THE MECHANISM OF THE NEPHRIDIAL APPARATUS
OF PARAMECIUM MULTIMICRONUCLEATUM

I. Expulsion of Water from the Vesicle

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ABSTRACT

Recent analysis of the mechanism of the nephridial apparatus of Paramecium multimicronucleatum by high-speed cinematography (300 fps at X 250) confirms the observations by electron microscopy (Schneider, 1960) that once the pore is opened, the vesicle is invaginated by adjacent cytoplasm and is emptied by collapsing under pressure from that cytoplasm, aided perhaps by pressure of the fibrils which anchor the ampullae to the excretory canal. There is no indication of active contraction of the vesicle or its membrane. There is no permanent pore to the vesicle. The vesicle is closed by a sealing of the ruptured membrane where it is in contact with the pellicular excretory canal. At onset of expulsion of vesicular fluid the membrane across the basal opening of the excretory canal is ripped along one semicircular portion of the excretory pore and is driven up against the opposite wall as a flap while the water rushes out. A constriction of the vesicular and cell membranes at the base of the excretory canal reseals the opening.

The discovery of the water-expulsion vesicle ("contractile vacuole") of Paramecium is usually attributed to Joblot in 1718, and the observation of the repeated appearance of the ampullary canals of Paramecium attributed to Spallanzani in 1776. These vesicles are and have been repeatedly the basis for study in protozoa dealing with their development and structure and their functions in filling and expulsion (see references: von Gelei, 1935; Kitching 1952, 1954, 1956, 1967; Lloyd, 1928, Weatherby, 1941). Unfortunately, little critical attention has been given to the mechanism by which the vesicle is emptied.

While turgor is still considered by some, e.g. Czarska, 1964, to be involved in the expulsion, Kitching (1956, 1967) dismisses body turgor as being essential to expulsion of the vesicle (described as a "systole"), and he assigns it only a possible contributory function. While no direct measurements are known for the pressure required to empty the vesicle, Kitching calculates a necessary hydrostatic pressure of 0.33 cm of water greater within the vesicle than outside it to empty it (Kitching, 1952). Such a pressure he assumes may possibly develop as a result of tension within or pressure upon the vesicular membrane. He further assumes that, in ciliates at least, the pressure is the result of tension in the vesicular wall which causes the wall to contract during evacuation. He admits, however, that there is no knowledge of whether an oriented protein layer around the vesicle is present or is involved in the process (Kitching, 1956). This assumed contractility of the water-excretory vesicle of protozoa has been generally accepted; ever since the studies of Claparède (1854) and Lachmann (1859) the
FIGURE 1 Photomicrographs from a motion picture (300 fps) showing the rupture and evacuation of the water-expulsion vesicle of *Paramecium multimicronucleatum* (side view). In frame 1, the pore-sealing membrane across the basal end of the excretory canal (internal pore) is still intact. Frames 2-6 show the ripping of that membrane and the subsequent opening of the internal pore. Frames 9-35 show the invagination of the bottom of the vesicle and the emptying of it. In frame 36, the cytoplasm beneath the internal pore of the excretory canal has constricted and formed a new pore-sealing membrane resealing the old vesicle. $\times$ 1000.
Figure 2. Photomicrographs from a motion picture (300 fps) showing the same sequence of the opening of the pore-sealing membrane as Fig. 1, but from a surface view. The pore, at first dark in the photograph, appears white as it opens. X 3200.

Figure 3. Sketches based on selected tracings of photomicrographs in Fig. 1 showing the invagination of the vesicle. The dotted line in the lower portion of frames 12–36 denotes the position of the vesicular membrane before evacuation started.

Structure has been and still is usually termed the contractile vacuole, not only in ciliates but in other taxonomic groups. However, there is some contradictory evidence. For example, Taylor (1923) wrote of the water-excretory vesicle of Euplotes patella as collapsing, and older drawings by Fauré-Fremiet (1905) of Campanella umbellaria show a collapse of the vesicu-
FIGURE 4

The frames from Figs. 1 and 2, that are equivalent, are shown together to clarify the sequence of the opening of the pore-sealing membrane from both views. In the lower row the membrane appears dark, and the open pore appears light. In frame 7 the flap is assumed to be the dark area at the lower left side of the pore. This dark area does not appear in frames 6 or 8 or in other frames of the same sequence. X 1600.

