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ROTARY MOVEMENTS AND FLUID MEMBRANES IN TERMITE FLAGELLATES

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SUMMARY

We previously described a remarkable type of cell motility that provided direct, visual evidence for the fluid nature of cell membranes. The movement involved continual, unidirectional rotation of one part of a protozoan, including the plasma membrane and cytoplasmic organelles, in relation to a neighbouring part. The cell membrane in the 'shear zone' appeared continuous with that of the rest of the cell. The rotary motor consisted, at least in part, of a non-contractile, microtubular axostyle which extended centrally through the cell. The protozoan was a devescovinid flagellate found in the hindgut of a Florida termite.

In this paper, we have confirmed earlier reports of this type of motility in other kinds of devescovinids from Australian termites. By demonstrating continuity of the plasma membrane in the shear zone of the Australian devescovinids as well, we have obtained additional examples that provide direct, visual evidence for fluid membranes. A comparative analysis of rotational motility in various devescovinids revealed 2 different kinds of rotary mechanisms. *Hyperdevescovina* probably have an internal motor, in which one part of the cell exerts forces against another part, as in the Florida termite devescovinid. *Devescovina* species, on the other hand, have an external motor, in which flagellar and/or papillar movements exert forces against the surrounding medium. The structure of the axostyle in different devescovinids was compared, and its role in rotational motility discussed with respect to the behavioural data.

INTRODUCTION

We recently described an unusual kind of cell motility involving continual, unidirectional rotation of one part of a protozoon in relation to a neighbouring part (Tamm & Tamm, 1974). Rotation includes the plasma membrane as well as cytoplasmic organelles. Since the plasma membrane at the shear zone appeared continuous with the plasma membrane of the rest of the cell, these observations were taken as direct, visual evidence for the fluid nature of cell membranes (Singer & Nicolson, 1972). We also showed that the rotary motor involves a rod-like axostyle which runs through the cell like a driveshaft.

This protozoan is an undescribed species of devescovinid flagellate that lives in the hindgut of a Florida termite, *Cryptotermes cavifrons*. To our surprise, we found that similar rotational movements had been reported more than 20 years ago in certain devescovinids, particularly from Australian termites (Kirby, 1941–1949). Although limited by the availability of living material, Kirby (1947) believed that such rotational motility occurred frequently among devescovinids kept *in vitro*.

Because of the potential usefulness of this phenomenon for revealing important properties of cell membranes and motile mechanisms, we have re-examined various

devescovinids from Australian termites. Our aim was to discover the extent to which such rotational movements occur, and to obtain comparative data that would be useful for analysing the nature of the membranes and rotary motors in cells exhibiting this unusual type of motility.

MATERIALS AND METHODS

Organisms

Various devescovinids of the genera *Devescovina* and *Hyperdevescovina* from Australian termites (Hill, 1942) were studied. These included *Devescovina lemniscata*, from *Neotermes insularis* collected in Canberra, A.C.T.; *Devescovina* sp., an undescribed species from *Cryptotermes gearyi* collected in south Queensland; *Hyperdevescovina mitrata*, from *Kalotermes convexus* and *Kalotermes pallidonotum* collected near Canberra; *Hyperdevescovina falcifera*, from *Kalotermes banksiae* collected on the coast of N.S.W.; and *Hyperdevescovina balteata*, from *Ceratokalotermes spoliator* collected near Canberra, Australia.

The unidentified devescovinid from *Cryptotermes cavifrons*, a Florida (U.S.A.) termite, will be referred to as the *C. cavifrons* devescovinid in this paper (see Tamm & Tamm, 1974, for details).

Light microscopy

Living flagellates were studied by opening the hindgut of a termite in a few drops of 0·6 % NaCl on a microscope slide, then sealing off the preparation from the air with a Vaseline-ringed coverslip.

A Zeiss Universal microscope with Nomarski interference-contrast optics (0·65 N.A. objective) was used. Ciné films were taken through the microscope with a Locam 16-mm camera (Redlake Labs, Santa Clara, Calif.) on Kodak Plus-X negative film (Eastman Kodak). A Photo-Optical Data Analyzer (L-W Photo, Inc., Van Nuys, Calif.) allowed frame-by-frame analysis of ciné films.

Electron microscopy

Termite hindguts were removed and opened directly in a modified Karnovsky (1965) fixative: 2 % paraformaldehyde, 1·25 % glutaraldehyde, 0·1 M sodium phosphate buffer, pH 7·0 (Kubai, 1973). Devescovinids were fixed for about 2 h. In some cases, cell shape was better preserved by initial exposure to a 1 : 1 dilution of this fixative for several minutes, followed by 2 h in the more concentrated solution. Cells were washed several times in 0·1 M phosphate buffer, then postfixed in 2 % OsO_4, 0·1 M phosphate buffer for 1–2 h. After washing in distilled water, devescovinids were treated with 0·5 % uranyl acetate in veronal-acetate buffer (Ryter & Kellenberger, 1958) for 2–4 h. All fixation procedures were done at room temperature.

Devescovinids were dehydrated in an acetone series, then flat-embedded in Araldite. Cells were mounted on stubs in known orientation, and sectioned with a Reichert Om U2 ultramicrotome using a diamond knife. Sections were collected on Parlodion- or Formvar-filmed grids, stained with uranyl acetate and lead citrate, and viewed in a Hitachi 11-A electron microscope at 50 kV.

RESULTS

Structure

Kirby (1941–1949) has presented a detailed, light-microscope account of the morphology of various devescovinid flagellates obtained from termites. In the following light- and electron-microscopic description, certain features relevant to rotational motility in Australian species of *Devescovina* and *Hyperdevescovina* are compared

with corresponding structures in the devescovinid reported earlier from *C. cavifrons* (Tamm & Tamm, 1974). We have mainly used *Devescovina* sp. and *Hyperdevescovina balteata* (or H. *mitrata*) as typical representatives of the two genera. Unless noted otherwise, descriptions apply to all devescovinids studied here.

