Brief Notes

The Fine Structure of the Contractile Vacuole in Ameba.*

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The contractile vacuole is an organelle found in most freshwater Protozoa. In ameba, as studied with the light microscope, it is usually described as spherical, with a rather thick limiting membrane and surrounded by a layer of mitochondria (Fig. 1). The contractile vacuole undergoes a continuous series of cyclic changes. It enlarges gradually (diastole), collecting water until it reaches a critical size, and then suddenly contracts (systole), expelling its contents to the outside of the cell, whereupon a new diastole begins. Although the precise function of the contractile vacuole is not known, it is believed to be primarily an osmoregulatory organelle (7).

The limiting membrane of the contractile vacuole of *Amoeba proteus* was estimated by Mast (11) to be as much as 0.5 micron thick—much thicker than the membrane of any other organelle. The cytoplasm surrounding the vacuole wall in *Pelomyxa carolinensis* has been described as being in a gel state (17), having a higher optical density (1), and extending for a depth of about 2 microns, with a layer of mitochondria embedded in this gel. With the higher resolving power offered by the electron microscope, it seemed likely that the so called thick limiting membrane and its surrounding gelated cytoplasm would show evidence of specialized structures not seen with the light microscope.

Three species of fresh water amebas were studied, *Amoeba proteus*, *Pelomyza carolinensis* (*Chaos chaos*), and *Hartmannella rysodes*. *P. carolinensis* has numerous contractile vacuoles, whereas *A. proteus* and *H. rysodes* have only one.

Methods

Amoeba proteus and *Pelomyxa carolinensis* (*Chaos chaos*) were obtained from Carolina Biological Supply Company; *Hartmannella rysodes* was generously supplied by Dr. S. H. Hutner (Haskins Laboratories, New York). The handling procedures have been previously described (14). The amebas were washed and packed into a pellet by centrifugation. They were then fixed for a period of 10 to 20 minutes in 1 per cent OsO_4 in veronal acetate buffer, pH 8.6, with 0.01 per cent calcium chloride added. Fixation was carried out at 4°C. In some instances, single cells of *P. carolinensis* were fixed and carried through the entire procedure individually.

After dehydration and embedding in *n*-butyl methacrylate, thin sections were cut and subsequently studied in the electron microscope (RCA EMU-3C).

Amebas were also prepared by freeze-drying, embedding in diethylene glycol distearate, and sectioning according to the procedure described by Brandt (3). These sections were examined by phase contrast microscopy.

OBSERVATIONS AND DISCUSSION

When observed with the electron microscope, the so called thick vacuolar membrane is seen to be, in contradistinction to a previous report (5), a single thin membrane surrounded by a densely packed layer of small vesicles. This layer of vesicles ranges from 0.5 to 2 μ in thickness. In turn, the vesicular layer is surrounded by mitochondria (Fig. 2). At a higher magnification (Fig. 3), the vesicles are seen to range in size from 20 to 200 millimicrons. The thickness of both the contractile vacuole membrane and the membranes of the vesicles is about 70 A. The vesicles, which are found in close proximity to the contractile vacuole, are probably similar to those recently described by Rudzinska (15) in the sessile protozoan, *Tokophrya*. We found no evidence of fibrillar material in association with the contractile vacuole as has been described by previous investigators (2, 9, 10). Although the electron micrographs (Figs. 2 to 4) are all of *P. carolinensis*, the contractile vacuole and surrounding structures appear essentially the same in all three species of amebas studied.

In Figs. 3 and 4, vesicles can be seen in different

* This investigation was supported, in part, by a research grant (No. B-1202 C) from the National Institute of Neurological Diseases and Blindness of National Institutes of Health, United States Public Health Service.

‡ *Received for publication, March 4, 1958.*

relationships to the contractile vacuole. Some vesicles are close to, some are in contact, and some are in colescence with the membrane of the contractile vacuole. In the last instance, there is continuity of membrane and content between the vesicles and the vacuole.

Light microscope observations on living amebas indicate that the contractile vacuole is formed by the fusion of smaller vacuoles. Electron microscope observations suggest that these smaller vacuoles in turn (see Fig. 4) are formed by the coalescence of vesicles.

