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The Contractile Vacuole and Related Structures in *Tetrahymena pyriformis**

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SYNOPSIS. The nephridial system of *T. pyriformis* consists of a contractile vacuole with its two pores and an elaborate network of tubules. Both the vacuole and its tubules constitute a continuous system of single membranes of approximately 70 Å in thickness. Tubular fibrils of about 250 Å extend from the pore wall to the vacuole membrane and appear to function in dilating the pore prior to systole. The nephridial tubules arise directly from the wall of the vacuole and are continuous with it throughout the vacuolar cycle. The tubules appear to be contiguous with the granular endoplasmic reticulum. A typical Golgi complex was not found. Some speculation about the interrelation of these structures as regards function is offered.

As our knowledge of protozoan ultrastructure becomes elucidated(1), the similarity between these organisms and metazoan cells becomes more apparent *(8,16)*. *Essentially* all of the structures long recognized in cells of higher animals are found in protozoa, modified to be sure, for special functions. Since the protozoa were the first eucells(16), it is highly likely that the basic structures found in higher animal cells were first organized in the protozoan and later become modified for special functions in metazoan cells. Further exploration of protozoan cytology can be fruitful in gaining a better understanding of common origin of all cell structures.

Among the cytoplasmic structures in protozoa one of the most conspicuous is the contractile vacuole, which has been the subject of both morphological and physiological studies for a long time(21). Whereas most protozoa possess a contractile vacuole, it is among the fresh water forms that it reaches its greatest complexity, probably because osmoregulation is most demanding in their hypotonic environment. Representatives from all groups of ciliates have been examined with the light microscope in regard to their contractile vacuole, and more recently the ultrastructure of several groups has been studied, namely, suctoria (19), hymenostomes(20), astomes(17), peritrichs(2, 6) and entodiniomorphs(14). While there is considerable variation among them, all show a system of vesicles or tubules intricately associated with the contractile vacuole. The most elaborate system examined so far is that of *Paramecium*(20). This system consists of a vast array of nephridial tubules distributed throughout the cytoplasm which appear to be contiguous with the endoplasmic reticulum. The fine structure of this organelle supports earlier evidence that perhaps the contractile vacuole, together with its ancillary structures, does more than function solely as an osmoregulator. Its continuity with the endoplasmic reticulum implies additional functions.

The purpose of this report is to describe the contractile vacuole apparatus in *T. pyriformis* and to attempt to show its relationship with the endoplasmic reticulum.

MATERIALS AND METHODS

Strain E of *T. pyriformis* was used throughout this investigation. The ciliates were grown axenically in 500 ml Erlenmeyer flasks in 2% proteose-peptone (Difco Laboratories, Detroit) supplemented with 0.5% glucose, liver extract and a mineral salt mixture. Both logarithmic (48-72 hr) and stationary (5 days) growth phases, incubated at 25°C were concentrated by centrifugation into a soft pellet. This pellet was then fixed for 30 minutes in 1% OsO_4 buffered with 9.14 M veronal acetate (pH 7.4) containing 45 mg of sucrose per ml. The ciliates were dehydrated with graded ethanol and embedded in Epon 812 according to the method of Luft(10). The blocks containing the pellets were polymerized for several days at graded temperatures (35°-60°C), sectioned with a Porter-Blum microtome and examined in an RCA EMU-3E electron microscope. Magnifications on the plate ranged from 2,700 to 10,600 and the micrographs were enlarged photographically as desired.

OBSERVATIONS

Light microscope observations of the contractile vacuole of *T. pyriformis* reveal its location, size, rate of pulsations, and little else. Electron microscope studies, however, demonstrate a highly complex organelle which is closely related to other cell structures. In addition to the obvious vacuole itself, there are two well-defined pores perforating the cortex which permit fluids to be discharged to the outside of the cell during systole and an elaborate network of branching tubules continuous with the vacuole wall (Figs. 1-6). The vacuole and tubules constitute an interconnecting system composed of single membranes of about 70 Å in thickness (Figs. 4, 8).

The pores are depressions in the cortex located on meridians 5 and 6 in the posterior region of the ciliate. They are complex structures composed of thick walls from which fibrils originate (Figs. 1, 3, 5, 6, 7). The

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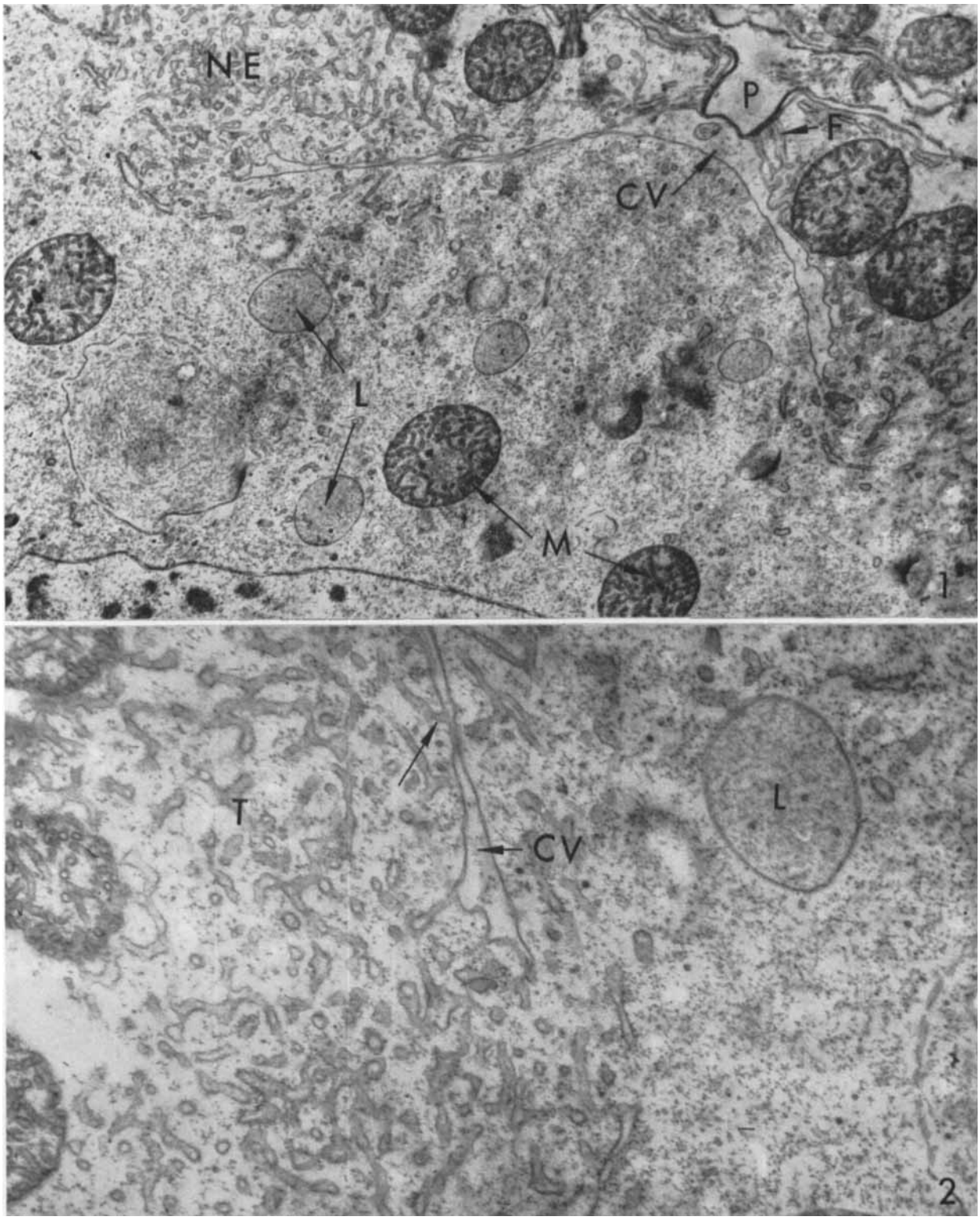