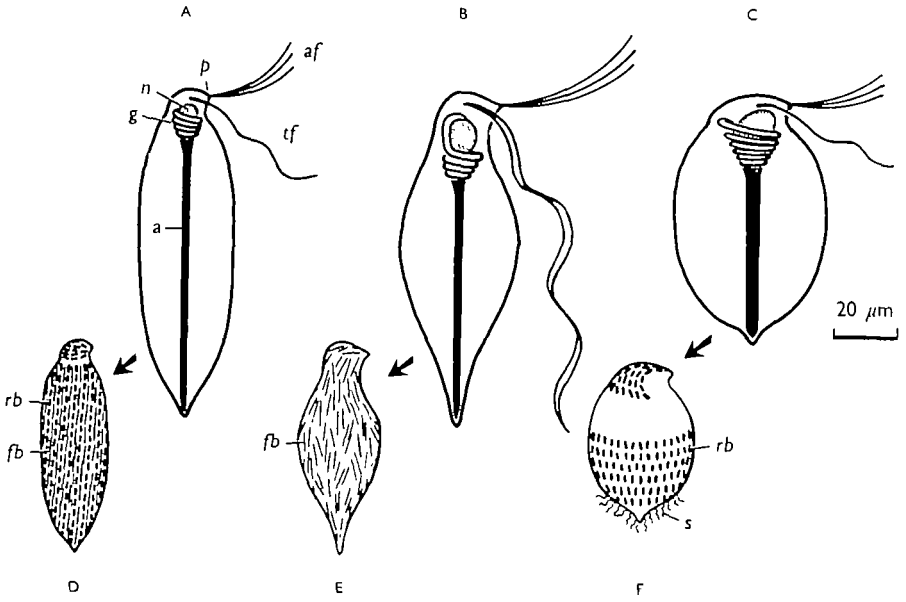

Fig. 1. General morphology of various devescovinid flagellates which show rotation of one part of the cell in relation to a neighbouring part. Main features (A–C) and bacterial patterns on the lower surface (D–F) are compared. A, D, devescovinid from *C. cavifrons*; B, E, *Devescovina* sp.; C, F, *Hyperdevescovina balteata*. Cell shape is typical of devescovinids freshly isolated from termites. Sizes are approximately to scale (20-μm bar applies only to A–C). *a*, axostyle; *af*, anterior flagella; *fb*, fusiform bacteria; *g*, Golgi; *n*, nucleus; *p*, papilla; *rb*, rod bacteria; *s*, spirochaetes; *tf*, trailing flagellum.

Fig. 1 shows the general morphology of the various devescovinids. The flagellates possess a papilla at the anterior end, from which 3 anterior flagella emerge. The basal bodies of these flagella lie at the ends of deep invaginations of the papillar surface (unpublished observations). A trailing flagellum arises from one side of the papilla. In the *C. cavifrons* devescovinid and in species of *Hyperdevescovina*, the trailing flagellum is similar in diameter to the anterior flagella; in *Devescovina*, however, the trailing flagellum is modified into a broad ribbon (Fig. 1).

The nucleus is located at the anterior end of the cell. In the *C. cavifrons* devescovinid and species of *Devescovina*, the nucleus is oval, with its long axis parallel to that of the cell. In *Hyperdevescovina*, the nucleus is rather oblong, and is tilted off the long axis of the cell in the papillar direction (Fig. 7).

A rod-like axostyle extends centrally through the cell body (Fig. 1). The main part of the axostyle begins at the base of the nucleus and runs posteriorly, tapering

to a point at the caudal end. In cells with a rounder shape, the caudal projection of the axostyle is surrounded by the plasma membrane.

Electron microscopy shows that the axostyle consists of a coiled sheet of microtubules which run parallel to one another in an antero-posterior direction (Fig. 2). In all devescovinids we have examined so far, the row of microtubules spirals inward in a clockwise direction as viewed from the anterior end. The spacing between successive turns of the sheet is often fairly uniform, but not nearly as regular as the distance between rows of microtubules in the double-coiled configuration of the heliozoan axopod, for example (Tilney & Porter, 1965). The number of microtubules, and hence the overall diameter of the axostyle, varies between different kinds of devescovinids (Fig. 2).

Microtubules in the sheet have a centre-to-centre spacing of about 30–40 nm. Each microtubule bears short projections (or a lateral flange; we have not yet detected evidence of longitudinal periodicity) that extend toward the next microtubule inward along the row, but do not appear actually to connect adjacent microtubules (Fig. 2). The projections do not lie on a line running between the centres of the tubules, but are tilted toward the outer side of the row.

A sheath is present around the axostylar microtubules in the *C. cavifrons* devescovinid, but is absent in *Devescovina* and *Hyperdevescovina* (Fig. 2). The sheath consists of a single layer of 5–7-nm-thick filaments which run at right angles to the long axis of the axostyle (Fig. 2A; S. Tamm, in preparation).

In all devescovinids some of the axostylar microtubules continue anteriorly alongside the nucleus, giving this end of the axostyle a notched appearance in some views (Fig. 7). This cup-shaped extension of the axostyle ends by overlapping with another set of microtubules, the pelta, which runs under the anterior surface (Fig. 4A). Unlike the microtubules of the axostyle, those of the pelta do not bear lateral projections.

The Australian devescovinids, like the one from *C. cavifrons*, possess a large Golgi complex coiled in a left-handed spiral around the base of the nucleus and trunk of the axostyle (Fig. 1). The coils are usually spaced closer together on the papillar side, giving the Golgi the appearance of a lop-sided spring (Fig. 6B). In *Devescovina* sp. the anterior part of the Golgi is C-shaped, extending along the side of the nucleus opposite the papilla, then curving toward the papilla above the nucleus (Fig. 8). *Hyperdevescovina* lack an anterior extension of the Golgi; instead, the most anterior gyre is wider in circumference and slightly separated from succeeding gyres (Fig. 6A).

A striated filament, the parabasal filament, runs along the inner surface of the Golgi helix.

Like the *C. cavifrons* devescovinid, Australian devescovinids of both genera possess an extensive tubular system of smooth membranes in the cytoplasm. These membranes resemble the agranular reticulum of other cell types. In the devescovinids, elements of the smooth endoplasmic reticulum occur close to the plasma membrane over much of the cell, but have never been seen to fuse with the cell membrane (Fig. 3).

The plasma membrane of the anterior part of the Australian devescovinids rotates

relative to the rest of the cell membrane (see below). The cell membrane in the intervening region (i.e., the surface shear zone) appears continuous with that of the rest of the cell, and has a trilaminar structure typical of unit membranes (Fig. 3). Similar continuity of the plasma membrane across the shear zone of the *C. cavifrons* devescovinid was reported earlier (Tamm & Tamm, 1974).

Ectobiotic bacteria of different types are arranged in characteristic patterns on the surface of devescovinids (Fig. 1 D–F). Both rod-shaped and slender, fusiform bacteria are found on the surface of the *C. cavifrons* devescovinid (Fig. 1 D). The rod bacteria are embedded in specialized invaginations of the cell membrane, whereas the fusiform bacteria are attached to ridges of the surface (Tamm & Tamm, 1974). An extremely narrow zone without bacteria separates the head and body of this devescovinid.

Only the fusiform type of bacterium is attached to the surface of most species of *Devescovina* (Figs. 1 E, 4 B). Because of their small diameter, these bacteria appear as fine surface striations in the light microscope. By light microscopy, we can detect no obvious, bacteria-free zone between head and body of *Devescovina* sp. fixed immediately after opening the hindgut (osmium tetroxide vapour fixation, wet-mount).

Species of *Hyperdevescovina* bear rod-shaped bacteria and spirochaetes on the cell surface, but no fusiform bacteria (Figs. 1 F, 4 A, 5). The rod bacteria are arranged in rows in a spiral pattern around the papilla and anterior end of the cell. This anterior cap of bacteria ends abruptly at a level about midway over the Golgi complex. A belt of more-or-less longitudinally oriented rod bacteria covers most of the posterior half of the body. In between these 2 regions is a wide zone of cell surface without bacteria. The rod bacteria in *Hyperdevescovina* are completely enclosed in pockets of the cell membrane (Fig. 4 A), whereas those in the *C. cavifrons* devescovinid are only partially enclosed (Tamm & Tamm, 1974). Spirochaete bacteria are also attached to the extreme posterior end of *Hyperdevescovina* (Fig. 1 F).

