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FEEDING IN CILIATED PROTOZOA

I. PHARYNGEAL DISKS IN *EUPLOTES*: A SOURCE OF MEMBRANE FOR FOOD VACUOLE FORMATION?

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SUMMARY

The ciliate *Euplotes* is able to expend a very large amount of membrane in the formation of food vacuoles. Calculations based on the rate of ingestion of the food organism *Tetrahymena* indicate that an amount of food vacuole membrane equivalent to approximately 50–150% of the total *Euplotes* cell surface area can be produced within 5–10 min. An aggregation of osmiophilic, membrane-limited 'pharyngeal disks' is found packed in the cytoplasm just beneath the cell surface membrane in the region of the cell mouth and cytopharynx. These disks, which can be seen also in living cells, have average dimensions of 2 μm diameter by 100 nm thickness, and contain tightly packed layers of a thin lamellar material. Electron micrographs have revealed the apparent fusion of the limiting membrane of disks with the cell's plasma membrane at the base of the gullet. The lamellar disk contents are thereby released to the exterior medium in the buccal cavity, where they form a loosely packed layer over the surface membrane. It is postulated that the pharyngeal disks represent a repository of preformed membrane for use in food vacuole formation. The disk contents may also play a role in food ingestion, although this is not well defined at present. The myeloid content of old food vacuoles is very similar to that of nearby disks in the cytoplasm, suggesting that the disks may form by pinching from shrinking food vacuoles during the digestive cycle. Thus a cycle of membrane flow is envisaged, with the pharyngeal disks (1) coalescing with the surface membrane during food vacuole formation, (2) reforming by pinching from these food vacuoles during digestion, and (3) migrating back to the oral region to serve as a membrane store for subsequent food vacuole formation.

INTRODUCTION

The interrelationship among various types of cell membrane systems in general, and the biosynthesis of the plasma membrane in particular, are areas of considerable current research activity. Protozoan cells offer several advantages for studies on the mechanism of formation of new cell membrane (Conner, 1972; McKanna, 1973). For example, when protozoa are feeding rapidly they can produce large amounts of surface plasma membrane, which is internalized in the formation of food vacuoles. Marshall & Nachmias (1965) have estimated that an amoeba feeding on *Paramecium* has the ability to consume the equivalent of its entire surface area in plasma membrane within 5 min. Similarly, Wetzel & Korn (1969) have studied the phagocytosis of latex beads by *Acanthamoeba*, noting that in 30 min 50–100% of the amoeba's surface membrane is converted to phagocytic vacuole membrane surrounding 250–300 ingested latex particles. Obviously, since these cells do not decrease in size, this means

that there must be a continuous replenishment of surface membrane during such rapid bursts of feeding.

From the point of view of studying the cellular mechanism of such plasma membrane production, the amoebae have the disadvantage that phagocytosis occurs over much of their surface area. This is not the case with most ciliated protozoa, which have a highly organized cortical surface and a specialized feeding apparatus. Ciliary beating creates currents in the water that sweep food particles and prey organisms into a gullet, or buccal cavity. In the region of the cell mouth (cytostome) the food particles are wrapped in a piece of membrane from the feeding cell and pinched off into the cytoplasm for subsequent digestion.

When observing a feeding ciliate, it is immediately obvious that the amount of membrane produced in this way must be substantial. It is thus all the more remarkable that this type of membrane turnover occurs at a very restricted location on the cell surface. My own calculations (Kloetzel, 1970; see text) indicate that the ciliate *Euplotes* can ingest sufficient *Tetrahymena* in 2 min to require the production of $2{\cdot}6 \times 10^4\ \mu m^2$ of food vacuole membrane, or roughly the equivalent of the *Euplotes*' entire surface area.

The present report provides some data on feeding rate in *Euplotes*, and describes a set of membranous organelles, the 'pharyngeal disks', found around the cytopharynx and cytostome of this cell. It is proposed that these organelles represent an internal repository of preformed cell membrane that is used in the formation of food vacuoles.

METHODS AND MATERIALS

Experimental organisms

Euplotes (probably *E. eurystomus*; Carolina Biological Supply Co., Burlington, N.C.) were maintained in wheat infusion containing a mixed population of bacteria. *Tetrahymena pyriformis* were grown in 2% proteose-peptone, washed in amoeba medium (Prescott & Carrier 1964), and fed to *Euplotes* daily.