Fig. 1. A low magnification micrograph of the contractile vacuole (CV) including the nephridial tubules (NE) and one of the pores (P). The vacuole has completed systole. The irregular nature of the pore fibrils (F) suggests that they are relaxed. Mitochondria (M) and lysosomes (L) are also present in the micrograph. × 7500.

Fig. 2. This is a higher magnification of the left end of the contractile vacuole (CV) shown in Fig. 1. Note that the nephridial tubules (T) are continuous with the vacuole (unmarked arrow). × 18,000.

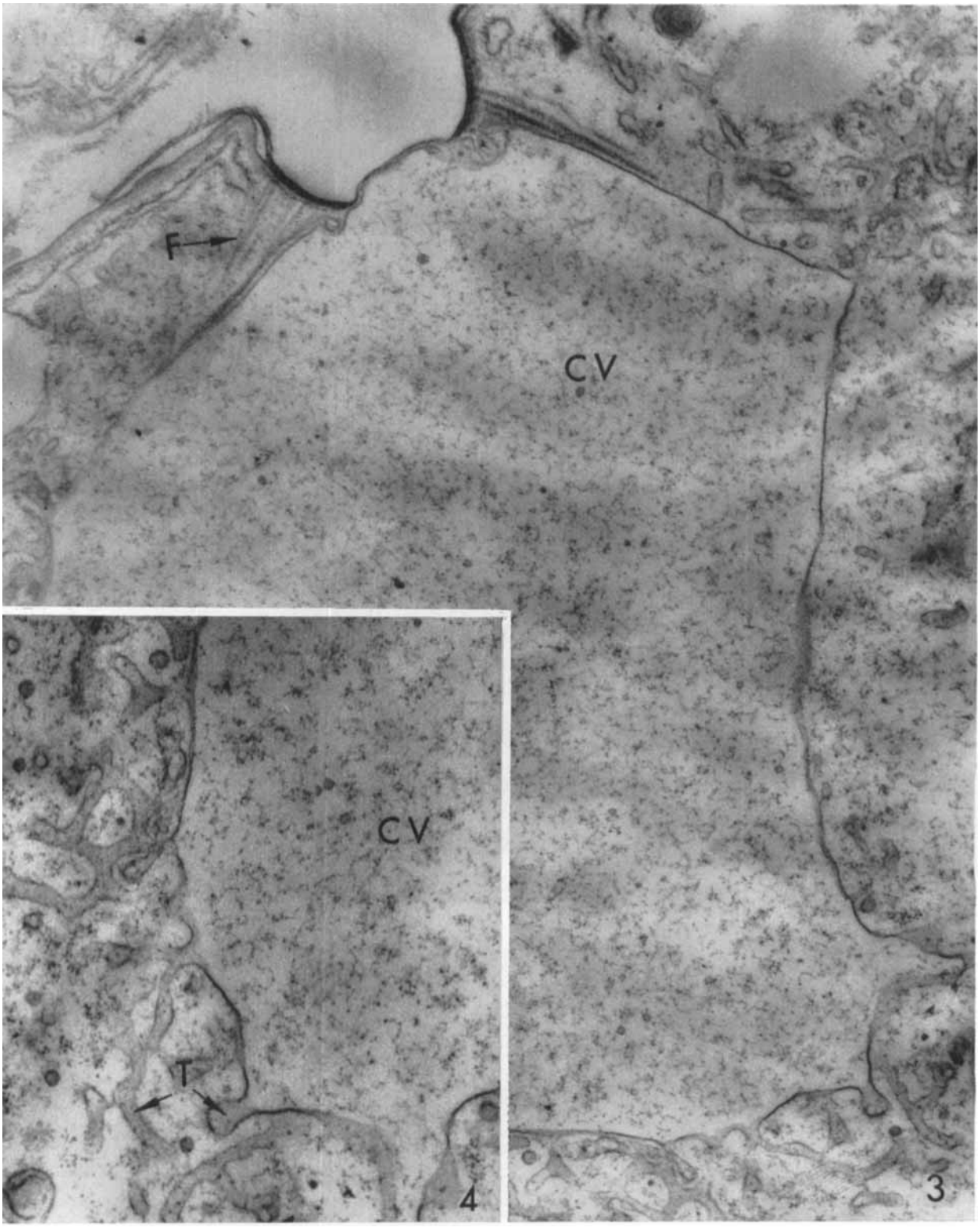


Fig. 3. The contractile vacuole (CV) containing finely granular material is shown in diastole just before discharge. The pore fibrils (F) are straight which indicates they are under tension. Note that the pore diameter is greater than in Fig. 1. × 22,500.

Fig. 4. An enlargement of the right portion of the vacuole (CV) of Fig. 3, showing the details of the tubules (T). Note that they are continuous with the vacuole. × 30,600.

