Motile Detergent-extracted Cells of *Tetrahymena* and *Chlamydomonas*

URSULA W. GOODENOUGH
Department of Biology, Washington University, St. Louis, Missouri 63130

ABSTRACT *Tetrahymena* and *Chlamydomonas* cells treated with high (0.25–0.5%) concentrations of the detergent Nonidet P-40 in appropriate buffers retain the shape of the intact cells but are devoid of any ciliary activity unless supplied with MgATP. ATP causes them to swim actively, with beat parameters and swimming patterns indistinguishable from those of intact cells. Both types of detergent-extracted cells are completely devoid of ciliary membranes. The *Tetrahymena* preparations also lack all cellular membranes, whereas cellular membranes remain intact in the *Chlamydomonas* preparations. Experiments demonstrating the effects of ATP, ADP, vanadate, erythro-9-[3-2-(hydroxynonyl)]-adenine, and Ca^{++} are described to illustrate the use of these detergent-extracted cells in research on ciliary motility.

Tetrahymena and *Chlamydomonas* have become established as key experimental organisms for research on cell motility. Each has been the subject of numerous studies on the ultrastructure and biochemical components of its cilia (1–12 and references cited therein); each has been induced to carry gene mutations that alter normal motility (13–17); and each can be readily grown in axenic culture and subjected to genetic analysis (18, 19). The analysis of these organisms has been hampered, however, by the lack of demembranated motile cells analogous to the detergent-extracted sea urchin sperm so elegantly exploited by the Gibbons laboratory (20–24).

I report the successful preparation of detergent-extracted *Tetrahymena* and *Chlamydomonas* cells that retain their axonemes. Electron microscopy shows that both are completely devoid of ciliary membranes. The *Tetrahymena* preparations carry only fragmented pieces of cell and organelle membranes, whereas both membrane systems appear intact in the *Chlamydomonas* cells. Both types of detergent-extracted cells display native forms of motility when supplied with MgATP. Experiments are presented to document that this motility can be manipulated by changing the ionic environment of the preparations and by using inhibitors of ciliary dynein ATPase. The contribution of membrane systems to observed motility patterns is also assessed. The extracted cells should be particularly valuable for the analysis of ciliary coordination.

MATERIALS AND METHODS

Culture and Extraction of Tetrahymena: Log-phase *Tetrahymena thermophila* cells, strain B-1868, mating type III, were grown at 28°C in axenic medium containing proteose peptone and yeast extract, as described by Gorovsky et al. (25). To prepare extracted cells, ~20 ml of cell culture is poured into a 50-ml plastic centrifuge tube (for batch isolates, many such tubes can be processed in parallel) and centrifuged gently (taken to 650 *g* and then down) to minimize cell packing and concomitant mucus release. The culture medium is decanted, and all further manipulations are carried out at 4°C. Each pellet is resuspended in 30–40 ml of HMDES (10 mM HEPES, 5 mM $MgSO_4$, 1 mM dithiothreitol (DTT), 2 mM EGTA, 4% sucrose, pH 7.4). (17). (In this and all subsequent steps, pellets are resuspended immediately after centrifugation is completed, and resuspension is effected by gently stirring with a glass rod, never by suction.) The cells are again centrifuged at 650 *g* as above, the supernatant is decanted, and the wash is repeated with fresh HMDES. These washes appear to be essential to remove all traces of Ca^{++} and thereby to minimize mucus release and prevent deciliation. The pellet from the second wash is suspended in 4 ml of HMDEK (30 mM HEPES, 5 mM $MgSO_4$, 1 mM DTT, 2 mM EGTA, 25 mM KCl, pH 7.4). (To this and all subsequent buffer solutions, 10 μl/ml aproteinin [Sigma Chemical Co., St. Louis, MO] is added just before use.) To this is added 4 ml of 0.5% Nonidet P-40 (NP-40, Particle Data Inc., Elmhurst, IL) in HMDEK to give a final detergent concentration of 0.25% (concentrations of up to 1% NP-40 yield reactivatable preparations but cell shape appears less well preserved; more dilute detergent concentrations cause the cells to secrete copious mucus and shed their cilia long before adequate extraction has occurred). The cells are gently swirled in the detergent for 1 min, then 25 ml of cold 0.5% NP-40 in HMDEKP (HMDEK plus 2% polyethylene glycol [PEG], 20,000 mol wt [Fisher Scientific Co., Pittsburgh, PA]) is added. The suspension is then pelleted at 650 *g*. This pellet should be translucent, in contrast with the pinkish-brown pellets of intact cells. The pellet is then subjected to one or two gentle washes in 30 ml of detergent-free HMDEKP to remove detergent, residual mucus, and any debris. The cells are then suspended at the desired concentration in HMDEKP. (More recently, the "mucus-free" strain of *Tetrahymena thermophila*, SB-255, has been obtained from Dr. Eduardo Orias, University of California, Santa Barbara; this generates excellent preparations and eliminates the problem with mucus.)

ATP reactivation is ordinarily performed by mixing equal volumes of extracted cells suspended in HMDEKP and MgATP dissolved in HMDEK; thus the cells swim in a final concentration of 1% PEG. A 0.1% PEG solution is also compatible with motility. For some samples, initial washes were carried out in HMDEKP as above, but the cells were then washed twice in HMDEK plus 0.5% NP-40 and reactivated in this detergent solution. Surprisingly, they swim almost as well in

THE JOURNAL OF CELL BIOLOGY · VOLUME 96 JUNE 1983 1610–1621

the detergent as they do in the HMDEKP; by contrast, they swim poorly in HMDEK alone and tend to lose their intact shape, suggesting that either PEG or NP-40 must be present to stabilize some aspect of their cortical integrity. Extracted cells maintained on ice in HMDEKP are reactivatable for at least 4 h after preparation; when refrigerated overnight, however, they cannot be reactivated the next morning, even though they appear intact by phase-contrast microscopy, suggesting that component(s) necessary for motility are slowly extracted or proteolyzed.

Culture and Extraction of Chlamydomonas: Wild-type *Chlamydomonas reinhardi* gametes, strain 137c mt^+ (strain CC-620 as maintained at the *Chlamydomonas* Genetics Center, Duke University, Durham, NC) were harvested from agar plates in nitrogen-free, high-salt minimal medium (26), washed once in this medium, suspended at $\sim 10^7$ cells/ml, and allowed to differentiate into fully motile gametes for 1 h (26). Vegetative cells were grown in liquid Tris-acetate-phosphate medium (27) to mid-log phase.

To prepare extracted cells, the protocol detailed above for *Tetrahymena* is followed exactly except that the preparations require centrifugation at 3,000 *g*. After exposure to detergent, the pelleted extracted cells appear green and the supernatants are clear. It is critical to the success of this procedure that the cells have intact cell walls: when, for example, the procedure is applied to our wild-type mt^- strain, which tends to have thin or absent cell walls in the gametic state, most of the cells lyse in the detergent and the wash supernatants are green. The extracted *Chlamydomonas* cells are even more stable than their *Tetrahymena* counterparts in HMDEKP, being partially reactivatable after overnight storage at 4°C; by contrast, they swim only briefly in 0.5% NP-40 in HMDEK, since prolonged exposure to detergent causes deflagellation and cell lysis.

Microscopy: For phase-contrast microscopy, intact cells were fixed briefly with glutaraldehyde to stop their swimming and allow photography; extracted cells were photographed unfixed. Films of reactivated *Tetrahymena* were made of fields magnified × 300, using a Zeiss photomicroscope equipped with phase optics and an Opti-quip Model 220 camera using Kodak 2415 film.

For scanning electron microscopy (SEM) of *Tetrahymena*, both cells and extracted cells were suspended in cold HMDEKP, to which was added an equal volume of 2% glutaraldehyde (Ladd Research Industries, Inc., Burlington, VT) and 0.2% tannic acid (Mallinckrodt Inc., St. Louis, MO) in HMDEKP. After a 1-h fixation, samples were washed once in 30 mM HEPES, pH 7.4, postfixed in 0.5% OsO_4 in 0.1 M sodium phosphate buffer, pH 6.0, for 1 h, and washed once in distilled water. The samples were then pipetted into the apparatus designed by Mesland (28), wherein disks of freshly cleaved mica are held at the bottom of small chambers, and overlying solutions can be rapidly exchanged by pipetting. Once in this apparatus, the samples were successively dehydrated with a graded ethanol series followed by extensive exchanges with 100% ethanol; they were then critical-point-dried using a Polaron E3000 device. The samples on the mica disks were coated with gold in a Polaron E5000 sputter coater and examined with a Hitachi S-450 SEM at 15 kV.

For transmission electron microscopy (TEM) of sectioned *Tetrahymena* material, portions of the samples prepared as above for SEM were stained *en bloc* with 1% uranyl acetate for 30 min after the osmium postfixation, washed and dehydrated in graded ethanol, and embedded in Epon-Araldite. Independent samples of *Tetrahymena* were prepared by freeze-substitution: cells or extracted cells were quick-frozen without fixation by the technique of Heuser (29); a scintillation vial containing 15 ml of frozen acetone was placed in a bath of liquid nitrogen; it was overlain first with 1 g of 70% OsO_4 and then with the frozen sample on its stage, filled with liquid nitrogen, capped, and stored overnight at −95°C; it was next transferred to −20° for 4 h and then brought to room temperature. The OsO_4/acetone mixture was decanted, replaced with 100% ethanol for 15 min, then with 1% uranyl acetate in 100% ethanol for 1 h, and then with ethanol, propylene oxide, and Araldite resin.

The *Chlamydomonas* material was prepared for TEM by the glutaraldehyde/tannic acid procedure described above for *Tetrahymena*.

Reactivation Reagents and Inhibitors: Reagents used for reactivation experiments are as follows. ATP was purchased from Sigma Chemical Co. (A-5394; vanadium <1 ppm) and prepared as a 1–20-mM MgATP stock solution in HMDEK that was retitrated to pH 7.4. ADP was purchased from Boehringer Mannheim Biochemicals, Indianapolis, IN; sodium metavanadate, from Fisher Scientific and prepared as a 10-mM stock solution in distilled water and diluted just before use in 10 mM Tris-HCl, pH 8.1. P^1, P^5-di-(adenosine-5′)-pentaphosphate was purchased from Sigma Chemical Co.; erythro-9-[-3-2-(hydroxynonyl)]-adenine (EHNA), from Burroughs Wellcome & Co., Raleigh, NC and prepared as a 5-mM stock solution in HMDEK. Protocols for reactivation experiments are detailed in Results.