When the growing contractile vacuole is small, the mitochondria are randomly distributed in proximity to the vacuole. As the vacuole enlarges, the mitochondria are displaced, until they form a layer surrounding the mature vacuole (17). Systole occurs abruptly when the vacuole reaches the critical size for the particular species (13).

On the basis of these observations with the light and electron microscopes, it is postulated that the various appearances of the vesicles are stages in a continual process related to the cyclic changes of the contractile vacuole. These findings suggest that the vesicles contribute both their contents and their membranes to the growing contractile vacuole during diastole.

In amebas, the contractile vacuole has been implicated as an organelle which segregates hypotonic solutions from the cytoplasm and discharges them outside the cell (6, 7). The method whereby the contractile vacuole collects water from the surrounding cytoplasm has not been elucidated. Kitching (8) proposed three theories: (a) that the vacuolar water separates from the cytoplasm because of the osmotic pressure of dissolved excretory matter, (b) that the vacuolar water is separated from the cytoplasm as a result of phase changes occurring in the cytoplasm, or (c) that water is secreted by an active transport mechanism. He further suggests that vacuolar fluid may be altered by active transport of solute across the contractile vacuole membrane.

Electron microscope observations of the contractile vacuole wall, as described in this report, suggest that it is formed by the coalescence of small vesicles. The segregation of solvent is probably across the membranes surrounding these small vesicles, since membrane area and volume considerations might make this a more efficient site than the contractile vacuole wall.

From a morphological point of view, the sequence of events whereby the contents of these small vesicles eventually communicate with the external environment, is similar to, but opposite in direction to pinocytosis. In pinocytosis the cell membrane invaginates, and frees submicroscopic vesicles into the cytoplasm (12). Recent studies on pinocytosis in the ameba (3, 16) demonstrated that it is not only a mechanism of incorporating fluids, but that a segregation of solutes and solvents occurs in the process. In these studies it was shown that ionized solutes are adsorbed on the cell surface in higher concentration than they are in the medium. Therefore, the cell surface that is going to form the wall of the pinocytosis vacuole concentrates solutes on it prior to pinocytosis.

The process by which the contractile vacuole segregates solute and solvent can be modeled on the mechanism of pinocytosis. In contractile vacuole function, small vesicles permeable to electrolytes and water separate from alveolar structures of the cytoplasm. These vesicles may be derived from pinocytosis vacuoles or components of the endoplasmic reticulum. The vesicles then become less permeable. A subsequent adsorption of the solute on the inner surface of the membrane would leave a hypotonic solution in the vesicle. The fusion of these small vesicles forms the large contractile vacuole. An example of a change in membrane permeability has been described in amebas in the case of the membrane of the pinocytosis vacuole. Studies by Chapman-Andresen and Holter (4) demonstrated that the plasmalemma is impermeable to glucose. However, when the plasmalemma is internalized forming the wall of the pinocytosis vacuole, it becomes permeable to glucose almost immediately.

The process underlying the function of the contractile vacuole outlined above for the segregation of water and solute permits the transfer of large amounts of water across the cell membrane. The mitochondria surrounding the contractile vacuole may supply the energy required for the water segregation.

SUMMARY

The fine structure of the contractile vacuole of three species of amebas (*Amoeba proteus*, *Pelomyxa carolinensis* (*Chaos chaos*), and *Hartmannella rysodes*) is found to be a single thin membrane surrounded by a dense layer (0.5 to 2 μ in thickness) of small vesicles 20 to 200 mμ in diameter. Peripheral to the layer of small vesicles is a layer

of mitochondria. The thickness of both the contractile vacuole membrane and the membranes of the vesicles is about 70 A.

These small vesicles appear to be emptying into the contractile vacuole by fusion of their membranes. A mechanism for the segregation of water from the cytoplasm into the contractile vacuole has been suggested, using pinocytosis as a model. It is suggested that the small vesicles surrounding the contractile vacuole are the loci of solute-solvent separation.

