INTERNALIZATION OF MACROMOLECULES FROM THE MEDIUM IN SUCTORIA

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ABSTRACT

Tokophrya infusionum like all other Suctoria lacks an oral cavity. Its feeding apparatus consists of tentacles, long narrow tubes through which the contents of the living prey are ingested. For normal growth, reproduction, and longevity of clones, *Tokophrya* needs supplements deriving from the medium in addition to living prey. Since *Tokophrya* lacks a mouth, these supplements can reach the cytoplasm only through the complex structure of the cortex, which is composed of a three-membraned pellicle and a dense epiplasm. In addition, external to the cortex, an extraneous coat covers the whole organism. Only the outer pellicular plasma membrane is continuous; the other two and the epiplasm are interrupted by the outer plasma membrane which invaginates at intervals forming the so-called pits. The invaginated plasma membrane dips down into the cytoplasm where it extends to form a saccule. Experiments with cationized ferritin and Thorotrast provide evidence that internalization of these macromolecules takes place through the pits by pinocytosis. The membrane of the saccules of the pits forms invaginations which pinch off giving rise to small, flattened vesicles containing the tracers. The tracers were never found free in the cytoplasm but exclusively in the flat vesicles. These vesicles are thus the vehicles transporting macromolecules from the medium to the cytoplasm. The saccules of the pits are the natural loci of pinocytosis and together with the flattened vesicles perform an important function in Suctoria, supplying the organisms with macromolecules from the medium.

KEY WORDS Suctoria · pinocytosis · electron microscopy · cationized ferritin · Thorotrast

The major food supply in Suctoria consists of living ciliates and they are essential for the survival of the predators and their maintenance in cultures. Little consideration has been given to the medium as a possible source of nutrients, because of the unusual way in which these voracious organisms feed. Suctoria do not have a mouth opening and their feeding apparatus is composed of tentacles

(Fig. 1, *inset*), long thin tubes protruding outside the body where they terminate in rounded knobs covered by a membrane (18, 19, 21, 23). A part of the tentacle extends deep into the cytoplasm (18, 21, 23) and there it remains open at its proximal end (Fig. 18). Each tentacle has the capacity of functioning as an oral apparatus when a swimming prey becomes attached to the knob of the tentacle (19–21). The attachment triggers a number of events resulting in the rupture of the prey's plasma membrane which merges instantaneously with the membrane of the tentacle (19). Thus the two or-

ganisms come to share a common surface. At the same time the membrane of the knob starts to invaginate into the tentacle carrying with it the cytoplasm of the still living prey (21). At the end of the intracytoplasmic part of the tentacle, the invaginating membrane inflates and pinches off to form a food vacuole which is followed by a succession of additional vacuoles (19, 20). In this way, the contents of the living prey are transported into the body of the predator. In the past, it had been assumed that the growth requirements of the predator were supplied entirely by the prey. In fact, attempts to test the effects of experimental substances on the predator had often been done by first feeding them to the prey and then allowing the Suctoria to ingest them through the tentacles along with the prey organism. Later observations on the suctorian *Tokophrya infusionum* suggested an alternative hypothesis, however. They showed that although *Tokophrya* could grow for some time in Chalkley's medium, a well balanced salt solution (29), or even in distilled water, the conditions for reproduction, longevity and duration of clones improved immensely in a medium containing a diluted yeast extract (17). Apparently, for normal growth *Tokophrya* needs some supplements deriving from the medium in addition to living prey. It became obvious that there must be some way that substances from the medium are able to enter *Tokophrya*. This began a series of experiments with macromolecular tracers, the results of which are presented here. Preliminary results of these studies have been published previously (24–26).

MATERIALS AND METHODS

Cultures

Bacteria-free cultures of *T. infusionum* and *Tetrahymena pyriformis*, the food organism, were maintained as described in previous papers (17, 20). For the experiments, young *Tokophrya* from newly established subcultures were used. Subcultures are routinely prepared by transferring a loopful of *Tokophrya* embryos and three dense drops of *Tetrahymena* into fresh yeast medium. After 2 d, all *Tetrahymena* are consumed by the rapidly reproducing *Tokophrya* and on the fourth or fifth day the organisms are translucent and devoid of food vacuoles; the majority of them have already been starving for 1–2 d. Such cultures give a good supply of young starved organisms.

Electron Microscopy Procedures after Treatment with Tracers and Trypsin

All experiments with the ferritin and Thorotrast as well as with the enzyme trypsin were performed on living *Tokophrya*. Before the treatment with tracers, the organisms were removed

from the walls of the culture vessel and pelleted by light centrifugation (~200 g for 5–10 min). Most of the supernate was removed and the organisms were suspended in the experimental medium.

CATIONIZED FERRITIN: supplied by Miles Lab., Inc., Research Division, Kankakee, Ill., was prepared according to Danon et al. (7) and used with some modifiations. To each milliliter of suspended living organisms was added 0.1 ml of ferritin diluted to 0.5 ml with Chalkley's medium (29) and not with saline as recommended by Danon et al. (7), and buffered with Veronal HCl, pH 7.2, at a concentration 1/50 M of the Veronal. Michaelis' Veronal HCl buffer produced a precipitate which did not form by lowering the molarity of the Veronal. In controls, the ferritin was left out; otherwise, the processing was the same. The preparations remained at room temperature (19°C) for different lengths of time ranging from 15 min to 16 h and were gently shaken periodically, thereafter pelleted, washed twice in Chalkley's medium with Veronal HCl buffer, pH 7.2, and fixed in 2% glutaraldehyde in 0.05 M Veronal HCl buffer in Chalkley's medium, pH 7.2, for 1 h at room temperature, followed by two washes in Chalkley's medium, buffered with the same buffer, and two washes in cold 0.1 M sodium cacodylate buffer, pH 7.2, with 0.18 M sucrose. In the last wash, the organisms remained overnight. The following day, they were postfixed in cold 1% OsO₄ (wt/vol) in 0.1 M sodium cacodylate buffer, pH 7.2, for 1 h and thereafter washed twice in distilled water. After dehydration in ascending alcohols (70, 95, and 100%) and in propylene oxide, they were left overnight in a mixture of propylene oxide and Epon 180 (1:1) and finally embedded in Epon as described in previous papers (20, 23). Polymerization took place at 60°C for 24–48 h and for further hardening the blocks were kept for 24 h at 95°C. Sections were double stained with uranyl acetate and lead citrate and examined in a Philips 300 electron microscope.

