal avoidance threshold

To determine whether the temperature at which thermal avoidance began was a function of the temperature at which cells were cultured, we compared thermotaxis in cells cultured at 15, 28 and 35 °C (Fig. 3). Cells cultured at these temperatures were pre-incubated in control solution for 5 min (rather than the usual 60 min) at room temperature, and then assayed for their ability to avoid regions of 37 to 42 °C (Fig. 3). The shorter pre-incubation reduced the chances that cells grown at 15 or 35 °C would 'acclimatize' to room tempera-

Fig. 3. Effect of growth temperature on thermal avoidance at six test temperatures. In all experiments the test solution was control solution with 0.5 mM-Na⁺ added. Cells were grown at 28 (●), 35 (□) or 15 °C (○). Mean values ± standard deviations are shown; the number of experiments, *n*, was 4, except for points with no error bars where *n* = 1. With no heat applied to the test arm: $I_T = 0.93 \pm 0.04$, $I_M = 0.07 \pm 0.01$ for cells grown at 28 °C; $I_T = 0.93 \pm 0.02$, $I_M = 0.09 \pm 0.02$ for cells grown at 35 °C; $I_T = 0.93 \pm 0.03$, $I_M = 0.13 \pm 0.04$ for cells grown at 15 °C.

Table 3. *Effect on thermal avoidance of shift to 28 °C after growth at 15 or 35 °C*

Cells were cultured for many generations at either 15 or 35 °C; they were then incubated at 28 °C in Cerophyl medium (with bacteria) for the period indicated and assayed for avoidance of a 40 °C region as described in Methods. For all experiments there were four independent trials.

Growth temperature (°C)	Incubation time at 28 °C (h)	I_T	I_M
15	0	0.02 ± 0.01	0.04 ± 0.02
15	1*	0.05 ± 0.02	0.03 ± 0.02
15	24	0.27 ± 0.04	0.07 ± 0.03
35	0	0.81 ± 0.03	0.19 ± 0.02
35	1†	0.49 ± 0.06	0.08 ± 0.02
35	24	0.23 ± 0.06	0.08 ± 0.03
28	0	0.21 ± 0.03	0.10 ± 0.02

* Cultures shifted from 15 to 28 °C had reached 22 °C after 1 h and 28 °C after 3 h.

† Cultures shifted from 35 to 28 °C had reached 30 °C after 1 h and 28 °C after 3 h.

ture, and control experiments with cells cultured at 28 °C showed that neither I_T not I_M was significantly altered by the shorter pre-incubation. The temperature dependence of thermal avoidance reflected the temperature at which cells were cultured; for example, cells cultured at 35 °C showed no avoidance at 38 °C, those cultured at 28 °C showed measurable but not strong avoidance at 38 °C, and those cultured at 15 °C showed strong avoidance. In all cases, avoidance increased with increasing temperature in the test arm, but for the cells grown at 35 °C, avoidance even at 42 °C was incomplete. No experiments were done at higher temperatures because above 42 °C gross changes in morphology and behaviour were observed.

The displacements in the thermotaxis versus temperature profiles (Fig. 3) were smaller than the differences in culture temperature; half-maximal thermal avoidance ($I_T = 0.45$) occurred at 37.7, 39.2 and 42 °C, respectively, for cells cultured at 15, 28 and 35 °C. In general, I_M for cells grown at 35 °C was somewhat higher than that for cells grown at 15 °C (0.13 ± 0.05 versus 0.04 ± 0.02 ; Table 3).

Fig. 4. Swimming behaviour on approach to a heated wire. Dark-field photographs (time exposures) were taken as described in Methods, with cells in 0.5 mM-NaCl. Wild-type (*a*, *c*) or Pawn B (*b*, *d*) cells were initially added to the left side and are swimming from left to right in the photographs. In (*a*) and (*b*), the wire was heated to give a temperature of about 40 °C at the wire; (*c*) and (*d*) are unheated controls. Light tracks represent the swimming path of individual cells. Bar marker (in *a*) represents 0.1 cm.

When cells in the exponential phase of growth at 15 or 35 °C were transferred to medium containing bacteria at 28 °C, they re-acquired within 24 h the thermal avoidance characteristic of cells cultured at 28 °C (Table 3).

Effects of pH and buffer

Preliminary experiments suggested that thermal avoidance behaviour was sensitive to pH, and in view of the strong dependence of the pK of Tris on temperature ($\Delta pK/\Delta^\circ C = -0.031$), it was necessary to establish whether the important gradient in these experiments was of pH or of temperature. When MOPS ($\Delta pK/\Delta^\circ C = -0.006$) was substituted for Tris in our assay, no change in either I_M or I_T was observed.