Rotational motility

We have observed rotation of the anterior part of the cell (head) while the posterior part (body) remains stationary in all the Australian devescovinids studied here. The following account is based mainly on direct observations and ciné film analysis of *Devescovina* sp. and *Hyperdevescovina balteata*. However, the kinds of rotational movements observed in these 2 species seem typical of motility in the 2 genera of Australian devescovinids.

Rotation of the head is always in a clockwise direction as viewed from the anterior end of the cell. This agrees with Kirby's (1941–1949) account of rotation in *Devescovina lemniscata* and *Hyperdevescovina mitrata*, as well as with our previous report on the *C. cavifrons* devescovinid (Tamm & Tamm, 1974). The speed of head rotation in the most vigorous *Hyperdevescovina balteata* is about 1 rotation/1–2 s (room temp.) – similar to that in the *C. cavifrons* devescovinid (Tamm & Tamm, 1974).

Rotation of the anterior end involves the plasma membrane of this part of the cell, using the papilla, flagella, and ectobiotic bacteria as visible membrane markers. In *Hyperdevescovina*, the entire anterior cap of rod bacteria turns as the head rotates,

while the posterior belt of rod bacteria remains stationary (Fig. 5). Because the bacteria-free surface zone between these 2 regions is much wider than the corresponding zone in the *C. cavifrons* devescovinid (cf. Fig. 1 D, F), the location of the surface shear zone in *Hyperdevescovina* cannot be defined as precisely by bacterial markers as it can in the *C. cavifrons* devescovinid (but see below).

Observations on *Devescovina* sp. showed that the fusiform bacterial pattern on the anterior end turned as this part of the cell rotated. Due to difficulties in visualizing these filamentous bacteria by light microscopy, we have not yet determined the nature of the bacterial pattern in the shear zone in this species. However, no discontinuity in the bacterial pattern is evident between the anterior and posterior parts of *Devescovina* sp. fixed *in situ* (Fig. 1 E). Therefore, some of these long bacteria are probably dislodged from the surface of the shear zone during rotation. Occasional observations of fusiform bacteria sticking off twisted devescovinids from *C. cavifrons* support this view.

Rotation of the head also includes the anterior cytoplasm and organelles, such as the Golgi and nucleus. More importantly, head rotation is accompanied by rotation of the axostyle within the body cytoplasm (Figs. 6–8). Analysis of ciné films shows that rotation of these structures takes place in the same direction and at the same speed as that of the surface (see also Tamm & Tamm, 1974).

Rotation of the helical Golgi complex is more evident in the Australian devescovinids than in the *C. cavifrons* devescovinid because the Golgi is relatively larger in the Australian cells (Figs. 6, 8). In addition, the C-shaped anterior extension of the Golgi in *Devescovina* provides a conspicuous marker for following rotation (Fig. 8).

Rotation of the nucleus is difficult to detect in the *C. cavifrons* devescovinid and *Devescovina* sp.; however, nuclear rotation is obvious in *Hyperdevescovina balteata*, because the nucleus is tilted away from the axis of rotation in this genus (Fig. 7).

Head rotation is accompanied by rotation of the axostyle. In devescovinids, the axostyle does not propagate undulatory waves as do the contractile axostyles of other flagellates (Grimstone & Cleveland, 1965; Mooseker & Tilney, 1973; McIntosh, 1973). Rotation of the axostyle in the *C. cavifrons* devescovinid was usually detected by following rotation of marker particles or fusiform bacteria on the surface of the axostylar projection in rounded cells (Tamm & Tamm, 1974). In *Hyperdevescovina* the anterior extension of the axostyle is more obvious than in other devescovinids, and provides a direct marker for demonstrating rotation of the axostyle (Fig. 7).

Besides the above-mentioned organelles, rotation of the anterior part of Australian devescovinids involves all of the ground cytoplasm to a level coinciding approximately with the most posterior gyre of the Golgi complex. This is particularly clear in *Hyperdevescovina*, where vacuoles and granules surrounding the Golgi rotate with the head, but cytoplasmic inclusions immediately posterior to the Golgi do not turn (Fig. 7). This indicates that the surface shear zone is relatively narrow in this genus, and located in the anterior region of the bacteria-free surface zone.

Comparison of motility in devescovinids

Table 1 summarizes similarities and differences in motility between the *C. cavifrons* devescovinid and Australian devescovinids, as well as between the 2 genera of Australian flagellates.

Table 1. *Comparison of motility in various devescovinids*

Rotation	*Devescovina* (*D.* sp.)*	*Hyperdevescovina* (*H. balteata*)*	Devescovinid from *C. cavifrons*†
I. Head CW, body stationary	Usually in non-swimming cells	In most cells	In most non-swimming cells
(*a*) Continuous	Doubtful	In swimming cells	In non-swimming and swimming cells
(*b*) Intermittent	Commonly	In non-swimming cells	Rarely
Pauses:	No papillar movements; trailing flagellum active	Papillar movements; trailing flagellum active	—
Rotation:	Jerky, matching papillar movements; trailing flagellum active	Smooth; no papillar movements; anterior and trailing flagella active	—
II. Head CW, body CCW	Never observed	Occasionally in swimming cells	Usually in swimming cells
III. Head stationary, body CCW	Never observed	Never observed	Often
IV. Whole cell CW	Usually in swimming cells	Never observed	Never observed
V. Head and body CW, but independently	Never observed	Rarely	Rarely

CW, clockwise; CCW, counterclockwise; as viewed from anterior end of cell.
* Observations made largely on these representatives of the 2 genera.
† Mainly from Tamm & Tamm (1974).

Rotation of the head is characteristically an intermittent process in both Australian genera, especially in non-swimming cells observed after 5–10 min *in vitro* (Table 1, section I*b*). The head (or body) of non-swimming *C. cavifrons* devescovinids, in contrast, usually rotates continuously for hours in slide preparations (Table 1, section I*a*).

In *Hyperdevescovina balteata*, for example, one complete turn of the anterior end is commonly followed by a brief pause, then another rotation around to the same position, a pause again, and so on. During the pauses, the body does not turn in the opposite direction (as it does in the *C. cavifrons* devescovinid when rotation of the head is temporarily prevented). There are thus brief periods without relative rotation between the anterior and posterior parts of the cell. The discontinuous nature of rotation in Australian devescovinids was also observed by Kirby (1941–1949).

However, in vigorous, swimming *Hyperdevescovina balteata* observed immediately

after opening the hindgut, the anterior end often rotates continuously, without intervening pauses (Table 1, section I*a*). Such cells are difficult to observe because they are typically found in a dense, writhing mass near the opened hindgut. Non-swimming cells which show intermittent rotation are commonly scattered singly near the fringes of the preparation, making them easier to observe and photograph (Figs. 5–7).

