MEMBRANE BEHAVIOR OF EXOCYTIC VESICLES

II. Fate of the Trichocyst Membranes in *Paramecium* after Induced Trichocyst Discharge

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ABSTRACT

A specific exocytic process, the discharge of spindle trichocysts of *Paramecium caudatum*, was examined by means of the electron microscope. This exocytosis is induced by an electric shock simultaneously in nearly all of the trichocysts (ca. 6,000–8,000) of a single cell. Single paramecia were subjected to the shock and then fixed at defined times after the shock so that the temporal sequence of the pattern of changes of the trichocyst membranes after exocytosis could be studied. The trichocyst vacuoles fuse with the plasma membrane only for that length of time required for expulsion to take place. After exocytosis, the membrane of the vacuole does not become incorporated into the plasma membrane; rather, the collapsed vacuole is pinched off and breaks up within the cytoplasm. The membrane vesiculates into small units which can no longer be distinguished from vesicles of the same dimensions that exist normally within the cell's cytoplasm. The entire process is completed within 5–10 min. These results differ from the incorporation of mucocyst membranes into the plasma membrane as proposed for *Tetrahymena*.

Exocytosis is the process in which cytoplasmic vacuoles fuse with the plasma membrane so that their contents can be released to the outside. This occurs in many excretory and secretory processes at all levels of eucaryotic life (31). During this event, in some cell types, the vesicle membrane is incorporated into the plasma membrane. In such cases the cell must have some means of regulating its total surface area so that it can maintain a constant size or shape; otherwise continuous exocytic activity would ultimately lead to an indefinitely large surface area.

Examples of exocytic processes in which the vesicle membrane becomes incorporated into the plasma membrane are found in mammals in the goblet cells of the digestive and the respiratory systems (13, 33), the exocrine pancreatic cells (14,

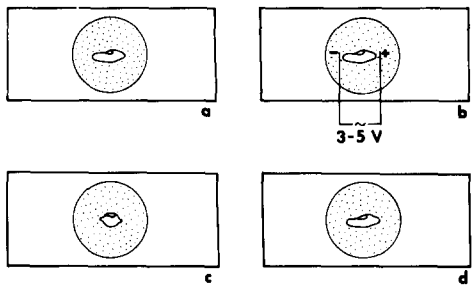
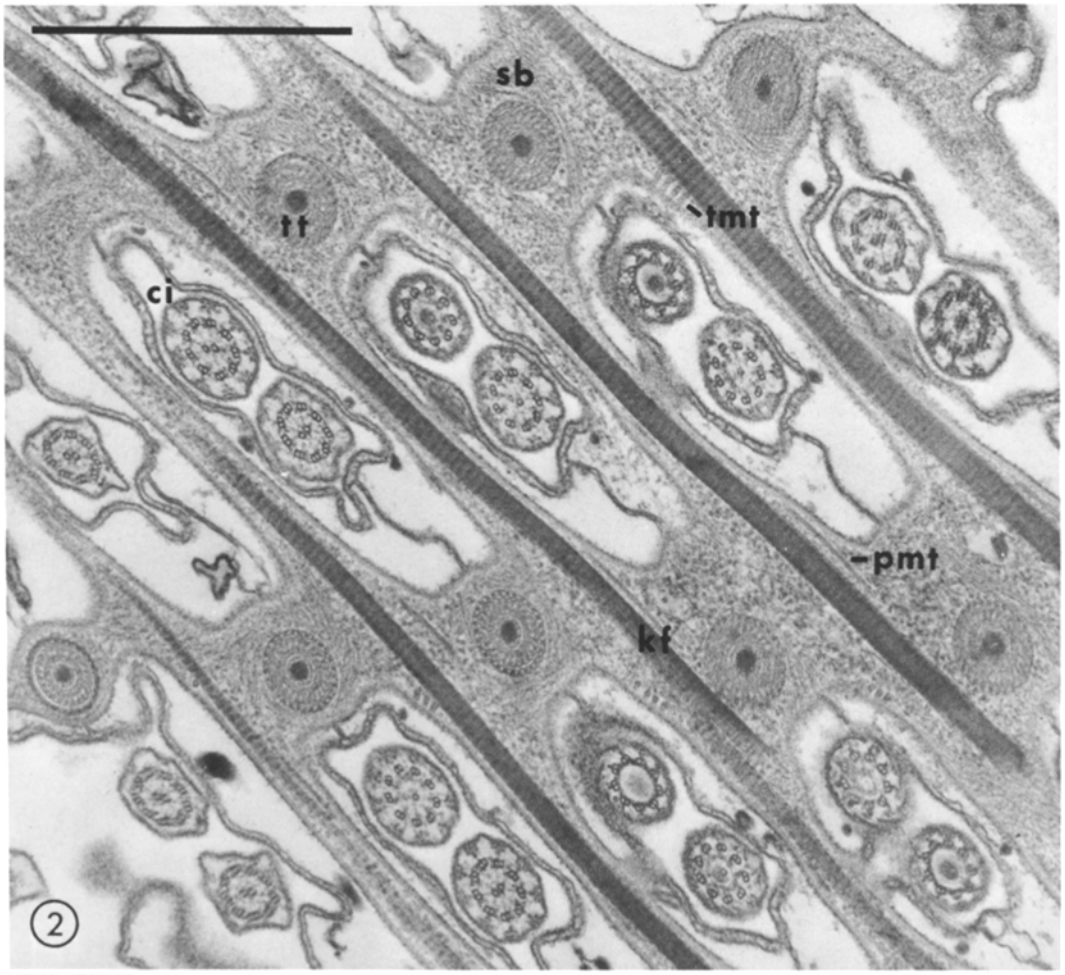