RESULTS

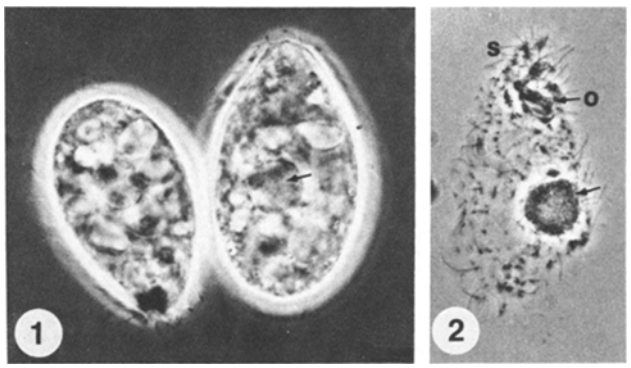
Structure of Extracted Tetrahymena Cells

Living *Tetrahymena* cells are phase-dense organisms (Fig. 1) that form a pinkish-brown pellet upon centrifugation. The extracted cells, by contrast, form a transparent pellet, and their only phase-dense inclusions are the macronucleus and one or two micronuclei (Fig. 2). They also retain both their oral and their somatic ciliature (Fig. 2, *O* and *S*). These can be better visualized by SEM: Figs. 3 and 4 compare an intact organism with an extracted cell. The surface of the intact cell carries cilia and occasional membrane depressions (arrows) left when the cilia snap off. The surface of the extracted cell carries ciliary axonemes and a few membrane vesicles (Fig. 4, arrows); its texture is otherwise lumpy and pockmarked.

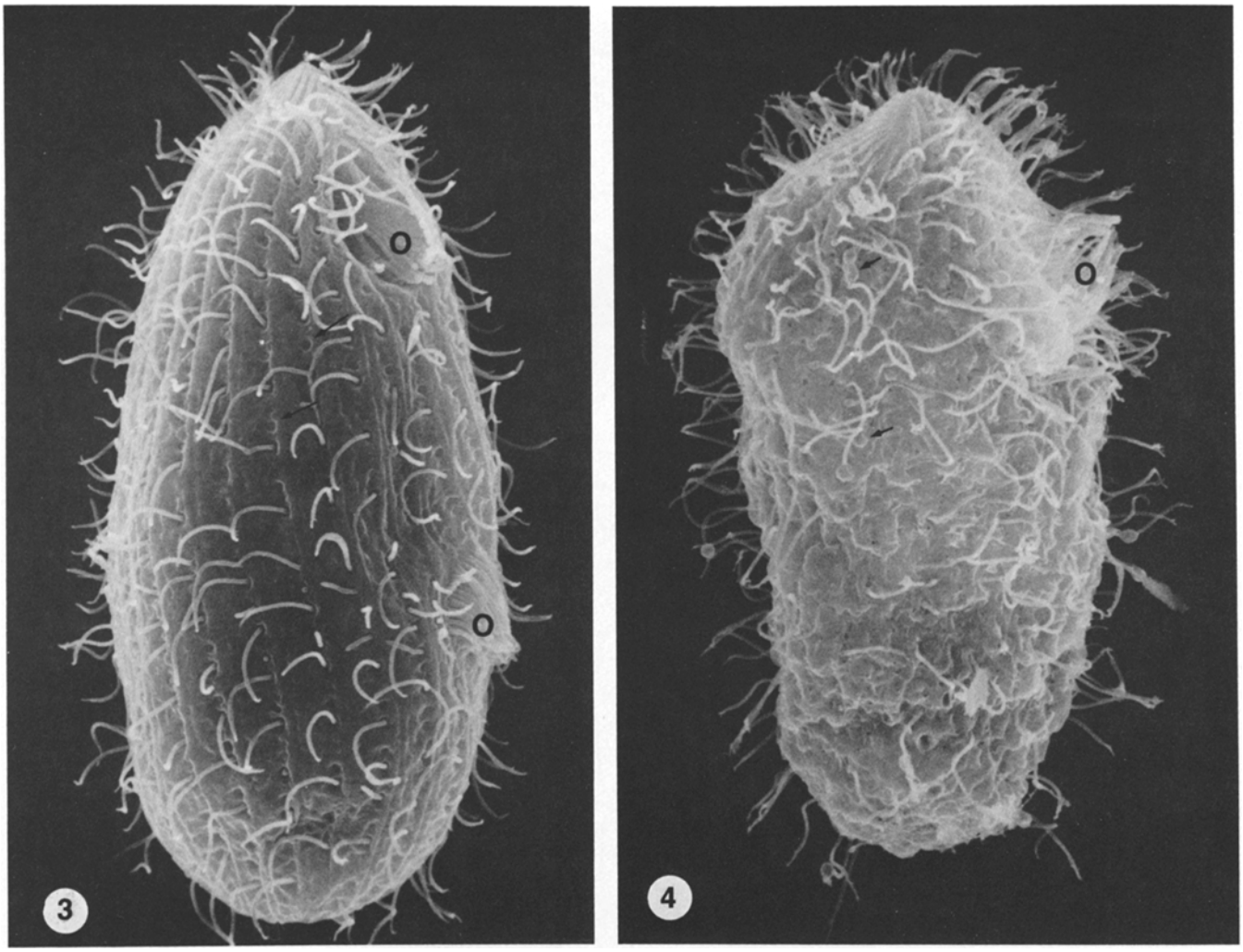
Figs. 5–10 show representative thin sections of intact *Tetrahymena* and corresponding regions of the extracted organisms. It is clear, first, that the internal membranous components (mitochondria, mucocysts, endoplasmic reticulum, etc.) of the intact cells (Figs. 7 and 9) have been completely solubilized, leaving only membrane fragments in the interiors (Figs. 5 and 6, arrows). Second, the ciliary and plasma membranes have also been solubilized, leaving only broken shards of membrane on the surfaces (Figs. 5, 6, 8, and 10, arrows). The integrity of the extracted cells is apparently provided by fibrous material known as the "epiplasmic layer" (30–32). In intact cells, this layer underlies the cell membrane (Figs. 7 and 9, *e*) and forms a dense "pellicular plate" as it approaches the distal region of each basal body (Figs. 7 and 9, *p*). Each extracted cell is found to be encircled by an epiplasmic layer (Figs. 5, 6, 8, and 10, *e* or *p*), buttressed from both without and within by bands of cortical microtubules (Figs. 6 and 8, *mt*) and striated fibers (Fig. 6, *sf*). Thus, the extracted preparations appear to possess an intact "cortical system" (33) but lack all the membranous systems of *Tetrahymena*. They differ from previously described pellicle preparations and isolated oral apparatuses (32, 34–36) in that they retain their ciliary axonemes and the shape of intact cells.

Structure of Extracted Chlamydomonas Cells

The *Chlamydomonas* and *Tetrahymena* extracted cells are prepared by identical protocols, but they prove to have very different properties. By phase-contrast microscopy, the *Chlamydomonas* preparations remain green and dense, differing from controls only in that they are immotile and that their flagella are much less refractile. By TEM, their cell membranes and internal organelles appear intact (Figs. 11 and 12), as though detergent had not penetrated the cell interior. Their flagellar membranes, however, are completely solubilized (Figs. 13 and 14). Solubilization terminates at the same location in every case, namely, at the distal end of the flagellar transition



FIGURES 1 and 2 Living *Tetrahymena* cells (Fig. 1) and an extracted cell (Fig. 2) viewed by phase-contrast microscopy. Arrows point to the macronuclei; a small micronucleus is visible above the macronucleus in the extracted cell. *O*, oral ciliature; *S*, somatic ciliature. × 630.



FIGURES 3 and 4 Living *Tetrahymena* cell (Fig. 3) and an extracted cell (Fig. 4) viewed by SEM. Two oral apparati (O) are visible in Fig. 3 (in preparation for cell division) and the surface membrane carries depressions (arrows) left where some somatic cilia have snapped off. The axonemes of one oral apparatus (*O*) are visible in the extracted cell, and two large contractile vacuole pores are seen near the posterior midline. Small holes perforate the extracted cell surface; arrows indicate membrane vesicles. Oral ridges are seen at the anterior ends of both the cell and the extracted cell. × 3,000 and × 3,500.

region (Figs. 11 and 12, arrows). A dense material lies between the doublet microtubules and the flagellar membrane at this level (Figs. 11 and 12; see also Figs. 34 and 35 of reference 10). Moreover, the cell wall is in close contact with the cell membrane at the bases of the tunnels (Figs. 11 and 12, arrowheads). It appears, therefore, that the dense material in the transition region may resist detergent activity, and/or that the wall may retard detergent's access to the cell during the course of the extraction period, the result being that the extracted cells have an apparently unperturbed cytoplasm, but naked axonemes that have lost access to internal stores of ATP.

It is difficult to state whether the extracted *Chlamydomonas* cells are "dead" or "alive". They evolve oxygen in the light and consume oxygen in the dark, at rates equivalent to those of control cells exposed to the same buffer solutions minus detergent (unpublished experiments). On the other hand, their plating efficiency is only 10^{-3} that of controls, indicating that some aspect of the treatment is incompatible with mitotic growth. They are also unable to regenerate new flagellar membranes, nor can they regenerate new flagella if their axonemes are removed by exposure to high calcium (see below), in contrast with the rapid regeneration effected by living cells (37).

