

THE MECHANISM OF THE WATER
EXPULSION VESICLE OF THE CILIATE
TETRAHYMENA PYRIFORMIS

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ABSTRACT

Analysis of high-speed (150 frames/sec) cinematographs of the filling and expulsion of the water expulsion vesicle of *Tetrahymena pyriformis* shows that the vesicle fills as water is pumped into it by contractions of at least four ampullary sacs which are continuous with the endoplasmic reticulum. When filled, the vesicle is pressed against its two excretory pores by cyclotic movements of the cytoplasm. This pressure closes the apertures of the ampullae, preventing backflow from the vesicle into them, and also spreads the pellicle of and at the pore, thereby stretching and rupturing the pore-sealing membrane. The vesicle is then invaginated by the cytoplasmic pressure, driving fluid out of the pore. The pore-sealing membrane then reforms, apparently by constriction, and the vesicle is again filled. Electron micrographs show that crisscrossed pore-microtubules extend from the pore to the openings of the ampullae, anchoring the vesicle in place. Each pore is surrounded by a stack of at least 11 ring-microtubules, to which the anchoring pore-microtubules are attached. The pore-microtubules appear to exert tension which assists in spreading the pore, aiding cyclotic pressures in rupturing the pore-sealing membrane. A possible mechanism for the cyclotic pressure and ampullary contraction is proposed.

The water expulsion vesicle (WEV or "contractile vacuole") of the freshwater and marine protozoa has been intermittently studied since the discovery of the WEV in *Paramecium* by Joblot (1718) and the observation of the repeated appearance of the ampullary canals of *Paramecium* by Spallanzani (1776). Investigators have repeatedly studied its development, structure, filling, and expulsion (see reviews by von Gelei, 1935; Kitching, 1952, 1954, 1956, 1967; Lloyd, 1928; and Weatherby, 1941), but until recently little critical attention has been given to the *mechanism* by which the vesicle is emptied or filled. Kitching (1967) suggested that high-speed cinephotographic techniques of the living state might provide valuable information.

Wigg et al. (1967) and Organ et al. (1968 a,

1969 a) used regular and high-speed cinematography, 200–300 frames/sec, respectively, and frame-by-frame analyses of their movies to show that the WEVs of *Amoeba proteus* and *Paramecium multimicronucleatum*, respectively, do not contract, but rather collapse as postulated by Metcalf (1910) and von Gelei (1939), and that the assumed contraction of the WEV of the protozoa, which had been generally accepted since the studies of Claparède (1854) and Lachmann (1859), does not occur. Organ et al. (1968 a) showed that during its expulsion the WEV of *P. multimicronucleatum* collapses in about 100 msec, and high-speed photography of 200–300 frames/sec is needed to capture all of the sequential events occurring in the evacuation of the WEV. Similarly, filming rates

of at least 150 frames/sec are required for adequate analysis of the filling and expulsion of the WEV of *Tetrahymena* (Organ et al., 1968 a, 1969 b).

The electron micrographs by Elliott and Bak (1964) show that the WEV of *Tetrahymena* is invaginated into itself during expulsion. Slow framing rate motion pictures (4–8 frames/sec) also strongly support invagination and collapse of the vesicle as it empties (Cameron and Burton, 1969). Our preliminary report based on motion pictures taken at 200–300 frames/sec also supports that assumption (Organ et al., 1969 b). In this paper we present the photographic evidence for that contention, including new electron micrographs as well as the printed motion picture sequences.

MATERIALS AND METHODS

The cultures of *Tetrahymena pyriformis* studied were grown axenically on proteose-peptone medium (Elliott and Hayes, 1955). Vigorous and numerous organisms were available at any time during the studies. The cinephotographed organisms were immobilized on a 1% agar film over glass as described by Organ et al. (1968 b).

The evacuatory movements of the WEV were photographed repeatedly in the same and in different organisms to depict the nature and mechanism of the movements and their sequences involved in emptying and filling the vesicle. A Lo-Cam (Redlake Laboratories, Inc., Santa Clara, Calif.) 16-mm motion picture camera (capable of 10–500 frames/sec) was used. The *Tetrahymena* were photographed through a Zeiss-Nomarski variable phase-contrast and interference microscope (Carl Zeiss, Inc., New York) at a total magnification of $\times 750$. The movements were photographed both in phase contrast and in interference contrast using as the light source a 60-w tungsten lamp. Motion pictures were taken at a filming rate of 150 frames/sec, and the films were professionally developed and printed.