Behaviour of Pawn mutants

Pawn mutants, selected for their inability to reverse swimming direction in response to stimulation, and known to be defective in the Ca^{2+} channel of the excitable membrane (Kung & Eckert, 1972), showed grossly defective thermal avoidance (Fig. 2). Pawn A, a slightly leaky mutant (Chang *et al.*, 1974), showed barely significant avoidance at 41 or 42 °C, and none at all at lower temperatures, and Pawn B (non-leaky) showed no avoidance at any temperature tested. Values of I_M were the same for both Pawn mutants, and were essentially unaltered by the test temperature (data not shown). In 1 mM- Na^+ , I_M for both Pawn mutants was two to three times higher than I_M for wild-type cells, presumably because the latter underwent frequent spontaneous changes of direction and therefore had shorter mean free paths.

Behaviour on approach to a hot wire

Dark-field time exposures (Fig. 4) allowed us to record the behaviour of individual cells as they approached an area warmed by a hot wire. Wild-type cells cultured at 28 °C performed a series of avoiding reactions as they entered a region of about 40 °C (Fig. 4*a*), but Pawn B mutants swam into and through the warm region with no interruption of their

paths (Fig. 4*b*). In a control experiment, wild-type cells swam past the wire when it was unheated (Fig. 4*c*), as did Pawn B mutants (Fig. 4*d*). Thermal avoidance in this experimental situation is accomplished by classical avoidance reactions involving temporary ciliary reversal, a fact well documented in the cine film by Schlieper (1937). We have not attempted to quantify the velocity of swimming from the dark-field photographs; it is possible that some alteration in velocity also resulted from the thermal stimulus.

DISCUSSION

The assay described here gives a quantitative and highly reproducible measure of thermal avoidance behaviour in *Paramecium*. The natural tendency to swim upward (negative geotaxis) is counterbalanced by the tendency to avoid a warm region, and when the upper arm of the apparatus is warmer than about 40 °C, the latter tendency dominates. The assay should be applicable to any free-swimming, geotactic organism.

The ionic composition of the assay medium proved to be an important factor in thermal avoidance; increasing concentrations of monovalent cations or decreasing concentration of Ca^{2+} resulted in stronger avoidance at a given temperature, and lowered the thermal avoidance threshold. More important than the concentration of a given ion was the ratio of $[\text{monovalent cation}]/[\text{Ca}^{2+}]^{1/2}$ (the Donnan ratio). Previous studies of excitability (Naitoh & Eckert, 1968) and behaviour (Kamada & Kinosita, 1940; Jahn, 1962; Grebecki, 1965) revealed a similar dependence on the Donnan ratio and led to the proposal (Naitoh, 1968) that monovalent cations displace Ca^{2+} from a surface binding site, making Ca^{2+} available to activate the reversal mechanism. High concentrations of monovalent cations displaced surface-bound Ca^{2+} (Naitoh & Yasumasu, 1967) and elicited classical avoidance reactions (Jahn, 1962; Grebecki, 1965) to an extent proportional to the Donnan ratio.

Thermal avoidance showed a remarkably sharp dependence on temperature; cells cultured at 28 °C showed barely measurable avoidance at 38 °C but virtually complete avoidance at 41 °C. We found that the exact temperature at which thermal avoidance occurred depended on the growth temperature; cells cultured at higher temperatures required higher temperatures to produce the avoidance behaviour. Thus, the thermosensory transducer is able somehow to acclimatize to different growth temperatures.

Glaser (1924) and Yapp (1941) studied the swimming velocity of *Paramecium* as a function of temperature and both reported that velocity increased with temperature. Yapp noted that when the temperature exceeded about 30 °C, the frequency of avoiding reactions increased dramatically, so that forward motion practically ceased. Tawada & Oosawa (1972) and Tawada & Miyamoto (1973) documented the occurrence of orthokinesis (alteration of swimming velocity) in *Paramecium* subjected to thermal stimulus near the culturing temperature, but more recently Nakaoko & Oosawa (1977) have reported that klinokinesis (alteration in the frequency of direction changes) is primarily responsible for the accumulation of *Paramecium* at an optimal temperature in at least certain strains of *Paramecium*.

Our results show that thermal avoidance is accomplished at least in part by klinokinesis. The most direct demonstration of this presented here is the dark-field photographs (Fig. 4) which show sharp changes in swimming direction as cells enter a region of about 40 °C. Schlieper (1937) previously used micro-cinematography to document heat-induced avoiding reactions in *Paramecium caudatum*, and Hildebrand & Dryl (personal communication) have also made the same observation with *Paramecium caudatum*. The failure of Pawn B to show thermal avoidance further suggests that classical avoidance reactions are involved; this mutant cannot reverse its swimming direction, but shows negative geotaxis (Kung, 1971).

