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# Direct Evidence for Fluid Membranes

**(membrane and organelle rotation/axostyle/termite flagellate)**

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**ABSTRACT** **We describe a new kind of cell motility that provides direct, visual evidence for the fluid nature of cell membranes. The movement involves continual, unidirectional rotation of one part of a devescovinid flagellate in relation to a neighboring part, at speeds up to one rotation/1.5 sec (room temperature). Rotation includes the plasma membrane, using the flagellar bases and ectosymbiotic bacteria embedded in pockets of the membrane as visible markers. The plasma membrane between the rotating and stationary surfaces is continuous, without fusions with other membranes, and has the typical trilaminar structure of other cell membranes. The nucleus, helical Golgi complex, and stiff central axostyle also rotate. The head of the flagellate always rotates clockwise (as viewed from the anterior end) in relation to the body, but when the head becomes stuck to debris, the body rotates counterclockwise. Evidence suggests that the microtubular axostyle generates the motive force for rotation.**

We report a new kind of cell motility that provides direct, visual evidence for the fluid nature (1, *) of cell membranes. The movement involves continual, unidirectional rotation of one part of a eukaryotic cell membrane in relation to a neighboring part.

## RESULTS

The protozoan described here is a devescovinid flagellate from the hind gut of the termite, *Cryptotermes cavifrons* Banks (8). It is apparently a new species of the genus Devescovina (9); in this paper we simply refer to the protozoan as the "devescovinid."

Devescovinids freshly isolated from the termite have an elongate shape with the axostyle completely enclosed by the cell body (Fig. 1a); however, *in vitro* most of the cells gradually become rounder, leaving the axostyle projecting posteriorly (Fig. 1b). The change in shape occurs in various NaCl concentrations (0.3–1.0 g/100 ml) in addition to that used routinely (0.6 g/100 ml). Unless noted otherwise, all observations apply to both "elongate" and "round" devescovinids. Figs. 1–5 present a relevant morphological description of the devescovinid.

The most striking feature of living devescovinids is the continuous, unidirectional rotation between head and cell body (see Fig. 6).

Clockwise rotation of the head involves visible markers of the plasma membrane, such as the ectosymbiotic rod bacteria, the papilla, and the bases of the flagella (Fig. 7). Foreign debris or particles that become attached to the surface of the head also rotate. A sharp demarcation is evident between the rotating surfaces, corresponding to the boundary between the bacterial patterns on the head and body (see Fig. 1c). The entire surface of the head anterior to this boundary rotates at the same velocity (one rotation/1.5 sec in the most vigorous cells at room temperature). An initially slower velocity of head