Feeding studies

To assess the rate of feeding and to estimate membrane turnovers, *Euplotes* were maintained in filtered pond water or old culture media without *Tetrahymena* for 1–2 days. Large amounts of washed *Tetrahymena* were then introduced into the fasted *Euplotes* cultures; cells were removed at intervals with a braking pipette (Stone & Cameron, 1964) and fixed in aceto-orcein or acetocarmine solutions to determine the number of food vacuoles formed.

For membrane calculations, a sample of the *Tetrahymena* to be used in a feeding experiment was slowed with methyl cellulose, and the length and width of living cells measured microscopically with an ocular micrometer. The volume of the average cell was determined using the formula for a prolate ellipsoid. Since food vacuoles are roughly spherical, and *Tetrahymena* are ingested individually, the surface area of a sphere of the same volume as the average *Tetrahymena* was then calculated, this figure serving as an index of the amount of membrane required for enveloping one *Tetrahymena*. The dimensions of the *Euplotes* to be used were obtained similarly. For approximating the surface area of an average *Euplotes* cell, the formula for the area of an oblate ellipsoid was employed; a value of (length + width)/4 was used as the major semi-axis. Membrane turnover was calculated this way, rather than directly from the measured diameter of freshly formed food vacuoles, because food vacuoles shrink quickly after formation (presumably due to the rapid loss of fluid from the enclosed *Tetrahymena*).

Light microscopy

In order to slow living *Euplotes* for observation and photography, the cells were either placed in methyl cellulose solution or compressed between a petroleum-jelly-ringed coverslip and the microscope slide. Bright-field, phase-contrast and Zeiss Nomarski interference-contrast optics were used, and photomicrographs were taken (some with electronic flash illumination) on Kodak Panatomic-X film.

Whole mounts of *Euplotes* fixed briefly with osmium tetroxide vapour were prepared in order to examine structures in the mouth region. For more intensive staining of osmium-binding structures the procedure of Fauré-Fremiet & André (1968) was used. Basically, this involves enhancement of the density of bound osmium by reduction with pyrogallol before mounting the fixed cells in an aqueous mountant.

Electron microscopy

Euplotes were fixed in either of 2 ways: in 3 % OsO_4 in 0·01 M s-collidine buffer, pH 7·4, with 0·5 mg/ml $CaCl_2$; or in a mixture of 0·5 % glutaraldehyde and 2 % OsO_4 in 0·05 M phosphate buffer, pH 7·6. Fixation (30–60 min), buffer rinses, and ethanol dehydration were carried out at ice-bath temperature, before embedding in Epon–Araldite (Mollenhauer, 1964). Thin sections were stained with uranyl acetate in ethanol, methanol, or a mixture, followed by lead citrate, and examined with either a Philips EM 200 or Hitachi HU-12 electron microscope.

OBSERVATIONS

Feeding studies

The results of several feeding experiments are presented in Fig. 1. Because of variations in size of the *Euplotes* in different experiments, and especially in size of the *Tetrahymena* (depending on how long they were suspended in washing medium before being used as food), the amount of food vacuole membrane produced is expressed in terms of percentage of the total *Euplotes* surface area. The measured sizes of *Euplotes* and *Tetrahymena* for these same experiments, the number of food vacuoles formed, and calculations of the actual membrane areas involved, are presented in Table 1. As an example of the rapidity of feeding, Fig. 3 shows a cell that was exposed to *Tetrahymena* for only 5 min before being fixed and stained. All 17 food vacuoles were formed within this brief feeding interval.

Light microscopy

Euplotes fixed with osmium tetroxide vapour and prepared as whole mounts reveal a variety of osmiophilic structures when viewed with the light microscope. The most prominent of these structures are food vacuoles, and a collection of structures in the oral region that correspond to the 'epipharyngeal organelle' of Fauré-Fremiet & André (1968). Using the pyrogallol reduction technique on osmium-fixed *Euplotes*, as described by these authors, the epipharyngeal bodies are revealed as very dense, apparently discrete units, wrapped in a layer approximately 30 μm long around the terminus of the gullet (Figs. 4, 5). With a 100 × oil-immersion objective and Nomarski optics, the cytoplasm around the mouth of living, unstained *Euplotes* can be seen to be populated with a large number of these oral organelles (Fig. 6). They appear to be arrayed in an ordered layer against the gullet wall, and are in constant motion, moving as individual units in and out of the rows making up this layer.