FIGURE 1 Method of inducing trichocyst expulsion in *Paramecium caudatum*. An electric shock of 3–5 V of AC current was applied along the posterior-anterior axis (*b*) of a single *Paramecium*. The cell is in a small drop of culture medium on a microscope slide (*a*). Trichocyst expulsion can be recognized by a simultaneous strong contraction of the cell (*c*). After some time the cell regains its normal shape (*d*).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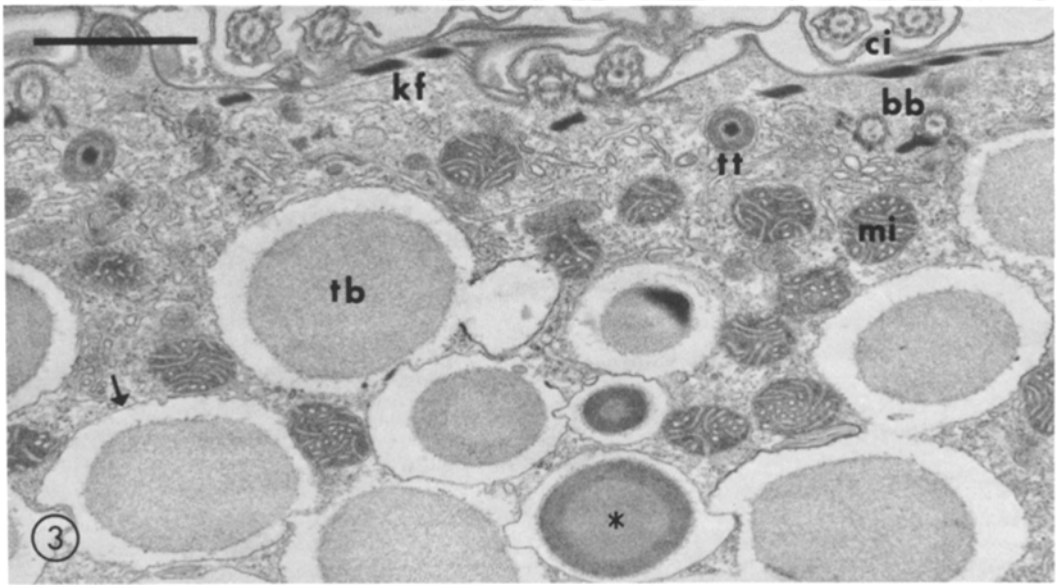
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23, 27), and nerve synapses (20, 21). No explanation of how the goblet cell's size is regulated has yet been proposed; however, a membrane recycling model has been suggested for exocrine pancreatic cells, although this has not yet been examined quantitatively. Heuser et al. (20, 21) have recently presented a detailed membrane recycling model based on morphometric investigations to explain the maintenance of a relatively constant surface area in nerve axons even though synaptic vesicles are continually fusing with the plasma membrane. They showed that the same amount of membrane surface is taken into the cell by endocytosis as is incorporated into the plasma membrane by exocytosis.

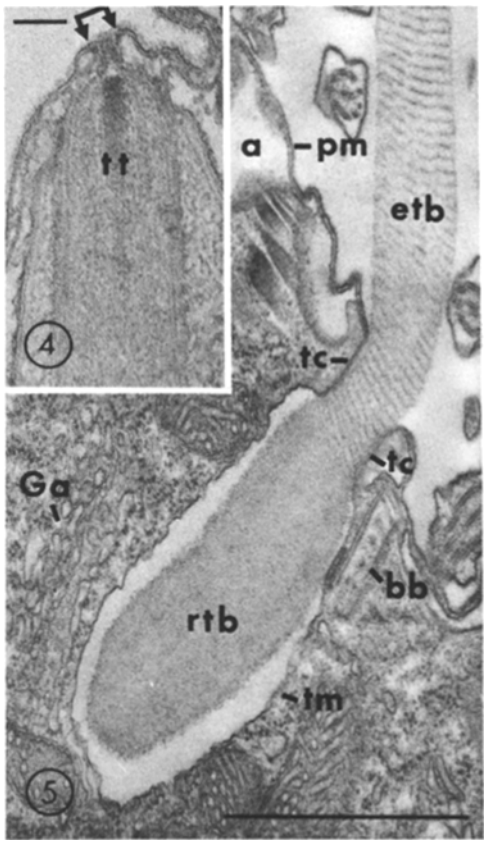


FIGURE 4 The first visible step of trichocyst expulsion is the fusion of the plasma membrane with the trichocyst membrane. A very narrow orifice is formed (arrows). *tt*, trichocyst tip. Bar indicates 0.1 μm. × 76,000.

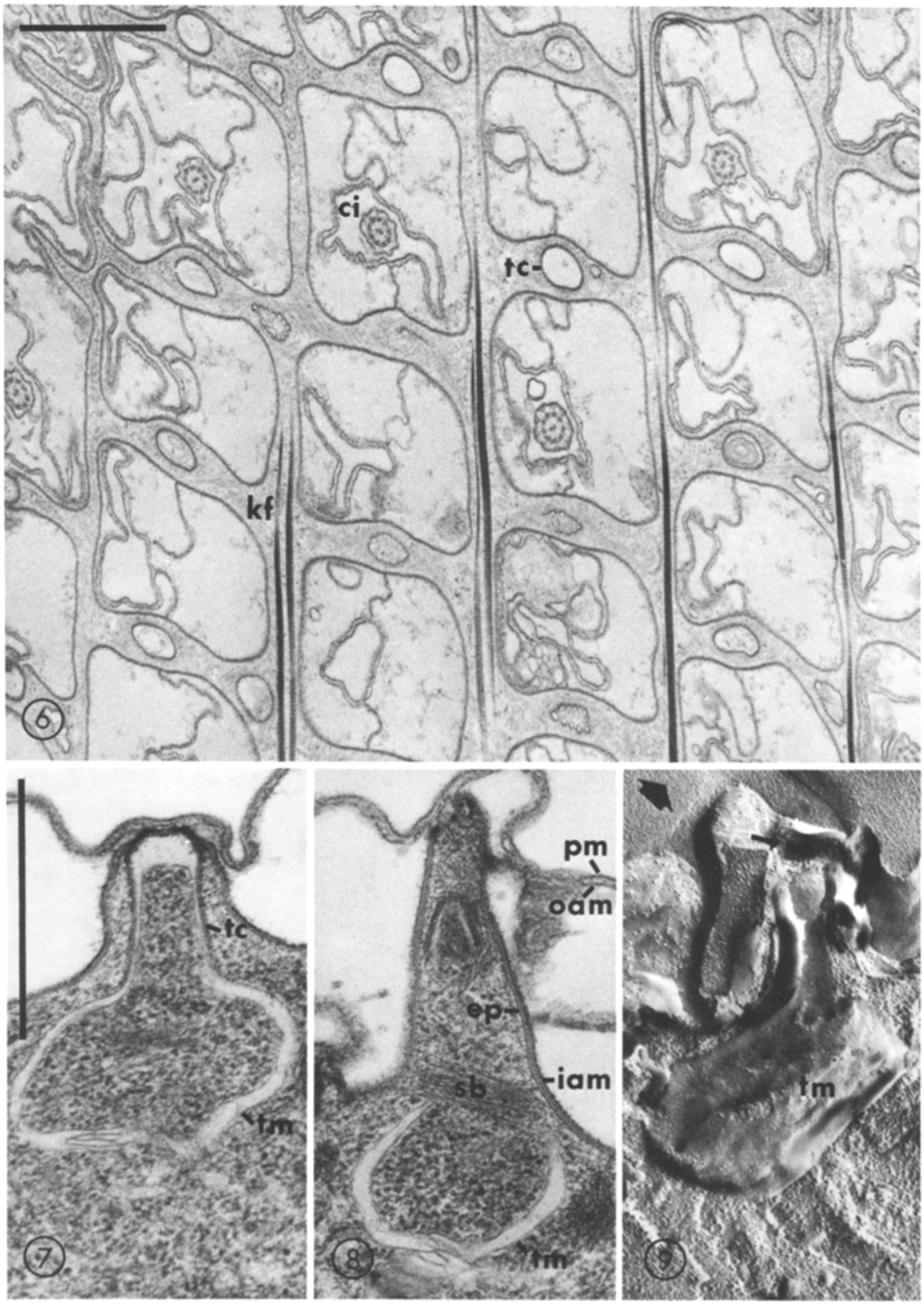
FIGURE 5 During expulsion the trichocyst is pressed through the tubular collar (*tc*) and at this level undergoes the well-known structural change to the 55-nm period. *a*, alveolus; *bb*, basal body; *etb*, ejected trichocyst body; *Ga*, Golgi apparatus; *pm*, plasma membrane; *rtb*, resting trichocyst body; *tm*, trichocyst membrane. × 33,000.