Other *Tetrahymena* were fixed in 2.5% glutaraldehyde buffered with Sörensen's phosphate (pH 7.4) for 1 hr, then postfixed with 1% OsO₄-phosphate buffer for another hour. The ciliates were dehydrated in graded ethanol solutions, centrifuged into a hard pellet, then embedded in an epoxy-resin (Epon 812) (Luft, 1961). The blocks were polymerized at 60°C for 3 days and gold sections were cut on a Porter-Blum ultramicrotome. The sections were further stained with lead citrate and uranyl acetate, then were viewed and photographed through an RCA EMU-3G electron microscope. Final magnifications were determined from the enlarged photographs.

OBSERVATIONS

Our observations confirm the indication by the electron microscopy of Elliott and Bak (1964), and

the contention based on 4–8 frames/sec cinematography by Cameron and Burton (1969), that the water expulsion vesicle (WEV) of *T. pyriformis* is collapsed, invaginating into itself during its evacuation, and does not of itself contract.

The evacuation of the vesicle is shown cinemographically in Fig. 1. The initiation of expulsion appears to be due to intravesicular pressure. This pressure develops in the WEV as it fills and is pressed upon by the moving cyclotic protoplasm. The pressure also causes a change in form of the vesicle toward the oblate, thus exerting tension on the pore-microtubules (PMT, Figs. 2 and 3) which anchor the vesicle to the pellicle and pull at the walls of the excretory canals. The high-speed cinematographs and electron micrographs strongly suggest that this pull causes an invagination of the pellicle, spreading the pore to which the vesicle is anchored. The pressure and tension cause rupture of the pore-sealing membrane (PSM, Fig. 4) which, until then, seals the excretory canal. The ruptured membrane appears to be driven up against the wall of the excretory canal, as a flap, while water rushes out, much as occurs in *Paramecium* (Organ et al., 1968 a). Once the membrane covering either one of the two excretory pores of the WEV is ruptured, the vesicle is invaginated by movements of underlying adjacent cytoplasm and is emptied by collapsing under pressure from that cytoplasm.

The electron micrographs (Figs. 2 and 3) show that the PMT appear to be anchored firmly to the pore, as Elliott and Bak (1964) indicate, and that their other ends are seen to be attached above the openings to the ampullae, holding them in place, too (Fig. 2). The probable functions of the pore-microtubules are anchorage of the vesicle to the pores and an aid to the rupture of the PSM.

There is a stack of at least 11 ring-microtubules (RMT, Fig. 4) around the pellicular canal of each excretory pore and to these the pore-microtubules each attach (Figs. 2–4). The PMT extend from the attachment sites at the RMT over that hemisphere of the WEV nearest the pore. Their other ends attach above the ampullary entrances, from which they also appear to radiate toward the pore. The diameters of both the RMT and PMT are approximately 196 Å.

Whether or not both pore-sealing membranes may be ruptured in the same evacuation of the WEV was not determined, but repeated observations of the rupturing of the pore-sealing membranes of *Tetrahymena* suggest that usually only