THOROTRAST (THORIUM DIOXIDE): is commercially no longer available and became accessible through the courtesy of Dr. Jean St. Germain, Sloan Kettering Institute for Cancer Research, N. Y. The suspension (most probably 25%) was diluted 1:20 or 1:40 with yeast medium. To a soft pellet of living *Tokophrya*, 1–2 ml of the diluted Thorotrast was added and the preparation was left for 3 h at room temperature, with shaking from time to time. Controls were prepared in the same way without Thorotrast. The preparations were centrifuged and fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.2, for 1 h, rinsed three times in the same buffer with 0.18 M sucrose, and left in the cold overnight. Postfixation in 1% cold OsO₄ in the same buffer followed, and further processing was the same as in the experiments with ferritin.

TRYPSIN: The purified enzyme, trypsin Type III from bovine pancreas, 2× crystallized, dialyzed, lyophilized, and salt-free was purchased from Sigma Chemical Co., St. Louis, Mo. Living organisms were incubated in trypsin (1–5 mg/ml) in Chalkley's medium in Sorensen buffer, pH 7.2, at room temperature or 37°C for various lengths of time (12–22 min). *Tokophrya* is a sessile organism firmly attached to the walls of the culture vessel by an attaching disk (Fig. 1, *inset*). It is therefore easy to stop the action of the enzyme by pouring off the trypsin medium and rinsing the vessel several times with Chalkley's medium. This can be done quickly because no centrifugation is necessary and there is no need to use a trypsin inhibitor. Controls with the omission of trypsin were processed in the same way. Fixation, dehydration, and embedding were done as described above.

EXPERIMENTS WITH TRYPSIN AND FERRITIN: In some experiments, incubation of living *Tokophrya* in trypsin was fol-

lowed by exposure to cationized ferritin. Further procedures were the same as in previous experiments.

RESULTS

Observations on Living Tokophrya

To find out whether *Tokophrya* is able to take up macromolecules from the medium, it was essential to design experimental conditions which would not harm the organisms. In the experiments, only those conditions were used in which *Tokophrya* did not change shape or size and in which the tentacles continued to function normally. The feeding ability of the tentacles is one of the most important criteria of *Tokophrya*'s viability. This required careful observations with the light microscope on living organisms exposed to various concentrations of experimental substances used at various temperatures for different lengths of time. Light microscope observations on living *Tokophrya* had therefore to precede the electron microscope study and became a valuable prerequisite for reliable results.

For example, *Tokophrya* exposed to cationized ferritin in saline as recommended by Danon et al. (7) became crenated. When saline was replaced by Chalkley's medium (29), an inorganic salt solution suitable for culturing of free-living Protozoa, no crenation occurred and the shape of the body remained unchanged.

Tokophrya incubated in Chalkley's medium with ferritin at a concentration recommended by Danon et al. (7) did not show any changes in the shape of the body throughout the duration of the experiment which lasted up to 16 h. To check the viability of the organisms, food was introduced at intervals to samples of the experimental culture. Attachment of the prey to the tentacles was normal, and several hours after feeding reproduction took place as it did in control cultures. Thorotrast appeared also to be nontoxic. No changes in the shape of the organism could be detected and feeding as well as reproduction appeared to be normal.

Trypsin at concentrations of 1 mg/ml at room temperature produced shrinkage of the body of *Tokophrya* after a few minutes of exposure. The reaction was reversible, however, and organisms regained their normal shape when transferred to a trypsin-free medium. Fast recovery took place also in organisms incubated in trypsin for 20 min at a concentration of 100 mg/ml. When the organisms were removed from the experimental medium, feeding in all experiments was normal.

However, incubation at a temperature +37°C for 10 min in a concentration higher than 10 mg/ml resulted in permanent damage of most of the organisms as indicated by the loss of their ability to capture prey.

Electron Microscopy Observations

THE STRUCTURE OF THE CORTEX: Since, as mentioned previously, *Tokophrya* like other Suctoria lacks an oral cavity, and since it utilizes nutrients from the medium in addition to its prey, the surface seems the most probable route for the uptake of these nutrients. Accordingly, the surface structure has been studied in detail.