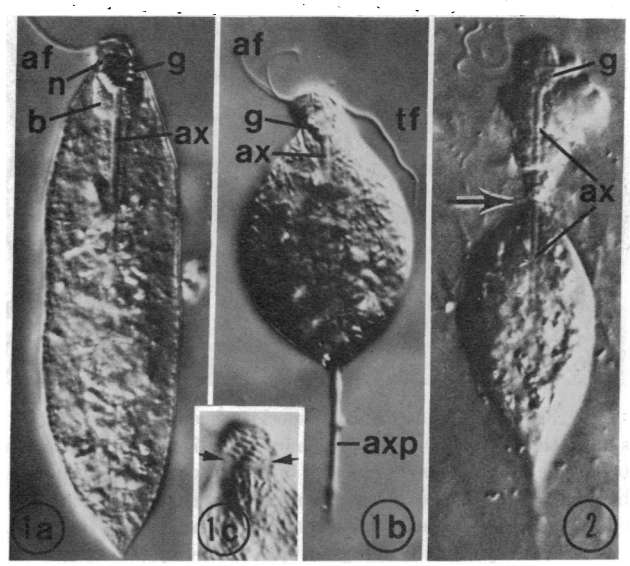



FIG. 1. (*a* and *b*) General features of elongate (*a*) and round (*b*) devescovinids (see *text*). Three anterior flagella (af) emerge from the papillar side of the head (facing reader's left in Fig. 1*a–c*). Trailing flagellum (tf) arises nearby. Rod-like axostyle (ax) runs centrally throughout length of cell, forming an axostylar projection (axp) in round cells (*b*). Golgi complex (g) is coiled in a left-handed spiral around nucleus (n) and trunk of axostyle, with the coils closer together on papillar side (see Fig. 8). b, endosymbiotic bacteria. ×480. (*c*) Ectosymbiotic rod bacteria on anterior surface of devescovinid. Bacteria on body are arranged in parallel rows, oblique to the cell's antero-posterior axis. The pattern changes abruptly on the head, particularly on side shown here (see Fig. 7). Narrow bacteria-free zone between head and body (arrows; see Fig. 3) corresponds to boundary between rotating surfaces. ×800.

Anterior end towards top in Figs. 1*a–c* and 2. Nomarski optics (0.65 N.A. objective). Median optical slice through cell in Figs. 1*a* and *b* and 2.

FIG. 2. Print from cine film of abnormal cell almost twisting itself in half. The entire region anterior to constriction (arrow) rotated clockwise, while the posterior part remained stationary. Axostyle (ax) runs through the constriction and connects the two parts of the cell (see *text*). g, Golgi. ×480.

* M. K. Jain and H. B. White, in preparation.