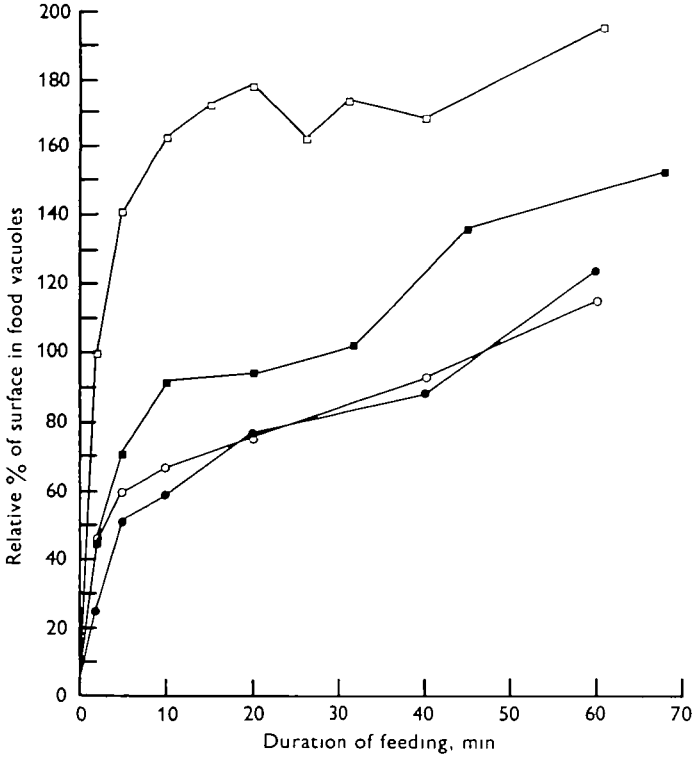

Fig. 1. Results of 4 feeding experiments. The amount of food vacuole membrane produced is expressed in terms of equivalent percentage of the entire calculated surface area of the *Euplotes* used for each experiment, as a function of the time the *Euplotes* were allowed to feed. Each point represents the mean of 20 cells counted for each feeding interval. Feeding experiments I, II, III, IV: □, ■, ○ and ●, respectively.

Electron microscopy

A view of the area near the base of *Euplotes*' buccal cavity is shown in Fig. 7. In the cytoplasm, just beneath the non-ciliated dorsal surface of the gullet, one or more rows of oral organelles can be seen, corresponding to those seen with the light microscope. In section these organelles appear rod-shaped, generally about 2 μm long and 0·1–0·15 μm wide. Their long axes are primarily perpendicular to the cell surface membrane; rows of microtubules (cytopharyngeal ribbons; Pitelka, 1969) extend between and appear to separate these organelles into units or small groups (Fig. 7; Fig. 10, inset).

These oral structures were originally termed 'cytoplasmic rods' (Roth, 1957; see Kloetzel, 1970). The observations of Fauré-Fremiet & André (1968) on these same structures led these authors to the opinion that the rods were actually leaves or sheets making up an ensemble that they called the 'foliated epipharyngeal organelle'. It is apparent from light-microscopic observations (see above) that these organelles could not be true rods, or they would be below the resolution of the light microscope, and thus not detectable as individual units in living cells. In order to resolve the size and

Table 1. *Food vacuole formation by Euplotes feeding on Tetrahymena*

Feeding experiment no.	*Euplotes* Dimensions, μm* Width	Length	Calculated surface area, μm^2	*Tetrahymena* Dimensions, μm* Width	Length	Calculated food vacuole surface area, μm^2	No. of food vacuoles formed after different feeding times† (Calculated total food vacuole membrane area, μm^2) 2′	5′	10′	20′	60–70′
I	94·6	143	26 818	27·2	44	3199	8·4 (26 712)	11·8 (37 743)	13·6 (43 506)	14·9 (47 655)	16·3 (52 144)
II	94·6	143	26 818	35	61·2	5592	2·2 (12 022)	3·4 (19 013)	4·4 (24 605)	4·5 (25 164)	7·3 (40 822)
III	84·7	130	22 527	19·4	35·9	1785	5·8 (10 264)	7·5 (13 388)	8·4 (14 994)	9·5 (16 958)	14·5 (25 883)
IV	84·7	130	22 527	27·1	45	3235	1·8 (5 824)	3·6 (11 648)	4·1 (13 265)	5·3 (17 148)	8·6 (27 825)