Swimming Behavior of the Extracted Tetrahymena Cells

Extracted log-phase *Tetrahymena* cells are totally immotile unless provided with exogenous MgATP. At appropriate ATP concentrations, detailed below, they swim in a fashion identical to that of intact cells: Their somatic axonemes beat in an anterior to posterior direction while their oral axonemes beat in a coordinated circumferential fashion, creating what appears to be a tiny vortex. They swim in a straight path but make frequent helical spirals about their long axes as they swim, just as the intact cells do (38). This rotation appears to be generated, at least in part, by the beat of the oral axonemes, which act in the fashion of a small propeller mounted parallel to the body surface. When intact cells change the direction of their swimming path, they first point their tapered anterior "noses" in a new direction, and the rest of the body then reorients accordingly. The extracted cells carry out this same behavior, their

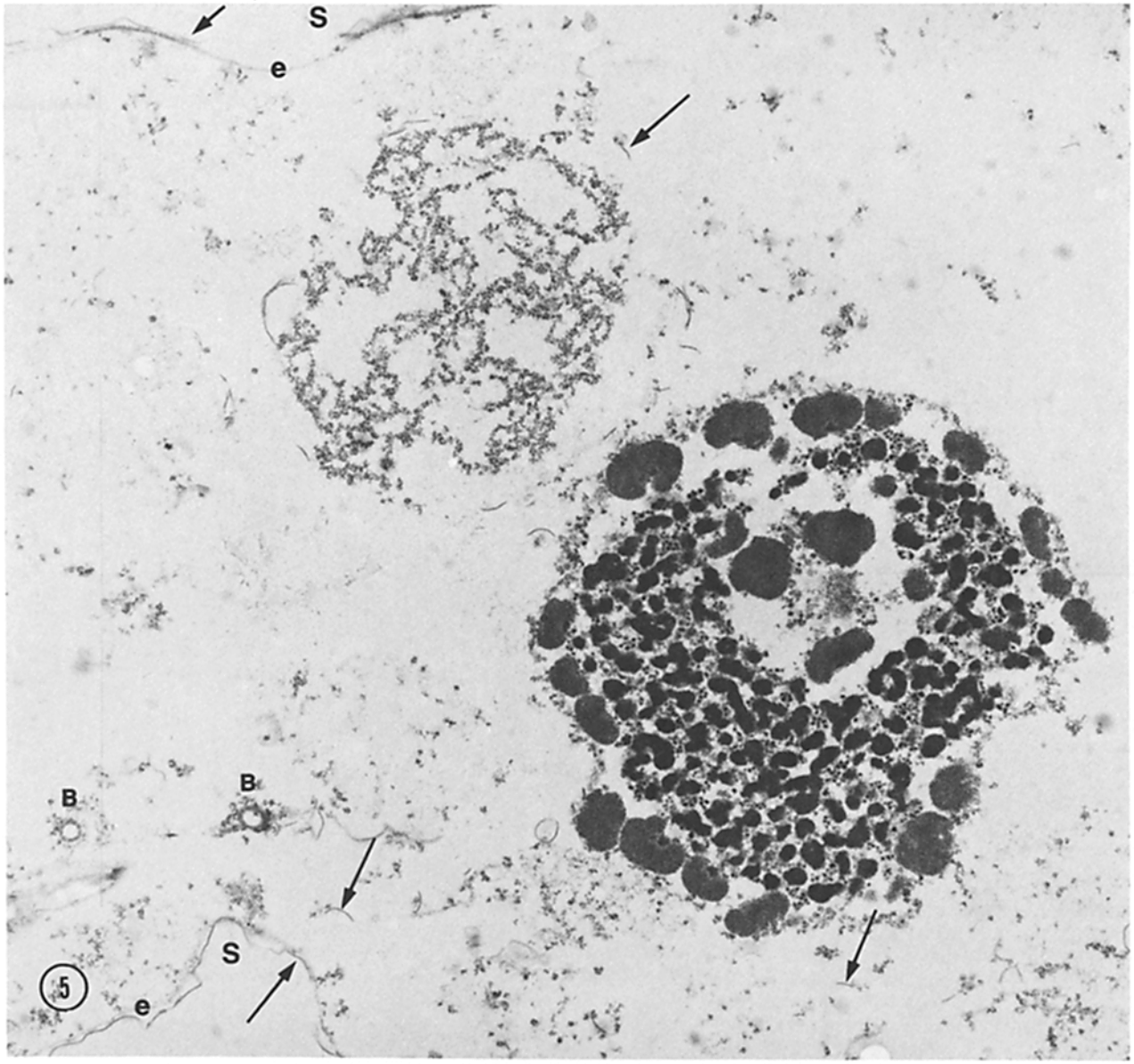


FIGURE 5 Interior of an extracted *Tetrahymena* cell. The macronucleus retains its black chromatin bodies and granular peripheral nucleoli, and the micronucleus above it retains its more fibrous chromatin, yet both are free of their nuclear envelopes. Fragments of the envelopes and of other intracellular and surface membranes are marked by arrows. The two surfaces (*S*) of the extracted cell carry a continuous epiplasmic layer (*e*). Basal bodies are indicated at *B*. × 14,000.

noses turning before the rest of the body turns. Fig. 15 presents a sequence of movie frames that document these features of in vitro *Tetrahymena* motility.

Rates of motility were determined by mixing extracted organisms with MgATP, placing a sample of the mixture in a hemacytometer chamber, and measuring the time (with a digital stopwatch) taken for individual cells to swim straight across a 200-μm grid. Any cell whose swimming veered significantly from a straight path was not scored, nor were any cells that stopped during the course of the "race". The data obtained for a given MgATP concentration proved to be highly reproducible from preparation to preparation. Table I *a* summarizes the optimal swimming rates obtained, expressed both in microns per second and as percentage of living-cell rates. The extracted cells swim best (39% of living rates) at 1 mM MgATP (no motility occurs in the absence of Mg^{++}). At higher MgATP concentrations, motility declines and, at >7 mM MgATP, it ceases altogether, the oral cilia no longer beating and the somatic axonemes trembling in an uncoordinated fashion. At <1 mM MgATP, motility decreases linearly with MgATP concentration until it ceases at ~30 μM. Below this concentration, the oral and somatic axonemes continue to wave slowly until the MgATP in the medium is depleted.

Even in a hemacytometer chamber, both *Tetrahymena* cells and extracted cells quickly adhere to the slide and coverglass so that motility can be observed only for a few minutes. Extracted cells in a test tube continue to swim for 15–30 min, but the proportion of motile cells decreases with time. Those that are not swimming continue to display an active oral and somatic axonemal beat for several hours, and the reason why this beat is inadequate to propel them through the medium is not obvious by visual inspection.

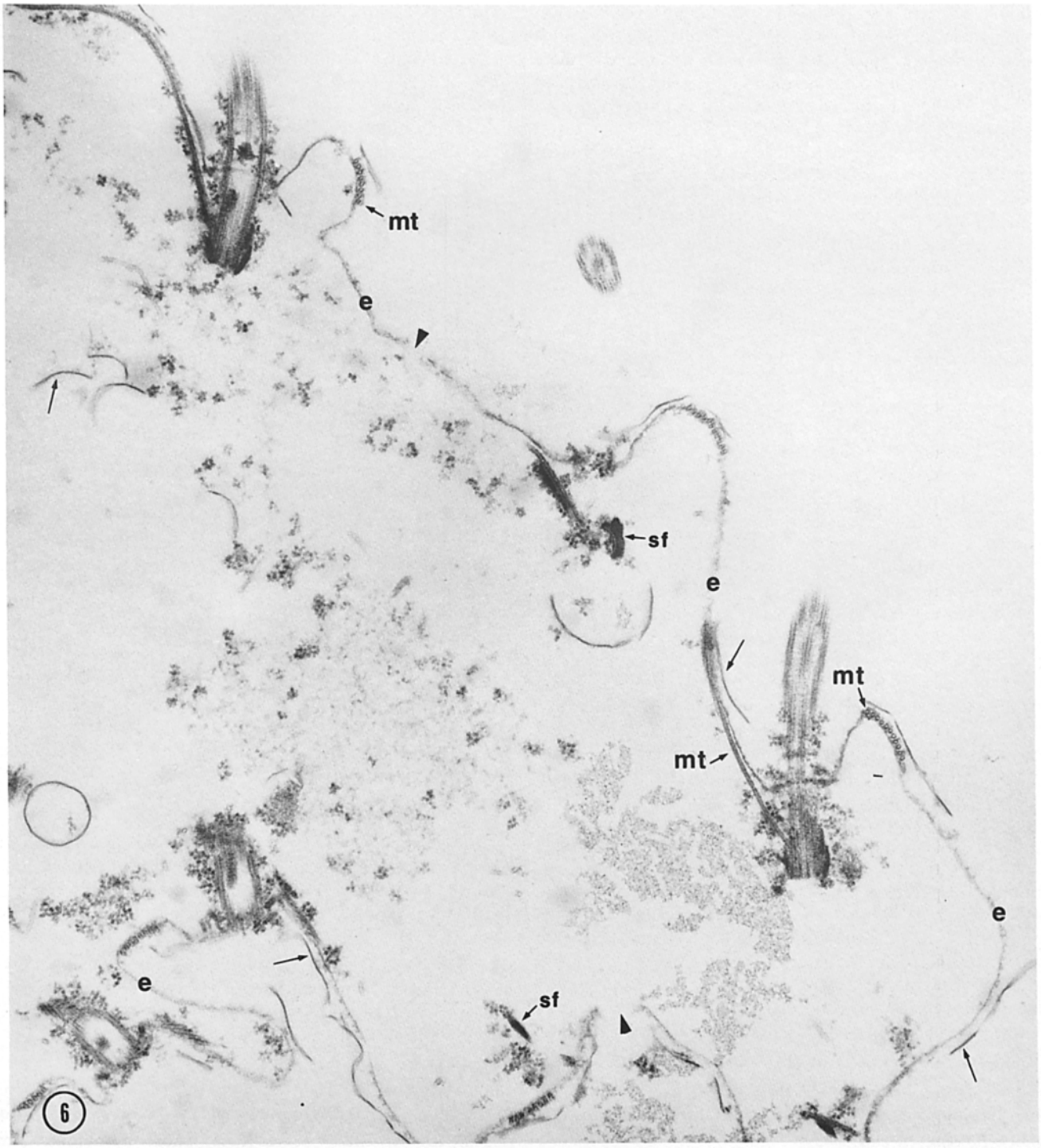


FIGURE 6 Surface of an extracted *Tetrahymena* cell in the region of somatic cilia. Membrane fragments are indicated by arrows. The continuous epiplasmic layer (*e*) carries occasional breaks (arrowheads) that may correspond to the holes in the surface of SEM preparations (Fig. 4). Cortical bands of microtubules (*mt*) and striated fibers (*sf*) associate with the epiplasm and the basal bodies. × 30,000.