In addition to this mechanism of exocytosis, after which the vesicle membrane is incorporated into the plasma membrane, a second mechanism of membrane disposition is possible. In this case the vesicle membrane will fuse only temporarily with the plasma membrane during exocytosis and then immediately be pinched off probably as a flattened cisterna and transported back into the cytoplasm (27). Such a method can be verified most easily in those types of cells in which the incorporation of new membrane would disturb an existing, highly ordered pattern of the plasma membrane which has a relatively constant surface area. Ciliated protozoans furnish such an example since their pellicle, consisting of an outer plasma membrane and underlying membrane-bounded alveolar layer, is sculptured into a relatively invariable pattern and its surface area normally changes only with cell growth.

In the ciliates, at least three exocytic processes are known: excretion of the contractile vacuoles (10, 25, 37), defecation of food vacuoles (5, 11, 38), and ejection of extrusomes (4, 12, 15, 17, 22, 26). Excretion (10, 37) and defecation (5) proceed by the second method of exocytosis, i.e., the membranes of the exocytic vesicles (contractile vacuole and defecation vacuole) are not incorporated into the plasma membrane after exocytosis. However, little is known about the fate of the extrusomal membrane after ejection of these organelles, except for the assumption that in *Tetrahymena* the mucocyst membranes become part of the plasma membrane (34, 35, 36). The purpose of this study is to

FIGURE 2 A grazing section through the pellicle of *Paramecium caudatum*. Besides the cilia (*ci*) and the kinetodesmal fibers (*kf*), the trichocyst tips (*tt*) represent the main components seen at this level of section. *pmt*, posterior microtubules; *sb*, striated microfibrillar band; *tmt*, transverse microtubules. In all micrographs the bar indicates 1 μm unless marked differently. × 42,000.

FIGURE 3 A tangential section through the cortex at a level deeper than Fig. 2. The most predominant elements are the trichocyst bodies (*tb*) which are surrounded by a membrane (arrow). Amongst the mature trichocysts one occasionally finds immature organelles which are characterized by a more intense stain (*). *bb*, basal body; *ci*, cilium; *kf*, kinetodesmal fiber; *mi*, mitochondrion; *tt*, trichocyst tip. × 21,000.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see what happens to the membrane of another extrusome, the trichocyst membrane of *Paramecium caudatum*, after trichocyst ejection.

Resting trichocysts are relatively large membrane-bounded bodies having a diameter of 0.5 μm and a length of 3–4 μm. Their long axis is divided into a tip and a body; only the body undergoes a transformation during discharge. There are some 6,000–8,000 trichocysts in each cell, and they are regularly distributed within the cell's cortex, alternating with single or paired basal bodies. Their tips closely underlie the plasma membrane (Fig. 21 *a*).

An electric shock was used to trigger the ejection of nearly all trichocysts within a single cell. The cells were then individually fixed at specific times after the shock and prepared for electron-microscope examination.

MATERIALS AND METHODS

Paramecium caudatum was cultivated in hay infusion medium at room temperature. The triggering of the trichocyst ejection was accomplished by a method developed by Wohlfarth-Bottermann (40) and modified by us for our study. Single cells in small drops of culture medium (Fig. 1 *a*) were placed on microscope slides. These cells were then irritated while being observed under a dissection microscope. The irritation, lasting 1–3 s, was applied with two fine wire electrodes connected to a 3–5 V AC source (Fig 1 *b*). Trichocyst discharge occurred only when the cell was oriented so that the electric current was administered along the cell's anterior-posterior axis (Fig. 1 *b*). During trichocyst discharge the cell body could be observed, even at a very low magnification, to undergo a strong contraction (Fig. 1 *c*). A shock administered when the cell was oriented perpendicular to the current flow resulted only in an altered swimming behavior rather than in trichocyst discharge, i.e., cells rotated around their long axis very rapidly but did not change position. The cells were able to regain their normal shape (see Results) after this irritation (Fig. 1 *d*).

Single cells were fixed at specific times after the application of the electric shock: 0, 5, 10, and 30 s. and 5, 10, 15, and 30 min. Control cells were fixed without the shock treatment.