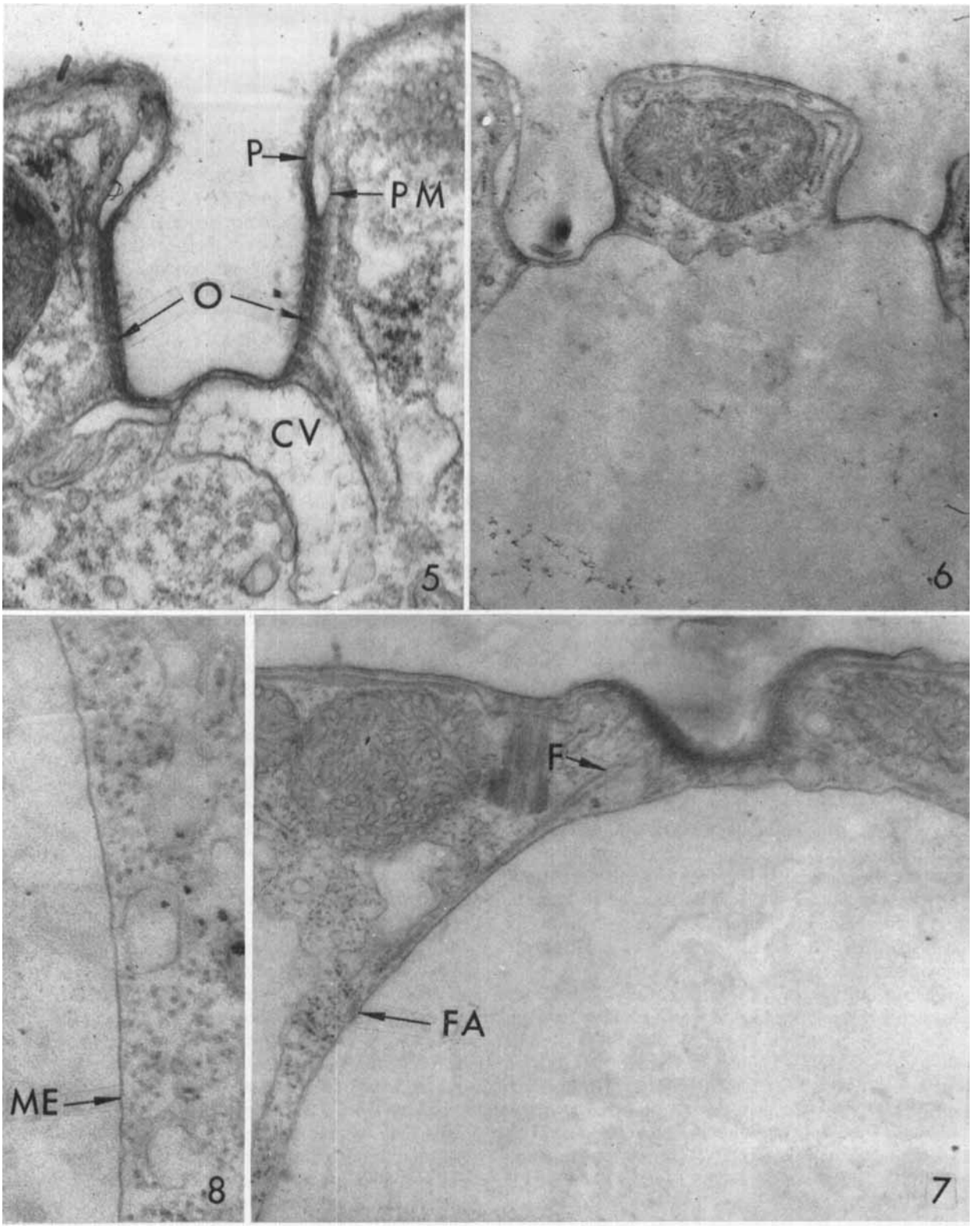


Fig. 5. This high magnification micrograph of a pore in section shows the eleven points of origin of fibrils (O). The pellicle (P) and plasma membrane (PM) coalesce and thicken to form the pore wall. The vacuole (CV) is in systole but shows only in part. × 36,000.

Fig. 6. *T. pyriformis* has two pores as shown in this micrograph, both of which function as channels for the discharged contents of the vacuole. Only rarely does a section pass through both pores. A mitochondrion lies between them. × 18,500.

Fig. 7. The pore fibrils (F) show well in this micrograph where the vacuole is fully distended. This section is taken just to one side of the pore and shows the attachment of the fibrils to the vacuole (FA). × 20,500.

Fig. 8. An enlargement of a portion of the vacuole from Fig. 7. It shows the single membrane of the contractile vacuole (ME). × 40,500.

fibrils are tubular in structure and are about 250 Å in diameter (Fig. 7). The resolution was insufficient to show periodicity. In longitudinal sections of the pore, points of origin of eleven fibrils can be seen in profile (Fig. 5). They appear as dense tubules embedded in the pore wall. In transverse section of the pore, approximately 20 rows of fibril bases can be counted. Consequently, there must be well over 200 fibrils originating in the pore and radiating over the posterior region of the contractile vacuole to which they are attached (Fig. 7). It seems highly likely that the fibrils function in the operation of the contractile vacuole.

The pore is closed at its proximal (anterior) end by a dense double membrane of approximately 400 Å in thickness. The inner membrane is continuous with the membrane of the contractile vacuole. The outer membrane is a continuation of the inner portion of the pore wall itself (Figs. 3, 5). The latter seems to be a result of the consolidation of both the pellicle and plasma membrane. Both proximal membranes seem to maintain their integrity throughout the vacuolar cycle except during the short period when the contents are being extruded. No observations have been made at the moment of rupture, consequently it is impossible to describe where the break occurs or how the membranes are re-established.

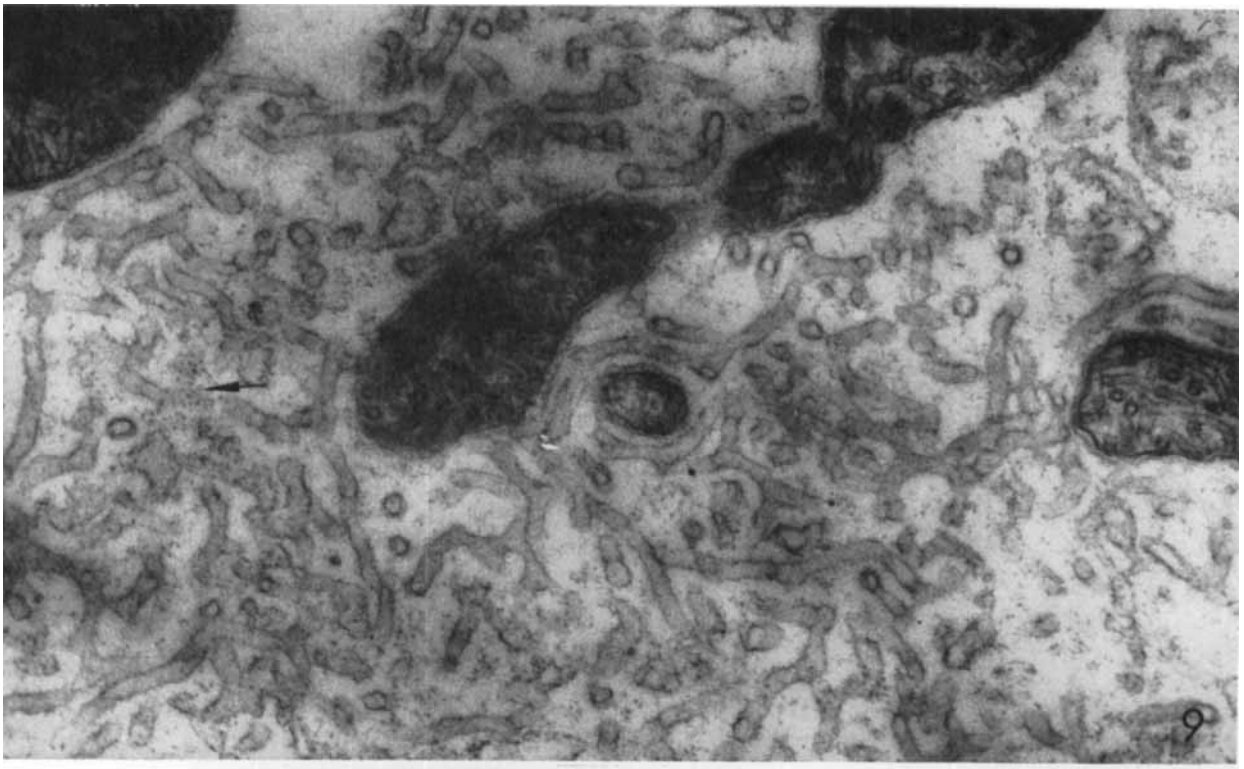
The diameter of the pore appears slightly smaller during systole than when the vacuole is filled, a difference which could be accounted for by the plane of longitudinal section (compare Figs. 1 and 3). As the intra-vacuolar pressure increases with the filling of the vacuole, tension is placed on the membranes, fibrils, and pore walls. Apparently as a result of this pressure, the membranes rupture, permitting the outward flow of fluids. The discharge appears to result from the combined action of the fibrils, the intracellular turgor, and the tensile strength of the proximal membranes.