* Each width and length represent the mean of 20 measured cells. Thickness of *Euplotes* was difficult to measure but was close to 40 μm for all experiments (the number used for calculations).

† Each reported food vacuole number represents the mean of 20 cells counted for each feeding interval.

For each of the 4 feeding experiments the sizes of the *Euplotes* and *Tetrahymena* are given, together with the calculated membrane areas involved.

shape of these structures, serial thin sections through the oral region of *Euplotes* were prepared. Reconstructions from these sections (Fig. 2) revealed that the epipharyngeal organelles are actually ovoid or disk-shaped in profile. The term 'pharyngeal disk' will be used, therefore, in subsequent descriptions of these organelles. Because of the thinness of the disks, and particularly because they are frequently bowed or curved, longitudinal sections showing the whole profile of a single disk are very rarely encountered.

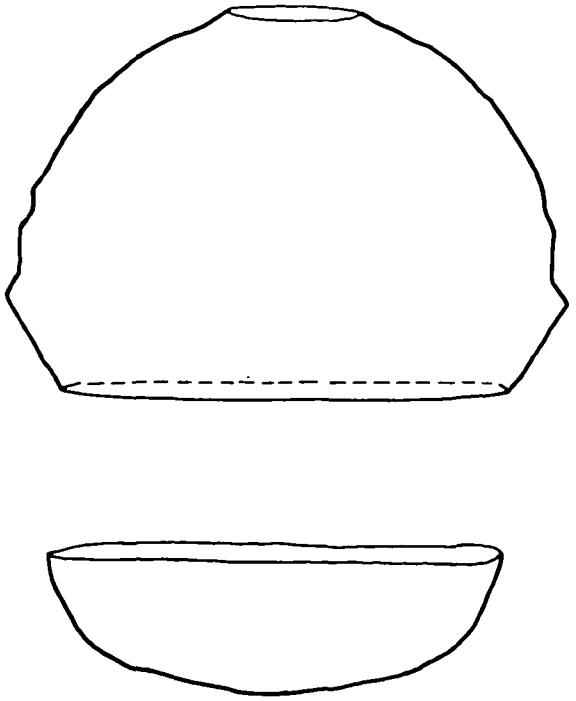

Fig. 2. Reconstruction of portions of 2 adjoining pharyngeal disks from a set of 18 consecutive serial sections, assuming an average section thickness of 80–90 nm. The upper disk of this pair has a maximum diameter of 2·2 μm.

At higher magnification the disks are seen to be limited by a prominent unit membrane measuring approximately 6 nm in thickness (Fig. 8). The contents of the disks are myeloid in appearance, apparently representing irregular concentric layers of electron-dense material. These internal layers are very thin and do not have the triple-layer appearance characteristic of unit membranes (cf. Fauré-Fremiet & André, 1968). Occasionally, for reasons not clear, a disk will be fixed in a swollen condition (Figs. 8, 9), in which case the multilayered nature of the contents is especially evident. At points, several layers converge at circular foci, which can be used as markers for the type of material found within the disks (see below).

On rare occasions micrographs have been obtained that illustrate apparent fusions between disks and the cell surface in the mouth region (Fig. 10). The membrane limiting such disks is directly continuous with the cell plasma membrane, and the disk contents are thus exposed to the external medium in the cell's buccal cavity.

Some of this layered material seems to form a coating loosely apposed to the external surface of the membrane. This feltwork is extensively developed in some sections (Fig. 11); circular foci can be noted in the layers (Figs. 10, 11), reminiscent of those seen within swollen disks in the cytoplasm (Fig. 9).