The papilla of devescovinids is often actively mobile. Its movements consist of jerky, lateral bendings, and are accompanied by lashing of the whip of anterior flagella. In *Hyperdevescovina balteata*, movements of the papilla and anterior flagella occur during the intermittent pauses, but not during head rotation (Table 1, I*b*). Rotation of the head is a fairly smooth, continuous motion in this devescovinid. Even though the papilla is not active, the anterior flagella beat rapidly during rotation. The rather inconspicuous trailing flagellum of *H. balteata* beats continuously during both rotation and pauses alike.

In *Devescovina* sp., vigorous movements of the large papilla coincide with rotation of the anterior end, but do not occur when rotation temporarily stops (Table 1, I*b*). Rotation itself is jerky, and is correlated with the periodic movements of the papilla. The long, ribbon-like trailing flagellum propagates waves posteriorly during rotational movements as well as pauses.

Weak papillar movements accompany rotation of the anterior end of the *C. cavifrons* devescovinid, but rotation is usually a smooth, continuous process in this devescovinid (Tamm & Tamm, 1974).

In freshly isolated, swimming devescovinids from *C. cavifrons*, the body usually rotates counterclockwise while the head turns clockwise. Similar counterclockwise rotation of the body has been observed in vigorous, fresh preparations of *Hyperdevescovina balteata*, but never in *Devescovina* sp. (Table 1, section II). Counterclockwise rotation of the body when the anterior end is stationary has never been observed in either genus of Australian devescovinids, although this type of motility commonly occurs in the *C. cavifrons* devescovinid whenever the head is stuck in debris (Table 1, section III).

Clockwise rotation involving the whole cell occurs in *Devescovina* sp., but has not been observed in *Hyperdevescovina balteata* or the *C. cavifrons* devescovinid (Table 1, section IV). We have been unable to confirm Kirby's report of this kind of motility in *H. mitrata* (Kirby, 1941–1949). In *Devescovina* sp., rotation of the entire cell is commonly found in vigorous, swimming individuals, whereas intermittent rotation of the anterior end alone typically occurs in non-swimming cells – especially those that are packed tightly together.

A very unusual kind of motility was seen rarely in preparations of *Hyperdevescovina balteata* and the *C. caviforns* devescovinid, but never in any of the other Australian devescovinids (Table 1, section V). In *H. balteata*, the usual intermittent rotation of the anterior end in a clockwise direction was accompanied by continuous rotation of the posterior part – without pauses – in the *same* direction. The posterior part (including surface bacteria as well as cytoplasm) rotated at a uniform velocity of about 1 rotation/s, regardless of whether the head was turning or not. Thus, although

the body turned in the same direction as the head, the 2 parts were clearly rotating independently of one another. A similar kind of motion was observed rarely in the *C. cavifrons* devescovinid, where rotation of the anterior end occurred intermittently instead of continuously.

DISCUSSION

After discovering a unique type of rotational motility in a devescovinid flagellate from a Florida termite (Tamm & Tamm, 1974), we re-investigated earlier reports of this phenomenon in related flagellates from Australian termites. In 5 monographs on more than 100 species of devescovinid flagellates from termites, Kirby (1941–1949) described rotation of one part of the cell relative to a neighbouring part in only a few species: *Devescovina lemniscata*, *Hyperdevescovina mitrata*, and *Macrotrichomonas restis*. Although limited by the shortage of living material, Kirby (1947) believed that this type of motility occurred commonly among devescovinids kept *in vitro*.

In this report we have confirmed Kirby's observation of partial cell rotation in *Devescovina lemniscata* and *Hyperdevescovina mitrata*, and, by noting similar rotational movements in other species of *Devescovina* and *Hyperdevescovina* from Australian termites, strengthened the view that this phenomenon is at least widespread within these 2 genera. It should be noted, however, that many smaller types of devescovinids belonging to other genera do not show rotation of one part of the cell. Instead, the whole cell commonly turns in a clockwise direction (unpublished observations; see below).

Kirby (1947) recognized that rotation of the flagella and anterior cytoplasm must mean that 'the surface of one part of the body turns in relation to the surface of another part'. Because he observed 'no more disruption of cytoplasmic structure . . . than when different parts of any fluid body move in relation to one another,' Kirby (1947) believed that this system demonstrated the 'fluidity and lability of the surface layer'. Kirby's view is remarkably prescient of current ideas on the nature of cell membranes (Singer & Nicolson, 1972).

In this report we have shown that relative movements of the surface layer in Australian devescovinids actually involve movements of the cell membrane, by using the papilla, flagella, and attached bacteria as natural membrane markers visible by light microscopy. Furthermore, we demonstrated that the membrane at the boundary between the rotating and stationary surfaces is continuous with the plasma membrane of the rest of the cell. Thus, the *C. cavifrons* devescovinid is not unique: other species of devescovinids show rotation of one part of the cell membrane in relation to a neighbouring part, and thereby provide direct, visual evidence for the fluid nature of cell membranes.

Mechanisms of rotational motility

The nature of the rotary mechanism in various devescovinids is an intriguing problem. We showed previously that the rotary motor in the *C. cavifrons* devescovinid is independent of flagellar activity, and must reside within the cell itself, since a temporary block to head rotation results in rotation of the body in the opposite

direction (Tamm & Tamm, 1974). That the axostyle is the rotary motor in the *C. cavifrons* devescovinid was suggested by various patterns of motility (Tamm & Tamm, 1974). Direct evidence that the axostyle generates torque in this cell was recently obtained by laser microbeam experiments (S. Tamm, in preparation).

Is the mechanism underlying rotational movements in the various Australian devescovinids similar to that in the *C. cavifrons* devescovinid? Two types of mechanisms, differing in the site of action of the motive force, can be distinguished: (1) an internal motor, as in the *C. cavifrons* devescovinid, in which one part of the cell (i.e. the axostyle) generates torque against another part, or (2) an external motor, in which flagellar and/or papillar movements exert forces against the surrounding medium. Comparison of various aspects of motility in different devescovinids (see Table 1) points to different mechanisms in the 2 genera of Australian flagellates.

An external motor seems the most likely cause of rotational movements in *Devescovina*. Certainly, rotation of the whole cell, which occurs commonly in this genus, must be caused by forces applied outside the cell itself. In many smaller kinds of devescovinids belonging to other genera, rotation of the entire cell is the *only* kind of motility observed. In these cases, rotation of the cell usually proceeds in vigorous jerks, correlated with lashing movements of the papilla and anterior flagella (unpublished observations). Although this point is not yet clear in *Devescovina*, we think a similar mechanism – i.e. papillar and/or anterior flagellar movements – is probably responsible for rotation of the whole cell.

Intermittent, jerky rotation of the head in species of *Devescovina* seems to be due to activity of the papilla and anterior flagella, since these 2 movements are so well correlated. The absence of counterclockwise rotation of the body in *Devescovina* sp. is consistent with the view that head rotation is caused by an external motor.

In *Hyperdevescovina*, on the other hand, the rotary motor is probably internal, as in the *C. cavifrons* devescovinid. We have never seen rotation of the whole cell in *Hyperdevescovina* (but see Kirby (1941–49) for an exception). Rotation of the anterior part of *Hyperdevescovina balteata* is smooth and is not accompanied by papillar movements. The trailing flagellum is continuously active during both rotation and intermittent pauses. Rotation of the head in *Hyperdevescovina* is therefore not due to papillar or trailing flagellum activity.

