An Electron Microscope Study of the Contractile Vacuole in *Tokophrya infusionum**

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ABSTRACT

Contractile vacuoles are organelles that collect fluid from the cytoplasm and expel it to the outside. After each discharge (systole), they appear again and expand (diastole). They are widely distributed among Protozoa, and have been found also in some fresh water algae, sponges, and recently in some blood cells of the frog, guinea pig, and man.

In spite of the extensive work on the contractile vacuole, very little is known concerning its mode of operation. An electron microscope study of a suctorian *Tokophrya infusionum* provided an opportunity to study thin sections of contractile vacuoles, and in these some structures were found which could be part of a mechanism for the systolic and diastolic motions the organelle displays.

In *Tokophrya*, as in Suctoria and Ciliata in general, the contractile vacuole has a permanent canal connecting it with the outside. The canal appears to have a very elaborate structure and is composed of three parts: (1) a pore; (2) a channel; and (3) a narrow tubule located in a papilla protruding into the cavity of the contractile vacuole. Whereas the pore and channel have fixed dimensions and are permanently widely open, the tubule has a changeable diameter. At diastole it is so narrow (about 25 to 30 mμ in diameter) that it could be regarded as closed, while at systole it is widely open.

It is assumed that the change in diameter is due to the contraction of numerous fine fibrils (about 180 A thick) which are radially disposed around the canal in form of a truncated cone, with its tip at the channel, and its base at the vacuolar membrane. It seems most probable that the broadening of the tubule results in discharge of the content of the contractile vacuole.

In the vicinity of the very thin limiting vacuolar membrane, small vesicles and canaliculi of the endoplasmic reticulum, very small dense particles, and mitochondria may be found. In addition, rows of closely packed vesicles are present in this region, and in other parts of the cytoplasm. It is suggested that they might represent dictyosome-like bodies, responsible for withdrawing fluids from the cytoplasm and then conveying them to the contractile vacuole, contributing to its expansion at diastole.

Contractile vacuoles are organelles that collect fluid from the cytoplasm and expel it to the outside. They have been extensively studied in Protozoa, in which they occur in almost all free-living, fresh water forms, and in some parasitic and sea water species. The presence of such vacuoles is not, however, limited to Protozoa; they have been described in fresh water algae (17), sponges (8), and in certain blood cells of the frog, guinea pig, and man (2). Their occurrence in such diverse forms as protozoa and vertebrates suggests that similar vacuoles are probably present in many other groups, but so far have not been noticed.

To identify the contractile vacuole with cer-

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tainty, it is necessary to see it in operation in the living cell. The rhythmic contraction of this organelle is the feature that distinguishes it from the other vacuoles of the cytoplasm. As is well known, each contraction results in the discharge of the fluid contained within the vacuole and is followed by the reappearance of the structure and its expansion until it is ready for a repeated contraction. The growing phase (diastole) may last for several seconds to several minutes, while the emptying (systole) of the vacuole is usually very rapid and lasts only 2 to 3 seconds.

In spite of the extensive work on the contractile vacuole, very little is known about the structural mechanisms involved in its systolic and diastolic movements. Its function is still debated (11, 14, 42). There is good evidence that it regulates osmotic pressure inside the cell, and there are also suggestions that it may function as an excretory organelle. For detailed information on the problem, Kitching's latest excellent review (14) should be consulted.

It was assumed that an electron microscope study of the contractile vacuole would provide new morphological information that might lead to a better understanding of the functioning of the organelle. Accordingly, the contractile vacuole of the suctorian *Tokophrya infusionum* was investigated. As a result, a number of structural details, which may play an important role in systole and diastole, were revealed for the first time and are described in this paper.

Materials and Methods

Several strains of *Tokophrya infusionum*, a fresh water protozoan from the class Suctoria, which have been in culture since 1948, were used for these studies (32). Stock cultures were maintained in a yeast medium 0.05 per cent (16) in bacteria-free conditions, and subcultures were made once a week. The food organism, axenic *Tetrahymena pyriformis* (3), was grown in a proteose peptone medium and introduced into the cultures of *Tokophrya* three times weekly.

Tokophrya is a small and for most of its life, sessile organism about 35 μ in diameter attached firmly to the substrate by a disc (35). For electron microscopy, sessile organisms were used. They were gently scraped off from the walls of the culture tubes and concentrated on the bottom by mild centrifugation. After the removal of the supernatant, the organisms were fixed in 1 per cent OsO_4 at pH 8.5 (20, 36) for about 30 minutes. During dehydration and impregnation with *n*-butyl methacrylate, mild centrifugation was applied before each change of the medium (36). The organisms were finally embedded by polymerizing the methacrylate at 45° for 24 hours, dichlorobenzoyl peroxide being used as a catalyst.

In order to avoid repeated centrifugation, an alternative procedure was sometimes used. After fixation, the organisms were centrifuged for about 10 minutes at 700 *g* until they formed a well packed pellet. The pellet was then cut into small pieces and handled as pieces of tissue until embedded (37).

Thin sections (about 50 mμ thick) were cut with a Porter-Bloom microtome (27), mounted on carbon coated grids (41), and examined with an RCA, type EMU 2B electron microscope equipped with an intermediate lens and a compensated objective. Micrographs were taken at original magnifications of 5,000 to 12,500 and enlarged further photographically.

OBSERVATIONS

Light Microscopy

The adult *Tokophrya infusionum* has usually one and sometimes two contractile vacuoles which, being deeply situated in the cytoplasm, are often seen in contact with the centrally located macronucleus. During their period of maximum expansion the organelles have an average size of about 10 μ and a shape that is more or less irregular. Just before contraction, however, the vacuole changes suddenly to a perfect sphere, as noted also in some other ciliates by MacLennan (18) and Kitching (14). In *Tokophrya* the expulsion of the vacuole content is not complete, since frequently a small residual vacuole $\sim$0.3 μ in diameter may be seen at the end of the systole. This small vacuole then expands by fusion with other vacuoles, which appear in the area immediately after systole, and by a continuing process of coalescence all the vacuoles of the region finally merge into a single large unit. The latter increases its size steadily until the next discharge. The systolic frequency, although relatively constant under stable conditions, varies with the age of the individual (38), the state of feeding and reproduction (as found also in other Suctoria (7, 13, 26, 31, 32)), and the temperature and tonicity of the medium (reported also in other Suctoria (10, 12, 13, 38)). Observations under phase contrast optics or with dark field illumination show that there is a distinct canal through which the contractile vacuole discharges its content into the surrounding medium. In *Tokophrya*, as in other Suctoria and Ciliata, the canal is a permanent structure persisting through the entire cycle of vacuolar activity.