The body of *Tokophrya* is covered by an amorphous extraneous coat of low density ~0.3 μm thick (Figs. 1, 2, and 3). Below it, the cortex is composed of the pellicle and the epiplasm. The pellicle consists of three unit membranes (Fig. 2), and the epiplasm is a dense homogeneous layer ~0.07 μm thick (Figs. 2 and 3). In some other Suctoria the epiplasm is a more complex layer, ~0.4–0.8 μm thick (10). The extraneous coat, three pellicular membranes, and the epiplasm surrounding the *Tokophrya* could act as a barrier isolating the cytoplasm from the medium. However, a more thorough analysis of the pellicle disclosed that only the outer pellicular membrane is continuous. The other two are interrupted at uneven intervals by the invaginations of the outer membrane which disrupt not only the two membranes but also the epiplasm (Fig. 2). The two interrupted pellicular membranes and the epiplasm invaginate also but only for some distance (Figs. 7 and 9). The outer membrane extends further into the cytoplasm where it terminates as a small saccule (Figs. 2 and 9). The whole structure is the so-called "pit" (12, 24). Each pit is composed of an opening, a short neck, and a saccule (Fig. 9). The neck has a diameter of ~100 nm and is surrounded by a thick wall composed of the pellicular membranes and of the epiplasm. The membranes surrounding the neck are tightly apposed and difficult to resolve as separate units. The saccule is embedded in the cytoplasm, is ~250 nm wide, and most of it is covered by a single membrane, the invaginated external pellicular membrane which lines the whole pit. Pits are present not only around the body of *Tokophrya* but also on the surface of the tentacular shaft (Fig. 19).

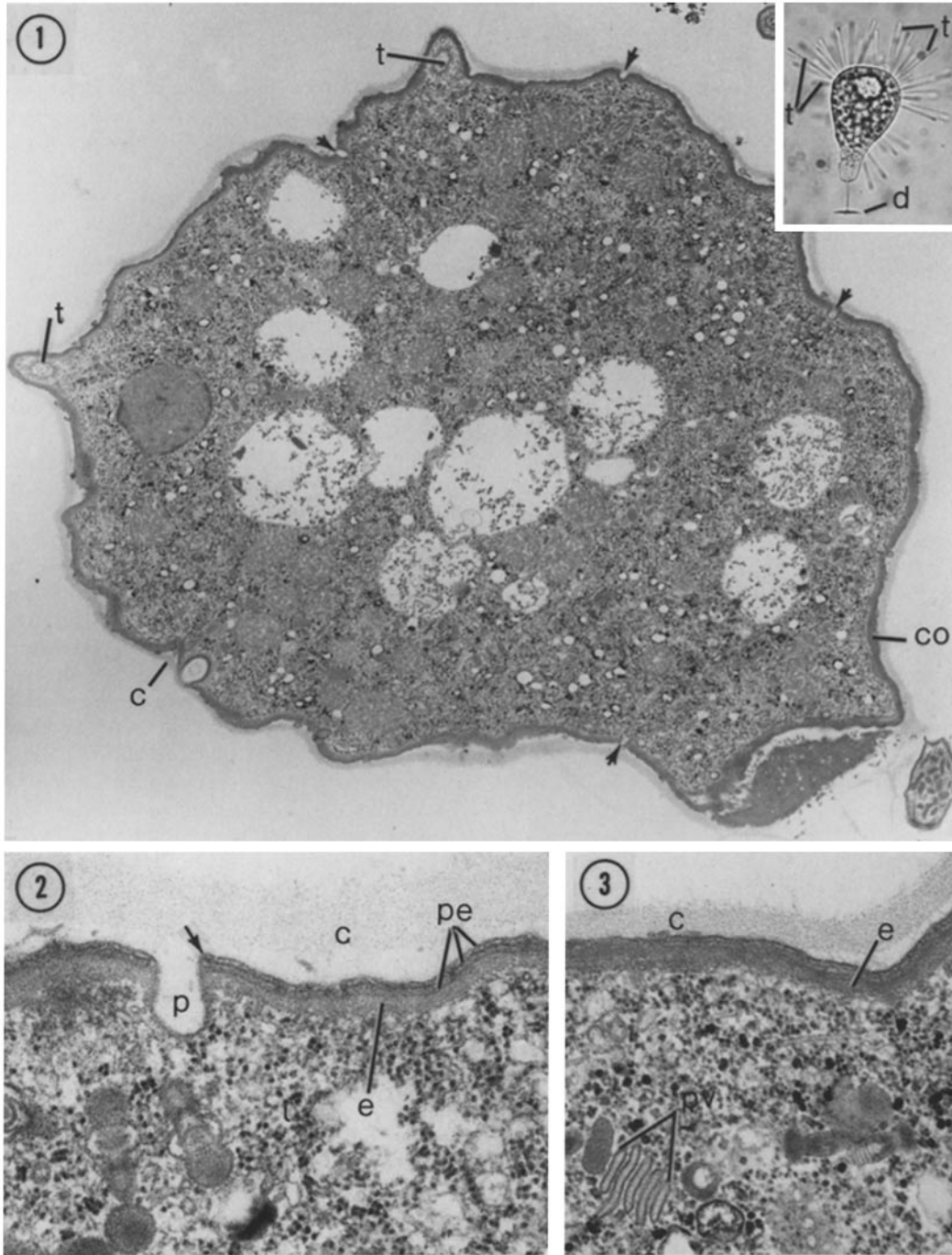DISTRIBUTION OF FERRITIN AND THOROTRAST: Ferritin is a regular, very dense,

FIGURE 1 Low-power electron micrograph of section of *T. infusionum*. The organism is surrounded by a dense cortex (*co*), in which pits can be seen at arrows. Outside the cortex is the extraneous coat (*c*). Cross-sections of tentacles can be seen at *t* and a part of the stalk at *s*. × 8,380. (*Inset*) shows a photomicrograph of a living *Tokophrya* with tentacles (*t*), the stalk terminating into an attaching disk (*d*). × 280.

FIGURE 2 Higher magnification of part of the cortex showing its structure. It is composed of a pellicle (*pe*) containing 3 unit membranes. The outer membranes invaginate at arrow and form a pit (*p*). Below the inner membrane is the dense epiplasm (*e*). Outside the cortex is the extraneous coat (*c*). × 44,850.