The possibility remains that the anterior flagella, which beat rapidly during head rotation, may cause this movement. However, the occasional cases of counterclockwise rotation of the body during clockwise rotation of the head in *Hyperdevescovina balteata* argue against such an external motor, but are easily explained by an internal mechanism. Likewise, the rare examples of clockwise rotation of the body independently of that of the head in *H. balteata* cannot be accounted for by flagellar or papillar activity.

To account for the intermittent nature of head rotation commonly observed in non-swimming *Hyperdevescovina*, an internal motor hypothesis requires that during the pauses either the motor is (1) turned off, or (2) its action is impeded by a block to rotation of both anterior and posterior parts of the cell. Since many of the most vigorous *H. balteata* show uninterrupted rotation of the anterior end, the intermittent

pattern may well be an artifact of *in vitro* preparations. If so, the second alternative – complete immobilization of the cell body, combined with periodic obstruction of head rotation – is the most likely explanation for the temporary pauses. The basic mechanism underlying rotational movements in *Hyperdevescovina* may thus be similar to that in the *C. cavifrons* devescovinid.

A critical experiment for determining whether the rotary mechanism is external or internal in the Australian devescovinids would be to prevent rotation of the anterior end mechanically, under conditions where the posterior part is free to move. If the motor is internal, the body should turn in the opposite direction, but if the motor is external, the body should not rotate. Thus far, we have not been able to do this experiment. It should be noted, however, that Kirby (1941–1949) reported counter-clockwise rotation of the posterior part of *H. mitrata* when the anterior end was wedged in debris – an observation that supports the hypothesis of an internal motor in this genus.

Comparison of axostyle structure in the *C. cavifrons* devescovinid, where the axostyle has been shown to be the rotary motor, and in Australian devescovinids with a presumed axostylar motor (i.e. *Hyperdevescovina*) or flagellar motor (i.e. *Devescovina*), revealed no obvious correlations between the structure of the axostyle and its function in motility. An active role of the microtubules in motility seems unlikely, since the microtubular pattern is similar in all devescovinids, regardless of the mechanism of rotation. However, the finding that the directions of head rotation and microtubular coiling are the same in all devescovinids indicates that the spiralling pattern of the axostyle may be related to its rotation direction, if only in a permissive or passive manner.

If further work confirms that the axostyle is indeed the rotary motor in *Hyperdevescovina*, which lack a filamentous sheath around the axostyle, then the sheath is obviously not an essential part of an axostylar rotary mechanism.

An experimental investigation of the nature of the rotary motor in the *C. cavifrons* devescovinid is now under way. Of particular interest is whether this motor works by a shearing mechanism comparable to that of striated muscle. Many diverse types of cell motility are thought to operate by a sliding filament mechanism (Pollard & Weihing, 1974; Satir, 1974), but possible exceptions to this scheme have been noted (Weis-Fogh & Amos, 1972; Tilney, 1975; Berg, 1974). We are currently using microsurgical, biochemical, ultrastructural, and cell model approaches to answer this question, and to study other properties of the axostylar rotary motor.

The question arises as to how 'natural' are the kinds of rotational movements observed in devescovinids kept *in vitro*. In *Devescovina* (and in many smaller types of devescovinids) it appears that rotation involving the whole cell is an ordinary kind of motility that occurs in the termite hindgut (cf. Table 1). Rotation of the anterior end of *Devescovina*, on the other hand, is probably an abnormality of *in vitro* conditions; we do not usually see this type of movement in fresh, vigorous cells, and there is no discontinuity in the pattern of surface bacteria between head and body of cells fixed *in situ*. Indeed, Kirby (1947) felt that rotation involving part of the cell was probably not the ordinary behaviour of the flagellates. However, we believe that rotation of

the head in *Hyperdevescovina* and the *C. cavifrons* devescovinid *does* occur in the termite, because this motility is found in the most vigorous individuals of these species observed immediately after opening the hindgut. In addition, a bacteria-free surface region, corresponding to the shear zone, exists between the head and body of *Hyperdevescovina* and *C. cavifrons* devescovinids fixed *in situ*.

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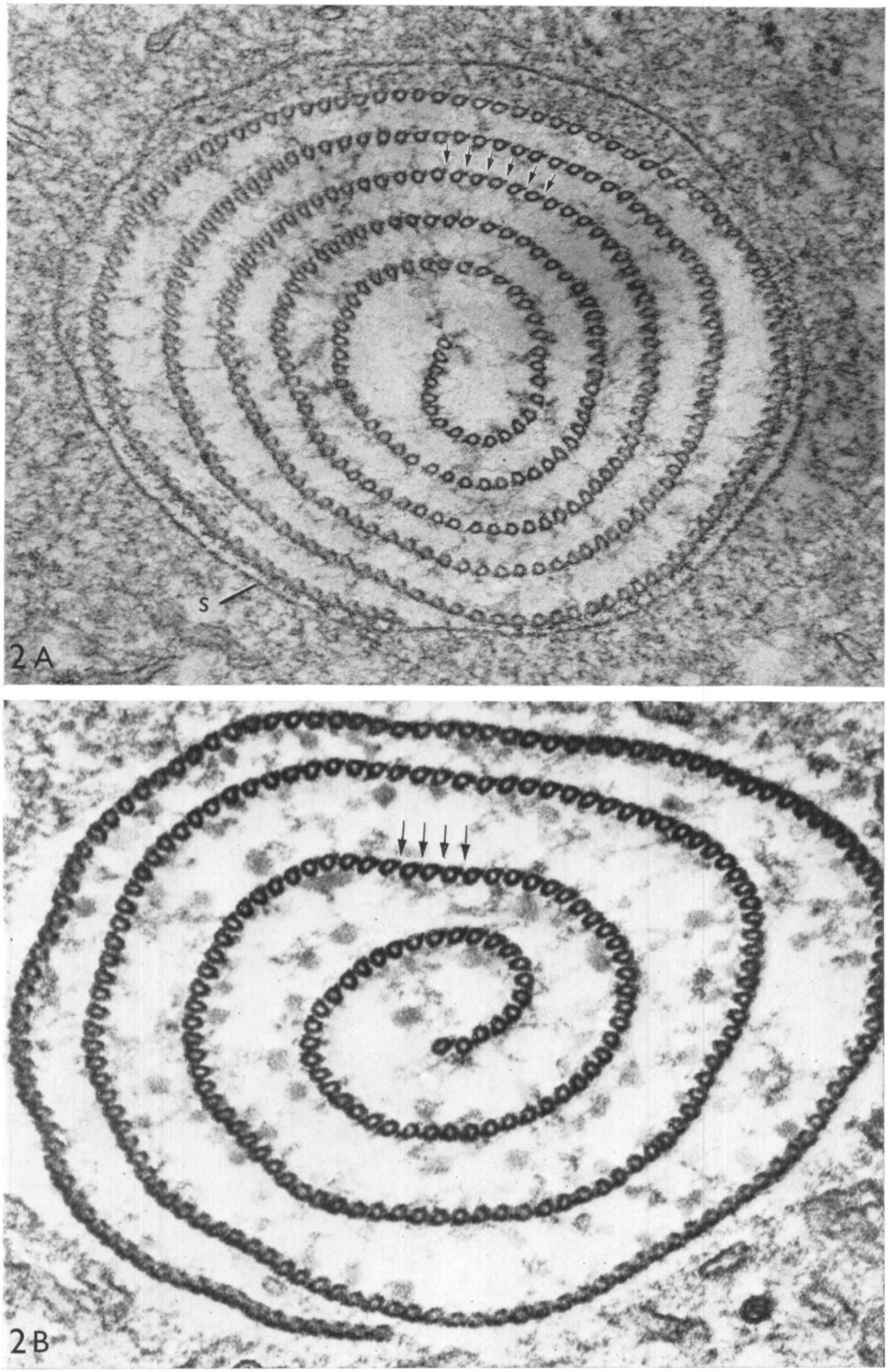