Electron Microscopy

Size and Shape of Contractile Vacuoles.—In sectioned material the size and shape of the vacuolar profiles vary greatly depending on the functional phase of the organelle, as well as on the angle and plane of section. The dimensions range from 2 to 6 μ for the shorter, and from 2.5 to 10 μ for the longer axis. The shape was found to be either regularly oval (Fig. 1), irregular (Fig. 19), or more or less deeply folded (Fig. 2). Oval profiles correspond to the spherical forms of contractile vacuoles seen in light microscopy, the change in shape being due to deformation during sectioning. On the basis of light microscope observations, it is assumed that the oval forms represent vacuoles just before expulsion; the irregular profiles correspond to various stages of diastole, whereas the profiles which show many folds in their limiting membrane belong to vacuoles collapsing at systole. In some instances, several profiles of small vacuoles (0.1 μ to 2 μ in diameter) were found assembled close to one another (Fig. 6). It is assumed that their presence coincides with an early diastolic stage, during which a new contractile vacuole is formed by the fusion of small contributory vacuoles. Similar small vacuoles were also found in the immediate vicinity of, and occasionally in continuity with, contractile vacuoles at later diastolic stages (Figs. 5 and 7).

Limiting Membrane.—Around the contractile vacuole there is a very thin limiting membrane, measuring about 100 A. In satisfactorily preserved cells this membrane appears as a continuous dense line (Figs. 1 and 2). Membrane discontinuities or "pores" of different sizes found in some preparations (Fig. 7) are most probably artifacts, and seem to represent breaks produced during fixation or embedding. Occasionally the limiting membrane shows evaginations (ranging from 15 mμ to 230 mμ in width) extending into the cytoplasm (Figs. 4, 5, and 7), and some of these appear to be connected with small vesicles. Fig. 5 shows a broad evagination continuous with a larger vesicle.

Canal Leading from the Contractile Vacuole to the Outside.—As pointed out above, the contractile vacuole is connected with the outer surface of the cell through a canal which is a permanent structure of great complexity. Three successive parts can be distinguished along this canal: (1) a pore, which is the outermost end and appears as an invagination of the cell surface (pellicle and cell membrane), and represents the broadest part of the entire structure (~500 mμ diameter); (2) a channel about 350 mμ in diameter and about 800 mμ in length; and (3) a very fine tubule about 25 to 30 mμ in diameter and 350 mμ in length, leading to the contractile vacuole proper. Figs. 8 to 11 show the three successive segments in serial sections in the same organism, whereas Figs. 12 to 19 show the canal sectioned at various levels and angles in other *Tokophrya.* The electron micrographs in Figs. 8 to 12, and in Fig. 15 demonstrate clearly the relatively abrupt change in diameter between the channel and the tubular segment. The funnel-like connection formed at this level, the tubule and the vacuole proper are all limited by a thin (~100 A) continuous membrane which contrasts strikingly with the thicker wall (~300 A) of the channel. The difference in thickness is apparently due to the fact that the channel, like the pore, is limited by both the cell membrane and the pellicle.

The tubule is usually located in a cytoplasmic papilla that protrudes into the cavity of the contractile vacuole (Figs. 8 to 13, 15, 16, 18, and 19). It is suggested that this papilla corresponds to the "plug" reported by several investigators (9, 11) in this part of the organelle.

Most of the canals encountered in electron micrographs have the general morphology described. Since they are associated with a vacuole of large size and more or less irregular shape, they are assumed to represent the canal as it exists during diastole. Occasionally, however, canals are encountered in which there is no differentiation between the wide channel and narrow tubule. It seems that the latter is missing. Apparently, its walls are pushed to the sides, becoming part of the contractile vacuole proper. The canal as a whole is shortened, and a direct, broad connection between the cavity of the vacuole and the outside is present (Fig. 14). Such appearances are accompanied by a flattened papilla. This structure is interpreted as representing the canal in systole.

Fibrillar Structures in the Vicinity of the Canal.—The cytoplasmic matrix surrounding the channel contains numerous fine fibrils, which in general appear radially disposed around this segment of the canal (Figs. 8 to 16, 18, and 19). Since they are present in all sections, it is assumed that they form a rather continuous array centered on the channel. The examination of many sections at various incidences shows that the array as a whole has the form of a truncated cone, with the

tip around the channel, and the base around the vacuolar membrane at approximately halfway towards the equator of the vacuole. In some places, the fibrils are grouped in bundles, sometimes as many as seven layers in depth (Figs. 8 and 9); in others, only one or two layers (Figs. 8 and 12) can be distinguished. It follows that the radial array is more or less continuous, with local "reinforcements" represented by the greater depth of bundles described. The inclination of the fibrils to the channel wall varies appreciably, the angle becoming more acute for fibrils more deeply located, especially for those confined to the papilla (Figs. 12 and 19).

The fibrils are about 180 A thick. At higher resolutions they appear to have a complex structure composed of a light core, surrounded by a dense wall (Fig. 12) which shows some suggestion of fine striations (Fig. 11), resembling the periodicity often encountered in protein fibers. The spacing is ~120 A. The electron micrographs do not supply information as to the way the fibers are anchored at their ends to the wall of the canal and to the vacuolar membrane.

In addition to the radial fibrils just described, a circular set of fibrils seems to be present around the fine tubular segment of the canal. In longitudinal sections of this segment these fibrils appear as two rows of regularly spaced, punctate profiles only ~20 mμ away from the wall of the tubule. It should be pointed out that such fibrils have been seen in a few specimens only, possibly because the conditions for their demonstration are more exacting. The evidence for the existence of this additional, circular set of fibrils is not considered definitive, only suggestive. By contrast, the existence of the radial set of fibrils is well established.

Cytoplasmic Structures in the Vicinity of the Contractile Vacuole

Granular and Vesicular Components.—Close to the vacuolar membrane, small particles are found scattered in the cytoplasmic matrix. Their size varies from 150 to 200 A. They represent most probably, Palade's small particulate containing ribonucleic acid (22, 25). Such particles appear more concentrated here than in other parts of the cytoplasm (Fig. 2).

Vesicles and canaliculi of different size and shape are also present in the perivacuolar region, and often appear disposed in small clusters (Figs. 3, 12, and 18). They are either rough or smooth surfaced (23), and apparently represent the endoplasmic reticulum, an interconnected vacuolar system which was first observed in electron microscopy by Porter in cells cultured *in vitro* (28). It was thereafter identified in thin sections in a variety of cells by Porter (27, 29), Palade and Porter (24), and many other investigators.

