NEGATIVE GEOTACTIC BEHAVIOR OF *PARAMECIUM CAUDATUM* IS COMPLETELY DESCRIBED BY THE MECHANISM OF BUOYANCY-ORIENTED UPWARD SWIMMING

KEIJI FUKUI AND HIROSHI ASAI
Department of Physics, Waseda University, 3-4-1, Okubo, Shinjuku-ku, Tokyo 160, Japan

ABSTRACT This paper presents evidence that the negative geotactic behavior of *Paramecium caudatum* takes place by the mechanism of buoyancy-oriented upward swimming. Photographs of swimming pathways of the organisms were completely described by two dynamic equations for the translational motion of the center of gravity of the organism's body and for the rotational motion of the organism's body about its center of gravity, where the rotational torque is induced by a slight difference in position between the center of gravity and the center of buoyancy. It now seems unlikely that complicated mechanisms such as the statocyst mechanism and the gravity-propulsion mechanism, which have been proposed by many investigators, need be considered for other protozoa since preliminary observation and analysis of other ciliates such as *Paramecium multimicronucleatum*, *Paramecium tetraurelia*, and *Tetrahymena pyriformis* also strongly suggested that their negative geotaxis is due to buoyancy-oriented upward swimming.

INTRODUCTION

In cultures of various protozoa, especially of *Paramecium caudatum* (*P. caudatum*), the organisms frequently tend to move upward and gather near the top of the culture medium. This phenomenon, called negative geotaxis, has been investigated by various approaches and various culture conditions from late in the last century up to the 1970's (1–15). However, there has been a long and inconclusive history of experiments and controversies. Five theories have been proposed at various times, i.e., the mechanical, pressure, resistance, statocyst, and gravity-propulsion theories. We previously showed that the first (mechanical) theory, proposed tentatively by Verworn (1) in 1889 and which unfortunately was disregarded, is in fact the most probable (16). Namely, we previously investigated the angle distribution of statically suspending posture of the Triton-treated immotile organisms at the sedimentation equilibrium point in sucrose density gradient and found that the suspending models of nonstarved organisms were directing their anterior part upward. Consequently, we considered that we could show evidence of the gravity-buoyancy moment, which causes rotational torque to turn the nonstarved organisms upward. This rotational torque and also an efficient enough propulsive force against the gravitational force bring about negative geotactic behavior. Also we showed by similar investigation that the lack of negative geotaxis of starved organisms is due to the smaller torque and propulsive force rather than those of the nonstarved organisms.

Here we present evidence that the tracks of smoothly swimming *P. caudatum*, which does not exhibit any trial and error, may be completely described by the dynamic equations based on the rotational torque, which turns the organisms upward.

MATERIALS AND METHODS

P. caudatum (wild type) was cultured at 20–22°C on hay infusion containing *Aerobactor aerogenes*. Organisms in the early stationary state of the culture were harvested by centrifugation and washed, then resuspended in test solution (1 mM $CaCl_2$, 1 mM KCl, and 5 mM Tris, adjusted to pH 7.0 with maleic acid) and used for experiments at a cell density of 5–10 cells/ml.

To observe and photograph the swimming motions, we prepared a glass vessel whose inside dimensions were 15 cm × 30 cm × 0.2 cm. The two-dimensional swimming pathways were recorded by multiple-streak low-magnification photographs under dark field. The multiple-exposure time for photography was divided by means of a rotating sector into five periods, consisting of two of 0.4 s and three of 1.0 s; between the 0.4-s exposure periods there was a 0.2-s unexposed period, and the other intervals were all 1 s. Therefore, if the exposure for the photographs is begun with the first 0.4-s exposure period, it is easy to identify the starting point or swimming direction of the photographed swimming pathways, as shown in Figs. 1 and 2. Thus, during four cycles of sector rotation, swimming motion could be recorded for up to 32 s, provided that the organisms swam in the observable photographic field.