Extracted cells stuck to glass surfaces are favorable preparations for observing the oral axonemal beat. Fig. 16, left panel, shows six consecutive movie frames of beating oral axonemes; for comparison, Fig. 16, right panel, shows six frames of an extracted cell not provided with MgATP, where the oral axonemes remain motionless (arrows). Each circumferential cycle of beating resembles the winking of an eye, and all the oral apparatuses in the field wink in unison at a rate proportional to MgATP concentration.

Swimming Behavior of Extracted Chlamydomonas Cells

The extracted *Chlamydomonas* cells swim about half as fast as their intact counterparts; Table I, b and c summarize the rates obtained for vegetative and gametic preparations. As with *Tetrahymena*, motility increases linearly with MgATP concentration to ~1 mM, with Mg^{++} again being required. At higher MgATP concentrations the proportion of motile cells decreases

dramatically until, at a 10-mM concentration, very few can swim, these moving at the 1-mM ATP rate. The rest circle about their axes, apparently because one axoneme is more functional than the other, and soon stop moving altogether. At 0.5 mM MgATP, motility continues in the test tube for at least an hour.

The pattern of swimming for extracted cells is very different from that for intact cells. Living cells are observed to swim for short distances, then stop and reorient in a new direction, sometimes at right angles to their previous path and sometimes in the opposite direction. (Indeed, most intact cells express this behavior during the course of the 200-μm hemacytometer race, meaning that most cannot be scored and the assay becomes quite tedious.) This swimming pattern is presumably a manifestation of the phototactic response of the intact cells (39), since they quickly accumulate over the illuminated portion of the microscope slide. By contrast, the extracted cells swim in completely straight paths, neither stopping nor turning except in very wide arcs, and they fail to accumulate in the illuminated sector of a slide. Therefore, it appears that detergent treatment of *Chlamydomonas* abolishes both the ability to reorient the swimming path and the ability to respond to light.

The use of the extracted cells for analyzing *Chlamydomonas* motility can be illustrated by the following two sets of observations. (*a*) Vegetative *Chlamydomonas* cells swim more slowly than their gametic counterparts, and this difference is mirrored by the extracted cells (Table I, *b* and *c*). Similarly, the mutant strain *sup$_{pf}$-1* swims more slowly than the wild type both in vivo (13, 15) and in vitro (Table I*d*). Thus the native *rate* of swimming, although not the pattern, appears to be preserved by the extracted cells. (*b*) The mutant strain *sup$_{pf}$-1* swims in a very jerky fashion, whereas the extracted mutant swims very smoothly, suggesting that this feature of its aberrant behavior requires an intact membrane system. Conversely, the mutant strains *pf-14* and *pf-18*, lacking radial spokes and central-pair microtubules respectively (17, 40), display an uncoordinated "twitching" (41) of their flagella in intact cells; the extracted cells fail to twitch, suggesting that at least a portion of this behavior requires an intact membrane system (cf. reference 41). In general, the extracted cells, requiring only ~10 min to prepare, are more likely than isolated axonemes to retain unstable components of mutant flagella, and are therefore more suitable for determining whether mutant phenotypes are membrane-mediated or axoneme-autonomous.

Responses to Nucleotides, Inhibitors, and Ions

The use of the extracted cells for analyzing the effects of various reagents on ciliary motility can be illustrated by the following observations.

ADP: Several investigators have noted that axonemes will beat in the presence of ADP, and have detected the presence of an adenylate kinase activity in their preparations (42–44). Both *Tetrahymena* and *Chlamydomonas* extracted cells will swim in >1 mM ADP, with a lag of ~2–5 min before beating begins and the rate of beating increases with time. This effect is completely abolished by the addition of 0.1 mM P^1, P^5-di-(adenosine-5′)-pentaphosphate, a specific inhibitor of striated muscle adenylate kinase (myokinase) (45), whereas the inhibitor has no effect on ATP-mediated motility. The presence of adenylate kinase activity in the highly extracted *Tetrahymena* preparations argues strongly that it resides in the axoneme and is not a contaminant. Its axonemal location warrants further study.

VANADATE: Extracted preparations of *Tetrahymena* and *Chlamydomonas* display equivalent sensitivities to vanadate, a noncompetitive inhibitor of dynein ATPase (46–48). Given 300–500 μM MgATP, both require 5 μM vanadate to stop all motility; in the 0.5–2-μM vanadate range, an occasional axoneme will propagate a slow, solitary beat; and below 0.5 μM vanadate, an increasing number of axonemes display an increasingly strong beat. Since the samples are in 2 mM EGTA, which may chelate some of the vanadate ions, the effective concentrations are possibly lower. The vanadate inhibition is fully reversible by the addition of epinephrine, as described by Gibbons et al. (46). Unextracted *Tetrahymena* and *Chlamydomonas* cells, by contrast, swim normally in the presence of millimolar concentrations of vanadate.

EHNA: Extracted *Tetrahymena* and *Chlamydomonas* cells also display equivalent responses to EHNA, a competitive inhibitor of dynein ATPase (49, 50). Given 200 μM MgATP, the addition of 1 mM EHNA stops all motility within 1 min. When an additional 200–400 μM of MgATP is added to the inhibited suspension, motility resumes.

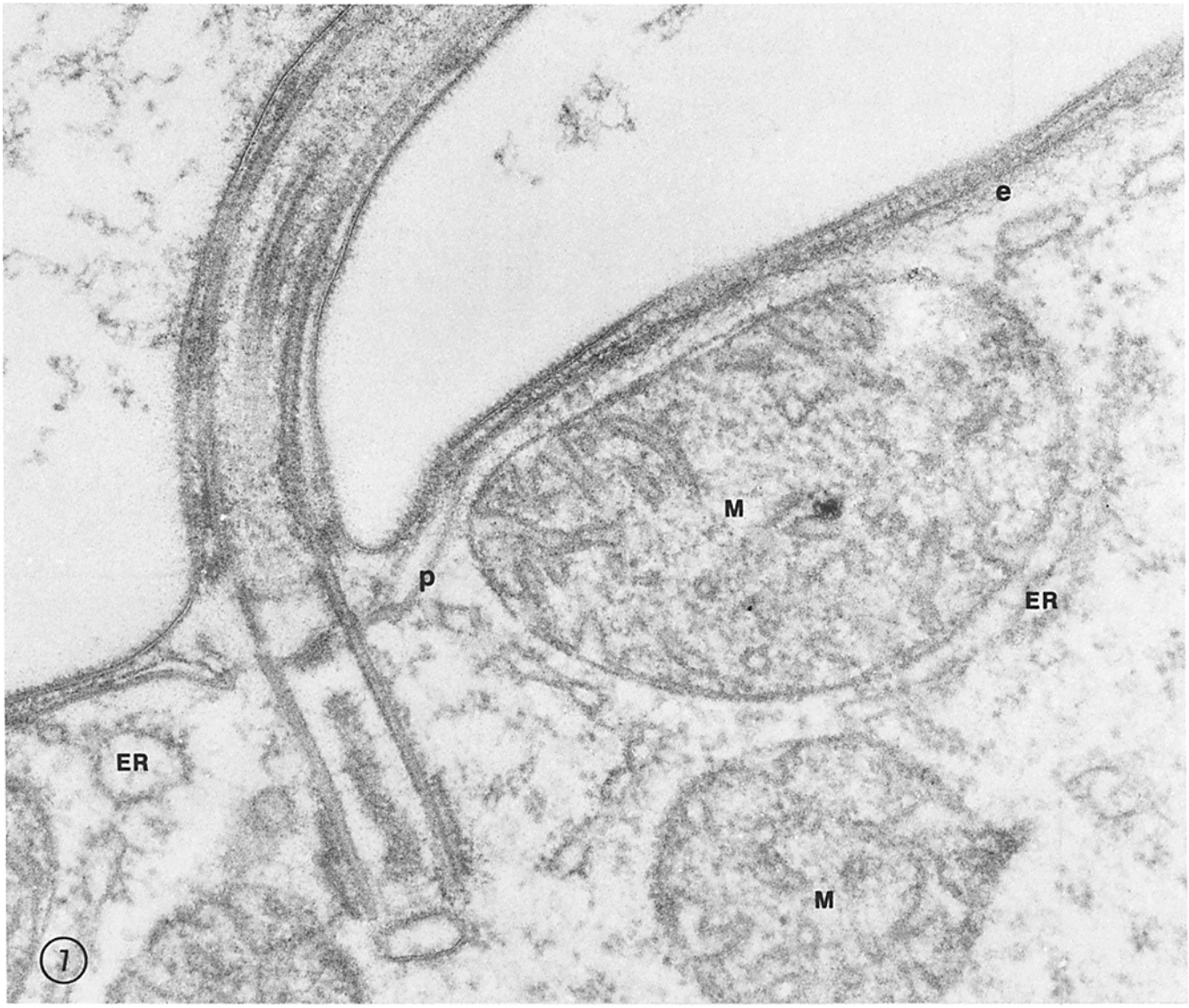
CALCIUM: Calcium ion has been reported to induce quiescence in extracted echinoderm sperm (24) and a reversal of beating in *Paramecium* and *Chlamydomonas* preparations (2, 5, 51, 52). The extracted *Tetrahymena* cells display a unique response to calcium. At $\leq 2.6 \times 10^{-5}$ mM free Ca^{++} (concentrations calculated with the computer program of Dr. Richard Steinhart, University of California, Berkeley), they swim actively; swimming slows considerably in the presence of 1×10^{-4} mM free Ca^{++}, and stops altogether at $\geq 1.6 \times 10^{-4}$ mM free Ca^{++}, even though the cilia continue to beat actively. The beat appears abnormal by visual inspection, but high-speed microcinematography will be required to determine whether it resembles the "hovering" motility executed by *Tetrahymena* during both cytokinesis and mating.