Fig. 2A and B. For legend see p. 632.

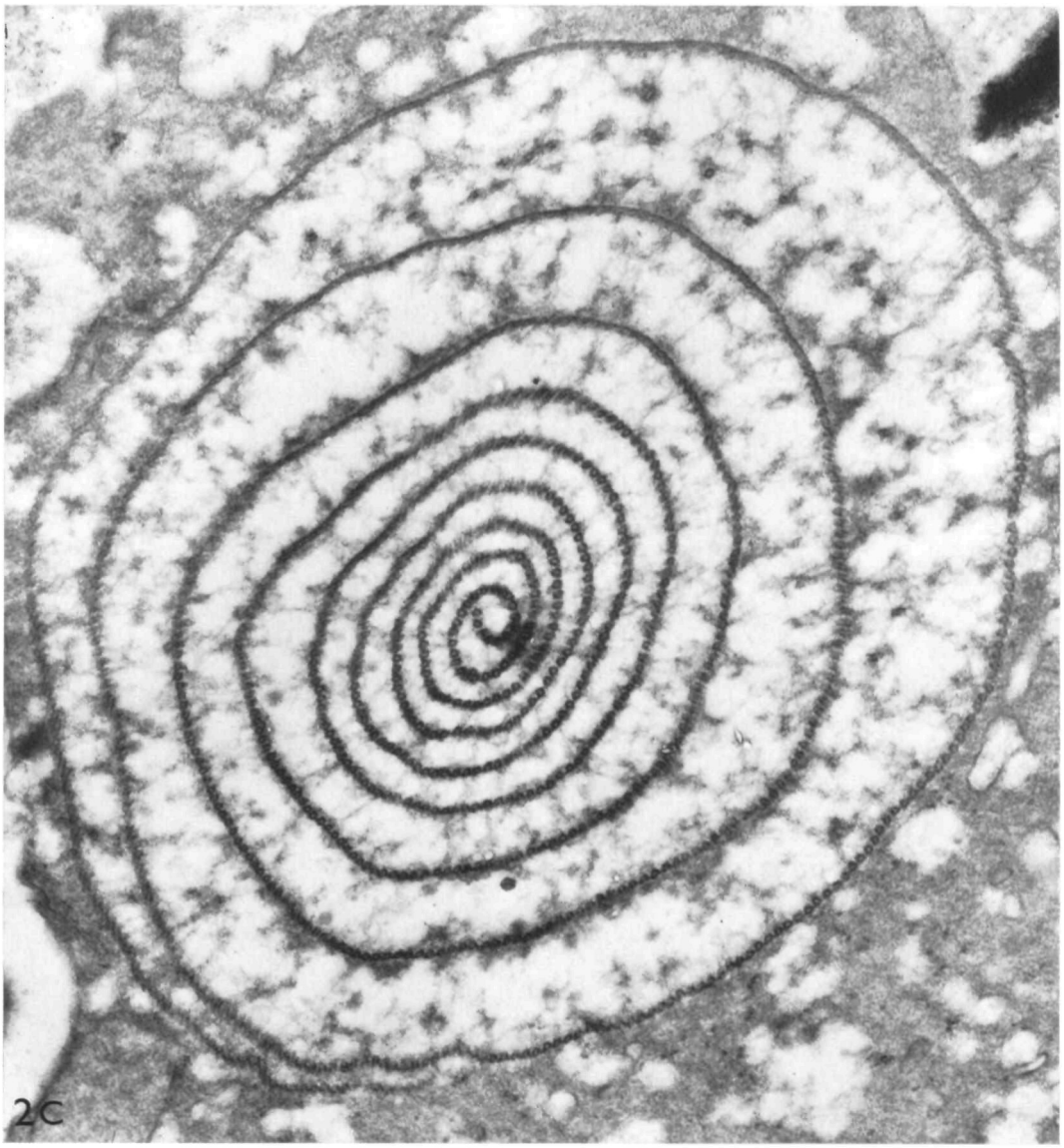


Fig. 2, A–C. Transverse sections through the axostyle of the *C. cavifrons* devescovinid (A), *Devescovina* sp. (B), and *Hyperdevescovina balteata* (C). Micrographs are printed so that the reader views the axostyle from its anterior end. In all devescovinids, the microtubular sheet spirals inward in a clockwise direction as viewed from the anterior end. Note the short projections (arrows) between adjacent microtubules in the row. The axostyle contains about 400 microtubules in the *C. cavifrons* devescovinid, about 300 in *Devescovina* sp., and 1200–1300 in *Hyperdevescovina balteata*. The innermost coils of the axostyle in *H. balteata* tend to be more tightly wound than the peripheral turns. A 5–7-nm-thick sheath (*s*) surrounds the axostyle of the *C. cavifrons* devescovinid, but is absent in *Devescovina* sp. and *Hyperdevescovina balteata*. A, ×76600; B, ×112000; C, ×42400.