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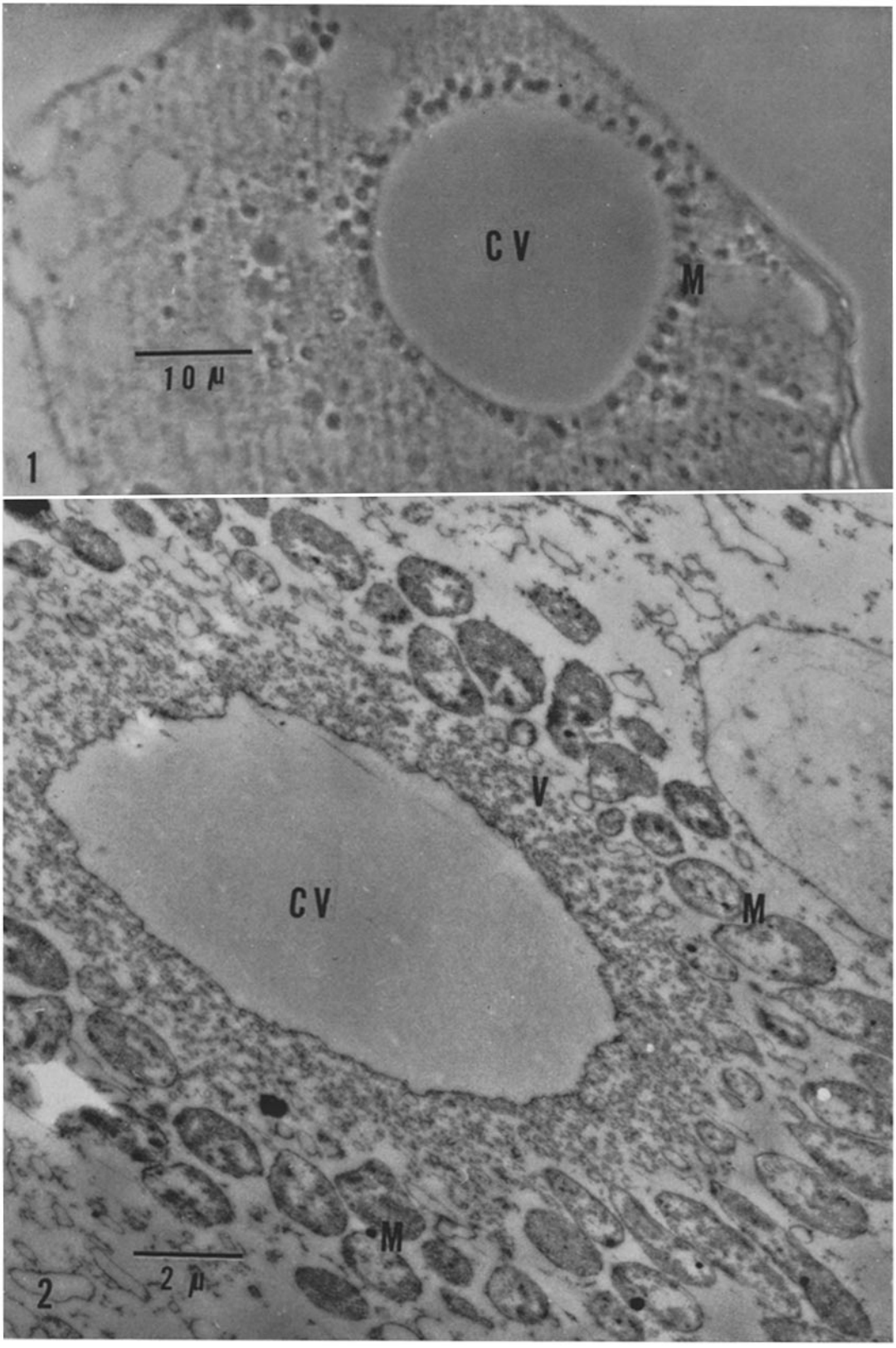
Explanation of Plates

Plate 236

Fig. 1. Phase contrast photomicrograph of a 1 micron section, cut through a contractile vacuole of *Pelomyxa carolinensis*. The contractile vacuole (*CV*) is surrounded with mitochondria (*M*). × 1,900.

Fig. 2. Electron micrograph of a section through a contractile vacuole of *P. carolinensis*. The contractile vacuole (*CV*) is surrounded by tiny vesicles (*V*) which, in turn, are surrounded by mitochondria (*M*). × 9,000.