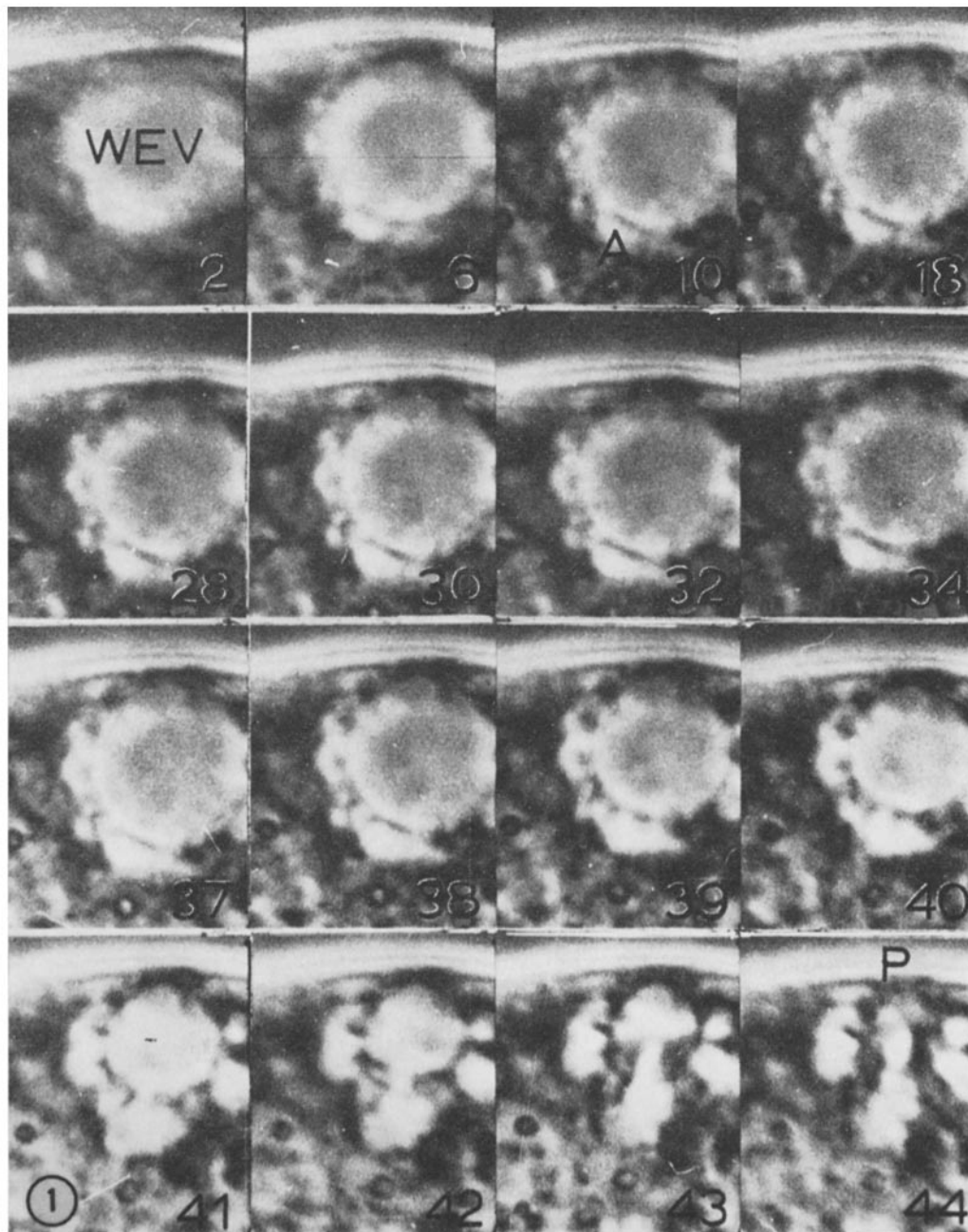


FIGURE 1 Expulsion of the water expulsion vesicle (*WEV*) of *Tetrahymena pyriformis*, a series of 16 pictures from a motion picture film photographed at 150 frames/sec. Pellicular indentation shown in picture 28. Filling of ampullae (*A*), pictures 6-44. Expulsion begins at picture 37, continues to picture 44, ampullae continuing to fill, meantime. *WEV* becomes slightly oblate at internal hemisphere and conically compressed toward the pore (picture 32) before expulsion. Oblate internal hemisphere of *WEV* invaginates into conically compressed pore-directed one; pictures 40-44. At least 4 ampullae are evident, filling throughout the sequence. $\times 5000$.