FIGURE 3 Shows clearly the extraneous coat (*c*) and the epiplasm (*e*). A pile of flattened vesicles (*pv*) can be seen in the cytoplasm. × 37,375.

small (7–10 nm) particle, easy to identify in electron micrographs. It is much smaller than ribosomes, which measure ~15 nm. *Tokophrya* incubated in cationized ferritin for 15 min to 16 h revealed a new unexpected structure extending outside the extraneous coat in the form of long (1–4 μm) wavy irregular fringes (Figs. 4–8). A similar structure has also been detected in *Tokophrya* treated with Alcian blue, Ruthenium red, and peroxidase (unpublished findings), and thus it is unlikely to be an artifact.

At the distal ends the fringes join together forming a kind of a pseudomembrane (Fig. 4). The whole structure has the appearance of a crown or halo closely adjacent to the extraneous coat and connected with it as if derived from it. In the region where the two structures meet, there is a dense accumulation of ferritin while the extraneous coat itself is often deprived of the tracer (Fig. 5) or is only slightly peppered with it in small areas (Fig. 6).

The presence and amount of ferritin granules in the coat depends upon the length of incubation, the pretreatment with trypsin, and the temperature of the trypsin medium. The longer the exposure to ferritin, the more ferritin granules are found in the coat, as seen in Fig. 7, incubated for 16 h, as compared with Figs. 5 and 6, treated with ferritin for 1 and 4 h, respectively. For example, the amount of ferritin in the coat of cells pretreated with trypsin at room temperature for 22 min (Figs. 9–15) or at 37°C for 12 min and then exposed for 3 h to ferritin (Fig. 8) is comparable to that of cells incubated for 16 h in ferritin without trypsin (Fig. 7).

Similarly, a dense aggregation of ferritin is found along the external membrane of the pellicle in organisms exposed only to ferritin for 16 h (Fig. 7) and also along the pellicle of organisms pre-

treated with 0.5% trypsin for 12 min at 37°C and then incubated with ferritin for 3 h (Fig. 8). The granules are not always attached to the membrane but very often at a distance of ~8–10 nm. Such a distance between ferritin and the membrane was also found in experiments with erythrocytes (4). Aggregates of ferritin are often found in the mouth and neck of the pit (Figs. 7–9, 12, and 14), and in the saccule (Figs. 10 and 14).

The most interesting finding is the presence and distribution of ferritin inside the organism. The tracer was never found free in the cytoplasm but always enclosed in vesicles. *Tokophrya* possesses two types of vesicles of very low density (23), one spherical with one or two osmiophilic caps (Figs. 6, 8, 9, and 12), the other flat, narrow, and elongate, about 380 × 40 nm (Figs. 3, 9–12, and 17). It is only the latter that contain the ferritin granules (Figs. 9–16). The flattened vesicles are scattered throughout the cytoplasm and are found often in the vicinity of the pits or connected with the saccules of the pits (Figs. 10–15). The saccules are usually regularly shaped as seen in Figs. 3 and 9. In many instances, irregular, asymetrical saccules deformed by one or more invaginations extend into the cytoplasm (Figs. 10–13). Some of these invaginations have a broad connection with the saccule (Fig. 10), others a very narrow one (Figs. 11 and 16), while still others form secondary and tertiary invaginations (Figs. 12 and 13). In Fig. 12, two flat vesicles with ferritin seem to be pinching off from one of the three invaginations. In Fig. 13, a small invagination of the saccule is connected with two vesicles containing ferritin which seem to be also pinching off. In this case, the two vesicles are ovoid rather than flat, but it is possible that flattening of some vesicles takes place after detachment. From time to time, a vesicle with ferritin connected directly with the saccule (Fig. 14) or a

FIGURE 4 Section through *Tokophrya* incubated in ferritin for 4 h. A new structure became visible in the form of a halo composed of long fringes (*fr*) surrounding the whole organism outside the extraneous coat (*c*). Arrows indicate the pits. × 7,840.

FIGURE 5 Section of organism incubated for 1 h in ferritin shows that the fringes (*fr*) are covered with ferritin granules. No ferritin is present in the extraneous coat. × 37,040.

FIGURE 6 After 4-h incubation in ferritin, some ferritin granules (*f*) appear also in the coat (*c*). The cytoplasm contains several vesicles with an osmiophilic cap (*o*). × 35,340.

FIGURE 7 *Tokophrya* incubated in ferritin for 16 h shows ferritin granules (*f*) in the coat and also along the outer membrane as well as at the entrance of the pit (*p*). × 37,375.