Piles of Packed Vesicles.—In addition to the vesicles mentioned, another so far unidentified vesicular structure was found. It is composed of elongated or oval, closely apposed vesicles arranged in long and narrow rows (Figs. 1 to 3, 6, 7, and 19). The individual vesicles are either flattened, about 30 mμ wide and 300 mμ long, or oval in shape, about 80 mμ wide and to 200 mμ long. Each vesicle is bound by a very thin, smooth surfaced membrane about 100 A thick. As many as 17 vesicles have been found arranged in a row which thus reaches 120 mμ in length. Such structures often appear close to the limiting membrane of vacuoles as depicted on Figs. 3, 6, and 7. In Fig. 7 rows of vesicles are at several points in close contact with the limiting membrane of one of the three major vacuoles. In Fig. 6 one of the several vesicles seems to be directly connected with a small vacuole (at arrow), suggesting perhaps that these arrays of vesicles take an active part in the formation and expansion of the contractile vacuoles. It should be pointed out, however, that such vesicles are not restricted to the perivacuolar cytoplasm, but are also found in other parts of the organism. It is probable that they represent dictyosome-like bodies, though they differ from the dictyosomes so far described (5, 6) as to their dimensions. The vesicles are shorter and the rows in which they are arranged are therefore narrower.

Mitochondria.—Numerous mitochondria may be found around the contractile vacuole at distances of 400 to 800 mμ from its membrane (Figs. 1 to 3, 7 to 11, 18, and 19). In a few cases mitochondria appear to be very close to the membrane of the vacuole (Figs. 1, 3, and 19), even touching it with their outer membrane. Their structure (Figs. 1 to 3, 5, 7, 11, 18, 19) is similar to that of mitochondria in higher organisms (21); *i.e.*, they possess an outer limiting membrane and an inner membrane which by its extensions forms the internal mitochondrial structures. There are, however, some differences (described in previous papers (34, 40)).

Basal Bodies.—Other structures, *e.g.* the basal bodies of the adult suctorian appear to be asso-

ciated with the contractile vacuole. They may be seen in all electron micrographs representing sections through the vacuole and its canal. In Figs. 8 to 11, 13 to 16, and 19 the basal bodies appear longitudinally sectioned. It is characteristic that they are always on one side of the pore. Figs. 17 and 18, which correspond to oblique sections cut close to the cell surface, show the basal bodies in cross-section. Such sections indicate that the basal bodies are arranged in an orderly fashion in six lines. Interesting in this respect is Fig. 18 which shows the beginning of formation of the brood pouch and demonstrates its relationship to the basal bodies and to the contractile vacuole. The basal bodies, assembled as usual in six rows, appear close to an invagination that involves both the pellicle and the plasma membrane. This invagination is an early stage in the formation of a brood pouch. The contractile vacuole and the narrow tubule can be also seen in the nearest vicinity of the developing pouch, but the channel and pore were missed by this section and do not appear in the micrograph.

DISCUSSION

Although contractile vacuoles occur also in a few other phyla (2, 8, 17), almost all information on these organelles is derived from studies on Protozoa, for the simple reason that the latter are the most convenient material for observations and experiments. It must be said at the outset that, from light microscopy, the contractile vacuole does not seem to be an organelle of uniform structure, even among Protozoa. There are great differences as to its shape, size, number, and presence or absence of a permanent canal. However, in spite of these variations all contractile vacuoles have some common features; they form by the fusion of smaller contributory vacuoles, and they have a remarkably fast contraction at systole. The contraction which results always in the emptying of the content lasts in almost all contractile vacuoles 2 to 3 seconds. The unusual speed of contraction, and the fact that it occurs at regular intervals when the general conditions remain the same, suggest that a very precise mechanism might be involved.

The present electron microscope study has disclosed several structures of possible importance for the mechanism of systole in *Tokophrya* and probably in other forms possessing a permanent outlet for the vacuole. This outlet or canal has a complex structure which involves differentiation along its axis and the presence of numerous orderly disposed fibrils. The canal consists of a pore, a broad channel, and a narrow tubule located in a papilla that protrudes into the cavity of the contractile vacuole. While the pore and channel have relatively stable dimensions and are permanently widely open, the diameter of the tubule changes over a wide range. During diastole its diameter is so small (about 25 to 30 mμ) that it might be regarded as being closed (Figs. 8 to 12, 15, 16, 18, and 19). At systole the tubule opens so widely that its identification as a distinct segment of the channel becomes rather difficult.

It is tempting to assume that the broadening and narrowing of the tubule is accomplished by the contraction and relaxation of the numerous fibrils located around the canal. At diastole when the fibrils are presumably relaxed, the papilla in which the tubule is located is prominent, and the tubule itself relatively long and narrow (Figs. 8 to 12, 15, 16, 18, and 19). Internal pressure of the vacuole on the papilla may constrict the tubule, thus keeping the vacuole closed. The contraction of the radial fibrils could have two effects. The deep fibrils implanted at a small angle could flatten the papilla, while those attached at a larger angle could presumably pull the walls of the tubule aside. As a result, a direct broad connection would be established between the contractile vacuole and the channel.

If, in addition to the radial array, circular fibrils are a regular feature of the outlet, then the presence of two sets of fibrils would suggest that they act like a combined dilator and constrictor in opening and closing the tubule. Such a mechanism would be similar to arrangements encountered at higher levels of organization, as for instance in the iris of the eye, where the broadening and narrowing of the pupil is accomplished by dilatory and constrictory muscles. At diastole the radial fibrils connecting the channel with the contractile vacuole would relax, while the circular fibrils contract, keeping the tubule as narrow as seen in Figs. 8 to 12, 15 and 16, 18, and 19. At systole presumably the circular fibrils might relax and the radial fibrils contract. The latter would pull the walls of the tubule to the side and open it widely. Once a broad connection was established between the vacuole and the channel, the vacuolar content would flow out, emptying the organelle. Assuming all this, then it could follow that the broadening

of the tubule is the releasing factor for systole. Kitching in his extensive work on contractile vacuoles, arrives at a similar conclusion (11, 14). He assumes that the critical process for the initiation of systole is probably the opening of the pore, which is plugged during diastole by remnants of the membrane from the previously discharged vacuole. The findings in *Tokophrya* do not support this assumed mechanism in all details, for no plug is found. What light microscope investigators have interpreted as a plug (9, 11) is in all probability the papilla in which the narrow tubule is located.