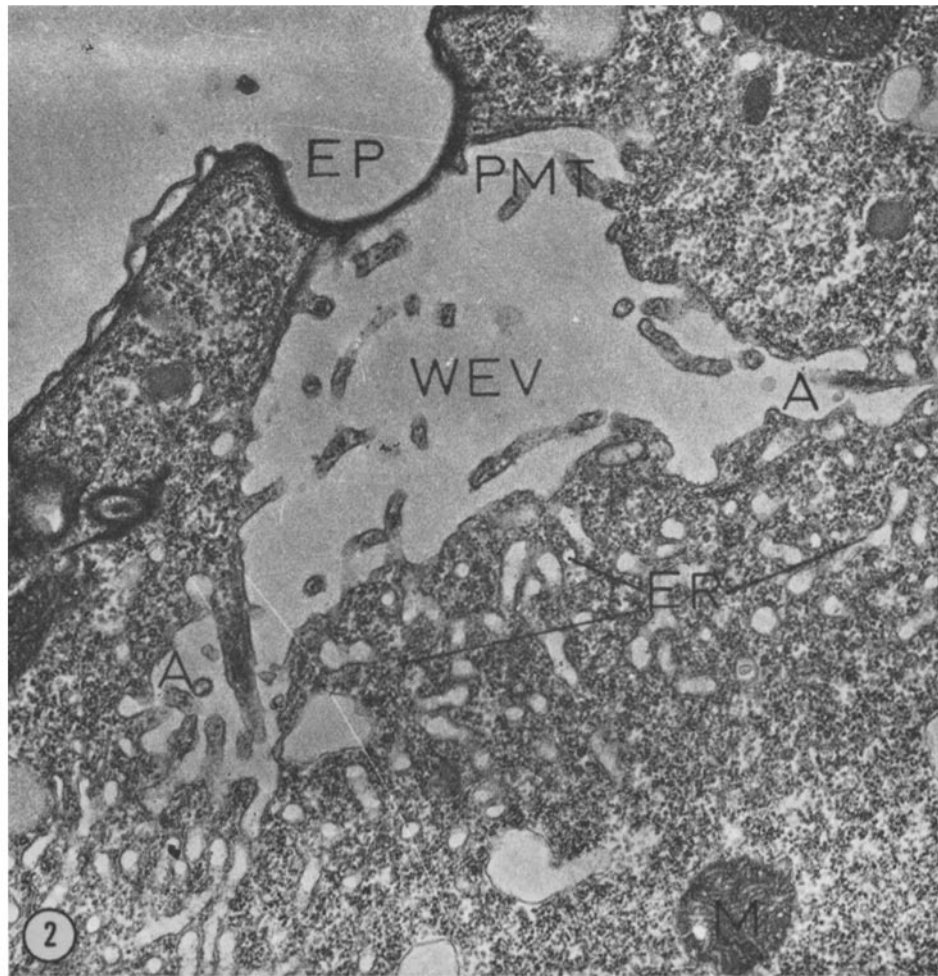


FIGURE 2 Vertical section through one pore of the WEV, filling via the ampullae. $\times 27,053$. EP, excretory pore, PMT, pore-microtubule; A, ampulla; WEV, water expulsion vesicle; M, mitochondrion; ER, endoplasmic reticulum.

one of the membranes is ruptured at a single expulsion (see also Organ et al., 1969 *b*). The same conclusion was reached by Cameron and Burton (1969) after observing motion pictures they took at 4–8 frames/sec. Presumably that pore ruptures which has the weakest, thinnest membrane. After the membrane has ruptured and the vesicle has invaginated and emptied, the vesicular membrane around the opened pore probably constricts and the pore of the WEV is resealed.

When the WEV begins to expel, its fluid content is forced out through the excretory pore (EP, Figs. 2–4). None is forced back into the ampullae

(see pictures 18–39, Fig. 1). Even before the WEV begins to expel its fluid, the ampullae already have begun to fill, possibly from the nephridial tubules of the ER. Fig. 2 shows smooth ER in the vicinity of two ampullae. Their injection pores (Fig. 2, proximal end of ampulla) are then *closed* by WEV pressures. Only after the expulsion is completed can the WEV be refilled via the ampullae.

The mechanism of filling appears to be a contraction of the ampullae (Fig. 6). Measurements taken from a frame-by-frame analysis (frames 48–61, (Fig. 6) suggest that the ampullae contract

since there is a visible decrease in the diameter of the ampullae and no invagination of the ampullae as water is pumped into the WEV. With the high-speed camera, the optical illusion that the vesicle is formed by the coalescence of several smaller vesicles (see Bishop et al., 1950; King, 1933, 1935) is shown to be wrong. The high-speed films show that each ampulla separately empties its contents into the WEV. The latter sags and fills below each ampullary entrance so that several small fluid-filled pouches develop. These meld together and disappear as the WEV further fills and distends.

The ampullae appear to be formed as a coalescence of a number of tubules of the endoplasmic reticulum, rather than having a discrete vesicular form (Fig. 2). When distended, however, they are ovate to nearly spherical, clearly acting as ampullae in collecting fluid and pumping it into the WEV proper.