The cells were fixed at room temperature either with a brief treatment in 0.1 M cacodylate-buffered 2% glutaraldehyde (1 min) followed by a longer time in 0.05 M collidine-buffered 2% glutaraldehyde (20 min) or with only collidine-buffered glutaraldehyde (20 min). The cells were then washed in buffer and postfixed in collidine-buffered 1.0% OsO_4 for 45 min, washed again, and dehydrated in increasing concentrations of ethanol. During the last dehydration step 100% propylene oxide was used. The embedding medium was Epon 812. A Sorvall MT-II ultramicrotome (DuPont Instruments, Sorvall Operations, Newtown, Conn.) and a diamond knife were used to make the thin sections. The sections were picked up on Formvar-supported or unsupported grids, stained successively in uranyl acetate (39) and lead citrate (32), and examined in a Hitachi HU-11A electron microscope operated at 75 kV.

The method for the freeze-fracture technique will be described in detail in another paper (4).

RESULTS

The spindle trichocysts of *Paramecium caudatum* are advantageous organelles for the examination of exocytic processes because of their large size and high number and their predictable arrangement in the cell's cortex. Superficial sections of the surfaces of control cells show the trichocyst tips cut transversely (Fig. 2, *tt*), whereas in deeper sections the trichocyst bodies are found to fill the cortical cytoplasm (Fig. 3, *tb*). The entire extrusome is surrounded by a membrane (Fig. 3, arrow).

FIGURE 6 Grazing section through the pellicle of *Paramecium* immediately after the electric shock. Instead of trichocyst tips, annular structures are found in the pellicle (*tc*). *ci*, cilium; *kf*, kinetodesmal fiber; *tc*, tubular collar. × 22,000.

FIGURES 7 and 8 Longitudinal sections through empty trichocyst vacuoles. The membrane of the distal end of the vacuole is inverted into the proximal end (fixed immediately after electric shock). *ep*, epiplasm; *iam*, inner alveolar membrane; *oam*, outer alveolar membrane; *pm*, plasma membrane; *sb*, striated microfibrillar band; *tc*, tubular collar; *tm*, trichocyst membrane. × 40,000.

FIGURE 9 In freeze-fracture pictures one recognizes that after trichocyst ejection the membrane (*tm*) of the trichocyst vacuoles covers large areas and is not tubular as it appears in thin-sectioned profiles. The big arrow indicates the direction of the shadow casting; the small arrow indicates the accumulation of particles at the trichocyst tip. × 40,000.

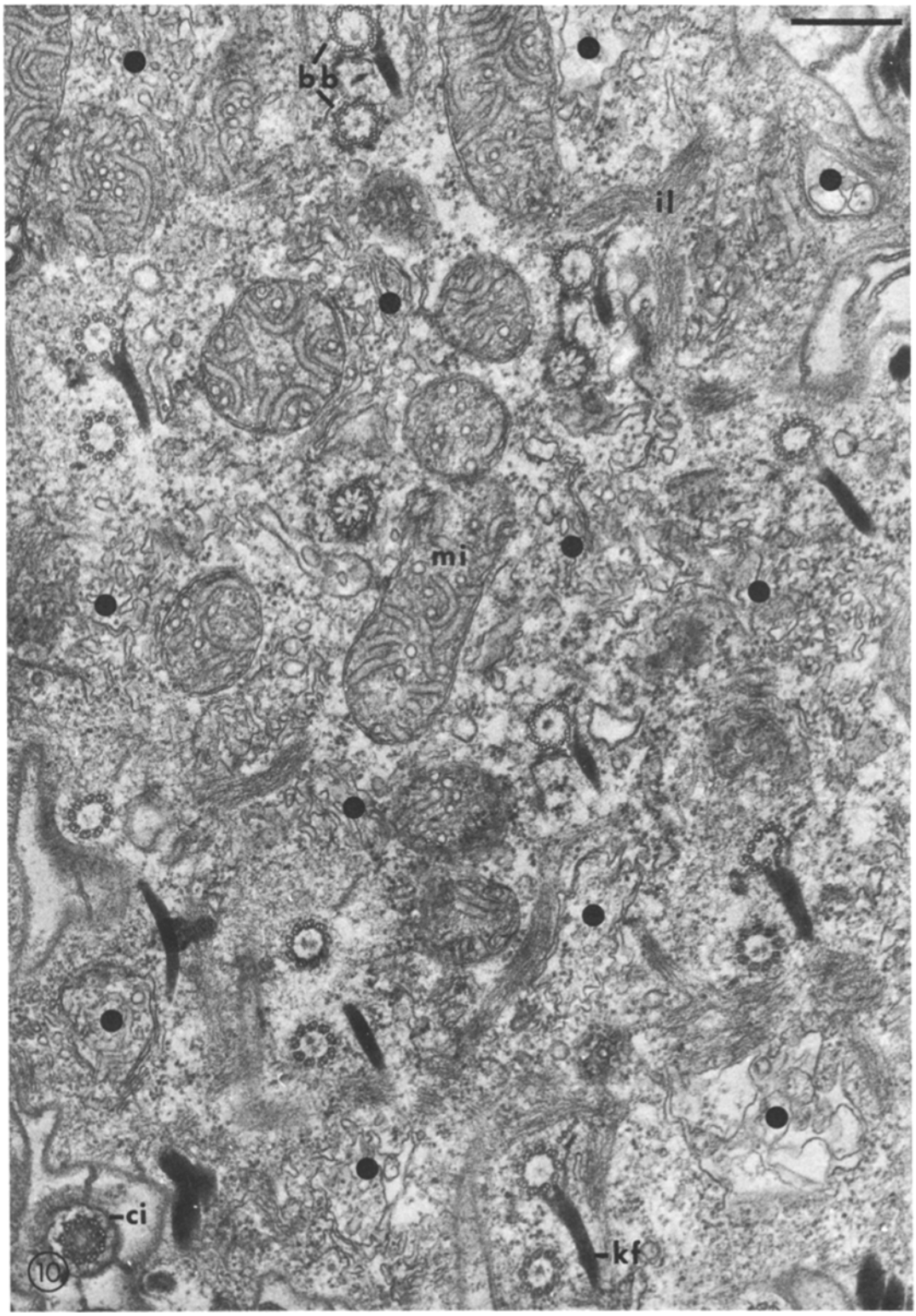


FIGURE 10 At a deeper level of the cortex of *Paramecium*, membranous accumulations can be observed at those sites at which the trichocysts are normally located (●) (immediately after the electric shock). *bb*, basal body; *ci*, base of cilium; *il*, infraciliary lattice; *kf*, kinetodesmal fiber; *mi*, mitochondrion. × 17,000.