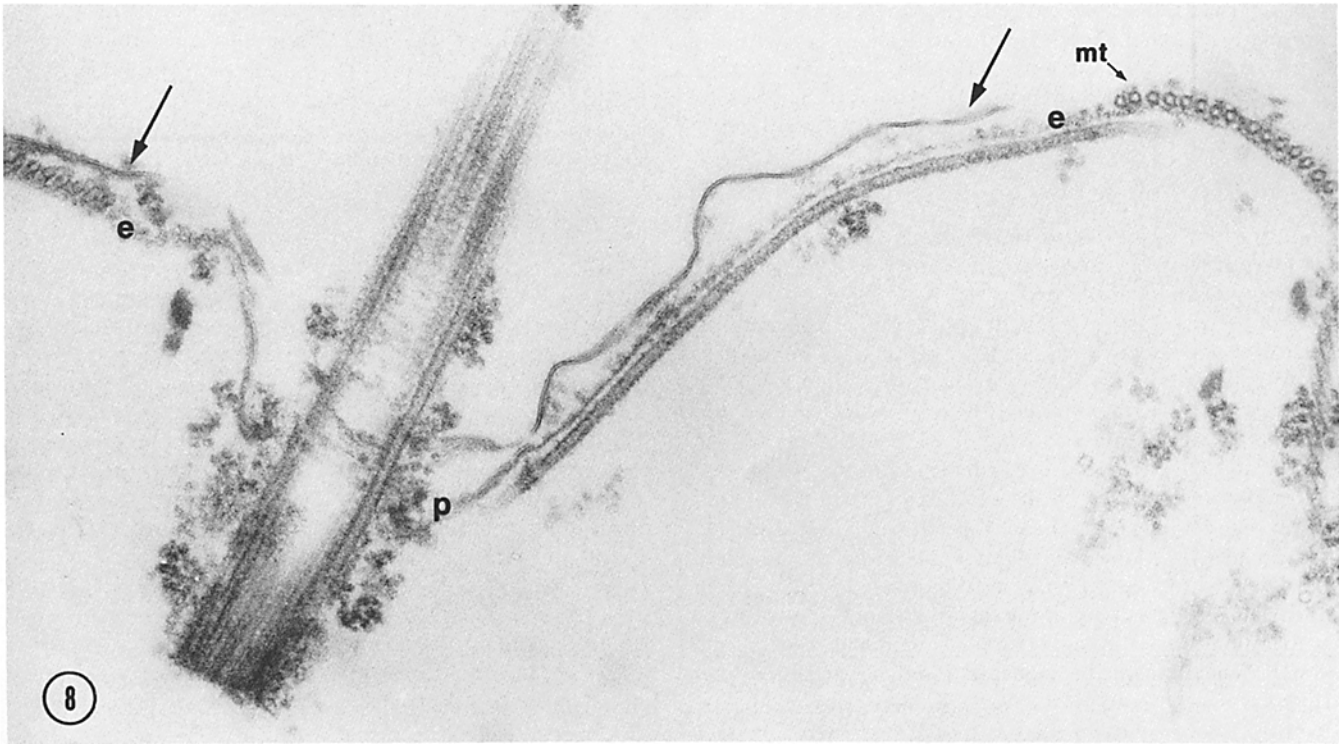
The extracted *Chlamydomonas* cells, both vegetative and gametic, continue to swim actively in a forward direction at free Ca^{++} concentrations up to 1.2 mM. At higher concentrations, the axonemes snap off the models at their proximal ends and are immotile in solution. By contrast, Bessen et al. (2) report that isolated *Chlamydomonas* axonemes are immotile at 1×10^{-2} mM free Ca^{++} and reverse their direction of beat at 0.1 mM free Ca^{++}, and Hyams and Borisy (5) describe backwards "swimming" of isolated flagella/basal body complexes induced by $>10^{-3}$ mM calcium. These discrepancies are puzzling and remain unexplained.

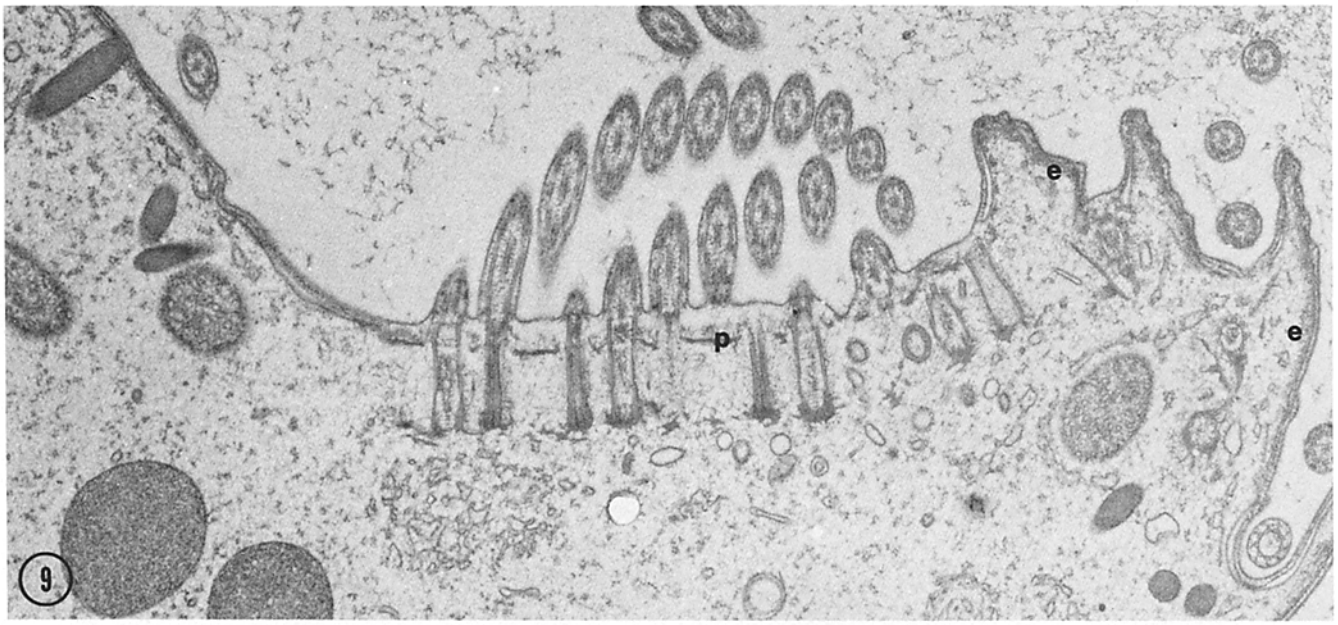
DISCUSSION

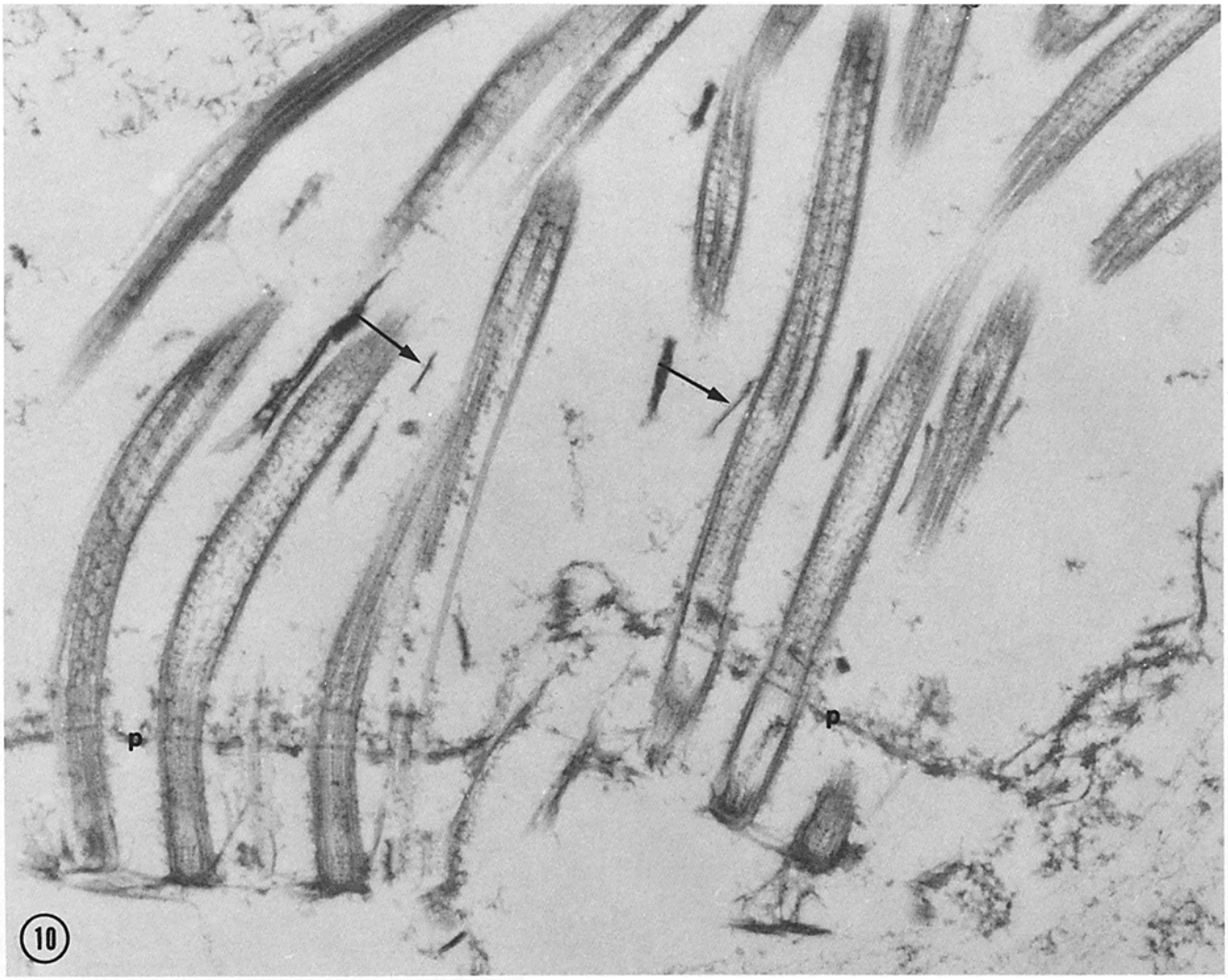
Research on the mechanism of ciliary motility has been greatly facilitated by ciliary preparations whose membranes have either been permeabilized to allow the passage of small molecules, or else removed altogether by detergent treatment. These preparations are of two general sorts. In some cases the cilia are first separated from their cells and then extracted, producing isolated demembranated "axonemes" that are induced to beat when provided with MgATP (1, 2, 17, 42). In other cases, the cilia are left in situ and the cells are permeabilized or demembranated to produce preparations whose motility requires the addition of exogenous MgATP.