RESULTS

A typical photograph of swimming pathways is shown in Fig. 1. The swimming pathways were recorded as described above. As illustrated in Fig. 2, the swimming direction was measured from enlargements of the nega-

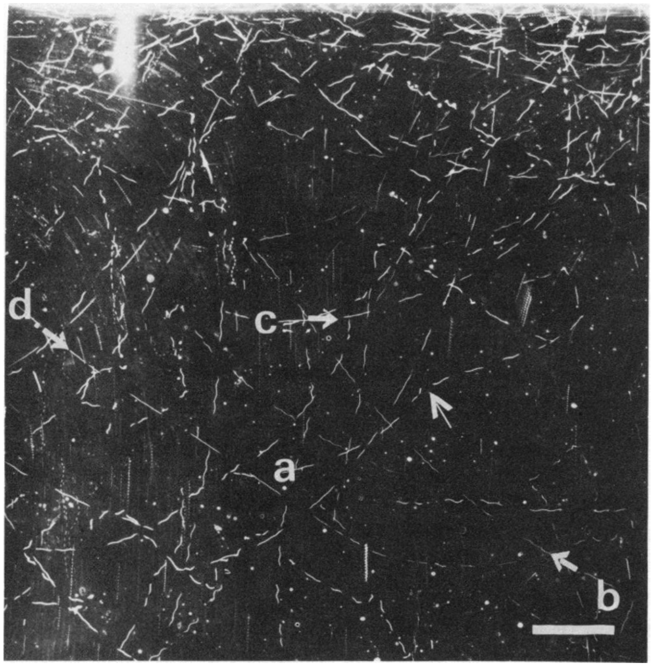

FIGURE 1 A typical photograph of swimming pathways. As illustrated in Fig. 2, the starting point of the photographed swimming pathways can easily be distinguished. The pathways *b*, *c*, and *d* were subjected to analysis in terms of a dynamic equation of rotation around the center of the gravity. Pathway *a* includes a sudden turning motion (the turning point is indicated by an arrow). Scale bar = 50 μm.

tives as the angle between the tangent of the swimming pathways and the horizontal plane.

As illustrated in Fig. 1, the swimming pathways were smooth curves that show that the swimming direction of each organism turned gradually upward, even when the organism was initially swimming downward as shown in Fig. 1 *c*. As also shown in Fig. 1 *a*, even when an organism made a sudden change of swimming direction, the swimming pathways before and after the turning point were smooth curves. We consider that these smooth upward swimming pathways reveal that a continuous torque was acting on each organism so as to turn the anterior of each organism upward.

To analyze the swimming pathways, the measured angles of the pathways were expressed as a time-dependent angular change as shown in Fig. 3 *a* and numerical analysis was performed. It is considered that the two-dimensional motion of the center of gravity of the organism (which, for simplicity, is assumed to be spherical) can be expressed by the following equations. Actually the organism has an ellipsoidal shape, so we also examined the solutions of the equations obtained when the organism was considered to be an ellipsoid. However, the results in the two cases were semiquantitatively similar. Therefore, the spherical model was used for the sake of simplicity. We have $M\,\mathrm{d}^2x/\mathrm{d}t^2 = -R\,\mathrm{d}x/\mathrm{d}t + P\cos\theta$, $M\,\mathrm{d}^2y/\mathrm{d}t^2 = -R\,\mathrm{d}y/\mathrm{d}t + P\sin\theta - mg$, and $I\,\mathrm{d}^2\theta/\mathrm{d}t^2 = -n\,\mathrm{d}\theta/\mathrm{d}t + T\cos\theta$, where x is the

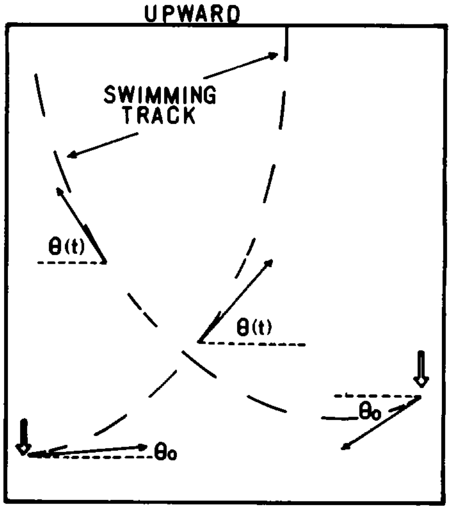

FIGURE 2 An illustration of swimming pathways traced from the photographed swimming pathways. Long arrows are the tangents of the pathways and the broken lines indicate the horizontal direction. The large arrows (➡) show the starting points of the photographed swimming pathways.