We considered it possible that what we call 'avoidance' is simply immobilization caused by elevated temperature. We have determined in separate experiments the temperature at which cells become immobile. Both Pawn and wild-type cells were motile at 40 °C, but

incubation at 41 °C or higher eventually (within 5 min) caused immobilization of both types. The fact that Pawn cells were not capable of thermal avoidance in our T-tube assay at temperatures that wild-type cells avoided completely indicates that avoidance is not due merely to immobilization.

The experiments described here with Pawn mutants indicate that the mechanism responsible for thermal avoidance shares at least some components with the mechanism(s) involved in responses to chemical and other physical stimuli. For example, a functional Ca^{2+} channel is apparently necessary for thermal avoidance.

What is the molecular nature of the thermoreceptor? The molecule(s) must undergo some change, presumably conformational, in response to a change of 1 to 2 °C in temperature. It must also be able to acclimatize to different culture temperatures by changing the absolute temperature at which this transition occurs. A protein could certainly change conformation with a change in temperature, but it seems highly unlikely that a single protein could acclimatize as the thermoreceptor must. Membrane lipids undergo sharp phase transitions or phase separations at temperatures near the physiological range, and the exact temperature of the transition depends on the type of lipid(s) present in the membrane (e.g. Overath *et al.*, 1970; McElhaney & Souza, 1976; Sinensky, 1971, 1974; Cossins, 1977; Wunderlich *et al.*, 1973; Kitajima & Thompson, 1977; Nandini-Kishore *et al.*, 1977). Cells cultured at higher temperature tend to have more saturated fatty acids in their lipids, and to undergo the 'solid' to 'fluid' phase change at higher temperatures, than cells cultured at lower temperatures (Overath *et al.*, 1970; McElhaney & Souza, 1976; Sinensky, 1971, 1974; Wunderlich *et al.*, 1973). Studies of a variety of prokaryotes (e.g. McElhaney & Souza, 1976; Sinensky, 1974; Gill & Suisted, 1978) and eukaryotes including the related ciliate protozoon *Tetrahymena* (Cossins, 1977; Nandini-Kishore *et al.*, 1977; Wunderlich *et al.*, 1973) show that cells alter their membrane lipid composition in response to factors such as changed growth temperature, apparently striving to maintain a constant fluidity of membrane lipid. There is ample evidence that thermally induced alterations in membrane lipid fluidity alter the activity of intrinsic membrane proteins (e.g. Farias *et al.*, 1975; Thilo *et al.*, 1977). Thus, membrane lipids have the basic properties required of a thermoreceptor. Poff & Skokut (1977) have also pointed this out in connection with their studies of thermotaxis in *Dictyostelium discoideum*.

Several lines of evidence suggest that in *Paramecium* some membrane lipid(s), perhaps associated with a specific ion channel, may be the primary thermoreceptor. The excitable portion of the *Paramecium* membrane is the ciliary membrane (Dunlap, 1977; Ogura & Takahashi, 1976), which also has a lipid composition distinctly different from that of other cellular membranes (Rhoads & Kaneshiro, 1979; Andrews & Nelson, 1979). Physical studies of the membranes of *Tetrahymena* suggest that the ciliary membrane is the least fluid of all cellular membranes of that organism (Thompson & Nozawa, 1977). Local anaesthetics, such as tetracaine, which may act by making membrane lipids more fluid (Lee, 1976; Hayden *et al.*, 1977) elicit strong avoidance reactions in *Paramecium* (Browning & Nelson, 1976). *Paramecium* grown at high temperature (35 °C) shows virtually no response at 15 °C to the chemical stimulant Ba^{2+} , although cells both cultured and assayed at 15 °C are normally responsive (Thiede, Kung & Fielder, unpublished observations). Finally, the experiments described here indicate that the thermal avoidance threshold varies with growth temperature.

Our present working hypothesis is this: some lipid species closely associated with an ion gate in the *Paramecium* membrane (e.g. the Ca^{2+} or K^{+} gate) can influence the opening and closing of the gate. Any factor that makes that lipid more fluid will tend to depolarize the membrane; this might be brought about, for example, by opening the Ca^{2+} channel or closing the K^{+} channel. One such factor is an increase in temperature; ionic composition of the medium may be another (Trauble & Eibl, 1974).

This research was supported by grants from the National Science Foundation (BNS 76-11490), the Graduate School of the University of Wisconsin, and a Dreyfus Foundation Teacher-Scholar Award to D.L.N. D.L.N. is the recipient of a Research Career Development Award (NIH 00085) and a Steenbock Career Advancement Award. T.H. is a pre-doctoral fellow in the Neurosciences Program, University of Wisconsin. We thank Ching Kung and Julius Adler for their helpful comments on this manuscript.

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