The nephridial tubules are continuous with the contractile vacuole membrane and extend throughout much of the cytoplasm in the posterior region of the cell (Figs. 1, 2, 9). Tubules of similar structure are seen in other regions of the ciliate (Fig. 12) and may well be a part of the nephridial system. The matrix within the tubules is of higher density than that of the contractile vacuole suggesting that molecular aggregates may be suspended in the contained fluid (Fig. 2). If this is so this material must be selectively reabsorbed into the cytoplasm before the fluid reaches the contractile vacuole. The tubules have a more or less uniform diameter of about 200 mμ (Figs. 4, 9). They remain attached to the contractile vacuole membrane throughout its cycle. A careful examination of the vacuole during both systole and diastole reveals that the tubules are never detached (compare Figs. 2 and 3), unlike the process in *Paramecium* where they are separated from the vacuole membrane during systole(20). The entire nephridial system in *T. pyriformis* seems to be a completely integrated structural unit during the vacuolar cycle.

The nephridial tubules appear to be contiguous with the endoplasmic reticulum. Nearest the vacuole they are without granules along their surfaces, hence are interpreted as being synonymous with the smooth or agranular endoplasmic reticulum (Figs. 2, 4, 9). Some distance from the vacuole, granules (ribosomes) aline the outer surfaces of the tubules (Fig. 10), which identifies them as rough or granular endoplasmic reticulum. Occasionally the transition from smooth to rough endoplasmic reticulum can be observed in some micrographs (Fig. 10). The granular endoplasmic reticulum may be seen in almost any region of the cell but it seems to concentrate near the plasma membrane and the peripheral mitochondria, particularly during logarithmic growth when they are elongated and are alined along the plasma membrane(5). Moreover, the outer membrane of the macronucleus evaginates, forming part of the granular endoplasmic reticulum (Fig. 11). Whether or not these membranes are an integral part of the nephridial system is impossible to determine from sectioned material. It is tempting to speculate that the entire membrane system is a structural unit.

The granular endoplasmic reticulum frequently balloons and appears to form vacuoles. This is particularly striking in the region of the plasma membrane (Fig. 12). Also, rough surfaced endoplasmic reticulum occasionally is organized into concentric patterns (Fig. 13) which, under the light microscope, appear as dense spheres or short rods. These could be interpreted as the Golgi complex by light microscopists (21). In such clusters of granular endoplasmic reticulum, clear vacuoles are seen. These appear to arise from the closed ends of the membranes (Fig. 14). Whether these vacuoles pinch off and become free in the cytoplasm or function as sites for the segregation of water, as well as other moelcules, is impossible to state. If the latter were true, water and other substances would be separated in the rough endoplasmic reticulum from the cytoplasm and conveyed via the nephridial tubules to the contractile vacuole. However, static micrographs can only suggest such a possibility and are no proof that segregation of cytoplasmic fluids actually takes place in the rough surfaced endoplasmic reticulum.

No typical Golgi complex has been identified in *T. pyriformis*. The agranular endoplasmic reticulum seems to be confined to the nephridial tubules. Stacked, flattened vesicles arranged as the Golgi complex of other protozoa(16) and metazoa have not been observed. Small granulated vacuoles ranging in size