horizontal component of the motion, y is the vertical one, M is the mass of the organism, m is the effective mass of the organism in water, R is the coefficient of friction for translational motion of the center of gravity, g is the gravity constant, θ is the angle, P is the propulsive force of swimming, I is the moment of inertia, n is the coefficient of friction for rotational motion around the center of gravity, and T represents the torque produced by the posterior density bias in the cell body. The actual motion is stationary and, consequently, we obtain the solutions as follows: $x - x_0 = nP/TR\cos\theta$, $y - y_0 = -nP/TR\ln\cos\theta - nmg/TR\ln\tan(\theta/2 + \pi/4)$, and $\tan(\theta/2 + \pi/4)/\tan(\theta_0/2 + \pi/4) = e^{Tt/n}$, where θ_0 is the initial angle and t is time.

Three typical swimming pathways with different θ_0 were analyzed by the equations. As shown in Fig. 3 *a*, the experimental angular change agrees with calculated values $\theta(t)$ very well, when a value of 0.13 s^{-1} is used for the parameter T/n. Further analysis was performed on six swimming pathways to obtain the parameter T/n as shown in Fig. 3*b*. It was found that similar values of the parameter T/n (0.13 ± 0.05) can be used generally for swimming pathways with various θ_0. We checked the tracks of upward orienting motion against the solution of the equation of translational motion. The values of the parameter were calculated or assumed as follows. A value of 0.13 was used for the ratio of T/n, based on the results of the analysis of time-dependent angular change. R was calculated from Stoke's approximation by taking 5×10^{-3} cm as the diameter of the organism. The propulsive force, P, was calculated as the product of resistance R and the mean swimming speed of the organisms in the horizontal plane; this gave a value of 7.07×10^{-5} dyn. The effective mass, m, was obtained as the product of the volume of the organism