Since the spindle trichocysts are unstable systems that discharge readily when subjected to various stimuli, some ejection stages can almost always be found after fixation even within control cells (Fig. 5). The first visible step of the ejection is a fusion of trichocyst and plasma membrane to form an extremely narrow opening (Fig. 4, arrows). This 40-nm diameter opening is smaller than the diameter of the rosette of particles which is found adjacent to the trichocyst tip in freeze-fracture preparations on the A face of the cytoplasm-adherent leaflet of the plasma membrane (see references 24, 30, and 35). As ejection continues, this opening widens until it reaches the diameter of the tubular collar (Fig. 5, *tc*); the structure of this collar has been described elsewhere (4, 6). As the trichocyst passes through this collar, as through a nozzle, it is transformed into an elongated shaft which has a 55-nm periodicity (Fig. 5, *etb*) (6, 18, 19).

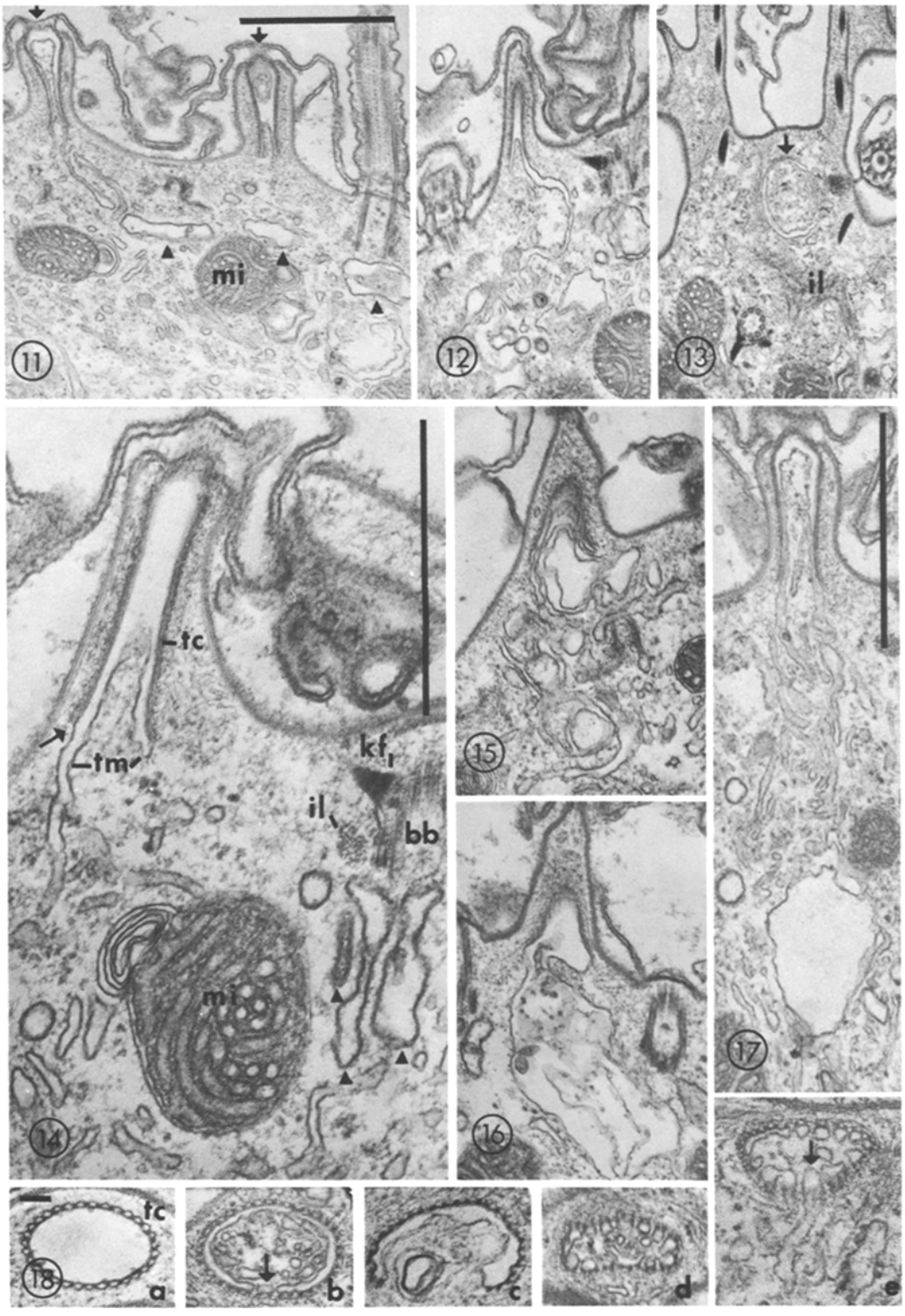
In grazing sections through the pellicle of cells fixed immediately after the application of an electric shock, one can recognize circular electron-transparent areas surrounded by cross-sectioned tubular collars in those places where the trichocyst tips are located in control cells (Fig. 6, *tc*). Sections made perpendicular to the cell surface confirm that the trichocysts are gone (cf. Fig. 6, *tc*, with Fig. 7, *tc*). Furthermore, one can see in these sections that the membrane of the trichocyst vacuoles (Figs. 7 and 8, *tm*) which had fused with the plasma membrane during trichocyst ejection (Fig. 5) is, after ejection, again separated from the plasma membrane and remains in the cell. Thus, the membrane of the trichocyst does not become part of the plasma membrane.

Due perhaps to the intracellular pressure and to suction arising from the rapid trichocyst ejection (a few milliseconds), the proximal end of the vacuole membrane is usually pulled up into the cavity of the tubular collar (Fig. 7), thereby forming a large cup-shaped flattened cisterna. With the freeze-fracture technique, it can be confirmed that the resultant structure which displays a tubelike profile when sectioned longitudinally (Figs. 7 and 8) is, in fact, a large flattened vacuole apparently formed by the inversion of the proximal half of the vacuole membrane into the distal half of the vacuole. In such pictures, one clearly recognizes large areas of the vacuolar membrane (Fig. 9, *tm*) rather than tubular channels.