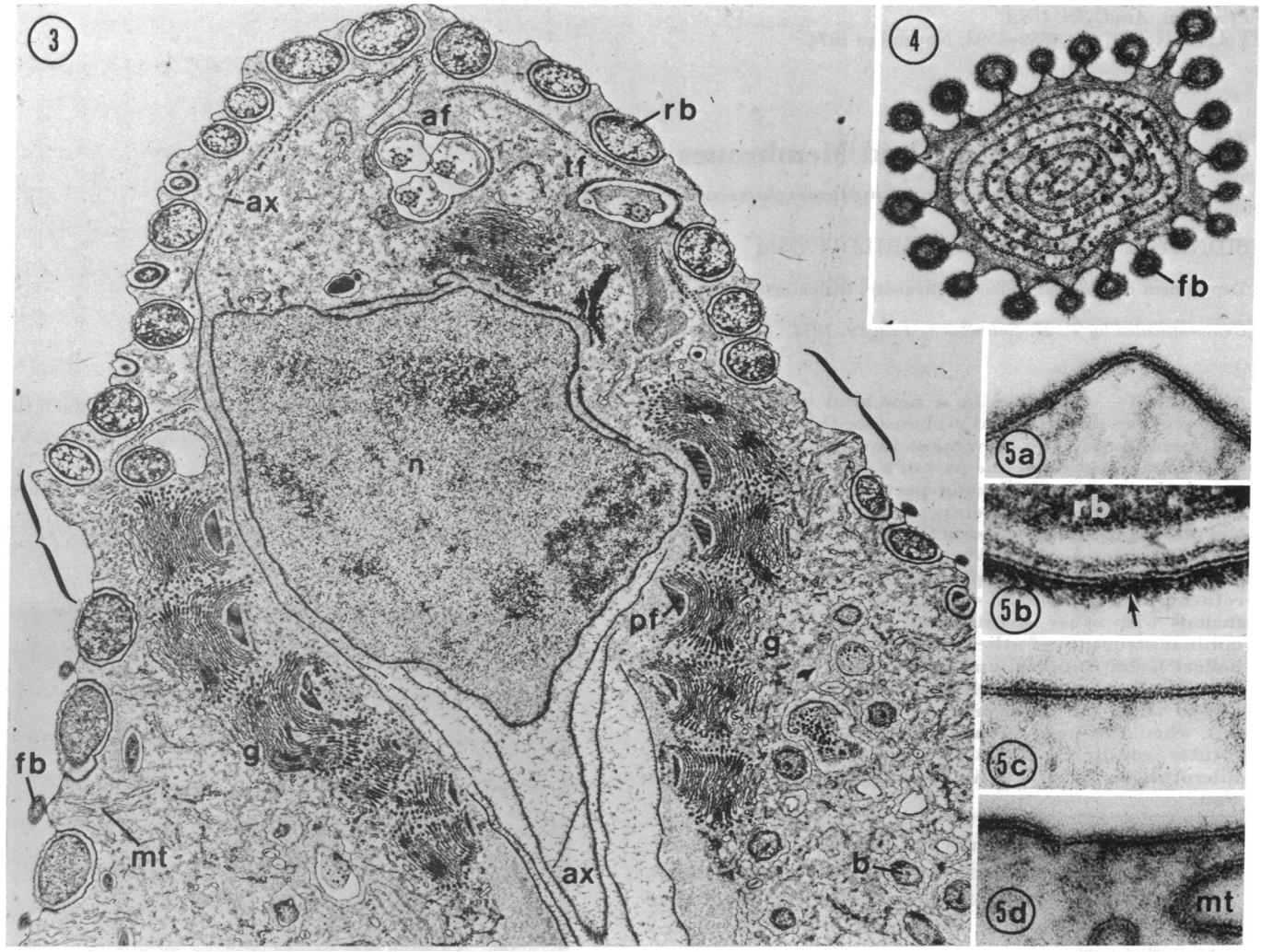



FIG. 3. Median longitudinal section through anterior end of devescovinid, almost perpendicular to plane illustrated in Fig. 1. Ectosymbiotic rod bacteria (rb) reside in specialized invaginations of the cell membrane. Note that cell membrane appears continuous at bacteria-free zone between head and body (brackets), corresponding to the rotational boundary between these two regions (see *text*). Coils of helical Golgi complex (g) and associated parabasal filament (pf) are cut transversely. Part of microtubular axostyle (ax) continues anterior to nucleus (n) and becomes associated with fibers in the head. A group of three anterior flagella (af) and the trailing flagellum (tf) are surrounded by surface invaginations. Numerous membranous tubules (mt) of unknown function occur close to the surface of the cell body; they apparently do not fuse with the cell membrane of Golgi cisternae. Slender, fusiformis-type bacteria (fb) are attached to ridges of cell membrane of body, but not head. b, endosymbiotic bacteria; anterior at top. Paraformaldehyde–glutaraldehyde fixation (10). ×11,300.

FIG. 4. Transverse section through axostylar projection of round cell. Axostyle consists of a spirally-wound sheet of microtubules. Fusiformis-type bacteria (fb) are attached to ridges of surrounding cell membrane. ×25,800.

FIG. 5. Structure of plasma membrane (*a*) of head, (*b*) of pocket surrounding rod bacterium (rb) on head, (*c*) between rotating surfaces of head and body (bracketed region in Fig. 3), and (*d*) of body. In all regions the cell membrane looks similar, with trilaminar structure typical of unit membranes. Membranous tubules (mt) near body surface do not fuse with plasma membrane (*d*). Dense layer (arrow) coats cytoplasmic side of membrane around rod bacteria (*b*). ×179,200.

rotation observed in some elongate cells may be an artifact of preparation.

Rotation of the head also involves internal structures, such as the helical Golgi complex (Fig. 8) and the nucleus (evident only in cells with enlarged nuclei parasitized by intranuclear bacteria).

Rotation of the axostylar projection in round cells can be detected by use of fusiformis-type bacteria attached to its surrounding cell membrane, or blebs and particles on its surface as markers (Fig. 9). The axostylar projection rotates in the same direction and at the same speed as the head, suggesting that the entire axostyle rotates relative to the body cytoplasm in both elongate and round cells, and that axostylar rotation is directly coupled with head rotation.

The group of endosymbiotic bacteria surrounding the Golgi and anterior end of the axostyle (Figs. 1a and b and 3) do not rotate, nor is any movement of body cytoplasm around the axostyle usually observed (but see below).