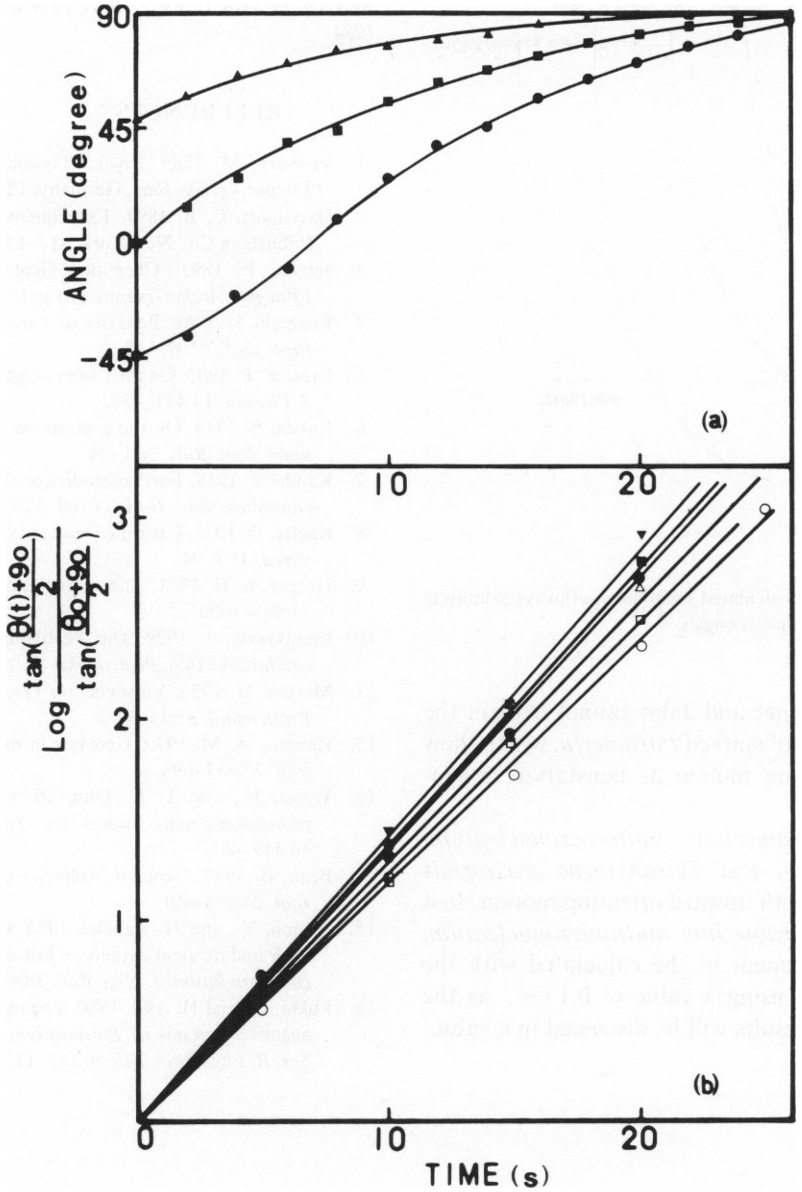

FIGURE 3 (*a*) Experimental and calculated angular changes of swimming pathways. The angular changes of the swimming pathways were measured from the photographed pathways that appear in Fig. 1*b* (▲), *c* (■), and *d* (●). The solid lines show the calculated angular change by using a value of 0.13 s^{-1} as the parameter T/n. (*b*) Linear plots of angular change. The parameter T/n is directly given as the slope of the best fitting lines of the plots. The plots correspond to pathways with various initial angles θ_0. From left to right, the value of θ_0 and the parameter T/n are as follows: −56°, 0.141; 30°, 0.135; 2°, 0.133; −47°, 0.129; 46°, 0.127; 62° 0.125, respectively.

and the difference of the specific gravity between the organism (16) (1.02) and the test solution. The resulting curves of motion are in good agreement with photographed actual swimming pathways of various initial angles θ_0 including the case where the θ_0 is negative, as shown in Fig. 4.

DISCUSSION

We can easily explain the negative geotactic behavior of *P. caudatum* by the simple physical mechanism. In this mechanism, we assumed essentially only the rotational torque that is reasonably predicted in our previous paper (16), as described already in the Introduction.

In 1974 Winet and Jahn (13) proposed the gravity-propulsion theory of negative geotaxis of *Tetrahymena pyriformis*, suggesting that the negative geotactic orientation was a physical consequence of the gyrational torque produced by geometrical asymmetry of the organism, but as shown in Fig. 1, we can conclude from detailed observation that the slight gyrating motion along the swimming pathways does not have any effect on the macroscopic upward orientation. Moreover, as shown in our previous

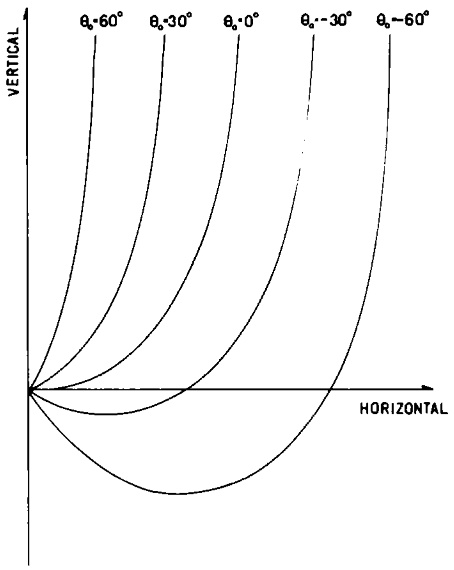

FIGURE 4 Illustration of the calculated swimming pathways exhibiting negative geotaxis with various initial angles.

paper, the theory of Winet and Jahn cannot explain the lack of negative geotaxis of starved *Paramecia*, which show almost the same gyrating motion as nonstarved organisms.

We found that *Paramecium multimicronucleatum*, *Paramecium tetraurelia*, and *Tetrahymena pyriformis* also showed similar smooth upward orienting motion. In a preliminary study of *Paramecium multimicronucleatum*, we obtained good agreement of the calculated with the experimental results by using a value of 0.17 s^{-1} as the parameter T/n. These results will be discussed in a subsequent paper.

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