The discharge of the contractile vacuole after the dilation of the tubule is most probably due to a difference in hydrostatic pressure between the contractile vacuole and the outside medium (10, 11, 14). Actually there seems to be a rapid increase in pressure at the end of diastole in *Tokophrya*, as indicated by the sudden rounding up of the contractile vacuole that precedes the systole (14, 18). It can be speculated that a sudden increase in intravacuolar pressure is not only responsible for the rapid discharge of the vacuole, but could also act as a trigger for the supposed contraction of the radial fibrils.

It is tempting to assume further that similar systolic mechanisms operate in all contractile vacuoles, but what is known from light microscopy does not seem to support such a supposition. A permanent canal is present in Ciliata and Suctoria organisms with a tough pellicle, whereas in other Protozoa having a thinner and less rigid external membrane (*e.g. Amoeba*) there is no visible pore. It is possible that in organisms of the latter class the whole canal narrows at diastole as the tubule does in *Tokophrya*, and thereby becomes too minute to be seen with the light microscope. Obviously an electron microscope study of the contractile vacuole in amebae could solve this problem.

The problem of whether the cytoplasm in the closest vicinity of the vacuolar membrane takes an active part in the discharge of the contractile vacuole remains unsettled. It may be assumed that the perivacuolar cytoplasm contracts actively during systole. For such activity fibrillar structures would seem to be necessary, but so far fibrils have not been found in the cytoplasm surrounding the vacuole in *Tokophrya*. The situation may be different in other Protozoa. For instance, Bairati and Lehmann (1, 15) describe a fibrillar network in the vicinity of the limiting membrane of the vacuole in *Amoeba proteus*. They assume that this network is capable of active contraction, and thus explain the mechanism of systole.

The fate of the vacuolar membrane during systole is difficult to ascertain. Some micrographs suggest that the membrane forms multiple deep infoldings (Fig. 2). These folds may gradually level off during the next diastolic phase, or the folded membrane of the collapsed vacuole may pinch off into small vesicles. The rapid appearance of a number of small vacuoles in this region immediately after the systole favors the latter supposition.

This study has uncovered a number of structural details which seem to be directly involved in one aspect of the systole, namely the opening of the discharging canal, but nothing comparable has been found so far for the mechanism involved in diastole. Some assumptions can be made, however, based on the preceding observations. For instance, the cytoplasm in the vicinity of the contractile vacuole contains numerous small vesicles that could be described as elements of the endoplasmic reticulum. Some of them are in immediate contact with the vacuole; others appear to open therein. Occasionally, rows of vesicles are found in the immediate vicinity of the contractile vacuole, and sometimes continuity between these vesicles and the vacuole can be observed. Such appearances could be interpreted as outfoldings (evaginations) in a still expanding membrane, or as phases in a fusion process of small vesicles into the contractile vacuole, or both. It is possible that the drainage of the cytoplasm takes place by small vesicles feeding into the contractile vacuole, but it cannot be assumed that all its fluid content reaches the contractile vacuole in this way.

Of special interest among the structures found in the vicinity of the contractile vacuole are the rows of vesicles, since they do not closely resemble any other structure so far described in other cells. It is assumed that they represent dictyosome-like bodies and are, therefore, related to the Golgi apparatus (5, 6). Close relationship between the Golgi apparatus and the contractile vacuole has indeed been reported by several investigators (4, 19). In *Tokophrya*, as already mentioned, these arrays of closely packed vesicles are not limited to areas around the contractile vacuole, but are found scattered at random throughout the cytoplasm, and it is possible that they contain fluid withdrawn from the cytoplasm.

The numerous mitochondria which are present around the contractile vacuole might be involved

in the many complex processes which take place during the formation and expansion of the contractile vacuole and during the expulsion of its content.

The association of basal bodies with the contractile vacuole is of some interest. They always appear on one side of the canal of the contractile vacuole (Figs. 8 to 11, 13 to 19). As already described in previous publications (30, 33), only the embryo of *Tokophrya* possesses cilia which disappear at the start of metamorphosis. However, the basal bodies remain. This electron microscope study has disclosed that in the adult suctorian they are assembled close to the contractile vacuole. Since it is this part of the body which gives rise to the future embryo, it might be, therefore, regarded as an "anlage."

It has been suggested by Weisz in his study on morphogenesis in *Stentor coeruleus* (43) that basal bodies give rise to a new contractile vacuole. This concept seems to be favored by the close relationship of the basal bodies with the canal of the vacuole, and it might well be that it applies to the formation of the two contractile vacuoles in the embryo.