In summary, the mechanism of elimination of fluid by the nephridial apparatus of *T. pyriformis* consists of three sequential visible events. These are: (a) the filling of the vesicle from the ampullae, which appear to be connected to the endoplasmic reticulum and collect fluid from it; (b) the indentation of the pellicle and rupture of the pore-sealing membrane of one of the excretory pores; and finally, (c) the emptying of the WEV by the movements of underlying cytoplasm, which cause it to collapse, evacuate, and invaginate. The WEV is then ready to be refilled by and from the ampullae (Figs. 1 and 6).

DISCUSSION

The filling of the WEV of *Tetrahymena* resembles that of *Paramecium* (Organ et al., 1969 a, 1969 b) but the ampullae which collect fluid from the nephridial tubules of *Tetrahymena* are not as easily detectable nor of so distinct a shape as the ampullae of *Paramecium* (see Schneider, 1960). The thin layer of cytoplasm around each ampulla of *Tetra-*

hymena is assumed to be a precariously balanced gel-sol organization of a β -actomyosinoid protein complex with adenosine triphosphatase (ATPase) activity as is the contractile cytoplasmic gel of some other protozoa (e.g., *Physarum polycephalum*, see Nakajima, 1964, and others; *Amoeba proteus*, see Bovee, 1952, and Simard-Duquesne and Couillard, 1962). The presence of such a gel network triggered into organization and contraction by cations and under tension could result in the contraction of the ampullae (Organ et al., 1969 a). Thus, no macroscopically (or microscopically) visible, discrete, and permanent set of fibrils (or microtubules) is required about the ampullae to elicit contraction; and no such microscopically discrete fibrils have been found in the electron micrographs of Elliott and Bak (1964), Cameron and Burton (1969), nor in this study (see Figs. 2 and 3). A contractile coacervate gel network should suffice in the absence of fibrils.

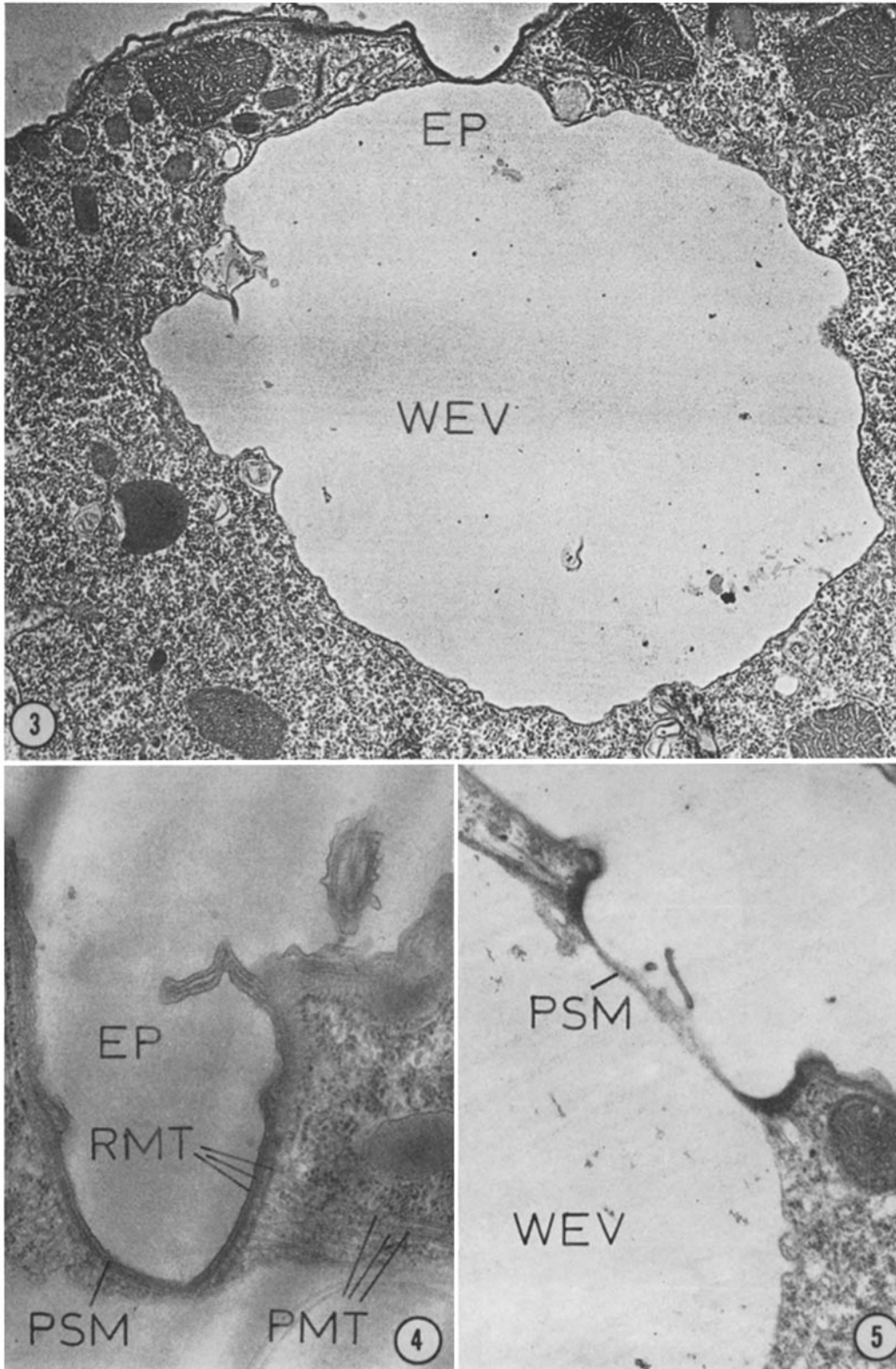
To some other investigators (e.g., King, 1935) the filling of the WEV of ciliates has appeared to be the result of coalescence of several smaller accessory vesicles into one large one. Cameron and Burton (1969) also mention this seeming phenomenon, based on their slow-speed (4-8 frames/sec) cinefilms. However, frame-by-frame analyses of high-speed films showing the filling of the vesicle (Fig. 6) reveal that this visible illusion is due to the irregularly sequential filling of regions of the intact WEV by fluid pumped into it from the ampullae. Nassanov (1924) and von Gelei (1939) also give this interpretation from direct observation of *Paramecium*.