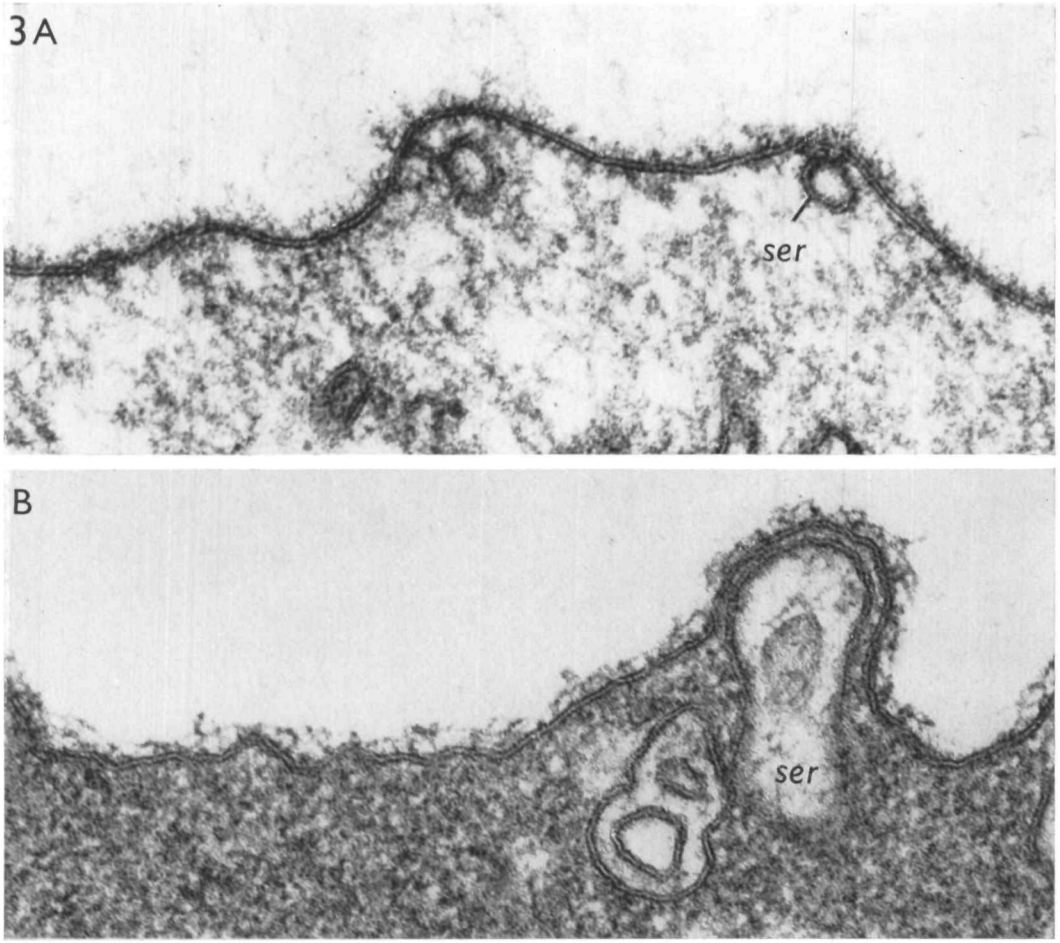
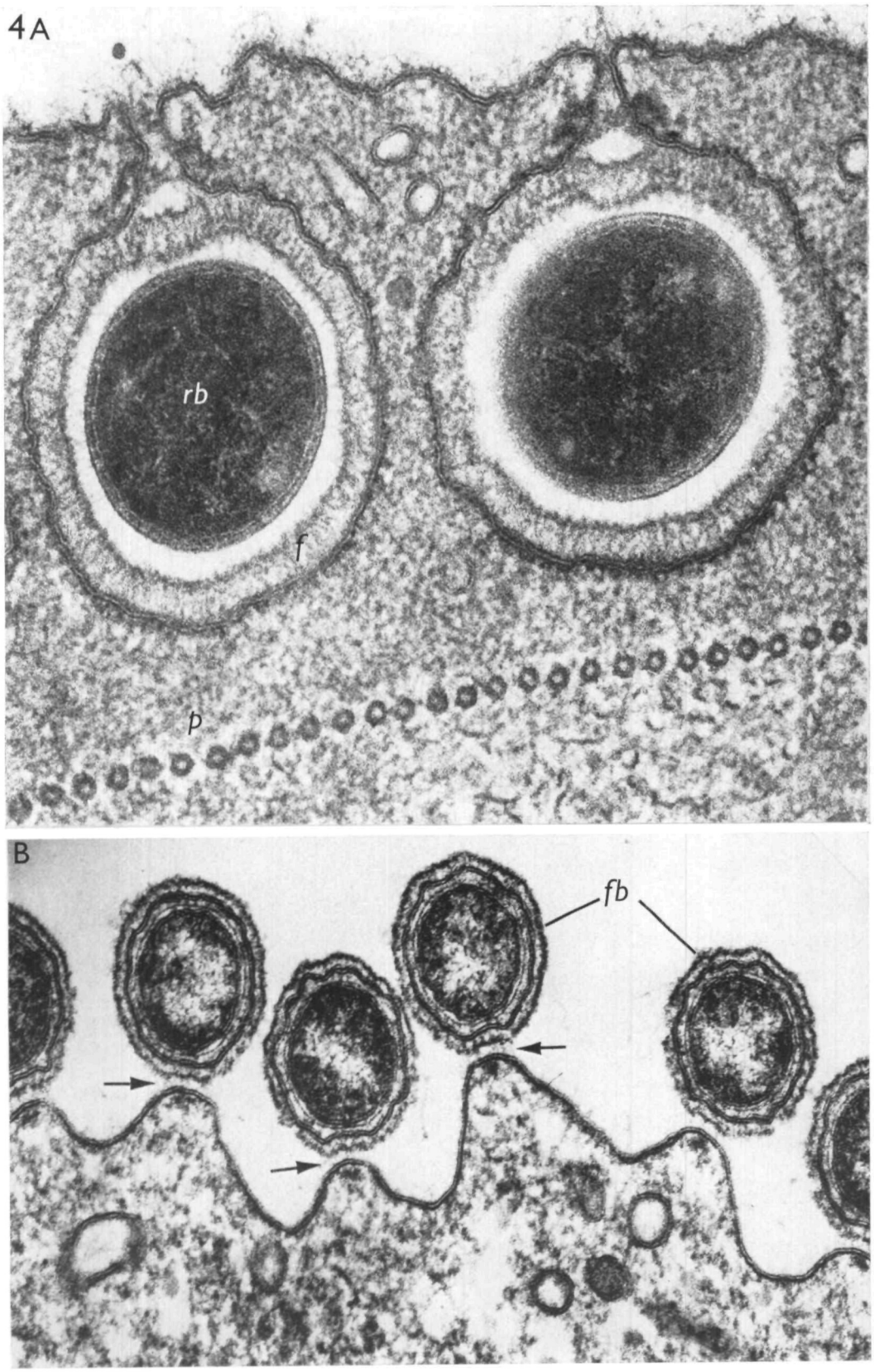
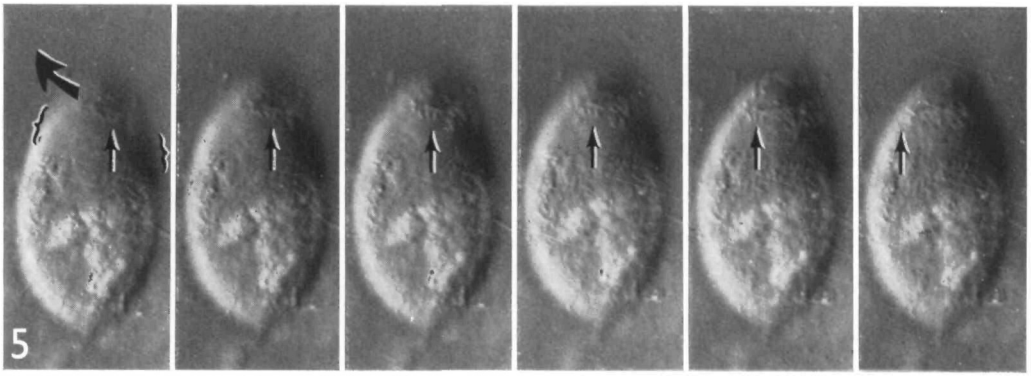


Fig. 3. Cell membrane in the region of the presumed shear zone between head and body of *Devescovina* sp. (A) and *Hyperdevescovina mitrata* (B). The plasma membrane appears continuous, and has the trilaminar structure typical of unit membranes. Elements of the smooth reticulum (*ser*) occur under most of the cell surface, but have never been seen to fuse with the plasma membrane. A, × 145 500; B, × 140 300.