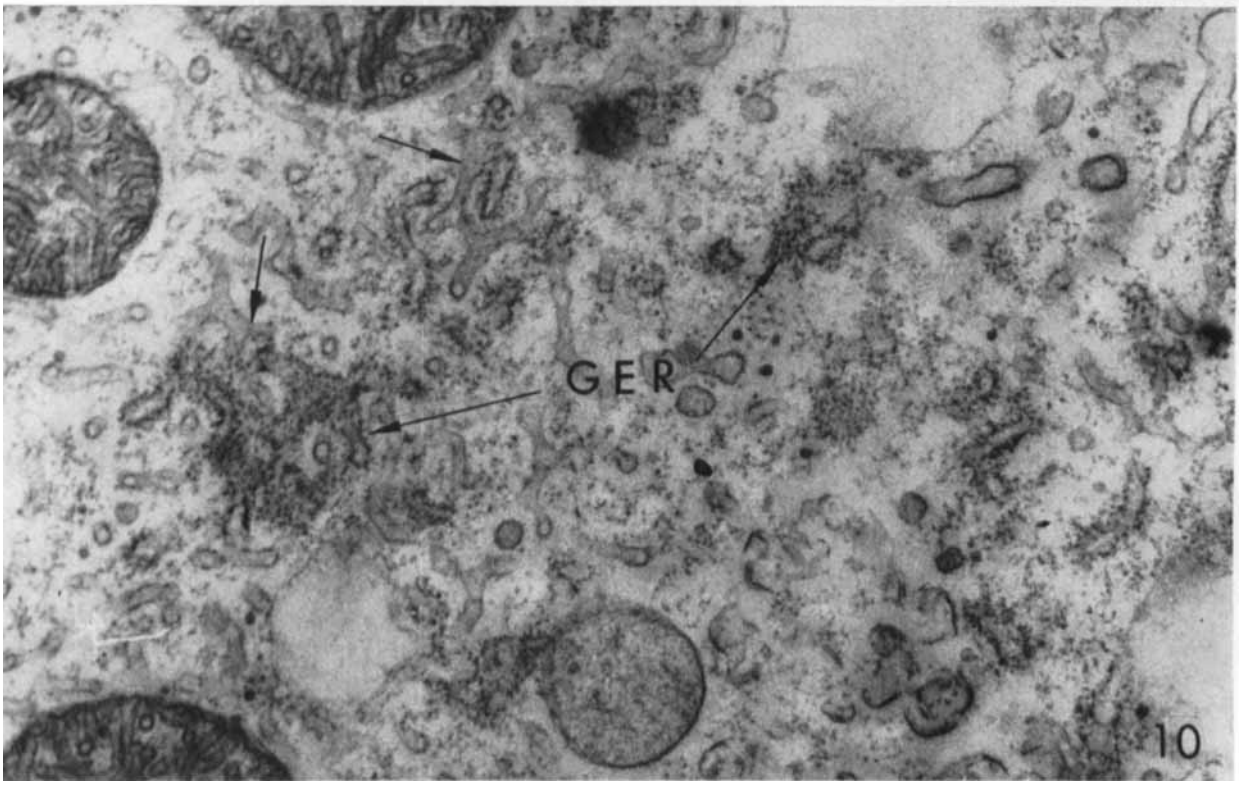
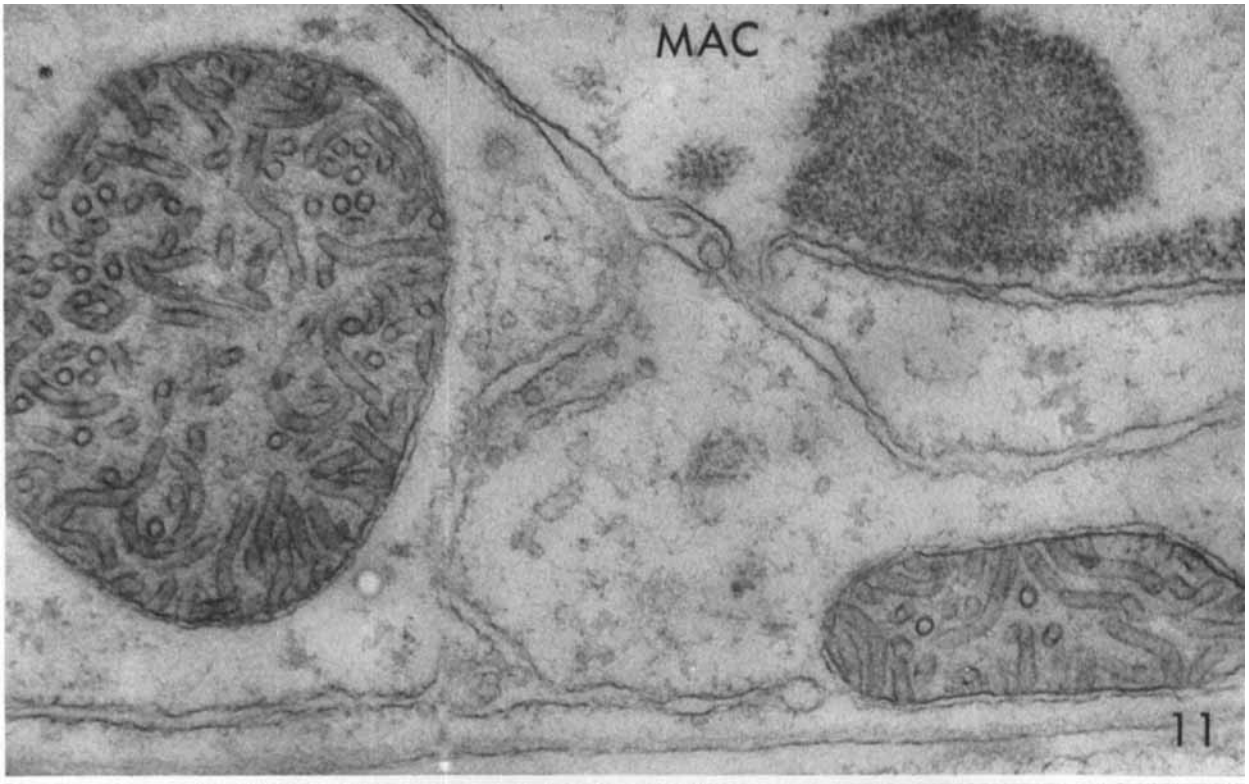


Fig. 9. An enlargement of the nephridial tubules showing their smooth surface and uniform diameter. The free ribosomes (unlabelled arrow) and mitochondria are randomly distributed among the tubules. × 29,500.

Fig. 10. Some of the nephridial tubules possess ribosomes on their outer surfaces which are interpreted as the granular endoplasmic reticulum. Regions of transition from nephridial tubules to granular endoplasmic reticulum are shown by unlabelled arrows. × 27,000.



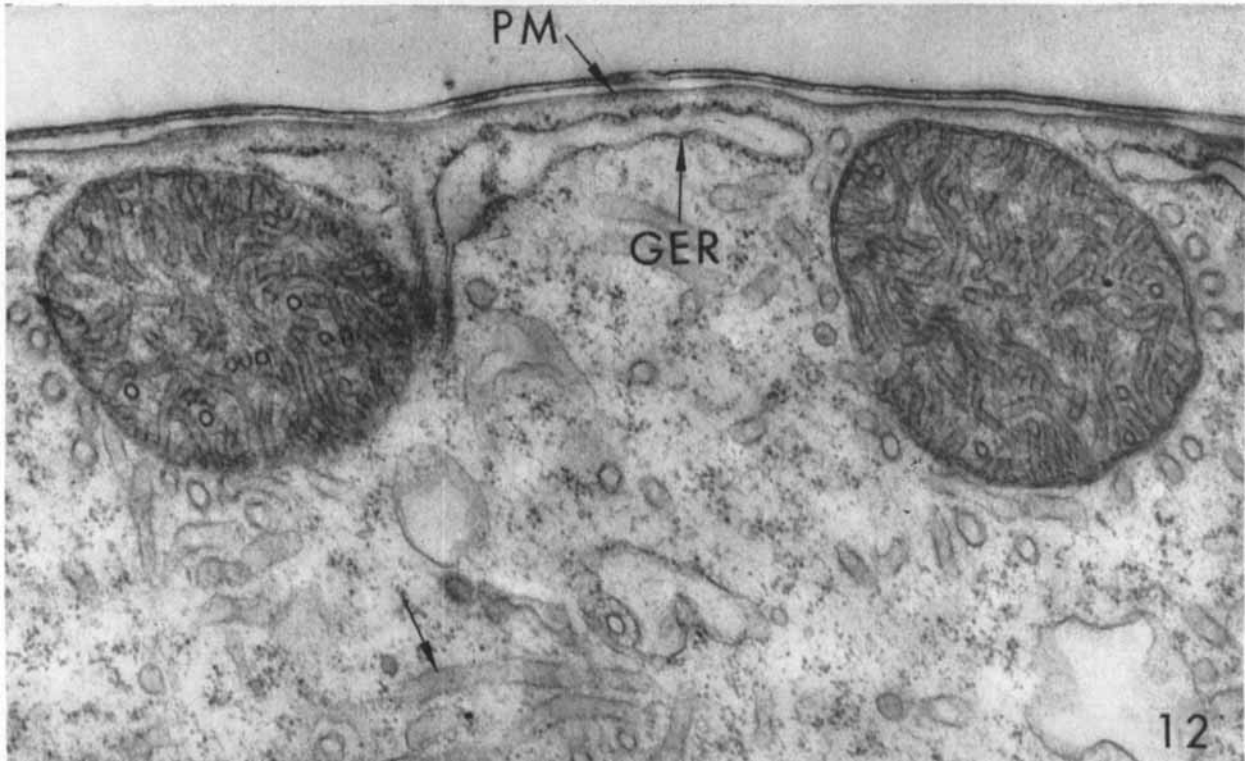


Fig. 11. The outer macronuclear membrane is continuous with the granular endoplasmic reticulum as shown here. The ribosomes do not show in this micrograph owing to poor fixation. Part of the macronucleus shows in the micrograph (MAC). × 27,000.