FIGURE 8 *Tokophrya* treated with trypsin (1 mg/ml) at 37°C for 12 min and incubated thereafter in ferritin for 3 h shows a similar distribution of ferritin as in Fig. 7. × 37,375.
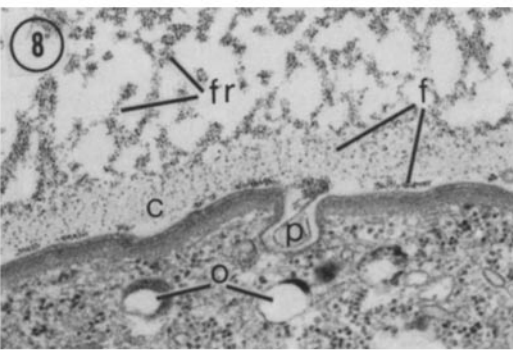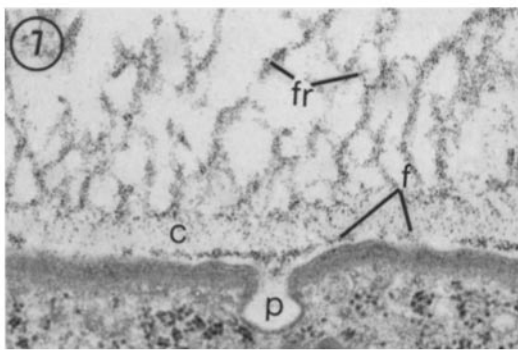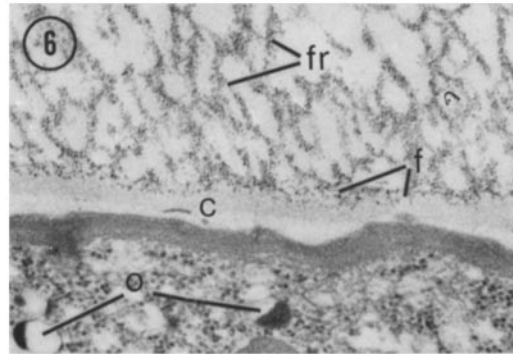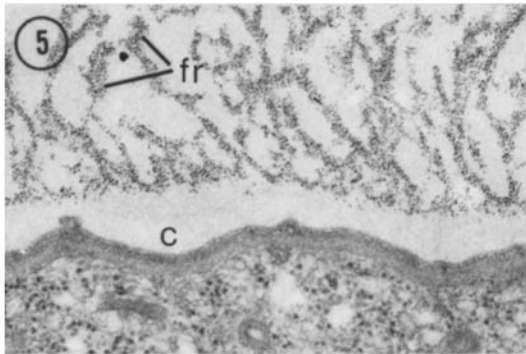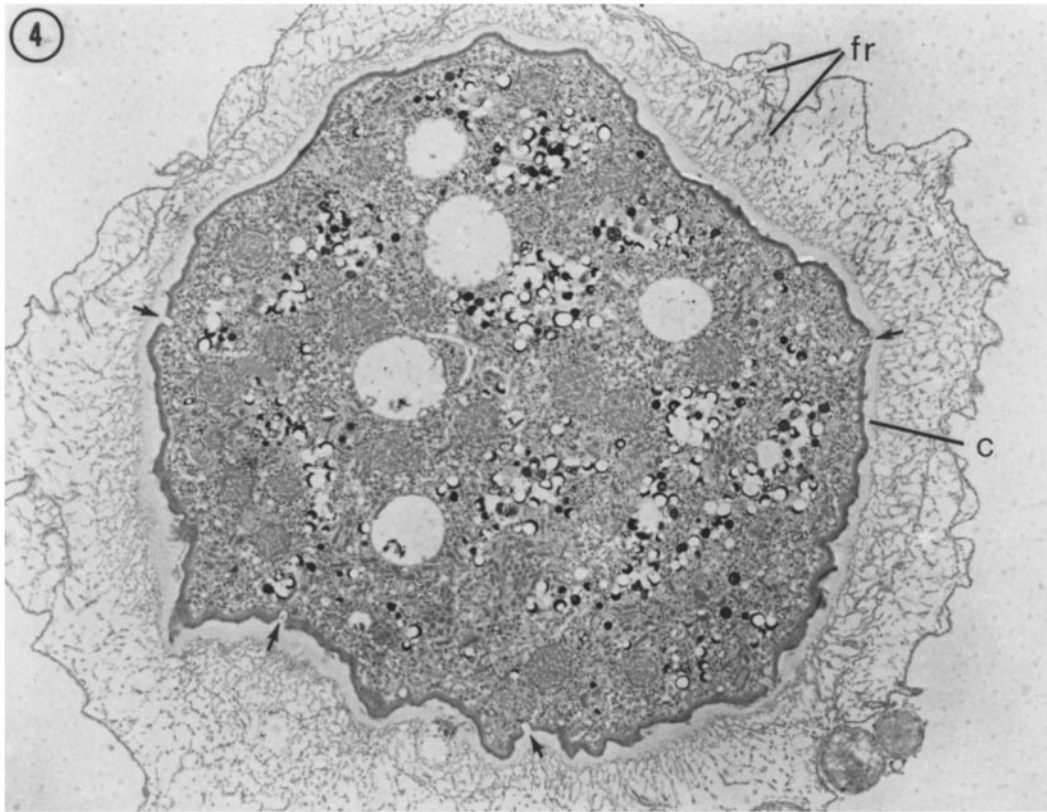
vesicle with ferritin in the last stage of pinching off from a saccule containing ferritin is seen (Fig. 15). The continuity of the membrane of a flat vesicle pinching off from a saccule is clearly visible in Fig. 16. The same figure also shows that the inner surface of the membrane in both these structures is coated by a fuzzy substance of low density. All these electron micrographs suggest strongly that the flattened vesicles derive from the saccule by invaginations of its membrane and supply evidence that ferritin enters from the medium to the vesicles through the saccules of the pits.

The flattened vesicles are often assembled in an orderly fashion in groups of three or more (up to 17) forming piles of vesicles (Figs. 3, 7, 17, and 20). Perhaps when the vesicles form one after another in close succession they pile up into heaps. The piles of vesicles are reminiscent of dictyosomes found in cells of plants and lower organisms. Since *Tokophrya* does not have a Golgi apparatus *sensu stricto*, the piles of vesicles have been considered as a primitive Golgi apparatus (17, 23).

The part of the tentacle projecting into the medium is also covered by three membranes (Fig. 19) and it too contains pits. Flattened vesicles with

ferritin are frequently found in the vicinity of the pits (Fig. 19). As described in previous papers (18, 20, 21), the tentacle is composed of an outer and inner tube (Fig. 18) separated by a wall of microtubules (Figs. 18 and 19), and the microtubules prevent the flattened vesicles from entering the inner tube.