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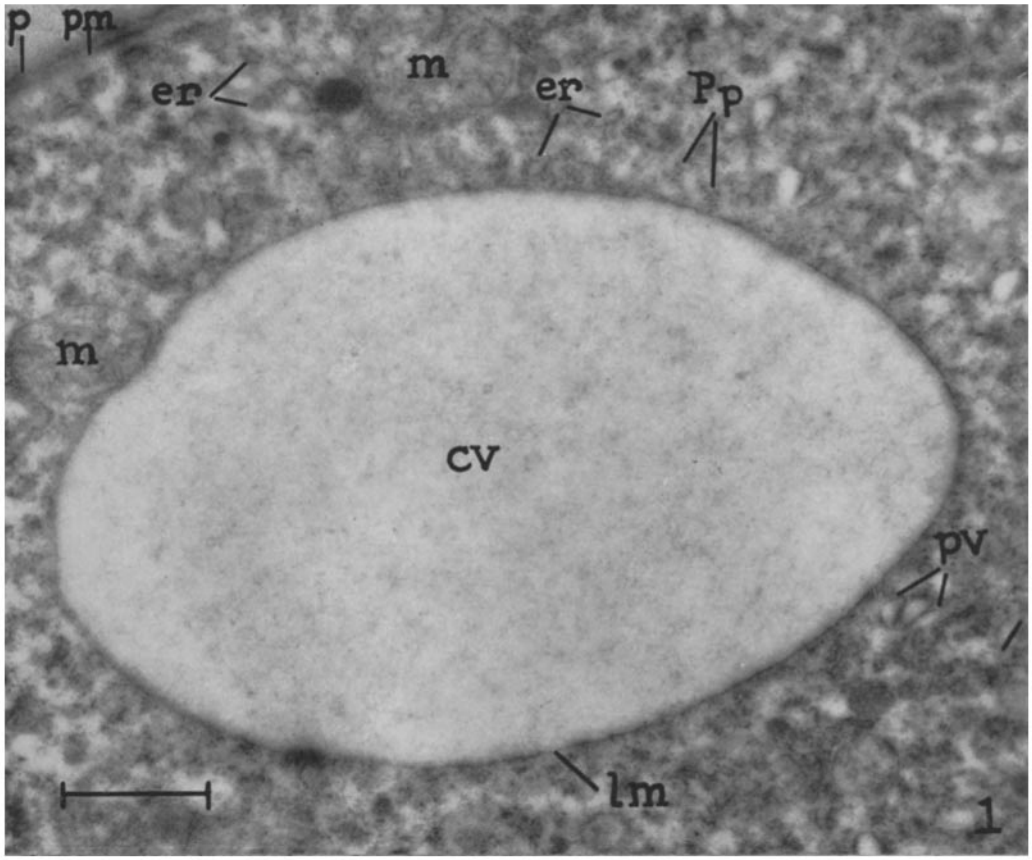
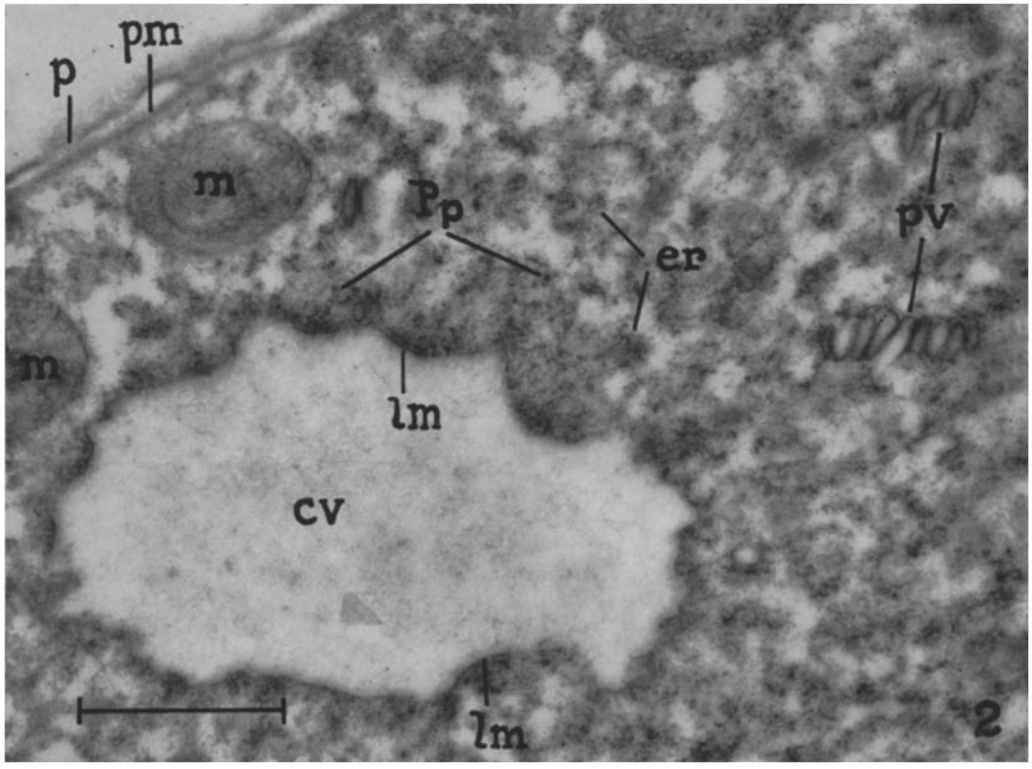
Explanation of Plates

Plate 99

Fig. 1. Electron micrograph of a section through a contractile vacuole (*cv*) at advanced diastole and just before expulsion. Note the thin limiting membrane (*lm*), which appears as a fine dense line. Small dense particles, Palade's particulate component (P_p), several mitochondria (*m*), vesicles of the endoplasmic reticulum (*er*), and three closely adjacent vesicles (*pv*) can be seen in the surrounding cytoplasm. The pellicle, *p*, and plasma membrane, *pm*, are also designated. × 19,250.

Fig. 2. Section through a contractile vacuole (*cv*), most probably at systole. The vacuolar limiting membrane (*lm*) is folded. Close to the limiting membrane an accumulation of Palade's small particles (P_p) may be seen. Rows of vesicles (*pv*) and also a few mitochondria (*m*) are evident in the surrounding cytoplasm. Endoplasmic reticulum is designated at *er*; pellicle at *p*; and plasma membrane at *pm*. × 27,500.





(Rudzinska: Contractile vacuole in *Tokophrya*)