When the anterior end of the devescovinid is unable to turn, the cell body rotates in the opposite direction at a slower speed (Figs. 6d and 10). Rotation of the body includes the surface (using rod bacteria as markers) as well as the internal cytoplasm (i.e., vacuoles, wood chips, etc.). The axostylar projection and Golgi complex do not rotate while the head is

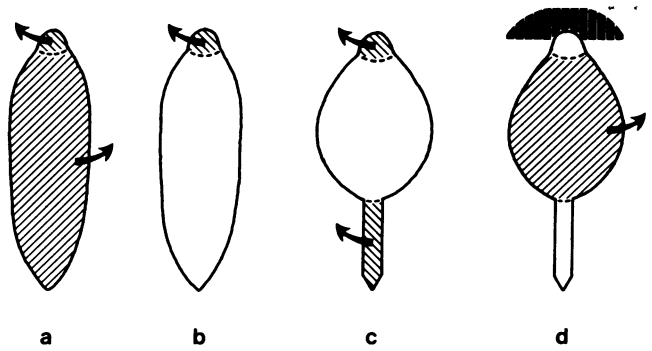



FIG. 6. Patterns of rotation observed in devescovinids. Only surface movements are shown; rotational directions relative to substrate are indicated by diagonal hatching and arrows, with dotted lines marking rotational boundaries. Relative speeds are not shown. (*a*) In swimming elongate cells, both head and body rotate in opposite directions (clockwise and counterclockwise, respectively, as viewed from anterior end of cell; this convention is followed throughout the paper). (*b*) In nonswimming elongate cells the head rotates clockwise but the body does not turn. (*c*) In nonswimming round cells both head and axostylar projection rotate clockwise at the same speed; this is the most common pattern in slide preparations. (*d*) When head is stuck to debris, axostylar projection is also immobilized, but body rotates counterclockwise. No exceptions to these directions of head compared with body rotation have been observed.

immobilized. The surface boundary between the rotating body and stationary head is identical in position and appearance to that described above for head rotation.

Abnormal types of rotation have also been observed. In many preparations the body surface of some elongate cells becomes twisted in the direction of head rotation, as if the cell membrane between head and body had become temporarily rigid, then fluid again. Occasionally, cells become constricted and almost twist themselves in half, with the entire region anterior to the constriction rotating in a clockwise direction (Fig. 2).

Another atypical kind of movement involves the rotation of body cytoplasm, but not the body surface, in a counterclockwise direction. Such motion has been occasionally seen in both elongate and round cells, with or without concomitant rotation of head and axostyle in the clockwise direction.

## DISCUSSION

This paper documents, in a flagellate protozoan, continual unidirectional rotation of one part of a cell membrane in relation to a neighboring part, by use of flagellar bases and surface bacteria as visible membrane markers. In contrast, the few examples reported of rotatory movements between cell parts (2–7) do not involve rotation of part of the cell membrane.

The plasma membrane at the boundary between the rotating surfaces of the devescovinid appeared continuous, without connections to internal membranes. It is difficult to imagine how the membrane of the head could continually turn in relation to that of the body, unless the intervening membrane were extremely fluid and mobile. Indeed, current research indicates that most cell membranes, at least in part, exist in a dynamic, fluid state (1, *). These studies rely mainly on sophisticated physical probes and extraneous membrane labels. The natural markers for the membrane used in our study provide an extraordinarily direct demonstration for the fluid model of membrane structure.