Fig. 4. Types of ectobiotic bacteria on the surface of devescovinids (see also Fig. 1 D–F). A, *Hyperdevescovina mitrata*. Rod bacteria (*rb*) are completely enclosed in specialized invaginations of the cell membrane on the head (shown here) and body. Note the extracellular fibrous layer (*f*) lining the membrane pockets. A single row of pelta microtubules (*p*) runs under the surface of the head. B, *Devescovina* sp. Slender fusiform bacteria (*fb*) are attached to ridges of the cell membrane by faint, fibrillar material (arrows). Dense aggregates underlie these attachments on the cytoplasmic side of each ridge. A, × 127 500; B, × 112 500.


4A
rb
f
p
B
fb



Figs. 5–8. Prints from 16-mm ciné films of head rotation in Australian devescovinids. In all figures, the upper surface of the rotating structure is in focus, and the anterior direction is towards the top of the photographs. Movement from the reader's right to his left thus represents rotation in a clockwise direction as viewed from the anterior end of the cell (large arrow in first or second print of each figure.) All figures are of non-swimming cells in which rotation was periodically interrupted by pauses (not shown here).

Fig. 5. Clockwise rotation of the surface of the anterior end in *Hyperdevescovina balteata*. The cap of rod bacteria on the head surface (see π-shaped configuration of 3 bacteria marked by arrows) rotates, but the bacterial pattern on the body surface does not turn. The surface shear zone is probably located in the anterior part of the bacteria-free zone (brackets). Time interval between prints is 0·125 s. × 540.

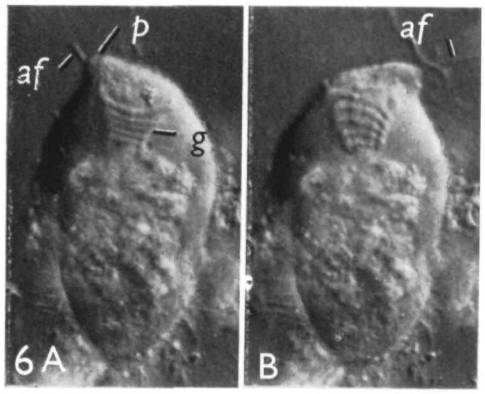


Fig. 6. Clockwise rotation of the Golgi (*g*), papilla (*p*), and anterior flagella (*af*) during head rotation in *Hyperdevescovina balteata*. The head has turned 180° from A to B. The body does not turn. × 560.

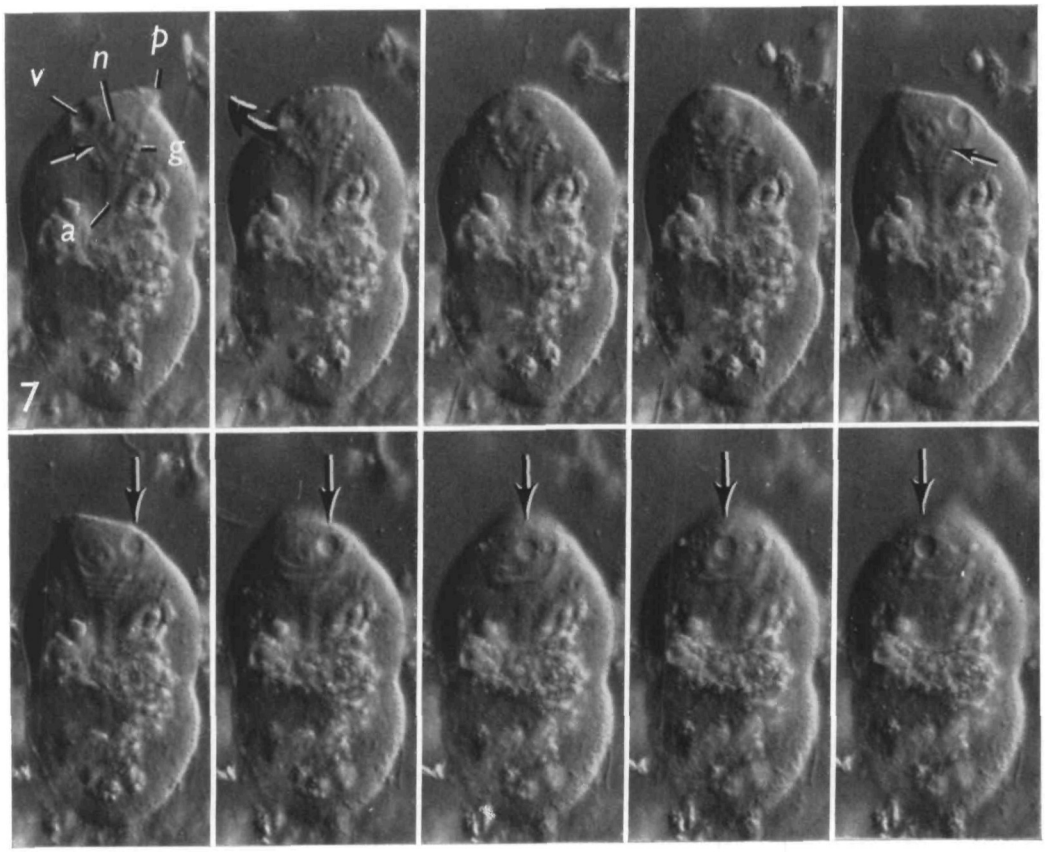


Fig. 7. Clockwise rotation of cytoplasmic structures during head rotation in *Hyperdevescovina balteata*. Upper row shows half of a revolution with focal plane midway through the cell. Tilting of the nucleus (*n*) off the axis of rotation provides an asymmetry for following its rotation. Likewise, the asymmetrical extension (small arrows) of the axostyle (*a*) along one side of the nucleus offers a built-in marker for following axostyle rotation. Papilla (*p*) and a vacuole (*v*) go out of the focal plane during rotation, and come back into focus after half a turn. The focal plane transects the gyres of the Golgi (*g*). Note that cytoplasm posterior to the Golgi does not turn. Lower row shows the second half of a different revolution of the same cell, with the focal plane gradually rising to the cell surface. The vacuole (arrowed) now remains in focus during rotation. Note again that the body cytoplasm does not turn. Time interval between prints is 0·4 s. × 540.

Fig. 8. Clockwise rotation of the Golgi complex (*g*) during jerky rotation of the anterior end of *Devescovina* sp. The C-shaped anterior extension of the Golgi provides a useful marker for following Golgi rotation. Note that vacuoles and inclusions in the body cytoplasm do not turn. Time interval between prints is about 6 s. ×400.