Fig. 12. The granular endoplasmic reticulum (GER) is also associated with the plasma membrane (PM) and the mitochondria. This micrograph also shows agranular endoplasmic reticulum which may be the nephridial tubules (unlabelled arrow). × 27,000.

from 0.01 to 0.1 μ are observed in close association with both the agranular and granular endoplasmic reticulum (Fig. 14) as well as throughout the cytoplasm (Figs. 1, 2). In another study, these have been identified as lysosomes. Whether they arise from one or the other type of endoplasmic reticulum has not been determined.

The relationship of the nephridial system with other cytoplasmic structures has been schematically presented in Fig. 15.

DISCUSSION

The nephridial system. The contractile vacuole has stimulated more research than any other protozoan organelle, beginning with the earliest microscopists (21). This is understandable because its striking rhythmic pulsations could be observed with even the crudest of light microscopes. With the advent of the electron microscope, its detailed morphology has been revealed in a number of protozoa(1, 16). As a result, its mechanism of operation is being elucidated.

Contractile vacuoles seem to possess certain structures in common, although some variation exists among the different groups of protozoa. They all are composed of a typical unit membrane and are surrounded by vesicles and tubules which open into the main vacuole. It has been proposed by Pappas & Brandt (15) that the vacuole wall of amoebae forms by the coalescence of small vesicles which contribute their membrane as well as their contents to the vacuole during diastole. Among the ciliates a well defined "nephridioplasm," made up of tubules and vesicles, provides channels for the flow of fluids from the cytoplasm to the contractile vacuole. The reported observations of numerous smaller vacuoles coalescing to form the contractile vacuole in ciliates(21) may be accounted for by the localized and irregular filling of the vacuole, frequently seen in *T. pyriformis* under the light microscope. These are not individual vacuoles as they appear to be. Rather, they are localized regions of the vacuole that fill at different rates. The apparent coalescence of smaller vacuoles results when the permanent channels between the various parts of the collapsed vacuole expand, forming the spherical vacuole which then increases in diameter until systole is initiated. There seems to be no unusual concentration of mitochondria in the tubular region, although they are clearly associated with the peripheral granular endoplasmic reticulum.

The structure of the nephridial system of *T. pyriformis* is less complicated than that of *Paramecium*, yet it may function in a similar fashion. Based on electron microscope studies of *P. aurelia* and *P. caudatum*, Schneider(20) concludes that systole is brought about by contractile fibers located in the walls of the vacuole, a view quite contrary to that held by earlier workers based on light microscope observations. Whereas views varied, both Nassonov(11,12) and von Gelei(7) were responsible for the generally accepted view that water enters the cell through the plasma membrane, increasing cell turgor until the intracellular pressure is sufficient to force the vacuole out of the pore, thus maintaining osmotic balance in the cell. The emptying was purely passive so far as the vacuole was concerned. Schneider(20) has clearly shown tubular fibers with a diameter of 200-250 Å extending over the surface of the vacuole. These are approximately the same size as cilia fibers (filaments) and the myofibrils of *Spirostomum ambiguum*(18), both of which are tubular and presumed to be contractile. In addition, Rudzinska(19) has described similar fibers of 180 Å in diameter associated with the contractile vacuole of *Tokophrya infusionum*. The contractile vacuole fibers in *T. pyriformis* possess about the same diameter (250 Å) as those described for other protozoa. However, they may function somewhat differently than those in *Paramecium*. Their location would imply that they may function not only in facilitating the emptying of the vacuole but also in opening the pore prior to the initiation of systole. As the vacuole becomes distended in diastole, the tension on the fibers increases. This pulls the walls radially, causing dilation of the pore (Fig. 3). Along with this action, the membranes closing the pore are stretched until they rupture, permitting the vacuolar contents to be discharged to the outside of the animal. It is impossible to generalize from the limited number of forms studied, but it may be that the rhythmic filling and emptying of the contractile vacuole of ciliates is closely associated with the contractile fibrils.

The nephridial tubules in the vicinity of the contractile vacuole seem to vary slightly in diameter during different phases of the vacuolar cycle. They have a diameter of approximately 200 mμ during systole (Fig. 2), whereas during diastole they are somewhat larger (Fig. 7). Apparently, as the vacuole fills a back-pressure is built up in the proximal tubules, causing them to enlarge in order to accommodate the fluid. The pressure is lowest at the termination of systole, hence the smaller diameter of the tubules. This system differs from that of *Paramecium* where the radial canals and ampullae intervene between the tubules and the contractile vacuole. These organelles apparently expedite the movement of fluids to the contractile vacuole. Perhaps the much larger *Paramecium* requires a more complex nephridial system than *Tetrahymena*.

Another striking difference between the nephridial systems of the two organisms is the discontinuity of the tubules and radial canals during systole in *Paramecium*, whereas in *Tetrahymena* there is no evidence