In several respects, extracted cells are the more desirable type of preparation, for the cilia remain tethered at one end via their basal bodies, and their beat parameters can therefore be assumed to be more native than when axonemes are flopping about in solution. The most widely studied are extracted echi-


e
M
p
ER
ER
M
7

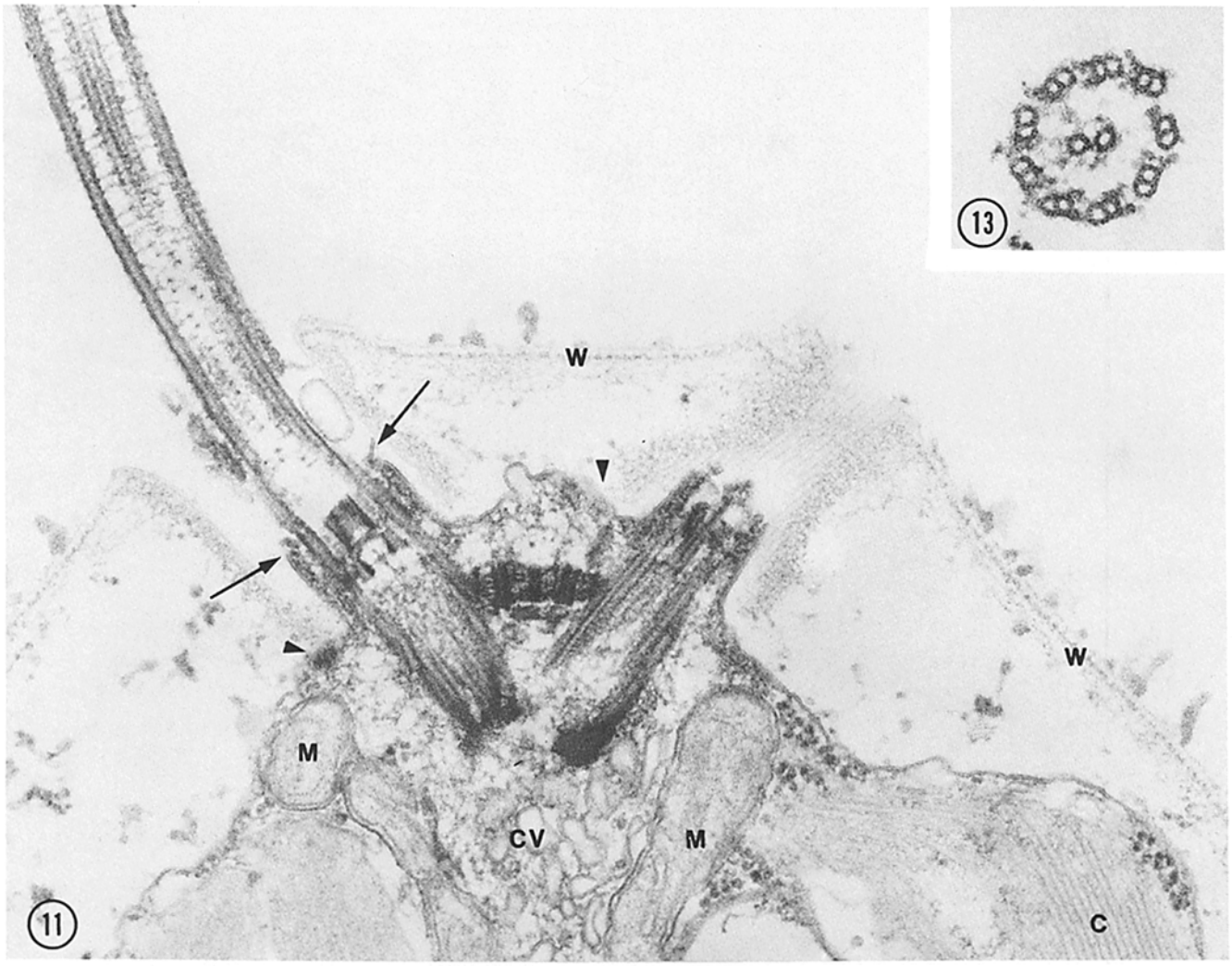

mt
e
e
p
8

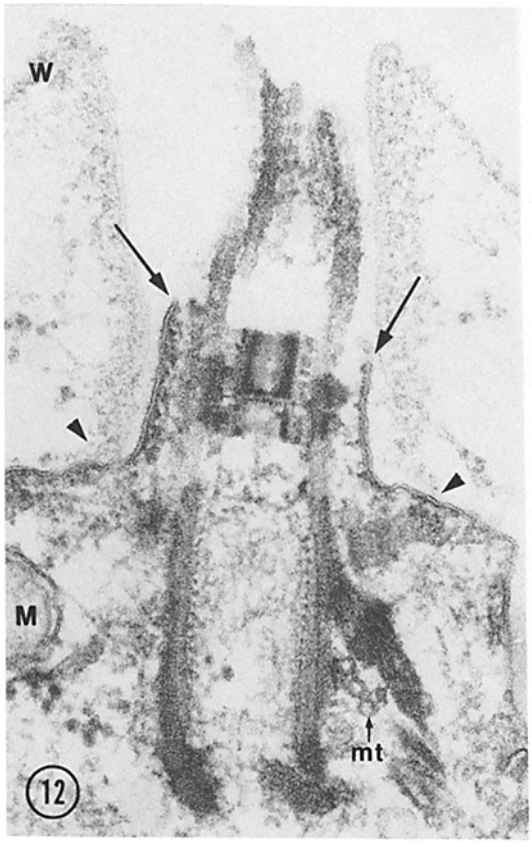


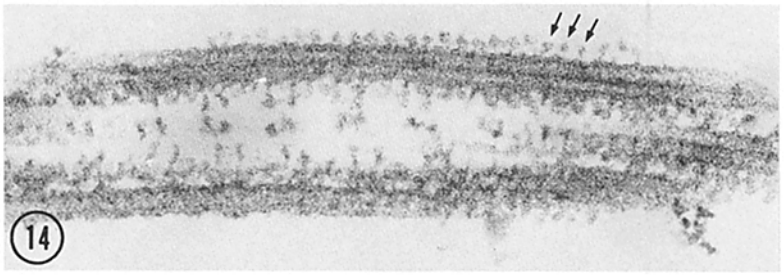


FIGURES 9 and 10 Oral region of a *Tetrahymena* cell (Fig. 9) and an extracted cell (Fig. 10) illustrating the extraction of cytoplasmic components and the absence of membranes around the axonemes. *e*, epiplasmic layer; *p*, pellicular plate; arrows, membrane fragments. Both figures, freeze-substitution. × 19,000 and × 37,000.

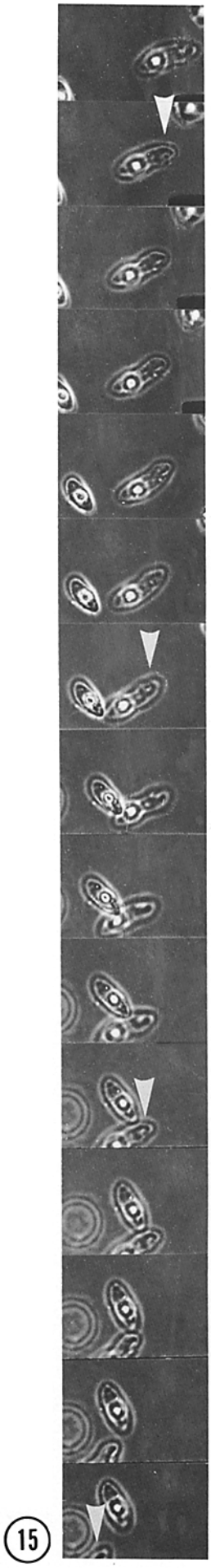
FIGURES 7 and 8 Somatic surface of a *Tetrahymena* cell (Fig. 7; freeze-substitution) and an extracted cell (Fig. 8), illustrating that such membranous components as mitochondria (*M*) and endoplasmic reticulum (*ER*) are completely extracted and that the cilium has been stripped of its membrane. *e*, epiplasmic layer; *p*, pellicular plate formed by epiplasmic layer at distal end of basal body. × 78,000 and × 64,000.







FIGURES 11–14 Extracted *Chlamydomonas* cells. Ciliary membranes are stripped from the axonemes down to the level of the H-shaped transition region (large arrows); below this region, the surface and intracellular membranes appear intact, and cytoplasmic ground substance is similar in density to unextracted cells. *W*, cell wall, perforated by "tunnels" that permit ciliary egress; arrowheads indicate sites where the wall appears to make contact with the cell membrane. *M*, mitochondrion; *cv*, membrane vesicles of contractile vacuole system; *C*, chloroplast thylakoids; *mt*, microtubules associated with the basal apparatus. Small arrows in Fig. 14 indicate a row of outer dynein arms. Fig. 11, × 58,000; Fig. 12, × 81,000; Figs. 13 and 14, × 105,000.