In tangential sections at the level of the proximal parts of the basal bodies (Fig. 10, *bb*), as is indicated by the appearance of the kinetodesmal fibers (Fig. 10, *kf*) and the infraciliary lattice (Fig. 10, *il*), accumulations of membrane can be observed at those precise sites where the trichocysts are normally located (Fig. 10, ●). (The former location of the trichocysts can be determined by using the basal bodies as markers.) These membranous accumulations represent sections through the empty inverted trichocyst vacuoles (Fig. 10; the section orientation is perpendicular to Figs. 7–9). The cortical cytoplasm does not, however, appear more electron transparent, which might have been the expected result of the discharge of the large trichocyst bodies from this area but, in fact, may even be more opaque than in control cells (cf. Fig. 3 with Fig. 10). This apparent contradiction may be produced by the strong contraction of the cell immediately after the electric shock (Fig. 1 *c*). At this time the cytoplasm fills the region vacated by the trichocysts.

Some time after the electric shock the cell shape returns, more or less, to normal (Fig. 1 *d*). In these paramecia, the trichocyst membranes are no longer as tortuous as they were immediately after the application of the shock (cf. Figs. 7–10 with Figs. 11–13). Also, the cytoplasm seems to be more transparent again. Long tubelike profiles extend from the empty trichocyst vacuoles into the cytoplasm. The trichocyst vacuoles can be easily identified by their tubular collars and their specific arrangement in the cortex (Figs. 11 and 12). At times, flattened cisternae can be found, with overall circular profiles, which resemble the inverted vacuoles of trichocysts that have just been discharged (Fig. 13, arrow). However, their outline is more regular than the highly irregular outlines of vacuoles which can be observed immediately after the shock treatment (cf. Fig. 13, arrow, with Fig. 10, ●).

Near the residual trichocyst vacuoles the cytoplasm is filled with small, thin-walled vesicles (Figs. 11, 12, and 14) which can be easily distinguished from nearby thick-walled vesicles (Figs. 11 and 14, arrowheads). These vesicles with thick membranes, which presumably have an origin separate from that of thin-walled vesicles, are frequently found in the cortical region of ciliates where they often lie beneath the basal bodies (see reference 1). Oblique sections through the empty trichocyst vacuoles at this stage sometimes reveal varying numbers of concentrically arranged mem-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branes (Figs. 15 and 16). At this late stage, vacuoles may also be found in which the tubular collar is still present, but the residual membrane has undergone vesiculation into small units (Fig. 17). These micrographs demonstrate that the trichocyst membrane is ultimately broken up into small vesicles.

The tubular collar follows a somewhat different pattern of decomposition. This structure, which covers the outer surface of the distal part of the trichocyst tip (Fig. 14, arrow, and Fig. 18 *a*, *tc*), gives a rigidity to this part of the membrane (6) and must be required for normal ejection. The collar is still intact shortly after ejection (Figs. 6–9, 11, 12, and 14–17). The collar then begins to break down either at one side (Fig. 18 *b*, arrow, and 18 *c*) or simultaneously around its entire circumference (Fig. 18 *d*). In oblique sections it is seen that elongated tubelike vesicles form (Fig. 18 *e*, arrow) which bear a similarity to the oblong vesicular profiles previously described (cf. Fig. 18 *e* with Fig. 17). The decomposition of the tubular collar occurs after the vesiculation of the rest of the vacuole membrane. This may be due to its more complex and probably more rigid structure.

After the complete destruction of the empty trichocyst vacuoles, a granular material can be found in regions where trichocyst tips were previously located (Fig. 19, arrows). In deeper sections, at a level comparable to that in Fig. 10, there are no large accumulations of membrane to be seen (Fig. 20). The vesicles present are identical to those normally found in untreated cells in this part of the cytoplasm.

In order to obtain information on the time required and the series of events leading to the breakdown of the trichocyst membrane, the cells were fixed at specific times after the electric shock was applied (see Materials and Methods). It can be demonstrated that the entire decomposition may require 5–10 min for completion and that the time necessary to complete the process in different cells may vary considerably. The intensity of the electric shock varied slightly, and thus its effect on the cell must have differed also. Variation in size of the *Paramecium* cells and the distance of the electrodes from the cell's anterior and posterior ends were variables that could not be rigidly controlled and may have had an effect on the time required for the cell to recover from the shock. However, disregarding such time variations, it is obvious from this study that the bulk of the trichocyst membrane remains inside the cell after the trichocyst is ejected and is decomposed within a relatively short time into fragments which can no longer be distinguished morphologically from normally occurring cell vesicles.

DISCUSSION

This study demonstrates that, like the membranes of contractile and defecation vacuoles, the membranes of spindle trichocysts of *Paramecium*

FIGURES 11 and 12 Longitudinal sections through empty trichocyst vacuoles (arrows) (30 s after trichocyst ejection). *mi*, mitochondrion. × 25,000.

FIGURE 13 Cross section through an empty trichocyst vacuole (arrow). (30 s after trichocyst ejection). *il*, infraciliary lattice. × 25,000.

FIGURE 14 A higher magnification of an empty trichocyst vacuole (30 s after trichocyst ejection) clearly reveals thickness differences between the trichocyst membrane (*tm*) and the membrane of the vesicles (arrowheads; see also Fig. 11, arrowheads) located below the basal bodies (*bb*). *il*, infraciliary lattice; *kf*, kinetodesmal fiber; *mi*, mitochondrion; *tc*, tubular collar. × 47,000.

FIGURES 15 and 16 Oblique sections through empty trichocyst vacuoles reveal concentric membrane layers in the region of the ejected trichocysts (Fig. 15) or large flattened vacuoles seemingly within the empty vacuole (Fig. 16). × 37,000.

FIGURE 17 A subsequent stage of trichocyst vacuole disintegration. The membrane is almost completely decomposed into vesicles. × 37,000.

FIGURE 18 Destruction process of the tubular collar (*a*, *tc*). The destruction begins either at one side (*b* and *c*) or at several places around the periphery of the collar at the same time (*d* and *e*). The bar indicates 0.1 μm. × 60,000.