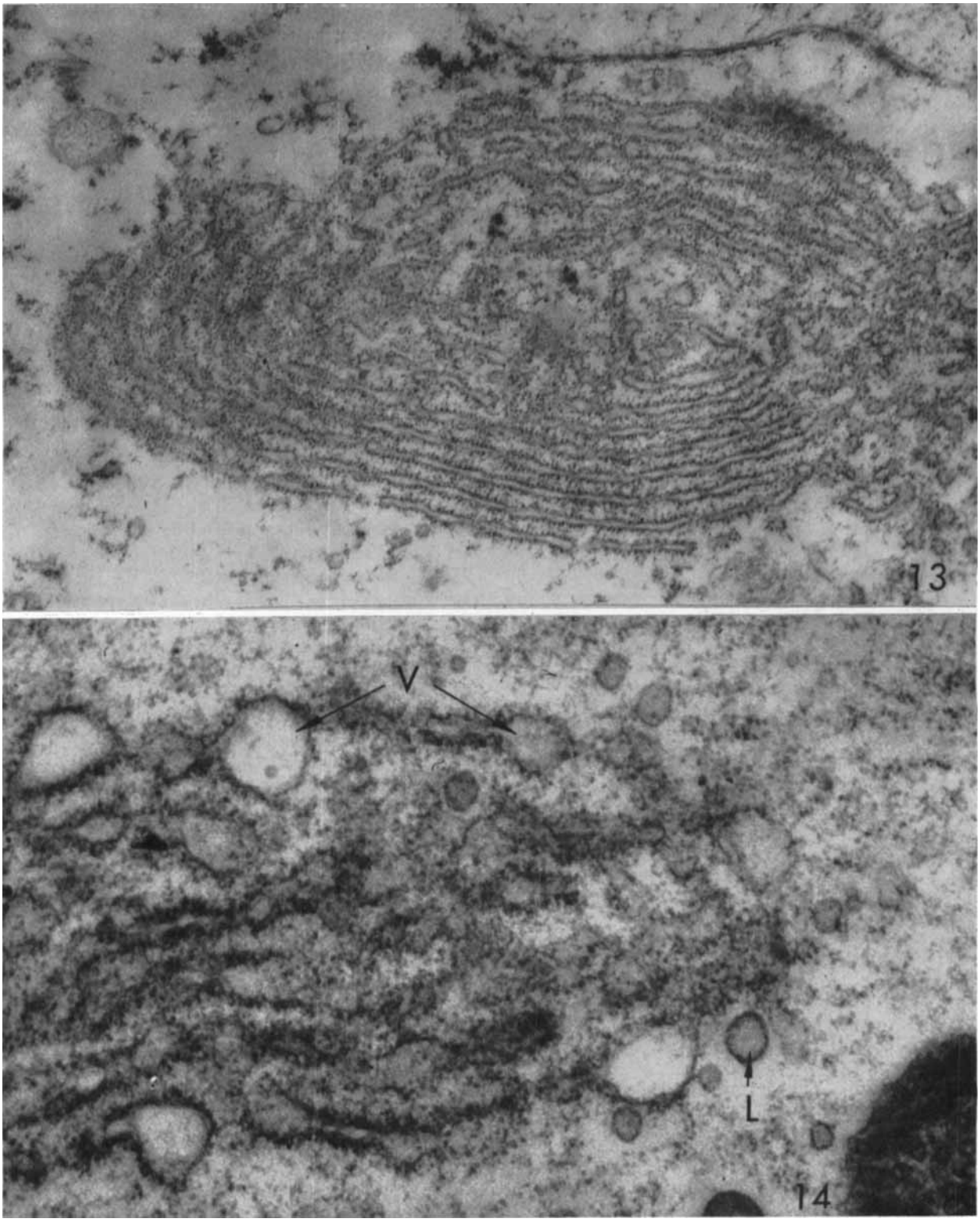


Fig. 13. The granular endoplasmic reticulum occasionally occurs in concentric patterns such as shown in this micrograph. × 28,000.

Fig. 14. A region of laminated granular endoplasmic reticulum showing vacuoles (V) at the closed ends. These may be water vacuoles. The smaller slightly dense granules are probably lysosomes (L). × 43,200.

that such discontinuity occurs. The tubules remain attached to the vacuole throughout the vacuolar cycle, thus constituting a continuous system of channels. The rhythmic breaking and mending of tubules with the radial canals in *Paramecium* as reported by Schneider(20) apparently facilitates the intermittent flow of fluids into the canals.

The endoplasmic reticulum and related vacuoles. The endoplasmic reticulum has been reported from representatives of all of the major groups of protozoa

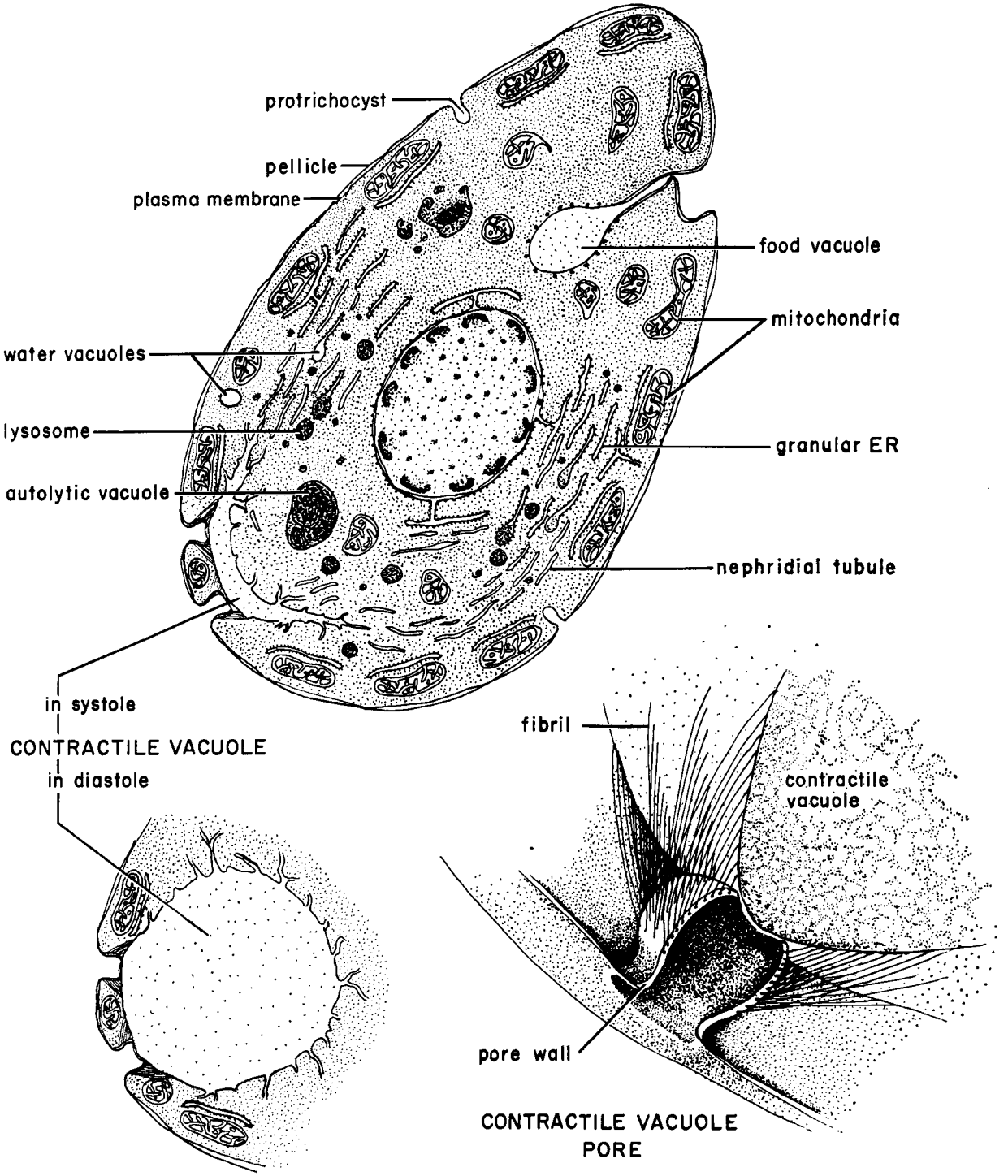


Fig. 15. A schematic representation of the nephridial system and related structures. The contractile vacuole is shown in sytole and diastole. The pore with its associated fibrils and contractile vacuole is shown in three diamensions. Other structures such as protrichocysts and autolytic vacuoles are shown. These are probably unrelated to the nephridial system.