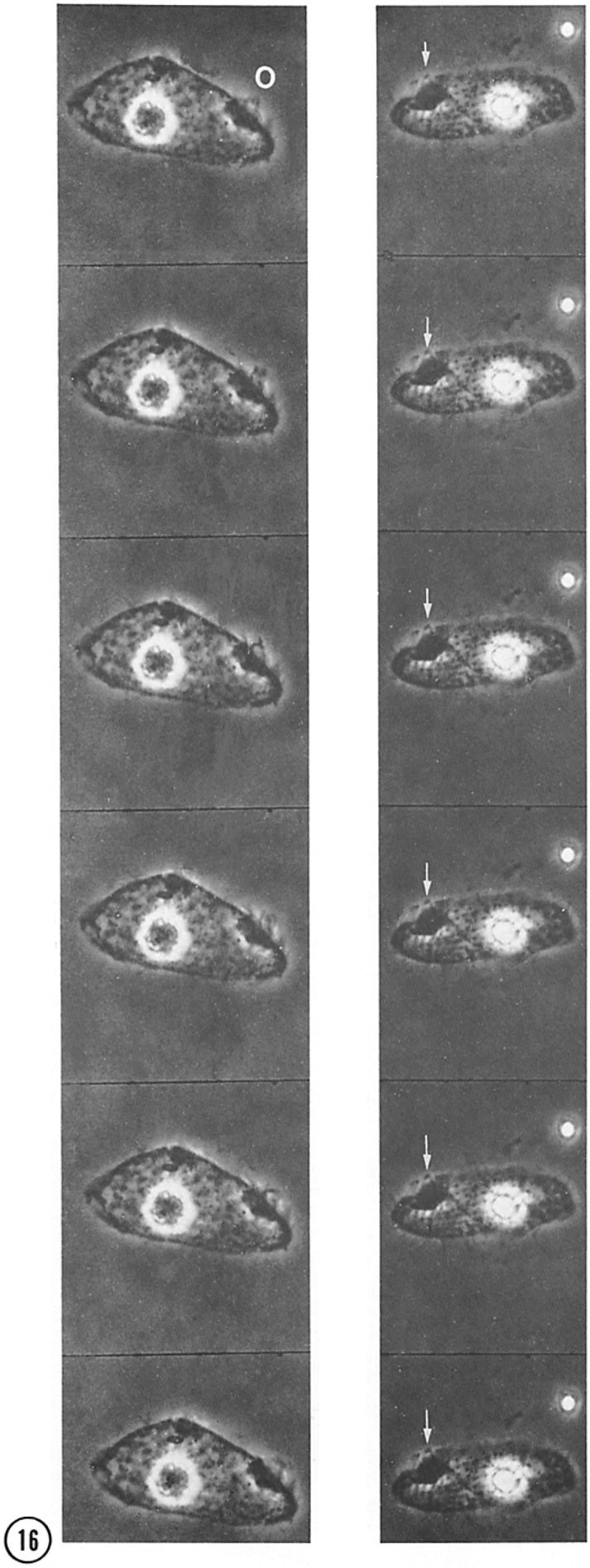


FIGURE 15 Motility of an extracted *Tetrahymena* cell. Montage shows every other movie frame in a 30-frame sequence filmed at 16 frames/s. Extracted cell appears in bottom frame (arrowhead) and swims across field to the right. Film strip is cropped to keep swimming cell centered; the immobilized cell included in the field serves as an absolute reference point.

FIGURE 16 Extracted *Tetrahymena* cells adherant to glass slide, incubated with 0.5 mM MgATP (left) or without ATP (right); six successive movie frames, filmed at 16 frames/s. The oral cilia (*o*) change their positions in each frame in the MgATP sample, and are motionless (arrows) in the ATP-free sample.

TABLE I

Swimming Rates of Extracted Cells

	[MgATP]	Swimming rate* μm/s	Swimming rate* % intact cells	Description of motility
(a) Tetrahymena (6×10^5 cells/ml)	Intact cells	247	100	—
	>7 mM	0	0	Trembling somatic, stationary oral
	5 mM	67	27	—
	2 mM	78	32	—
	1 mM	84	34	—
	0.5 mM	76	31	—
	0.2 mM	53	21	—
	0.1 mM	39	16	—
	0.05 mM	17	8	—
	0.02 mM	0	0	—
	0.01 mM	0	0	Feeble beat; stops within 1 min
(b) Chlamydomonas vegetative (2×10^6 cells/ml)	Intact cells	133	100	—
	10 mM	65	49	Most immotile or circling
	5mM	64	48	Many immotile or circling
	1 mM	58	44	Nearly all motile
	0.5 mM	37	28	—
	0.3 mM	31	23	Many circling
	0.15 mM	0	0	All circling
(c) Chlamydomonas gametes (2×10^6 cells/ml)	Intact cells	174	100	—
	10 mM	99	57	Most immotile or circling
	1 mM	90	52	Nearly all motile
	0.5 mM	70	40	—
	0.1 mM	28	16	Most circling
	0.01 mM	0	0	All circling
(d) Chlamydomonas mutant sup_{pf}-*1* (gametes)	Intact cells	74	100	—
	10 mM	0	0	All circling
	2 mM	21	28	—
	0.5 mM	0	0	All circling

* At least 10 intact or extracted cells were scored, as described in Results, for each [MgATP]. The five fastest swimming rates were then selected and averaged to yield the rates shown, the assumption being that maximum rates are least prone to error due to diagonal paths and/or mechanical obstacles.

noderm sperm, first produced by Gibbons and Gibbons (20), whose membranes are disrupted and whose native beat parameters can be almost fully reactivated in vitro. While invaluable for many purposes (reviewed in reference 53), these preparations have three drawbacks: they require a reliable source of sea urchins; they cannot be subjected to genetic dissection in the fashion of laboratory protozoa; and they are obviously not suited to the study of ciliary coordination since each bears but one flagellum.

Several investigators have produced extracted preparations of ciliated epithelia from oviduct (54, 55), trachea (56), and gill (57). Most of these are prepared in dilute detergent concentrations (0.04–0.1% Triton X-100), and, although electron micrographs are not included, they are reported to retain much of their membrane in permeabilized form. Dentler and LeCluyse (56) have recently made extracted trachea preparations in 0.5% Triton X-100, and include electron micrographs to document that most of the membranes have been disrupted. These preparations are very important for analyses of epithelial cilia, but they are relatively difficult to study by light microscopy compared with single cells.

Finally, Naitoh and his colleagues (51, 52) have reported making *Paramecium* models that undergo both forward and reverse swimming in the presence of ATP and appropriate cations. These models are prepared in 0.01% Triton X-100 (the critical micellar concentration for this detergent) and retain all their membranes, including ciliary membranes (52). The preparations are demonstrably permeabilized to ATP and cations (51, 52), but for many experimental purposes it is desirable to have unfettered access to the axonemal components.

The *Tetrahymena* and *Chlamydomonas* preparations described here have a number of experimental advantages. (*a*) They require only ~10 min to prepare, and very few cells are needed. (*b*) All the cells in a preparation are similar in structure and give uniform responses to experimental manipulation. (*c*) The axonemes are completely devoid of membranes, allowing reagents to be added and washed out without ambiguity. (*d*) The axonemal beat is optimal at ~1 mM MgATP, equivalent to estimated intracellular concentrations, but can be studied at micromolar MgATP and/or vanadate concentrations, allowing analysis of very slow axonemal waveforms. (*e*) They retain aspects of native motility that can be labeled "axonemal," and lose aspects that can be labeled "membrane-mediated." Specifically, the membrane-free *Tetrahymena* cells continue to rotate and change swimming direction in a fashion apparently identical to that of intact cells, while the ciliary-membrane-free *Chlamydomonas* cells retain the relative swimming rates of their intact progenitors but lose the "stop-turn" motility thought to be related to the phototaxis response.

The extracted *Tetrahymena* cells appear to represent the first

reported case where active ciliary swimming continues in the absence of cell membranes and cytoplasmic contents. Therefore, the coordinated axoneme beating of these preparations must be mediated entirely by the cortical components that remain. Conditions are described wherein the oral and somatic axonemes beat at different rates and where the axonemes continue to beat but cannot generate motility. Such conditions, and others that will doubtless be discovered by additional experimentation, should be excellent subjects for cinematographic analysis of the loss and recovery of ciliary coordination.

Two additional applications for these preparations can be noted. First, it may be possible to restore motility to extracted mutant cells by adding back purified wild-type components, much as motility can be restored to salt-extracted echinoderm sperm preparations by adding back dialyzed outer dynein arms (23). Second, the extracted cells should provide a convenient and sensitive bioassay for the effects on motility of monoclonal antibodies raised against axonemal components (58–60).

Dr. John E. Heuser prepared and examined quick-freeze, deep-etch replicas of the protozoan preparations; the resultant micrographs (to be included in a separate report) were very valuable in developing the final protocols described here. I thank Dr. Bessie Huang (Baylor College of Medicine) for generously providing the *sup$_{pf}$-1* strain, Dr. Jerry Bryant for filming the swimming cells, Michael Veith for the SEM micrographs, Robyn Carmody for carrying out the freeze substitution, and Amy Papian for typing the manuscript.

This work was supported by National Institutes of Health grant GM-29647 to Dr. Heuser and grants GM-30982 and GM-26150 to Dr. Goodenough.

Received for publication 22 November 1982, and in revised form 7 March 1983.