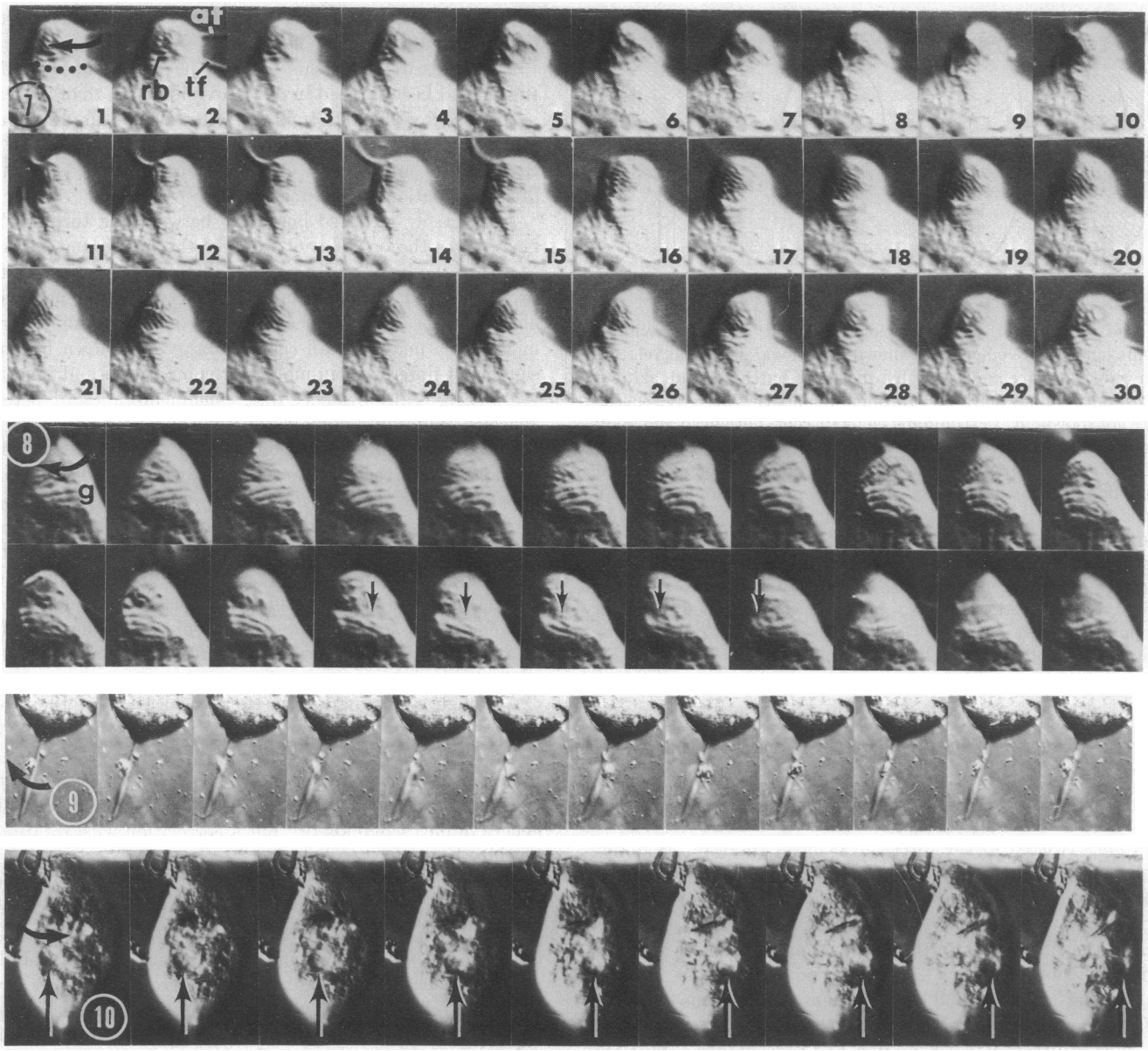
After "discovering" this unusual phenomenon, we found that it had been noticed in devescovinids more than 20 years ago by Kirby (9, 11). It is an interesting example of prematureness in scientific discovery (12) that Kirby's view of the "fluidity and lability of the surface layer" (11) did not attract attention at his time.

The sharply localized boundary between the turning surfaces raises the question of whether the membrane between head and body is biochemically different from that of the rest of the cell. Although no ultrastructural differences were observed (see Fig. 5), the apparent similarity in electron microscopic images of all cell membranes is known to mask significant differences in molecular composition and function (13). There is also evidence that some membranes are heterogeneous, containing domains of rigid and fluid lipids (*, 14, 15). However, the observation that an additional rotational boundary, between the axostylar projection and cell body, appears as the devescovinid changes shape *in vitro* argues against an intrinsically more fluid membrane between head and body. Instead, fluidity may be a basic property of the entire cell membrane, but only expressed in certain places for geometrical and/or mechanical reasons (i.e., cell shape). Attachment of the membrane to cytoplasmic structures may also be important in determining rotational boundaries on the surface.

Although the remarkable size and location of the Golgi complex suggests a possible function in rotation, its absence at secondary rotational boundaries (see Figs. 2 and 9) indicates no direct requirement of the Golgi for surface rotation to occur. A similar Golgi complex is found in many genera of devescovinids (9), but a correlation with rotational motility has not been shown.