PLATE 100

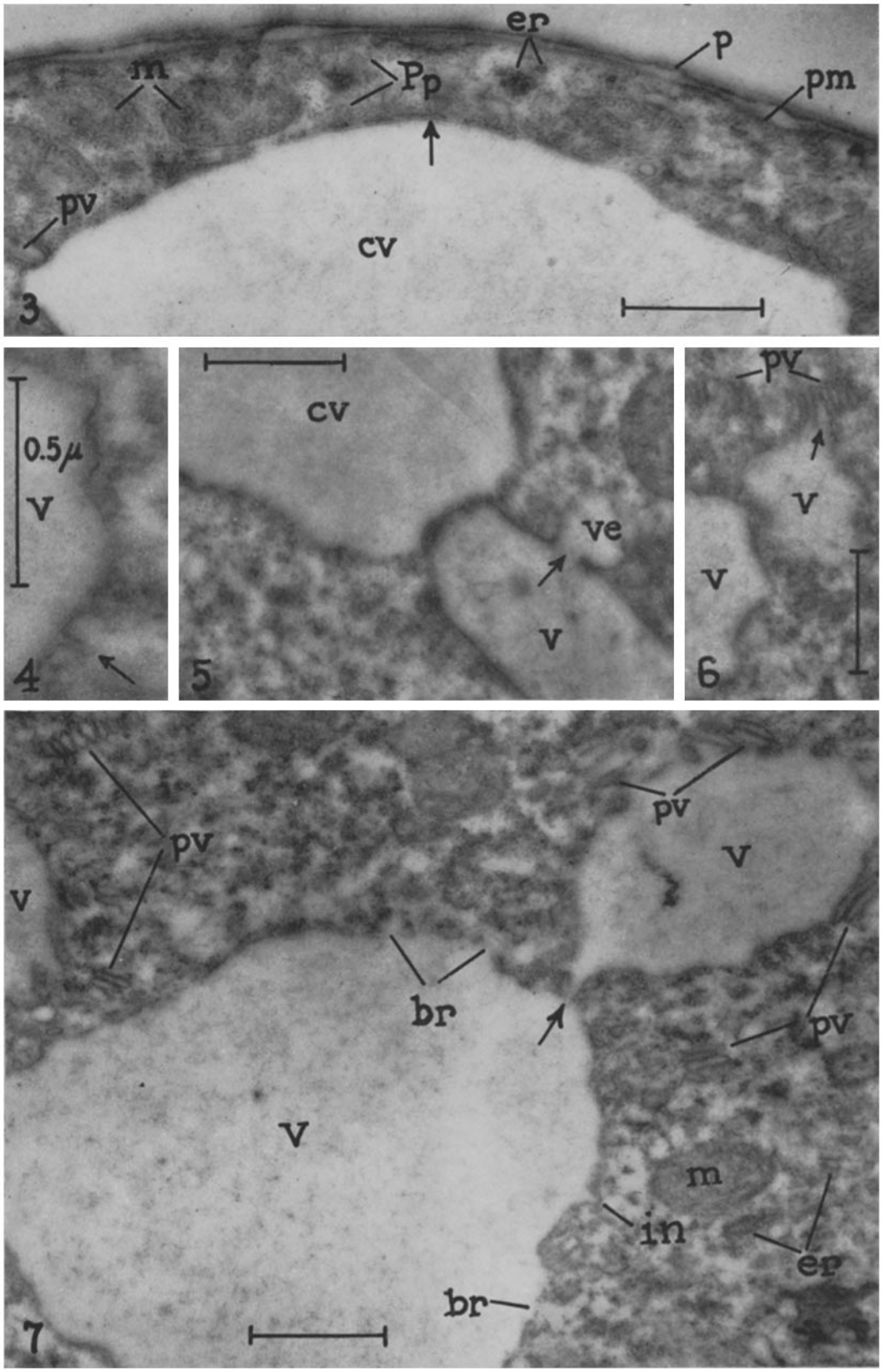
FIG. 3. Section through part of a contractile vacuole (*cv*). Close to the limiting membrane (at arrow) dense dots may be seen. They probably represent cross-sections through radial fibrils. Several mitochondria (*m*) are evident in the close vicinity of the vacuole. Endoplasmic reticulum is designated by *er*; Palade's small particles at P_p; rows of vesicles at *pv*; pellicle at *P*; and plasma membrane at *pm*. × 22,000.

FIG. 4. High power micrograph, showing part of vacuole (*v*), with a distinct evagination (at arrow) of its limiting membrane. × 66,000.

FIG. 5. A vesicle (*ve*), merging at arrow with a small vacuole (*v*), which is closely adjacent to a contractile vacuole (*cv*). × 22,000.

FIG. 6. Section through two adjacent small vacuoles (*v*), and neighboring stacks of vesicles (*pv*). At arrow one of the vesicles is merging with a vacuole. × 19,500.

FIG. 7. A smaller vacuole merging with a larger one. The connection (at arrow) has been already made; close to the membrane of the smaller vacuole in several places are stacks of vesicles (*pv*), which may be seen in other parts of the cytoplasm also. The vacuolar membrane shows in some places evaginations (*in*) and breaks (*br*); mitochondria are indicated at *m*, vacuoles at *v*, and endoplasmic reticulum at *er*. × 22,000.

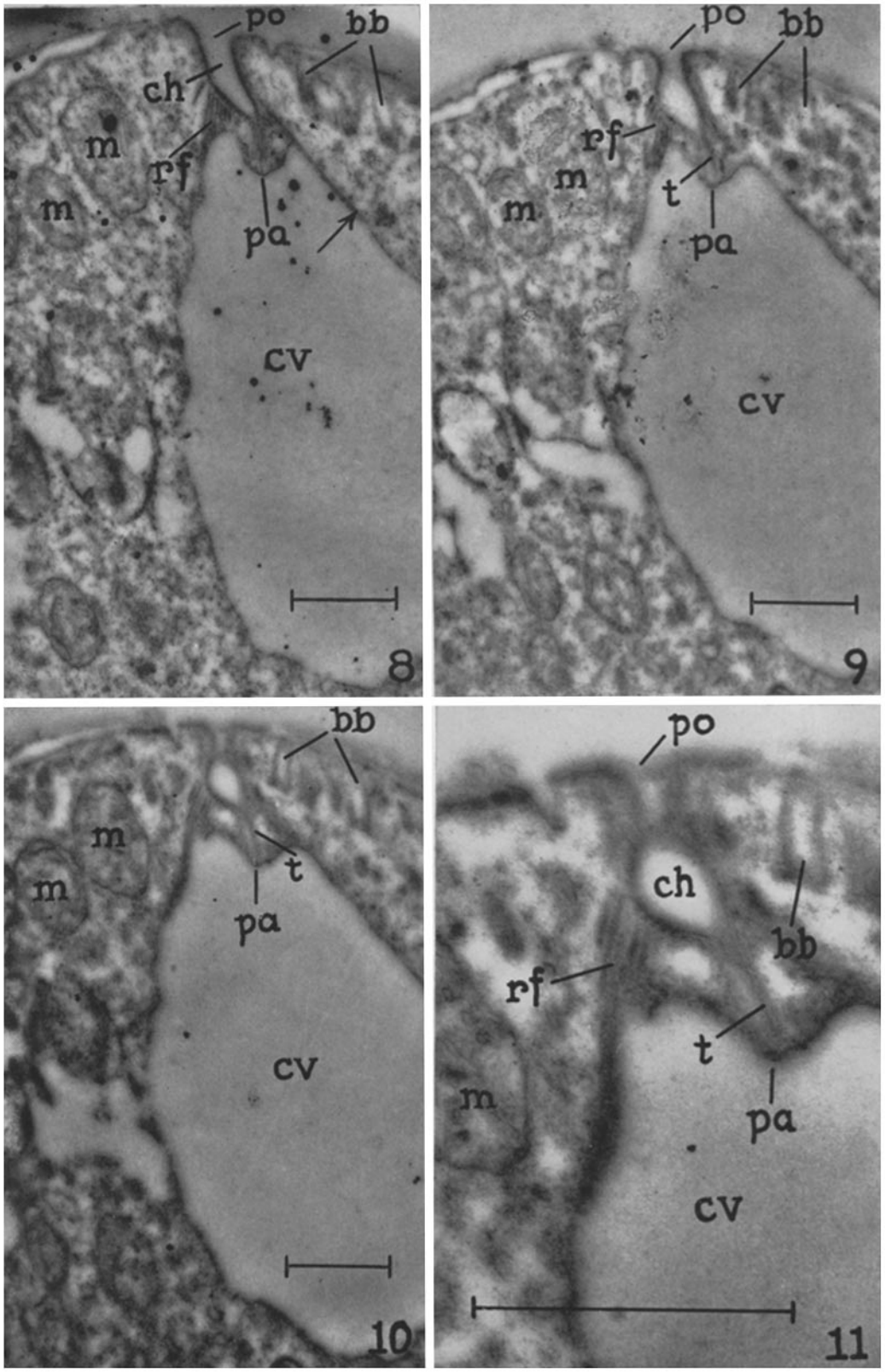


(Rudzinska: Contractile vacuole in *Tokophrya*)