Upon examination of *Euplotes* that have recently ingested *Tetrahymena*, it is apparent that some of the layered extracellular material found in the oral region is incorporated into the food vacuole along with the food. Between the food vacuole membrane and the enclosed *Tetrahymena* in freshly formed food vacuoles, regions are found that contain packed arrays of the lamellar material, frequently with cilia from the enclosed prey embedded in the layers (Fig. 12).

By far the vast majority of pharyngeal disks found within any single *Euplotes* is found congregated around the cytostome. Those disks found elsewhere in the cytoplasm are frequently near food vacuoles. Fig. 13 represents what is interpreted to be a terminal food vacuole, from which essentially all the digestible material has been removed, leaving only residue. Such food vacuoles usually display myeloid contents that greatly resemble the material found within the adjacent pharyngeal disks, although no sections have been obtained that show clear continuity between food vacuoles and disks. It may be significant to note that the limiting membranes of food vacuoles, pharyngeal disks, and the surface membrane all exhibit a clear trilaminar appearance of similar thickness (about 6 nm) in osmium-fixed preparations; all other cytoplasmic membranes are thinner and not distinctly trilaminar.

DISCUSSION

Feeding and membrane turnover in Euplotes

Simple observation of feeding *Euplotes* is sufficient to demonstrate that these cells are capable of very rapid production of food vacuole membrane. An attempt has been made in the present study to quantitate this membrane turnover, using the rate of ingestion of *Tetrahymena* as an index of food vacuole membrane formation. There are at least 2 difficulties that affect the accuracy of these calculations. First, the irregular contours of the *Euplotes* cell (and to a lesser extent those of *Tetrahymena*) make cell surface areas difficult to measure precisely. Second, newly formed food vacuoles are not always spherical, as assumed here. Nevertheless, even allowing for these approximations, the data certainly support the contention that membrane representing a large percentage of the *Euplotes* surface area can be rapidly formed and moved inside the cell. In extreme cases (with small *Tetrahymena*, which are eaten much more rapidly than large ones) a *Euplotes* cell can ingest 17 *Tetrahymena* within 5 min, representing an area of food vacuole membrane approximately twice that of the entire *Euplotes* surface. Certain obvious questions thus arise concerning the source of such an amount of food vacuole membrane.

In theory there are only 2 basic mechanisms for the production of new cell surface membrane: molecular or micellar insertion (*de novo* membrane synthesis); or the fusion of preformed cytoplasmic membrane-limited bodies with the cell surface. For a cell growing slowly or undergoing only moderate endocytosis, *de novo* synthesis of

new cell surface might suffice. According to classical concepts of membrane flow and phagocytosis, even in rapidly feeding ciliates such molecular addition could conceivably occur all over the cell surface, with a concomitant flow of surface membrane into the gullet to accommodate vacuole formation. However, certain considerations would seem to weigh against such molecular surface membrane construction in this case. First of all, the observed rate of membrane utilization in food vacuole formation would require an extremely rapid rate of insertion of new membrane molecules for surface replacement, particularly since the new membrane is required at one specialized location on the cell surface. However, the pellicle covering most of the surface of *Euplotes* (and many other ciliates; see Pitelka, 1969) is a complex structure. The plasma membrane (where *de novo* synthesis and associated membrane flow would presumably have to occur) is usually separated from the bulk of the cytoplasm by a mosaic of flattened membrane-limited alveoli, layers of fibrous material, and microtubule arrays. This 'isolation' of the plasma membrane would not appear to favour rapid exchange of membrane precursor molecules with the cytoplasm. Even in *Acanthamoeba*, in which there is neither a localized feeding area nor a multilayered pellicle, Goodall, Lai & Thompson (1972) found that phagocytosis did not stimulate a more rapid incorporation of precursor molecules into the plasma membrane, suggesting that pre-existing membrane is used to replace the ingested surface membrane.

In the amoebae (Wise & Flickinger, 1970) as well as in other cell systems the Golgi apparatus has been implicated as a source of new surface membrane (see Whaley, Dauwalder & Kephart, 1972). As is true of many ciliates, *Euplotes* does not possess any Golgi bodies. However, the pharyngeal disks may represent highly modified functional equivalents of Golgi cisternae, at least in so far as cell surface addition is concerned.