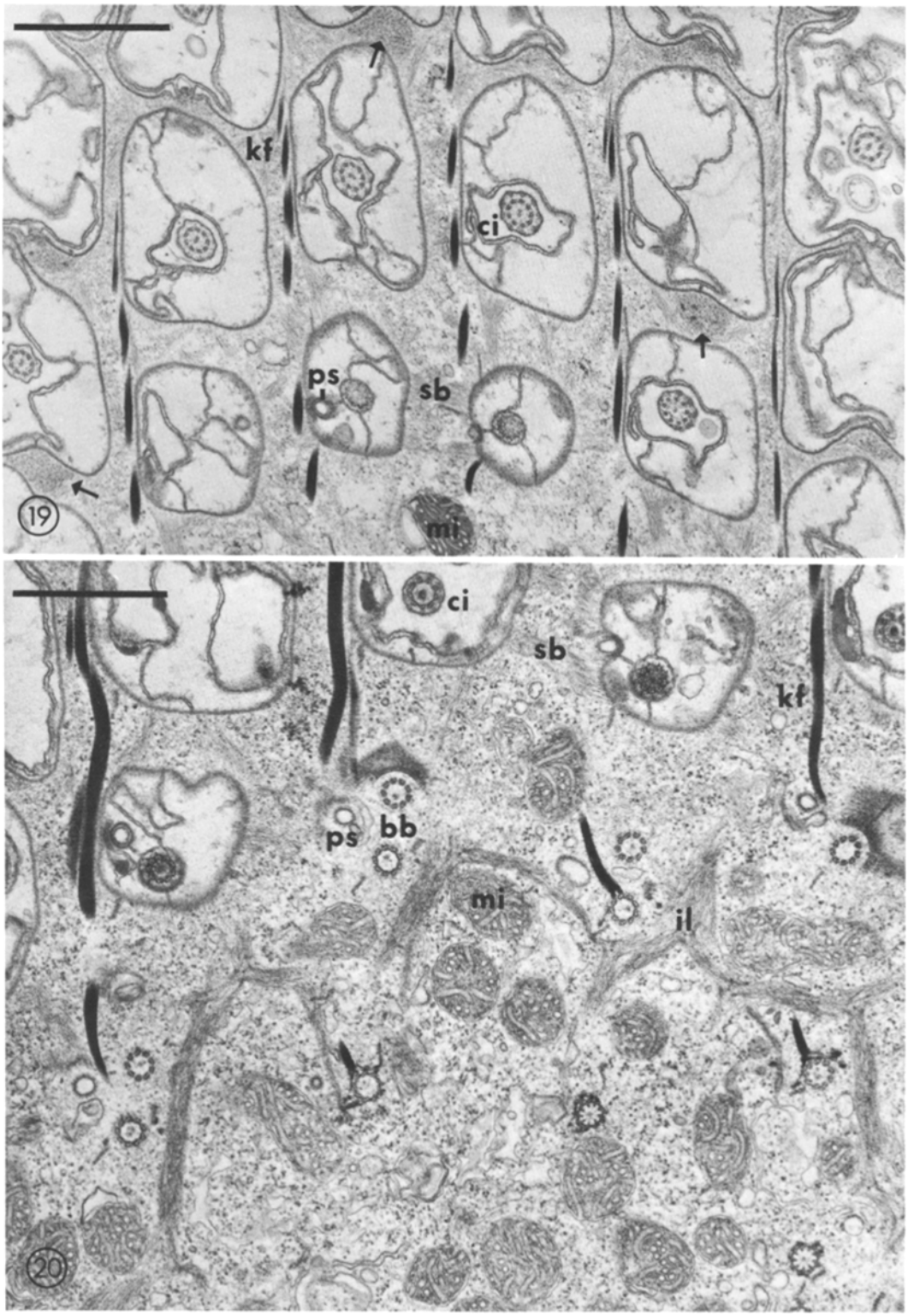
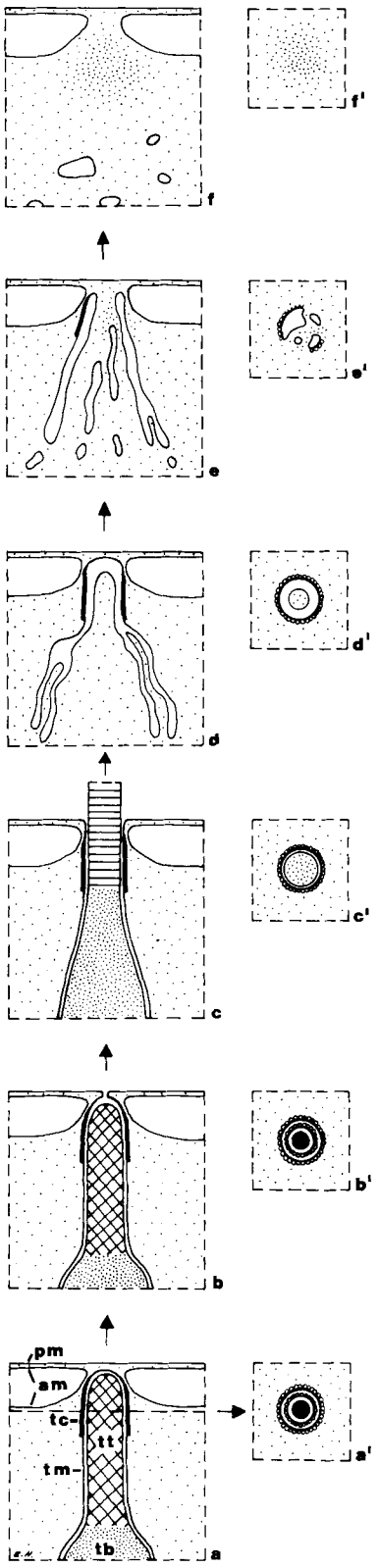


FIGURE 19 Grazing section through the pellicle of *Paramecium* (5 min after electric shock). Granular material is now located at the former sites of the trichocyst tips (arrows). *ci*, cilium; *kf*, kinetodesmal fiber; *mi*, mitochondrion; *ps*, parasomal sac; *sb*, striated microfibrillar band. × 25,000.

FIGURE 20 Section deeper in the cortex similar to the level in Fig. 10 (5 min after electric shock). Only an occasional accumulation of thin-walled membranes can now be seen at the level of the basal bodies. *bb*, basal body; *ci*, cilium; *il*, infraciliary lattice; *kf*, kinetodesmal fiber; *mi*, mitochondrion; *ps*, parasomal sac; *sb*, striated microfibrillar band. × 25,000.



caudatum are not incorporated into the plasma membrane but are pinched off into the cytoplasm after exocytosis. Before expulsion, the trichocyst membrane fuses with the plasma membrane (Fig. 21 *a* and *b*). Immediately after expulsion, the membrane of the vacuole is released back into the cytoplasm (Fig. 21 *d*). This process must take place very rapidly since we were never able to detect any stages of this pinching-off process but only the separated, flattened vacuoles. A similar situation exists in the "compound trichocysts" of the Microthoracidae (16). In these cells, only empty pinched-off vacuoles can be found after trichocyst ejection (Hausmann, unpublished observation).