PLATE 101

FIGS. 8 to 10 represent three serial sections through a contractile vacuole (*cv*) at diastole. The canal shows clearly its three parts; the pore (*po*), the channel (*ch*), and the tubule (*t*). The latter part comprises a papilla (*pa*) extending inward toward the contractile vacuole. Note the radial fibrils (*rf*) connecting the channel with the contractile vacuole. They appear in all three figures in the same area. In Fig. 8 a long single radial fibril is depicted at arrow, and a few fibrils in the papilla. On the right side of all electron micrographs close to the canal, basal bodies (*bb*) may be seen in the longitudinal section. Note mitochondria (*m*) in the upper left side in all three sections. × 16,500.

FIG. 11. High power electron micrograph of Fig. 10, demonstrating the fine structure of the radial fibrils (*rf*). They show a fine periodicity. The pore is designated at *po*; channel at *ch*; tubule at *t*; papilla at *pa*; contractile vacuole at *cv*; basal body at *bb*; and mitochondrion at *m*. × 50,000.



(Rudzinska: Contractile vacuole in *Tokophrya*)

PLATE 102

FIG. 12. Section through canal and part of contractile vacuole (*cv*), showing clearly the pore (*po*), channel (*ch*), and the tubule (*t*) located in the papilla (*pa*). Note the funnel-like connection between channel and tubule at arrow. The fibrils (*rf*) show a fine structure composed of a light core surrounded by a dense wall. Some of the fibrils (*rf*) appear to be very long. A single fibril seen in the papilla runs parallel to the wall of the tubule. Endoplasmic reticulum is shown at *er*; pellicle at *p*; and plasma membrane at *pm*. × 48,000.

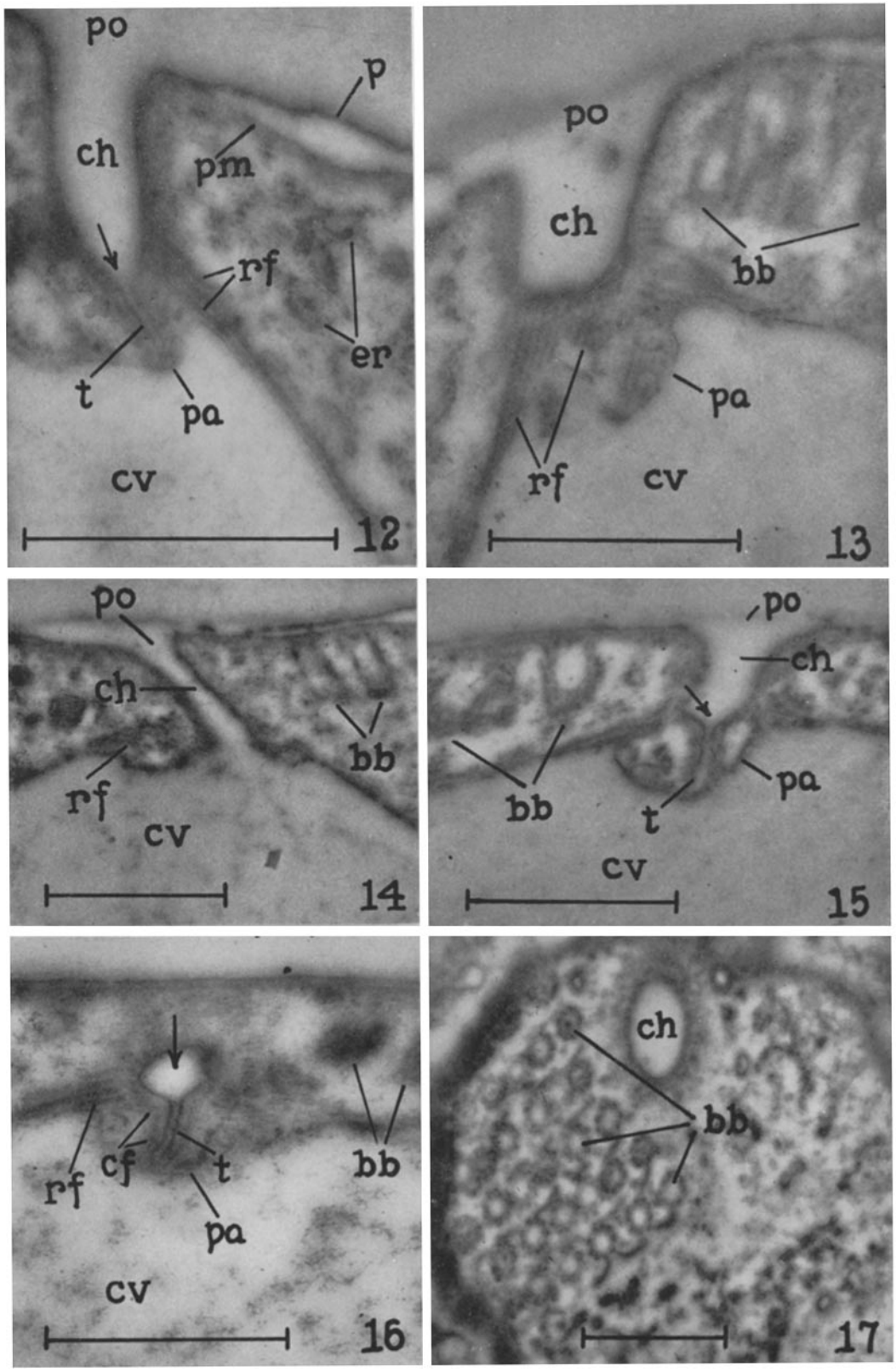
FIG. 13. Part of the canal and contractile vacuole (*cv*). The section shows only the pore (*po*) and channel (*ch*), and not the tubule. The papilla (*pa*) shows several radial fibrils (*rf*). Note the basal bodies (*bb*) on one side of the canal. × 38,500.