Figure 4

FIGURE 5

Sketches based on Fig. 4.

lalar membrane against itself in ciliates. Furthermore, older observations by Penard (1902) and Metcalf (1910), as well as recent cinephotomicrographs of the water expulsion vesicle of Amoeba protes by Wigg et al. (1967), show that the water expulsion vesicle of Amoeba protes does not contract. These studies and others to be mentioned later have led us to reinvestigate the evacuation of the water-excretory vesicle in Paramecium multimicronucleatum by cinephotomicrography.

MATERIALS AND METHODS

Paramecium multimicronucleatum (Gittleson strain), grown on lettuce medium and buffered to a pH of 7.2 with CaCO₃, was studied. The lettuce medium was made with Chalkley's solution and was inoculated with Bacillus subtilis. Vigorous and numerous paramecia were thus available at any time during these studies. The observed paramecia were immobilized on No. 1 thickness microscope slides coated with an even, thin layer of 1% nonnutrient agar.

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FIGURE 6

Top (surface) view of the evacuation of the water expulsion vesicle of Paramecium multimicronucleatum. Shows that the vesicle retains its circular shape in the horizontal plane as the bottom of vesicle becomes invaginated, and the vesicle decreases in volume. The frames shown are numbers 8, 28, 38, 48, and 58 respectively of the same sequence as the lower row of Fig. 4. X 2400.

The paramecia adhered by the tips of their cilia and were thus just immobilized, but otherwise normal.

The evacuatory movements of the water-excretory vesicle were repeatedly photographed in the same and in different, individual paramecia for determination of the nature and mechanism of the movements, and their sequences, involved in emptying the vesicle. Fastair and Hycam 16-mm motion picture cameras with variable framing rates (up to 500 fps) were used with a Carl Zeiss variable phase contrast microscope at X 250 magnification. The light source was a carbon arc lamp. Motion pictures were taken at 300 fps. The film was professionally developed and printed.

Analysis of the evacuatory movements was made by repeated viewing of the films at varied framing rates (2-24 fps) with a flickerless L-W Photo-optical analyzer and by frame by frame analysis of the single pictures printed on Kodak Polycontrast paper (Eastman Kodak Co., Rochester, N.Y.).

OBSERVATIONS

As has been suggested by the electron micrography done by Schneider (1960) on paramecia and by Mosevitch (1965) on Ichthyophthirius multifilis, the water-excretory vesicle of Paramecium multimicronucleatum is collapsed during its evacuation and does not of itself contract.

The evacuation of the vesicle is shown photographically in Figs. 1 and 2, and semidiagrammatically in Fig. 3. The photographs are a sequence of frames from a motion picture which clearly shows the evacuatory process which takes only milliseconds for completion. Our best estimates, based on several sequences, indicates an average time of 108.89 msec, or about 3/10 sec.

The vesicle becomes invaginated by adjacent cytoplasm (Fig. 1, frames 9-35; Fig. 3, frames 12-36), and it appears to collapse under the pressure created by the adjacent cytoplasm and perhaps some pressure created by the fibrils which anchor the ampullae to the excretory canal.

At the onset of expulsion of vesicular fluid, the pore-sealing membrane across the basal opening of the excretory canal is ripped along one semicircular border, and is driven up against the opposite wall as a flap while the water rushes out (Fig. 1, frames 1-5; Fig. 2, frames 1-8; and Fig. 3, in which the upper row represents stages seen in Fig. 1 and the lower row those from Fig. 2).

Fig. 4 shows frames from Figs. 1 and 2 which are equivalent in order to clarify the sequence of the opening of the pore-sealing membrane from both the side and surface views. Fig. 5 is a series of diagrams based on the photographs of Fig. 4.

The membrane opens approximately 15 msec before the expulsion of the vesicular fluid by means of cytoplasmal pressure which can be detected in the photographs. After the pore opens, the top (outermost) portion of the vesicle becomes rigid as the pressure of the cytoplasm causes the vesicle to invaginate upon itself. Initially, as the vesicle commences its expulsion, there is a small pressure exerted by the fibrils which are attached to the ampullae and wind over the top hemisphere of the vesicle. As the expulsion continues, the pressure exerted by those fibrils increases as the fibrils are stretched while the ampullae fill with more water.

Contrary to the description by King (1935) in his observations of the expulsion of fluid from the vesicle in P. multimicronucleatum, the bottom of the old contractile vacuole does not form the new membrane over the pore as it is pushed up toward...
of the movements of the water-expulsion vesicle of *Electron* micrographs and reconstruction diagrams DISCUSSION membrane. There is no indication that the vesicle whether the vesicle stayed circular throughout the focus; so it was impossible to determine accurately above-mentioned frames, the vesicle went out of section in diameter of the vesicle (Fig. 6). After this has occurred, the ampullae refill the vesicle. This constriction has completely blocked the pore in frames 37–42 and has partially blocked the pore in frames 32–36 and to a lesser extent in earlier frames.