The ampullae in *Tetrahymena* appear to be connected to the endoplasmic reticulum via the nephridial tubules (see Fig. 2). These connections also appear to remain open throughout the expulsion as the proximal end of the ampullae (closest to WEV) are closed off by pressure of the vesicular walls.

FIGURE 3 Nearly vertical section through a distended water-expulsion vesicle (WEV) and one pore. No ampullae evident, the section being between them. EP, excretory pore. $\times 17,770$.

FIGURE 4 Vertical section showing overlap of pore-microtubules anchored to ring-microtubules (11 evident on right side). $\times 74,500$. RMT, ring-microtubules; PSM, pore-sealing membrane. EP, excretory pore; PMT, pore-microtubule.

FIGURE 5 Vertical section through a completely filled water expulsion vesicle (WEV), showing the stretching of the pore just before rupture of the pore-sealing membrane (PSM). $\times 43,284$.



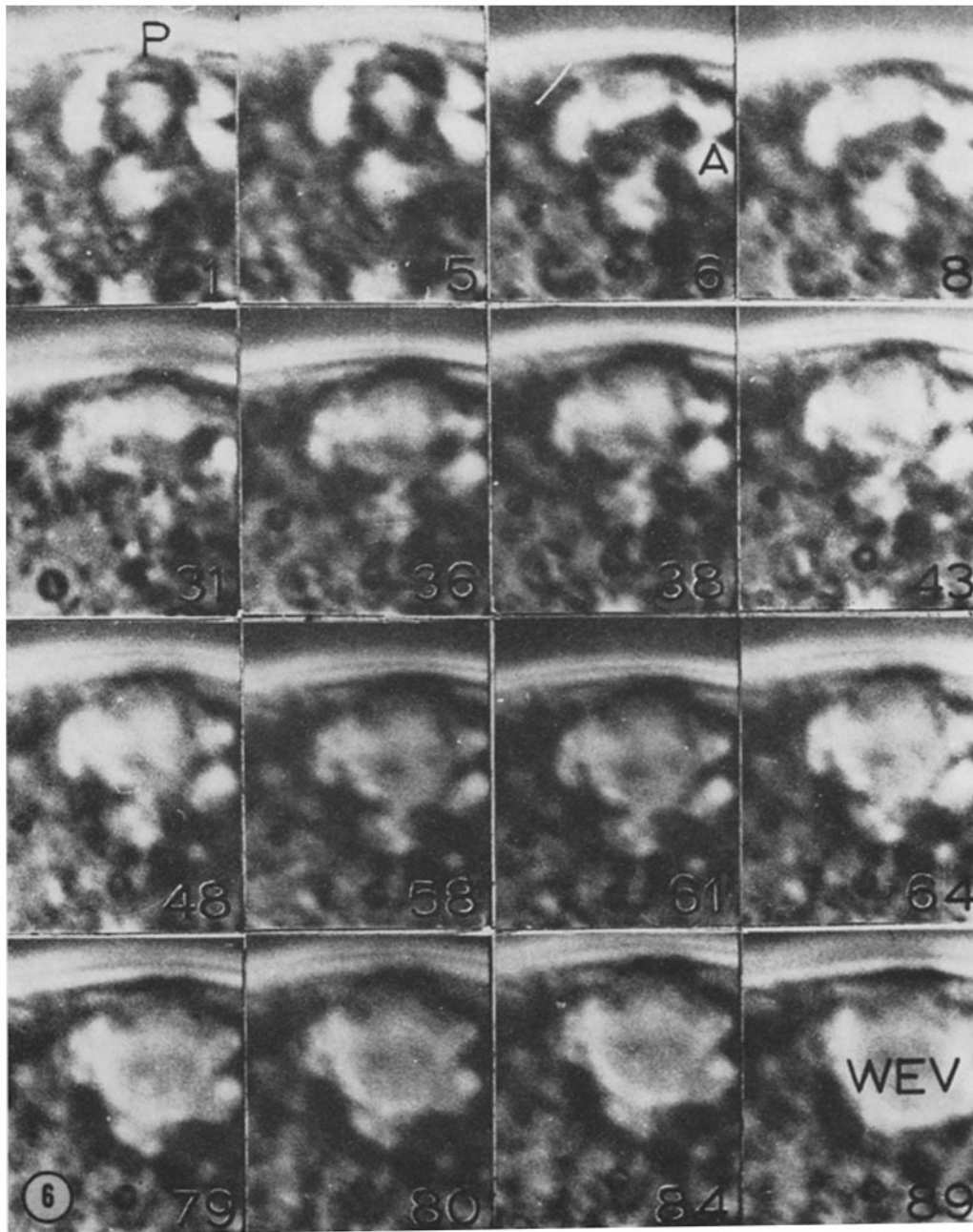


FIGURE 6 Series of selected pictures from a 90-frame sequence of a motion picture taken at 150 frames/sec, showing filling of the water expulsion vesicle (WEV) with fluid pumped into it from the ampullae (A). P, location of pore. $\times 5000$.

There is another theory of how enlargement of the WEV occurs in the protozoa. If the vesicular fluid were hypertonic to that of the cytoplasm (Schmidt-Nielsen and Schrauger, 1963), then movement from the cytoplasm into the vesicle could account for its enlargement. Hopkins (1946) assumed that such a hypertonicity may be created by secretion of metabolic excretory products into the WEV. Kitching (1956) pointed out that the amount of excretory products assumed to be produced is probably excessive and that such a "reverse osmosis" is not likely to occur.