In *Tokophrya* incubated in Thorotrast, the marker is found in the extraneous coat but not in the fringes which are therefore not visible. In the cytoplasm, it is present exclusively in the flattened vesicles (Fig. 20). The pits are the places where the granules of Thorotrast accumulate (Fig. 20), and presumably the Thorotrast would pass from the pits to the vesicles by the same pathway as does the ferritin.

DISCUSSION

The Role of the Pits

The pits are permanent structures and are characteristic for the subphylum Ciliophora to which Suctoria belong (30). Suctoria possess cilia as do all other members of Ciliophora. Each cilium is surrounded at its proximal end by a pit. The cilia disappear early in the life cycle of Suctoria, but

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FIGURES 9–15   are from organisms pretreated with 0.1% trypsin for 22 min at room temperature and incubated in ferritin for 3 h.

FIGURE 9   Regularly-shaped pit (*p*) with ferritin (*f*) at its entrance. Ferritin is present also along the outer membrane in the extraneous coat (*c*) on the fringes (*fr*) and in the cytoplasm, in a vesicle (arrow) belonging to a pile of flattened vesicles (*pv*). Several vesicles with an osmiophilic cap (*o*) are present in the cytoplasm. They do not have ferritin. × 37,375.

FIGURE 10   Two pits (*p*) with ferritin inside. One of the saccules of the pits has a large invagination on its side. The arrow shows in the latter the continuity of the outer membrane with the membrane lining the pit and the invagination of the saccule. In a group of three flattened vesicles, two contain ferritin (*f*). × 44,850.

FIGURE 11   Saccule of a pit with a narrow invagination (arrow). Several flat vesicles contain ferritin (*f*). × 59,800.

FIGURE 12   Ferritin entering a pit with three invaginations. From one of them (arrow) two flattened vesicles with ferritin (*f*) seem to be pinching off. Several vesicles with an osmiophilic cap (*o*) do not contain ferritin. × 37,375.

FIGURE 13   Saccule with an invagination (arrow) from which two vesicles with ferritin are pinching off. × 63,360.

FIGURE 14   A small vesicle containing ferritin is attached (arrow) to the saccule of the pit (*p*). × 59,800.

FIGURE 15   A small vesicle with ferritin is in the last stage of pinching off (arrow) from a saccule containing ferritin. × 56,370.

FIGURE 16   Higher magnification electron micrograph to show continuity between the membrane of the pit's saccule and a flat vesicle still connected by a narrow bridge (arrow). Note that a fuzzy coat covers the inner surface of the membrane of the pit, of the bridge and of the flat vesicle. × 113,000.
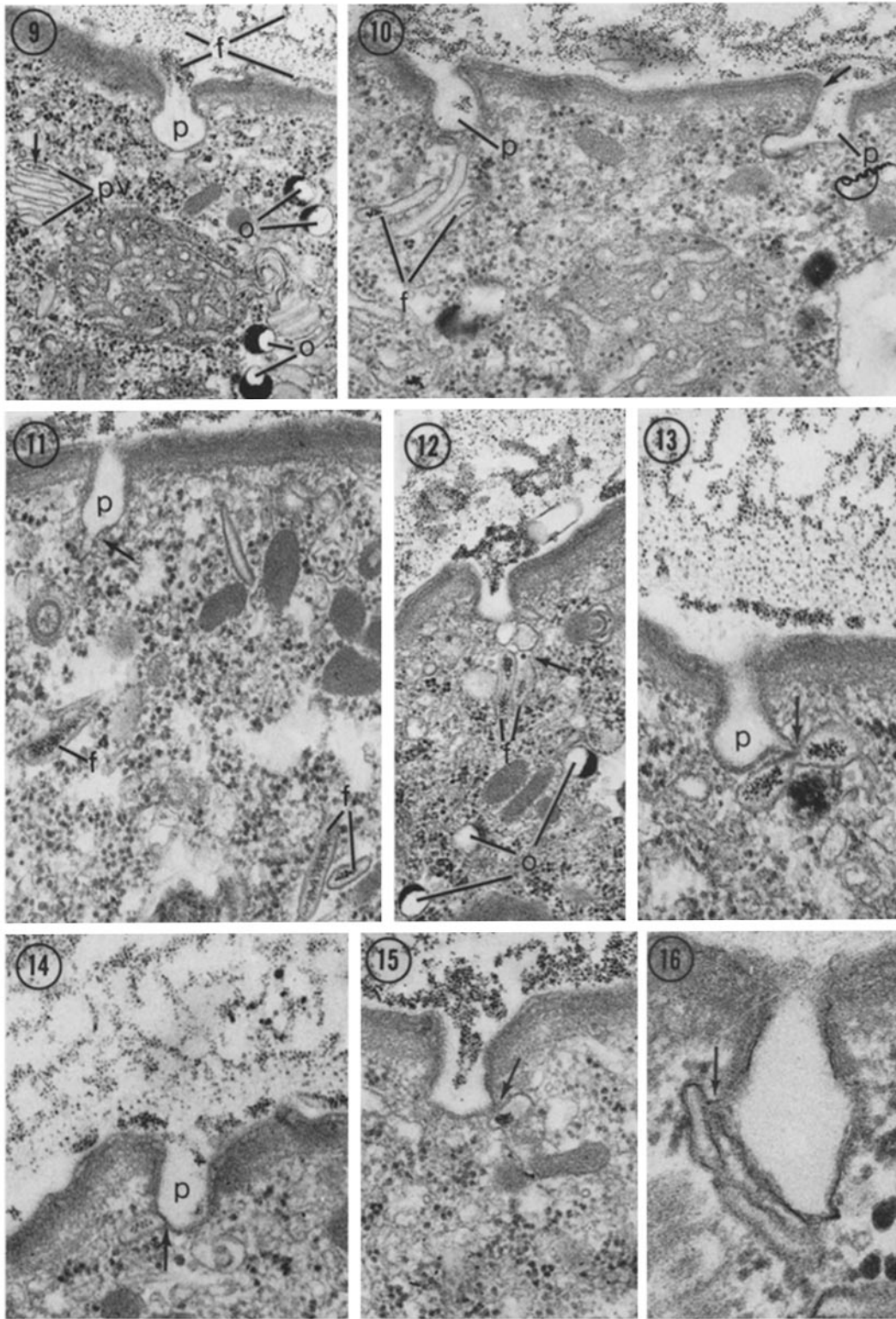
the pits remain throughout the adult sessile life.