REFERENCES

1. Allen, C., and G. G. Borisy. 1974. Structural polarity and directional growth of microtubules of *Chlamydomonas* flagella. *J. Mol. Biol.* 90:381–402.
2. Bessen, M., R. B. Fay, and G. B. Witman. 1980. Calcium control of waveform in isolated flagellar axonemes of *Chlamydomonas*. *J. Cell Biol.* 86:446–455.
3. Blum, J. J., and A. Hayes. 1978. Effects of sulfhydryl reagents on the ATPase activity of solubilized 15S and 30S dyneins and on whole ciliary axonemes as a function of pH. *J. Supramolec. Struct.* 8:153–171.
4. Goodenough, U. W., and J. E. Heuser. 1982. The substructure of the outer dynein arm. *J. Cell Biol.* 95:798–815.
5. Hyams, J. S., and G. G. Borisy. 1978. Isolated flagellar apparatus of *Chlamydomonas*: characterization of forward swimming and alteration of waveform and reversal of motion by calcium ions *in vitro*. *J. Cell Sci.* 33:235–253.
6. Johnson, K. A., and M. E. Porter. 1982. Transient state kinetic analysis of the dynein ATPase. *Cell Motility.* 1(Suppl.):101–106.
7. Mitchell, D. R., and F. D. Warner. 1980. Interactions of dynein arms with B subfibers of *Tetrahymena* cilia: quantitation of the effects of magnesium and adenosine triphosphate. *J. Cell Biol.* 87:84–97.
8. Piperno, G., and D. J. L. Luck. 1979. Axonemal adenosine triphosphatases from flagella of *Chlamydomonas reinhardtii*: purification of two dyneins. *J. Biol. Chem.* 254:3084–3090.
9. Piperno, G., and D. J. L. Luck. 1981. Inner arm dyneins from flagella of *Chlamydomonas reinhardtii*. *Cell.* 27:331–340.
10. Ringo, D. L. 1967. Flagellar motion and fine structure of the flagellar apparatus in *Chlamydomonas*. *J. Cell Biol.* 33:543–571.
11. Takahashi, M., and Y. Tonomura. 1978. Binding of 30S dynein with the B-tubule of the outer doublet of axonemes from *Tetrahymena pyriformis* and adenosine triphosphate-induced dissociation of the complex. *J. Biochem.* 84:1339–1355.
12. Warner, F. D., and D. R. Mitchell. 1978. Structural conformation of ciliary dynein arms and the generation of sliding forces in *Tetrahymena* cilia. *J. Cell Biol.* 76:261–277.
13. Brokaw, C. J., D. J. L. Luck, and B. Huang. 1982. Analysis of the movement of *Chlamydomonas* flagella: the function of the radial spoke system is revealed by comparison of wild-type and mutant flagella. *J. Cell Biol.* 92:722–732.
14. Huang, B., G. Piperno, and D. J. L. Luck. 1979. Paralyzed flagella mutants of *Chlamydomonas reinhardtii* defective for axonemal doublet microtubule arms. *J. Biol. Chem.* 254:3091–3099.
15. Huang, B., Z. Ramanis, and D. J. L. Luck. 1982. Suppressor mutations in *Chlamydomonas* reveal a regulatory mechanism for flagellar function. *Cell.* 28:115–124.
16. Takahashi, M., H. Onimaru, and Y. Naitoh. 1980. A mutant of *Tetrahymena* with non-excitable membrane. *Proc. Jpn. Acad.* 56(Ser. B):585–590.
17. Witman, G. B., J. Plummer, and G. Sander. 1978. *Chlamydomonas* flagellar mutants lacking radial spokes and central tubules. *J. Cell Biol.* 76:729–747.
18. Bruns, P. J., and T. E. B. Brussard. 1981. Nullisomic *Tetrahymena*: eliminating germinal chromosomes. *Science (Wash. DC).* 213:549–551.
19. Ebersold, W. T., R. P. Levine, E. E. Levine, and M. A. Olmsted. 1962. Linkage maps in *Chlamydomonas reinhardi*. *Genetics.* 47:531–539.
20. Gibbons, B. H., and I. R. Gibbons. 1972. Flagellar movement and adenosine triphosphatase activity in sea urchin sperm extracted with Triton X-100. *J. Cell Biol.* 54:75–97.
21. Gibbons, B. H., and I. R. Gibbons. 1973. The effect of partial extraction of dynein arms on the movement of reactivated sea urchin sperm. *J. Cell Sci.* 13:337–357.
22. Gibbons, B. H., and I. R. Gibbons. 1974. Properties of flagellar "rigor waves" formed by abrupt removal of adenosine triphosphate from actively swimming sea urchin sperm. *J. Cell Biol.* 63:970–985.
23. Gibbons, B. H., and I. R. Gibbons. 1976. Functional recombination of dynein 1 with demembranated sea urchin sperm partially extracted with KCl. *Biochem. Biophys. Res. Commun.* 73:1–6.
24. Gibbons, B. H., and I. R. Gibbons. 1980. Calcium-induced quiescence in reactivated sea urchin sperm. *J. Cell Biol.* 84:13–27.
25. Gorovsky, M. A., M.-C. Yao, J. B. Keevert, and G. L. Pleger. 1975. Isolation of micro- and macronuclei of *Tetrahymena pyriformis*. *Methods Cell Biol.* 9:311–327.
26. Martin, N. C., and U. W. Goodenough. 1975. Gametic differentiation in *Chlamydomonas reinhardtii* I. Production of gametes and their fine structure. *J. Cell Biol.* 67:587–605.
27. Gorman, D. S., and R. P. Levine. 1965. Cytochrome *f* and plastocyanin: their sequence in the photosynthetic electron transport chain of *C. reinhardi*. *Proc. Natl. Acad. Sci. USA.* 54:1665–1669.
28. Mesland, D. A. M. 1976. Mating in *Chlamydomonas eugamatos*, a scanning electron microscopical study. *Arch. Microbiol.* 109:31–35.
29. Heuser, J. E. 1980. Three-dimensional visualization of coated vesicle formation in fibroblasts. *J. Cell Biol.* 84:560–583.
30. Tokuyasu, K., and O. H. Scherbaum. 1965. Ultrastructure of mucocysts and pellicle of *Tetrahymena pyriformis*. *J. Cell Biol.* 27:67–81.
31. Peck, R. K. 1977. The ultrastructure of the somatic cortex of *Pseudomicrothorax dubius*: structure and function of the epiplasm in ciliated protozoa. *J. Cell Sci.* 25:367–385.
32. Williams, N. E., P. E. Vaudaux, and L. Skriver. 1979. Cytoskeletal proteins of the cell surface in *Tetrahymena*. I. Identification and localization of major proteins. *Exp. Cell Res.* 123:311–320.
33. Allen, R. D. 1967. Fine structure, reconstruction, and possible functions of components of the cortex of *Tetrahymena pyriformis*. *J. Protozool.* 14:553–565.
34. Wolfe, J. 1970. Structural analysis of basal bodies of the isolated oral apparatus of *Tetrahymena pyriformis*. *J. Cell Sci.* 6:679–700.
35. Smith, H. E. 1982. Oral apparatus structures in the microsomal form of *Tetrahymena vorax*. *Trans. Am. Microsc. Soc.* 101:36–58.
36. Williams, N. E., and J. Bakowska. 1982. Scanning electron microscopy of cytoskeletal elements in the oral apparatus of *Tetrahymena*. *J. Protozool.* 29:382–389.
37. Rosenbaum, J. L., J. E. Moulder, and D. L. Ringo. 1969. Flagellar shortening and elongation in *Chlamydomonas*. *J. Cell Biol.* 41:600–619.
38. Blake, J. R., and M. A. Sleigh. 1974. Mechanics of ciliary locomotion. *Biol. Rev. Camb. Philos. Soc.* 49:85–125.
39. Schmidt, J. A., and R. Eckert. 1976. Calcium couples flagellar reversal to photostimulation in *Chlamydomonas reinhardtii*. *Nature (Lond.).* 262:713–715.
40. Warr, J. R., A. McVittie, J. T. Randall, and J. M. Hopkins. 1966. Genetic control of flagellar structure in *Chlamydomonas reinhardtii*. *Genet. Res.* 7:335–351.
41. Hoffman, J. L., and U. W. Goodenough. 1980. Experimental dissection of flagellar surface motility in *Chlamydomonas*. *J. Cell Biol.* 86:656–665.
42. Brokaw, C. J. 1961. Movement and nucleoside polyphosphatase activity of isolated flagella from *Polytoma uvella*. *Exp. Cell Res.* 22:151–162.
43. Brokaw, C. J., and I. R. Gibbons. 1973. Localized activation of bending in proximal, medial, and distal regions of sea-urchin sperm flagella. *J. Cell Sci.* 13:1–10.
44. Raff, E. C., and J. J. Blum. 1968. A possible role for adenylate kinase in cilia: concentration profiles in a geometrically constrained dual enzyme system. *J. Theor. Biol.* 18:53–71.
45. Lienhard, G. E., and I. I. Secemski. 1973. P^1, P^5-di-(adenosine-5′)pentaphosphate, a potent multisubstrate inhibitor of adenylate kinase. *J. Biol. Chem.* 248:1121–1123.
46. Gibbons, I. R., M. P. Cosson, J. A. Evans, B. H. Gibbons, B. Houck, K. H. Martinson, W. S. Sale, and W. Y. Tang. 1978. Potent inhibition of dynein adenosinetriphosphatase and of the motility of cilia and sperm flagella by vanadate. *Proc. Natl. Acad. Sci. USA.* 75:2220–2224.
47. Kobayashi, T., T. Martensen, J. Nath, and M. Flavin. 1978. Inhibition of dynein ATPase by vanadate, and its possible use as a probe for the role of dynein in cytoplasmic motility. *Biochem. Biophys. Res. Commun.* 81:1313–1318.
48. Sale, W. S., and I. R. Gibbons. 1979. Study of the mechanism of vanadate inhibition of the dynein cross-bridge cycle in sea urchin sperm flagella. *J. Cell Biol.* 82:291–298.
49. Bouchard, P., S. M. Penningroth, A. A. Cheung, C. Gagnon, and C. W. Bardin. 1981. Erythro-9-[3-(2-hydroxynonyl)]adenine is an inhibitor of sperm motility that blocks dynein ATPase and protein carboxymethylase activities. *Proc. Natl. Acad. Sci. USA.* 78:1033–1036.
50. Penningroth, S. M., A. Cheung, P. Bouchard, C. Gagnon, and C. W. Bardin. 1982. Dynein ATPase is inhibited selectively *in vitro* by erythro-9-[3-2-(hydroxynonyl)]adenine. *Biochem. Biophys. Res. Commun.* 104:234–240.
51. Naitoh, Y., and H. Kaneko. 1972. Triton-extracted models of *Paramecium*: modification of ciliary movement by calcium ions. *Science (Wash. DC).* 176:523–524.
52. Naitoh, Y., and H. Kaneko. 1973. Control of ciliary activities by adenosine triphosphate and divalent cations in triton-extracted models of *Paramecium caudatum*. *J. Exp. Biol.* 58:657–676.
53. Gibbons, I. R. 1981. Cilia and flagella of eukaryotes. *J. Cell Biol.* 91 (3, Pt. 2):107s–124s.
54. Eckert, R., and A. Murakami. 1972. Calcium dependence of ciliary activity in the oviduct of the salamander *Necturus*. *J. Physiol. (Lond.).* 226:699–711.
55. Torres, L. D., F. L. Renaud, and C. Portocarrero. 1977. Studies on reactivated cilia. II. Reactivation of ciliated cortices from the oviduct of *Anolis cristatellus*. *Exp. Cell Res.* 108:311–320.
56. Dentler, W. L., and E. L. LeCluyse. 1982. Microtubule capping structures at the tips of tracheal cilia: evidence for their firm attachment during ciliary bend formation and the restriction of microtubule sliding. *Cell Motility.* 2:549–572.
57. Walter, M. F., and P. Satir. 1978. Calcium control of ciliary arrest in mussel gill cells. *J. Cell Biol.* 79:110–120.
58. Asai, D. J., and C. J. Brokaw. 1980. Effects of antibodies against tubulin on the movement of reactivated sea urchin sperm flagella. *J. Cell Biol.* 87:114–123.
59. Mitchell, D. R., and J. L. Rosenbaum. 1982. Monoclonal antibodies against *Chlamydomonas* dyneins. *J. Cell Biol.* 95(2, Pt. 2):323*a*. (Abstr.)
60. Piperno, G. 1982. Monoclonal antibodies to dynein arm components. *J. Cell Biol.* 95(2, Pt. 2):315*a*. (Abstr.)