In *Tetrahymena*, as the WEV fills and distends, pressure created within the vesicle causes the pore-microtubules (PMT, Figs. 2-4) anchoring the vesicle to the pellicle to pull at the walls of the excretory pore. As these taut microtubules exert tension, they probably pull the walls of the excretory pore radially, causing its dilation and apparent shortening of its length (the invagination mentioned by Organ et al., 1969 *a*, and the indentation noted by Dunham and Stoner, 1969). The dilation noted here of the excretory pore before evacuation of the WEV (see Fig. 5) has already been depicted and noted by Elliott and Bak (1964) and Cameron and Burton (1969) for *Tetrahymena*. A similar spreading of the pore also occurs in *Paramecium* and *Colpoda* (Organ et al., 1968 *a, c*). The pore of the WEV is opened by the hydrostatic pressure of the vesicular fluid which breaks the tautly stretched pore-sealing membrane.

At the onset of expulsion, the pore-sealing membrane is broken on one side by the hydrostatic pressure, and it is pushed outward as a flap. This has also been demonstrated in *Paramecium* (Organ et al., 1968 *b*) and in *Colpoda* (Organ et al., 1968 *a*). Also, the pore-microtubules present around the pore in *Tetrahymena* (Elliott and Bak, 1964; Cameron and Burton, 1969; and Figs. 2-5 in this paper) and *Paramecium* (Schneider, 1960) may help maintain the location and geometry of the pore and pore canal.

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