Role of the pharyngeal disks in the feeding process

The pharyngeal disks in *Euplotes* have been noted previously, as mentioned above. Originally described by Roth (1957) and termed 'cytoplasmic rods', these organelles were much more fully characterized by Fauré-Fremiet & André (1968). These authors first emphasized the flattened leaf-like arrangement of the disks, their myeloid contents, and their preferential localization around the base of the gullet in an array they christened the 'foliated epipharyngeal organelle'. The term 'pharyngeal disk' is used here to designate each of the component organelles making up the array, emphasizing their shape and individual nature. None of the authors above speculated on the role of the disks in the physiology of the cell. However, in the light of the discussion above, it appears very likely that the pharyngeal disks in *Euplotes* serve the function of providing the membrane, in pre-assembled form, necessary for rapid food vacuole formation. Several factors support this contention. (1) The predominant localization of the disks around the oral region suggests their involvement with some aspect of the feeding process. The presence within newly formed food vacuoles of lamellar material resembling the contents of the disks (Fig. 12) further supports this relationship. (2) From a theoretical standpoint, the shape of the disks provides a high surface-to-volume ratio. Taking an idealized disk 2 μm in diameter and 0·1 μm thick as an example, one finds

that a sphere occupying the same volume of cytoplasm as this disk (0·21 μm^3) would have only about one-fourth as much surface membrane (1·72 μm^2 vs. 6·34 μm^2). Conversely a sphere with the same surface area as this disk would occupy approximately seven times as much volume. The shape of the disks also facilitates their packing into ordered layers immediately next to their proposed site of fusion with the plasma membrane of the gullet. It is interesting to note that the rows of microtubules in this region may play a role in the stacking of the disks by serving as a 'gate', allowing insertion of disks into the array in only one preferred orientation. (3) The disks are limited by a clearly trilaminar unit membrane 6 nm thick, of a type seen elsewhere in the cell only in the plasma membrane and in the membrane enclosing food vacuoles. This similarity of membrane appearance between disks, food vacuoles and cell surface suggests some interrelationship among these membrane systems, as discussed below. (4) Although rare, pictures can be obtained that reveal disks apparently fused with the cell surface in the buccal cavity. Such micrographs could be interpreted as representing the *formation* of disks by pinching inward from the surface membrane. However, clumps of layered material that greatly resemble the contents of cytoplasmic disks are frequently found free in the buccal cavity, making it much more likely that the disks do indeed fuse with the plasma membrane, empty their contents, and supply membrane for cell surface expansion in the cytopharyngeal region of the cell. Certainly the most plausible use of this expanded cell surface would be to provide for the phagocytosis of food particles.

While this hypothesis of disk function is thus quite attractive, one other possibility should be entertained: the disk membranes could conceivably serve to permit a *transient* stretching of the gullet and cytopharynx while relatively large prey organisms (like *Tetrahymena*) are being squeezed through the ordinarily narrow cytopharyngeal opening, following which the disks could reform as the cytopharynx collapsed to its normal dimensions. In this case the disks could play a role similar to that postulated for the thick-membraned fusiform vesicles in the transitional epithelial cells of the mammalian urinary tract during cycles of stretching and contraction (Hicks, 1966; see Falk, 1969, and Bardele, 1972, for descriptions of similar vesicles in algal cells and pseudoheliozoan protozoa, respectively). This possibility is made less plausible because of the myeloid material ejected from the disks following their fusion with the cell membrane; it again would seem that recapture of this material by disks forming endocytotically from the wall of the buccal cavity might be difficult.

The nature and possible role of the layered contents of the disks require some comment. In this discussion, primary focus has been placed on the limiting membrane of the disks with respect to membrane turnover during food vacuole formation. It should be realized, however, that the disk contents could play a more important role in the feeding process than the membranes enclosing them. The osmiophilia of the disks would suggest that their contents are rich in lipids. Indeed, Fauré-Fremiet & André (1968) reported staining the disks with Sudan black, although I have not been able to do so. The lamellar appearance of the disk contents is also suggestive of lipoidal layers, although they are much thinner than the true unit membranes in the *Euplotes* cytoplasm. The volume of extruded disk material in the cytopharynx can be extensive;