The role of the pits is of great interest. The first attempts to unravel their function were undertaken on a ciliate, *Trichodinopsis paradoxa* (13). Thorotrast used in these experiments revealed the presence of the marker inside and outside the pits, as well as in the cytoplasm in vesicles and in food vacuoles. The results although notable were not conclusive because *Trichodinopsis* also possesses an oral apparatus by which the tracer could have been packaged into food vacuoles. In ciliates during the course of digestion, small vesicles containing digested material or undigestable particles such as Thorotrast are pinched off from the food vacuoles. In the *Trichodinopsis* experiments, these vesicles containing Thorotrast may have been derived from food vacuoles and may have fused with the membrane of the saccules. To reach conclusive results concerning the role of the pits, the organism used for such experiments should lack an oral cavity. This requirement is met by Suctoria, which do not have a buccal cavity. Thus, whatever enters the organism from the medium must pass through the pellicular sheaths covering the body. In a mutant strain of *Tetrahymena* in which no food vacuoles are formed, the pellicular membranes apparently do not act as a barrier to the entrance of small molecules (15, 16); however, macromolecules cannot traverse the membranes (15, 16). These findings are undoubtedly applicable to *Tokophrya* also since the fine structure of its cortex is very similar to that of *Tetrahymena*.

The experiments with ferritin and Thorotrast provided convincing evidence that, in *Tokophrya*, macromolecules from the medium reach the cytoplasm through the pits. Supplements from the medium are essential for normal growth and reproduction as well as for the longevity of the clones. No work has been done on the identification of these supplements; however, some of them might well be macromolecules and as such may be unable to pass through the pellicular membranes. As demonstrated in this paper, the pits provide such a route.

## Structures Involved in Internalization of Macromolecules

The pits are permanent specialized organelles composed of an opening, a neck, and a saccule. While the opening and the neck are surrounded by the pellicular membranes and by the epiplasm, the saccule itself is lined by a single membrane. This membrane is a continuation of the plasma membrane covering the whole body of *Tokophrya*. The regular shape of the saccule becomes distorted by invaginations of its membrane, which eventually pinch off to form flattened vesicles. Since it is generally accepted that attachment of particles to the plasma membrane is a prerequisite for endocytosis (3, 27), it is reasonable to assume that when ferritin or Thorotrast bind to the membrane of the saccule, invagination of its membrane may be stimulated as well as interiorization of the tracers into the flattened vesicles. This is a simplification of the very complex process of endocytosis which is still not fully explored and understood (3, 27). The sequence of events and structures involved, however, are better known. The saccules can be safely regarded as the natural loci of pinocytosis and the flattened vesicles as the vehicles transporting macromolecules to the cytoplasm.

FIGURE 17 Piles of flattened vesicles (*pv*), most with ferritin. × 58,900.

FIGURE 18 Section through tentacle (*t*) shows that the tentacle is a tube which extends not only to the outside but also deep into the cytoplasm. It shows also that the part of the tentacle extending to the outside is composed of an inner (*i*) and an outer (*ou*) tube, separated by a wall (*w*) of microtubules (seen in Fig. 19,under *m*). No coat (*c*) covers the tentacle. Ferritin is attached to the outer membrane, to the fringes (*fr*) and can be seen also in the pits (arrows). × 17,670.

FIGURE 19 Higher magnification of the part of the tentacle extending to the outside shows that the pellicle (*pe*) covering the tentacle is composed of three membranes continuous with the membranes of the body. The outer membrane forms pits (*p*), from which most probably originate the flattened vesicles located nearby. In one of them ferritin (*f*) is present. Ferritin is present along the outer membrane and in the entrance of the pit. Microtubules (*m*) indicate that the plane of sectioning passes through the wall separating the inner and outer tube. As described in Fig. 18, this wall is composed of microtubules. × 46,975.