Irritation of the cells with electricity does not appear to qualitatively alter the sequence of changes during or after trichocyst discharge; it effects only the discharge of a larger number of trichocysts at one time. The discharged trichocyst shaft, after electrical stimulation, has an appearance identical to that when fired under natural conditions. Also, the various stages of vacuolar disintegration seen in electrically shocked cells can be identified in control cells.

We can be fairly certain that most of the trichocyst membrane in *Paramecium* does not become incorporated into the plasma membrane since the presence of the tubular collar, sometimes recognizable for several minutes after expulsion, indicates that that part of the trichocyst membrane which lies closest to the plasma membrane does not change position. Incorporation of large amounts of membrane in a short period of time into only one of the membranes of the highly ordered and intimately associated pellicular membrane system of *Paramecium* (2) would surely cause large blisters in the cell's plasma membrane. Since no evidence, under normal conditions, for such an extensive separation of the plasma membrane from the underlying alveoli has been observed, we can be even more confident that trichocyst membranes are not incorporated into the

FIGURE 21 Schematic presentation of the trichocyst expulsion process (*a–c* and *a'–c'*) and of the destruction process of the trichocyst membranes (*d–f* and *d'–f'*) in longitudinal (left column) and in cross sections (right column) in *Paramecium*. For explanation, see text. *am*, alveolar membrane; *pm*, plasma membrane; *tb*, trichocyst body; *tc*, tubular collar; *tm*, trichocyst membrane; *tt*, trichocyst tip.

plasma membrane. On the other hand, our micrographs do not allow us to rule out the possibility that a small number of membrane subunits may be exchanged between the plasma membrane and the trichocyst membrane or that some lipid molecules or protein particles may be preferentially transferred from one membrane to the other.

These results for *Paramecium* differ from the hypothesis of Satir (34, 35) for *Tetrahymena* in which the mucocyst membrane, at least the lipid portion, is said to be incorporated into the plasma membrane. Satir and Satir (36) calculated that the membrane surface area of all the mucocysts of one cell would be sufficient to produce the plasma membrane of a daughter individual (including that covering the cilia). The conclusion is drawn that the mucocysts may be a source of new plasma membrane during cell growth and division. And yet, how the growth of other intimately associated pellicular membranes (12), i.e., the alveoli, is accomplished was not discussed. Experimental work will be required before it can be confirmed that this hypothesis for the fate of mucocyst membranes of *Tetrahymena* is correct.

The shape of the trichocyst vacuoles which persists briefly after normal expulsion also persists after the application of the electric shock (Figs. 7, 8, 11, 12, 13, and 21 *d*); this shape has already been described by other authors (7, 28, 30). However, only Pitelka (28) and Plattner et al. (30) have interpreted these observations in the manner confirmed by our experiments. De Haller and Heggeler (7) interpreted the vacuoles, which they saw after irradiating the cells with UV light, as being aberrant trichocysts caused by the effect of the UV light on the genes which control trichocyst development. In their opinion, trichocyst development at first occurs normally as already reported (8, 41, 42), but ultimately the trichocyst bodies, which are unstable due to a genetic disturbance, are resorbed, and only the empty trichocyst vacuoles migrate to the pellicle where they assume their normal position in the cortex. A quantitative study of this phenomenon has not been made. Ehret and McArdle (9) recently adopted this interpretation and used the term "cytoplasmic fingers" to describe the vacuoles, a term which has no meaning in our interpretation of the origin of these vacuoles. The vacuoles observed by de Haller and Heggeler were, in our opinion, the remnants of the trichocyst membranes left behind after trichocyst discharge which must have been induced by the fixation procedure used to prepare the cells for electron microscopy.

Within 5–10 min the empty trichocyst vacuoles undergo membrane vesiculation (Fig. 21 *e*, *e'*, and *f*). The collar-encased distal end of the trichocyst membrane seems to withstand disintegration for the longest time, probably because of its more complex structure (4, 6), but eventually this membrane segment also disintegrates. It is possible that the accumulations of granular material, which are found under the pellicle at the sites of the trichocyst tips 5–10 min after application of the electric shock (Fig. 19, arrows, Fig. 21 *f*), represent the macromolecular components that have previously formed the tubular collar that coats the outside of the membrane of the trichocyst tip. This granular material may be the same as the granulofibrillar material previously reported in the apical regions of the ridges of the pellicle (2), and the foregoing observations may explain the origin of this material.

The process of vesiculation of the trichocyst membrane which we have shown, and particularly the breakdown of the tubular collar region (Fig. 18 *b–e;* Fig. 21 *e*), can also explain Plattner's (29) observation that the intramembranous aggregations of particles on the A face of the plasma membrane disappear after experimentally induced exocytosis. The particles, which are arranged in annular rings in the plasma membrane of *Paramecium* at those sites adjacent to the trichocyst tips (24, 30, 35, 36), are interpreted as membrane-to-membrane attachment sites (30). When the trichocyst membranes normally resting below these sites are decomposed, the attachment sites are no longer necessary, and the particles presumably become randomly distributed within the plasma membrane.

The disintegration of the trichocyst vacuoles requires a longer period of time than some other vacuolar destruction processes. The defecation vacuole of *Paramecium caudatum*, for example, is decomposed into vesicles immediately after, or even during, the defecation process (5). However, in this system such a rapid transformation may be necessary since the membrane of the defecation vacuole appears to be recycled for new food vacuole formation (3). On the contrary, there is no evidence that the trichocyst membranes are involved in such a direct recycling process. It is possible, however, that these membrane vesicles may be incorporated into the endoplasmic reticu-

lum (ER) and thus be used again to form trichocyst membranes in a less direct recycling process. Spindle trichocysts have been shown to originate from the ER, and after a period of maturation, requiring up to 7 h in *Paramecium* (41) or even longer in other ciliates (42), they ultimately assume their final position in the cortex of the cell.

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