The nature of the rotatory mechanism is an intriguing problem. Flagellar activity does not cause the head to turn, because when the head is immobilized, the body rotates in the opposite direction. Convincing evidence that the microtubular axostyle generates the motive force for rotation comes from abnormal cells that almost twist themselves in half. The two parts that turn in relation to each other are connected by little else except the central axostyle (Fig. 2). Active rotation of the axostyle inside the posterior part of these cells must cause the observed rotation of the anterior part. If the plasma membrane were abnormally rigid, twisting of the cell should occur. A similar rotational movement of the axostyle in cells with a more fluid membrane should be accompanied by rotation of organelles closely associated with the axostyle, i.e., the nucleus, Golgi, and other structures in the head, including the surface.

If the axostyle exerted forces at right angles to its long axis, and in a counterclockwise direction against the surrounding cytoplasm, it should turn clockwise. Providing the internal viscosity of the body cytoplasm were sufficiently high, the cytoplasm should not be turned in the opposite direction. However, in abnormal situations the cytoplasm may become less viscous to varying degrees. The axostylar forces should then cause the body cytoplasm to rotate counterclockwise, reducing or eliminating the clockwise rotational component of axostyle and head. This type of rotation was, in fact, observed, giving further support to the axostyle as the source of the rotational movements.




FIGS. 7–10. Prints from 16-mm cine films of devescovinids (0.6 g/100 ml of NaCl, Vaseline-ringed coverslip preparations; Nomarski optics, 0.65 N.A. objective). In all sequences the upper surface of the rotating part is in focus, and anterior direction is towards the top of photographs. Movement from reader's right to his left thus represents rotation in a clockwise direction as viewed from anterior end of the cell (Figs. 7–9); left-to-right motion represents counterclockwise rotation (Fig. 10) (see arrows in first print of each figure). Successive prints arranged from left to right. Time interval between prints is 1/6 sec in Figs. 7, 9, and 10; 1/12 sec in Fig. 8.

FIG. 7. Clockwise rotation of head (surface features in focus). Papilla, anterior flagella (af), trailing flagellum (tf), and rod bacteria (rb), all rotate from reader's right to left; body surface and cytoplasm (see vacuole at lower left) do not turn. Dotted line indicates surface shear zone between head and body. One complete turn is shown, beginning with papilla facing reader's right in print 1. On the side of the head between the anterior flagella and base of the trailing flagellum there is a clear region without bacteria. As the papilla turns towards the reader, the clear area moves to the left (prints 1–10). Bacteria appear at the right in a horizontal pattern, which becomes most evident when the papilla faces the reader's left (prints 13–15; see Fig. 1*c*). As the papilla turns away from the reader (prints 16 and following), the bacterial pattern on the head becomes more oblique, as on the body. The clear region returns to view when the papilla once again faces to the right (print 30). ×830.

FIG. 8. Clockwise rotation of the Golgi complex (g). Head surface (out of focus here, but evident in other parts of film) rotated in same direction and at same speed as Golgi (about 1 rotation/2 sec); the body is stationary. First and last prints show same position of Golgi at start and end of one complete rotation. Coils of Golgi appear as thick, oblique lines. Due to its asymmetry (see Fig. 1*a* and *b*), the Golgi helix appears to "wax" (upper row) and "wane" (lower row) from right to left in successive prints. Note anterior end of Golgi helix (arrows). ×830.

FIG. 9. Clockwise rotation of particle attached to axostylar projection of round cell. One complete turn is shown. Focal plane remains fixed slightly above axostyle. The particle goes out of focus during its passage around the lower side of axostyle, then comes back into focus as it completes the rotation on the upper side. Clockwise rotation of the particle (about 1 rotation/2 sec) was accompanied by rotation of the head in the same direction, at the same velocity. Note that cell body does not turn. ×290.

FIG. 10. Counterclockwise rotation of cell body when head is temporarily stuck to gut wall (at top of figure) and unable to turn. Vacuoles (arrows) and inclusions in body cytoplasm, as well as body surface (not in focus), turn from left to right. ×430.

The unusual kind of motility described here raises many questions. One question which probably will never be answered satisfactorily is: why do parts of the cell rotate at all?

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