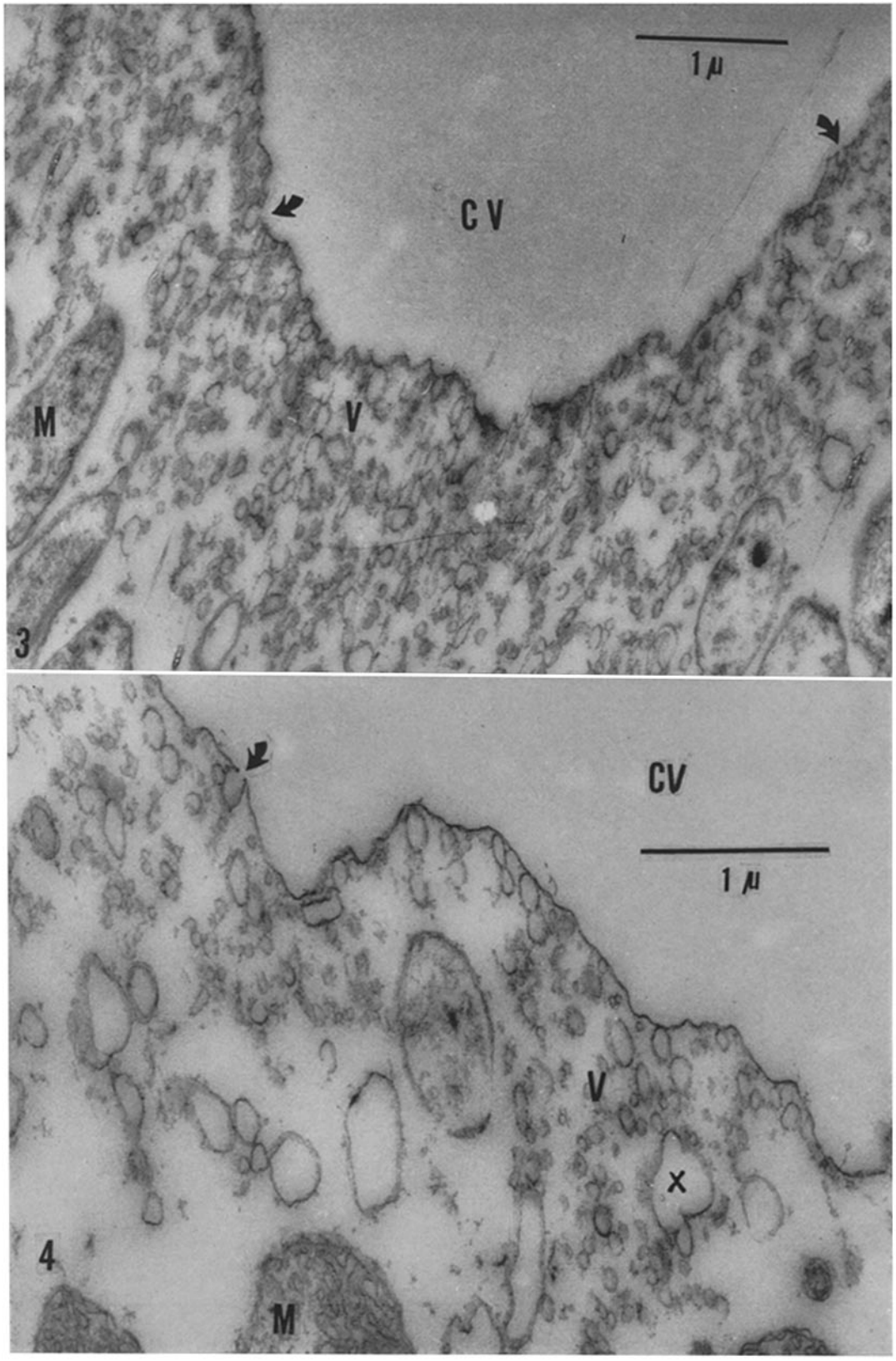


(Pappas and Brandt: Contractile vacuole in ameba)

PLATE 237

FIG. 3. Higher magnification of part of the same section shown in Fig. 2. The contractile vacuole (*CV*) is surrounded by many vesicles (*V*). The membranes of vesicles (at arrows) are continuous with the membrane of the contractile vacuole. × 23,700.

FIG. 4. Electron micrograph of section through part of a contractile vacuole. The membrane of the vesicle (at arrow) and the membrane of the contractile vacuole are continuous. It is suggested that the vesicles (*V*) contribute both their contents and their membranes to the contractile vacuole during diastole. An alveolar structure (*X*) showing a close relation with the surrounding vesicles can be seen. × 30,000.



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