FIG. 14. Section through part of contractile vacuole (*cv*), most probably at the beginning of systole. The canal is widely open on both sides and does not show differentiation into channel and tubule. The latter is absent, and it might be assumed that its walls became part of the contractile vacuole proper. The pore is shown at *po*; the channel at *ch*; basal bodies at *bb*; and radial fibrils at *rf*. × 27,500.

FIG. 15. Electron micrograph of part of contractile vacuole (*cv*), with canal showing distinctly its three parts: the pore (*po*), channel (*ch*), and tubule (*t*). The funnel-like connection may be seen at arrow; basal bodies at *bb*. × 33,000.

FIG. 16. Electron micrograph of part of contractile vacuole (*cv*), with tubule (*t*) and the funnel-like structure (at arrow). Note small darker dots at regular intervals along the wall of the tubule and funnel; it is suggested that they might represent cross-sections through circular fibrils (*cf*) surrounding the tubule. Radial fibrils are designated at *rf* and the papilla at *pa*. × 33,500.

FIG. 17. Oblique section cut close to the cell surface of *Tokophrya* showing the channel (*ch*) in cross-section, and six rows of basal bodies (*bb*), also in cross-section. × 22,400.

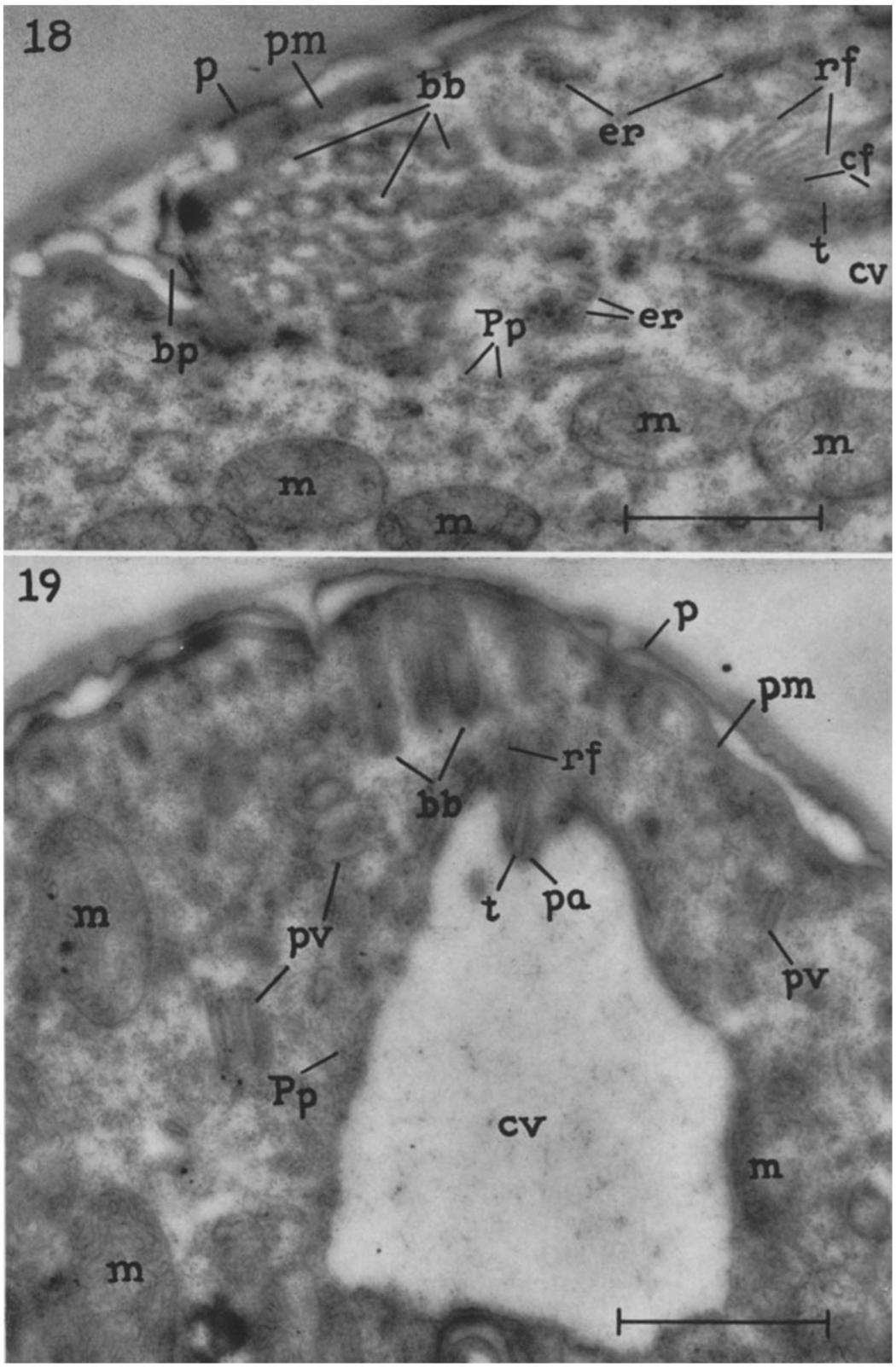


(Rudzinska: Contractile vacuole in *Tokophrya*)

PLATE 103

FIG. 18. Oblique section through *Tokophrya*, illustrating part of contractile vacuole (*cv*) and tubule (*t*). On both sides of the slender tubule small, dark dots may be seen. It is suggested that they represent cross-sections through circular fibrils (*cf*). In the vicinity several rows of basal bodies appear in cross-section, close to an invagination of the pellicle (*p*), and plasma membrane (*pm*), which represents the beginning of the formation of a brood pouch (*bp*). Radial fibrils as marked at *rf*; endoplasmic reticulum at *er*; Palade's small particulate component at P_p; basal bodies at *bb*, and mitochondria at *m*. × 30,000.

FIG. 19. Electron micrograph of contractile vacuole (*cv*), showing only part of the canal, namely the tubule (*t*) located in the papilla (*pa*). Radial fibrils (*rf*), seen in the papilla, run parallel to the long axis of the tubule. Several basal bodies (*bb*) in longitudinal section may be seen in the vicinity of the contractile vacuole. Mitochondria are designated at *m*; rows of vesicles at *pv*; Palade's small particles at P_p; pellicle at *p*; and plasma membrane at *pm*. × 33,000.



(Rudzinska: Contractile vacuole in *Tokophrya*)