FIGURE 20 *Tokophrya* incubated in Thorotrast for 3 h does not display fringes. The particles of Thorotrast (*Th*) are present along the coat (*c*), in the pits (arrow) and in some flat vesicles. × 47,120.
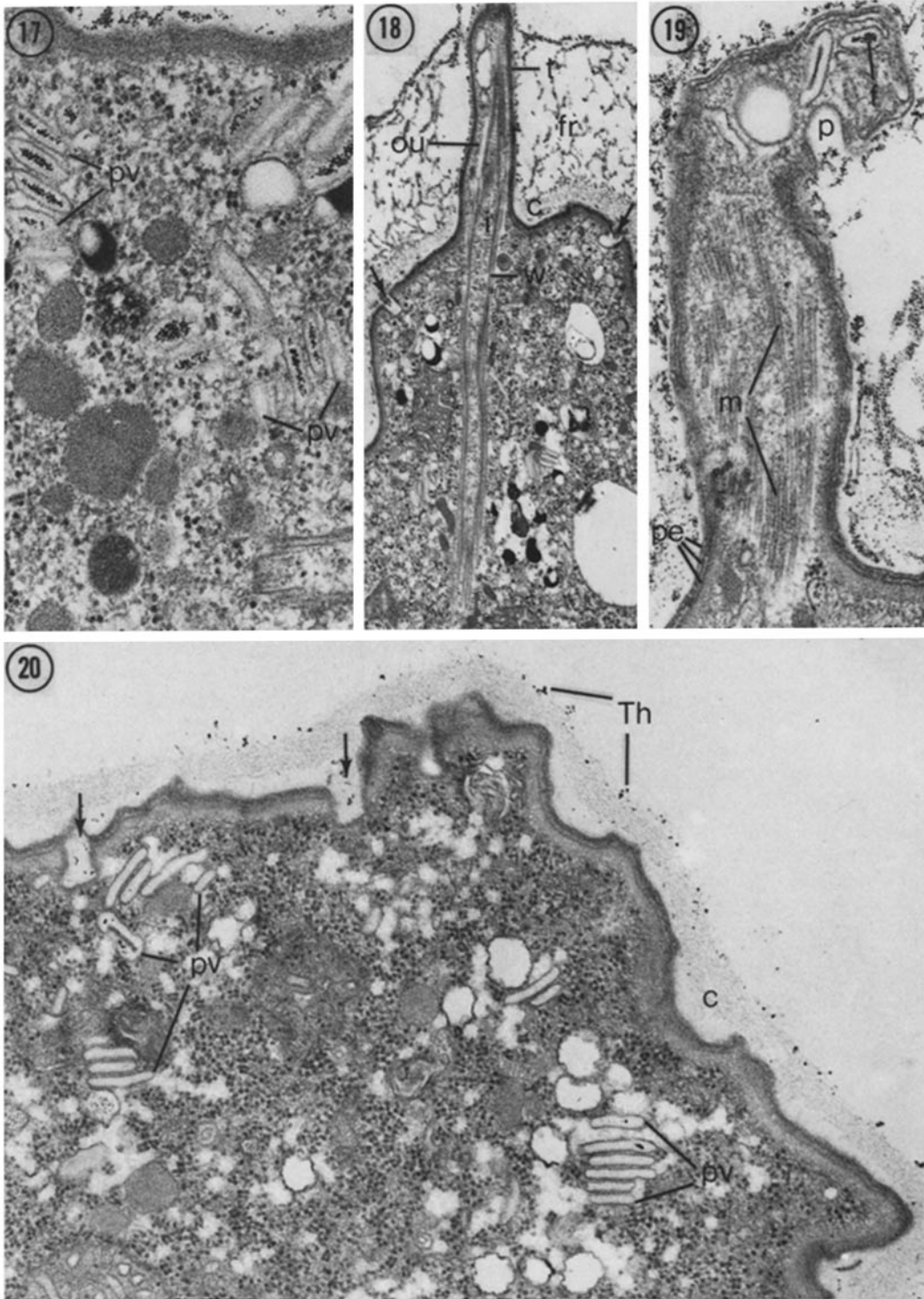
The membrane of the saccule is the only part of the surface of *Tokophrya* that is exposed on its inside to the cytoplasm. In all other areas, the plasma membrane is separated from the cytoplasm by two pellicular membranes and the epiplasm. It seems that for endocytosis to take place the direct contact of the plasma membrane with the cytoplasm is necessary. Bundles of cytoplasmic acto-myosin-like microfilaments are associated with the plasma membrane during phagocytosis (3, 9). No such filaments have been detected during pinocytosis in cells so far examined. It is, however, still possible that some contractile proteins are involved, since actin and myosin have been found in non-muscle cells (6) in Phyla as distantly related as Protozoa, Echinodermata, and Chordata (2, 14).

In the suctorian *Heliophrya erhardi*, the epiplasm is composed of numerous microfilaments 6–8 nm thick arranged in a regular although very complex pattern (10). It seems that the epiplasm in *Tokophrya* also contains microfilaments which are difficult to resolve and are seen only occasionally. The epiplasm surrounds the upper part of the saccule of the pit and thus could provide the motive force for the invagination process if it is in fact composed of contractile proteins.

Origin of Endocytic Membranes

The process of endocytosis requires a supply of plasma membranes. The supply needed may be very extensive when numerous vesicles or vacuoles are formed in rapid succession. The question arises, from what are the membranes derived. Is the plasma membrane the only source of such membranes? Are new membranes synthesized or are the membranes of cytoplasmic vesicles utilized? Most investigators agree that the pre-existing plasma membrane is the main, and some believe the only, contributor. This is particularly plausible when interiorization of plasma membranes proceeds at a rapid pace. In *Acanthamoeba*, the turnover rate of the entire plasma membrane is 2–10 times/h (5). In macrophages, the equivalent of the whole surface area is interiorized within 33 min (28). No cell could possibly synthesize new membranes with such speed. Recycling of small pinocytic vesicles is proposed by many investigators (8, 27).

In several species of ciliates an elaborate system of recycling of membranes has been described (1, 11). Vesicles of uniform size and shape are assembled in an orderly fashion in the cytopharyngeal region. They derive from food vacuoles which

condense during digestion and are apparently transported to the cytopharyngeal area where they fuse with the membrane of newly formed and growing food vacuoles.

It is of interest that the recycling vesicles in ciliates are in size and shape strikingly similar to the flattened vesicles in *Tokophrya*, suggesting that the latter might originate also from food vacuoles and be a part of a similar recycling system. There is so far no direct evidence of such a system in *Tokophrya*. To find an answer to this problem, further investigations are necessary, the most important being experiments on the rate of internalization of the plasma membrane during pinocytosis.

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