(1,9). Both smooth and rough surfaced membranes are present and the cytoplasm contains numerous ribosomes. The granular endoplasmic reticulum observed in *T. pyriformis* resembles that seen in *Paramecium* (20). In both, it is continuous with the tubules of the nephridial system. Perhaps such a relationship exists in all ciliates. However, this arrangement is quite different from that found in sarcodinids and flagellates(1) where no such nephridial tubules are found. Perhaps this constitutes a major difference in these groups of protozoa.

The classical Golgi complex is found in the sarcodinids, flagellates, and sporozoa(1,16) but in ciliates it is not well defined. Noirot-Timothee(13) has demonstrated typical Golgi material in *Opalina ranarum*. This is additional evidence that this protozoan, long classified with the ciliates, is, in reality, a flagellate. The typical stacked, smooth surfaced membranes with terminal vesicles are not observed in *T. pyriformis*. However, the granular endoplasmic reticulum does occasionally become oriented into lamellae with vacuoles at their closed ends. These regions are osmophilic and may be the ones interpreted by light microscopists as the Golgi complex.

Since no typical Golgi complex can be identified in *T. pyriformis* and since the only smooth endoplasmic reticulum observed seems to be a part of the nephridial system, it may be that the latter actually constitutes the Golgi complex as was suggested by earlier workers (11). It fulfills the usual description of being composed of smooth surfaced membranes which are contiguous with the rough endoplasmic reticulum. However, it is not composed of stacked, flat sacs but rather is a branching system of tubules. Moderately dense vacuoles appear among the tubules which have been identified as lysosomes (unpublished). It has not been possible to determine whether they originate from the tubules or are in any way associated with them. Therefore, this study has thrown little, if any, light on the ciliate Golgi complex. Cytochemical techniques are presently being employed in an effort to elucidate this problem.

The rough endoplasmic reticulum could be the site for the initial segregation of water and other molecules in the cytoplasm. The ballooned rough endoplasmic reticulum near the plasma membrane, where water probably enters the cell in greatest quantity, would be immediately segregated from the cytoplasm. It could then flow through the channels of both the rough endoplasmic reticulum and the nephridial tubules to the contractile vacuole. Other products of metabolism could likewise be absorbed, some of which must be reabsorbed before reaching the contractile vacuole. Some essential metabolic products, as well as enzymes, may not be reabsorbed, thus appearing in the environment of the cell. There is ample evidence that *T. pyriformis* excretes amino acids(22) and such enzymes as proteinases(3) and ribonucleases (2). It is possible that the port of exit for these molecules is by way of the contractile vacuole. Enzymes produced in the rough endoplasmic reticulum could most directly reach the outside of the cell via the nephridial system. Moreover, it may be that all metabolic products are not reabsorbed and that some, particularly amino acids, are lost from the cell, possibly through the contractile vacuole. Obviously, more than morphological studies will be required to substantiate these speculations.

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Intraepithelial Protozoon, *Klossiella boae* n. sp. in the Kidneys of a Boa constrictor

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THE occurrence of intraepithelial protozoan parasites of the genus *Klossiella* in the kidneys of different mammals has long been recognized.

Type-species of this genus is *K. muris*(6). A detailed study on the life cycle of *K. cobayae* was carried out by Stevenson(7). *K. equi* was named by Baumann (1) who found it in the kidney of a horse from Hungary, and has later been reported from the American Jack(5), the zebra(3), and from the Mexican burro(2).[1]

According to Reichenow(4) the genus *Klossiella* is grouped within the order of Coccidia, suborder Adeleidea.

In guinea pigs two forms of schizogony have been recognized: the first in the endothelial cells of the glomerular capillaries resulting in the formation of merozoites which either infect more endothelial cells or, after arrival in the convoluted portion, infect the tubular epithelium, and the second in the tubules where schizonts are once more formed and gametocytes produced. The gametocytes infect in pairs the cells in more distal parts of the tubules. One parasite of each pair enlarges more than the other; the former appears to be the macrogametocyte. The other, the microgametocyte, divides once and produces two microgametes of which one fertilizes the macrogamete(7). In *K. equi* this stage has been called the "uninuclear phase"(5) and is seen as a mass of reticulated protoplasm in a cytoplasmic vacuole of an enlarged tubular epithelial cell. The nucleus of the parasite is situated at the periphery. This stage is followed by the "uninuclear enlargement phase" in which the protoplasmic mass becomes enlarged while chromatin particles gradually assume a peripheral arrangement. When the growth of the parasites continues, small buds are formed along the periphery of their protoplasms. This phase of development has been called the "peripheral budding phase." In the next stage, the "oocyst phase," the buds are cast off from the central mass and transformed into free sporoblasts. Finally, the sporoblasts enclose themselves in sporocysts. Within the sporocyst, numerous sporozoites are formed(1,2,7).

Differentiation of various species has been based on the number of sporoblasts in an oocyst. The highest number of sporoblasts is 40 and was found in *K. equi* (5), whereas *K. muris* produces 12-16 and *K. cobayae* 8-20(4).

MATERIAL

A *Boa constrictor* was brought for post mortem examination by a fakir. The animal had been suffering from a necrotizing stomatitis and subsequently died.

Microscopical findings

Although the degree of parasitism was low, it was possible to find the characteristic stages of the parasite in the kidneys.

The smallest forms observed were located near the apical tips of epithelial cells of some collecting ducts (Fig. 1, arrows). In most cases two small round or oval uninuclear parasites were found in each cell. They lay in separated vacuoles of the cell protoplasm. In some cases two parasites, of which one was larger than the other, were found in the same vacuole and were situated in the center of the epithelial cells (Fig. 2, arrow).

Uninuclear enlargement phases were found in several collecting ducts. The parasite protoplasm stained moderately basophilic and was vacuolated. In some, the chromatin particles were situated centrally, while others showed a more peripheral arrangement (Fig. 3) or even a distinct ring of nuclei along the borders. The nucleus of the host cell was sometimes compressed by the enlarged parasite in its cytoplasmic vacuole.

The peripheral budding phase was seen only once (Fig. 1). The central protoplasm of the sporont stained basophilic and showed some vacuoles. Many

[1] After preparing this paper I was kindly informed by the editor about another species, *K. tejerai* which occurred in the kidneys of an opossum *Didelphis marsupialis aurita*. (Scorza, J. V., Torrealba, J. F. & Dagert, C., 1957. *Klossiella tejerai* nov. spec. y *Sarcocystis didelphidis* nov. sp. parasitos de un *Didelphis marsupialis* de Venezuela. *Acta Biol. Venezol.* **2**, 97-108). Unfortunately, this journal is not available in the Netherlands.