As the vesicle is emptied it remains circular, as seen in surface view, at least until its volume is reduced by more than 50%. This is shown in Fig. 6, a sequence of surface views, approximately equivalent to Fig. 3, frames 1–16. In this sequence, volume reduction is brought about mostly by invagination of the innermost wall of the vesicle (shown in Fig. 3) and only secondarily by a reduction in volume of the vesicle (Fig. 6). After the above-mentioned frames, the vesicle went out of focus; so it was impossible to determine accurately whether the vesicle stayed circular throughout the evacuation. However, the impression still remains that the vesicle does remain circular throughout as seen in frames 25–36 of Fig. 1. There is no indication of active contraction of the vesicle or its membrane.

**DISCUSSION**

Electron micrographs and reconstruction diagrams of the movements of the water-expulsion vesicle of *Amoeba proteus* (Penard, 1902; Metcalf, 1910; Wigg et al., 1967), *Campaillonia umbellaria* and *Ophrydium versatili* (Fauré-Fremiet, 1903; Fauré-Fremiet and Rouiller, 1959), *Tokophrya infusionum* (Rudzinska, 1958), *Paramecium aurelia* and *Paramecium caudatum* (Schneider, 1960), *Tetrahymena pyriformis* (Elliott and Bak, 1964), and *Ichthyophthirius multifiliis* (Mosewich, 1965) explicitly show an invagination of the vesicle during expulsion. This invagination is probably due to movements of the endoplasmic gel pushing on the vesicular membrane. All of the above authors except Elliott and Bak (*Tetrahymena*) refer to the vesicle as collapsing rather than contracting. Metcalf (1910) referring to the water-expulsion vesicle of *Amoeba proteus*, and Taylor (1923) describing the water-expulsion vesicle of *Euploites*, also indicate a collapse of the vesicle. All of the above investigators, with the exception of Elliott and Bak, interpret the evacuation of the vesicle as due to force developed in the movements of the endoplasmic gel, and not to contraction of the vesicle itself. Elliott and Bak (1964) invoke the action of unidentified fibrils and suggest that these fibrils have only a "facilitating" function while implying that expulsion requires another principal mechanism.

If discrete fibrils are involved in a contraction of the vesicle, as Elliott and Bak (1964) suggest, then, in electron micrographs they should be observable surrounding the vesicle. According to the electron micrographs of Schneider (1960) many (20–30) long fibrils form a spiral band from each ampulla and its injection tubule. These fibrils wind clockwise over that hemisphere of the vacuolar cavity which is adjacent to the pellicular pore; then they wind internally also around the short cylindrical tube of the pellicular pore and fasten to the pore and inner surface of the pellicle where the latter invaginates to form the pore. There are also some fibrils adjacent to the membrane of the ampullary segment of the tubule, but there are no discernible fibrils in or adjacent to the internally hemispheric segment, when filled, of the vesicular space.

It is evident that the ampullary fibrils associated with the expulsion vesicle of *Paramecium multimicronucleatum* cannot be wholly responsible for a "contraction" of the vesicle. It is now obvious, from our observations and from evidence in the literature, that the water-excretory vesicle of *Paramecium multimicronucleatum* is not a truly contractile organelle. Since the water-excretory vesicle of *P. multimicronucleatum* does not contract, it cannot properly be called a contractile vacuole. As already has been proposed (Wigg et al., 1967), we also propose that a better term for it might be the water-expulsion vesicle, since any method of expul-
sion is in accord with that term. We also believe that the term “systole,” which implies contraction, might better be replaced by the term *evacuation*, and “diastole” by *enlargement*; thus we would avoid the implication of a repetitive cycle of contraction and relaxation which does not exist.

The pore-sealing membrane, which breaks on one side and thereby becomes a flap (Fig. 3, frames 1–6), is shown clearly in the electron micrograph of Schneider (1960, Fig. 8 a). Schneider’s figure shows the membrane closed; the area in the center of the pore canal is very thin, while that near the pore wall is much thicker, especially on one side, and irregular in outline. Our assumption is that the thin area breaks under pressure and that the thicker portion is pushed outward as the flap. Therefore, evidence of existence of the flap does not consist entirely of the present photographs, which, alone, may not be entirely convincing. However, the flap can be seen better in the original motion picture when projected than in single frames, and also it can be deduced from Schneider’s electron micrographs.

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