these layers could serve as a lubricant when large prey organisms are squeezed through the narrow cytopharyngeal passage, or perhaps aid in immobilizing the cilia of captured prey. Nilsson (1972) reports that the mucus extruded from the oral mucocysts of *Tetrahymena* can bind solutes from the medium for subsequent ingestion. Since bacteria form a staple part of the *Euplotes* diet, ejected disk layers conceivably could play a role in binding and collecting bacteria until sufficient number have been accumulated for inclusion within a food vacuole. In Fig. 10 (inset) it can be noted that at points over the crests of ridges in the gullet wall, ejected disk lamellae are tightly packed against the plasma membrane in highly ordered arrays. The significance of this ordering is not obvious, but does perhaps permit the speculation that material from extruded lamella layers (possibly lipid) is somehow added to the plasma membrane itself during the membrane expansion accompanying phagocytosis of food. One fact is clear: some disk contents are taken into the food vacuole, forming a layer between the vacuole membrane and the ingested prey organism(s). It would seem most efficient if the disks could supply not only the membrane for the envelopment of the food particle, but also hydrolytic enzymes to be included within the food vacuole for initiation of the digestive events. Although preliminary cytochemical investigations indicate that the *Euplotes* disks do not contain acid phosphatase, Dembitzer (1968) finds that some of the cytopharyngeal vesicles in *Blepharisma* are acid-phosphatase positive. Further work is needed on this question.

Since many ciliates besides *Euplotes* are able to undergo rapid bursts of food vacuole formation, and with the preceding discussion in mind, it is not surprising to find that collections of membrane-limited vesicles, vacuoles, disks, or tubules have been reported frequently near the cytopharynx of a number of ciliates (Randall & Fitton-Jackson, 1958; Fauré-Fremiet, 1961; Miller & Stone, 1963; Jenkins, 1973; see Pitelka, 1969, for discussion). Such structures are usually found between rows of microtubules beneath that portion of the gullet wall limited by a single membrane, as in *Euplotes*. Although most authors have not speculated on the functions of these oral organelles, Schneider (1964) attempted to relate the disk-shaped structures found near the cytopharynx of *Paramecium* (cf. Jurand & Selman, 1969) to some secretory process associated with food vacuole formation. Kennedy (1965) and Elliott & Clemmons (1966) suggested that the oral vesicles of *Blepharisma* and *Tetrahymena*, respectively, might contribute structural material to developing food vacuole membranes. Very recently, Bradbury (1973) has described elongate 'apostome organelles' underlying the cytostome area of *Hyalophysa*, proposing likewise that these structures join the surface of the cell to replace the membrane and associated filamentous coat 'used up' in the pinocytotic formation of food vacuoles. In these other ciliates examined to date, the cytopharyngeal vesicles appear to be less prominent and less numerous than are the disks in *Euplotes*.

A final point concerns the *origin* of the pharyngeal disks. Those disks not found in the array around the gullet are usually seen near food vacuoles in the cytoplasm. As the ingested food organisms are digested, the food vacuoles gradually become smaller; at the end of the digestion cycle, only myeloid 'residual bodies' are left, whose contents greatly resemble those of the disks nearby. Taken together, these observations suggest

that disks may pinch off from shrinking food vacuoles and migrate back to the cytostome area; the disks could be looked upon as the intermediate vehicles in a closed cycle of membrane flow, from buccal cavity to food vacuole and back again. Since the *Euplotes* are continually growing and dividing, a mechanism for the production of new disks must exist; nevertheless, the thickness and prominent trilaminar nature of the membranes of the cell surface, food vacuoles, and disks suggests some interrelationship among these membrane systems, perhaps of the cyclical nature mentioned above. McKanna (1969) has proposed that a similar recycling of membrane occurs during feeding in the peritrich ciliate *Epistylis*, with cup-shaped vesicles serving the role of intermediate membrane carriers, although the photographs to illustrate this have not yet appeared. Bradbury (1973) finds membranous structures in *Hyalophysa* that she interprets as 'the collapsing walls of former food vacuoles', which are apparently transformed into the 'apostome organelles' that fuse with the cell surface near the cytostome during pinocytosis. Clearly, more complete information is required to substantiate the concept of membrane cycling in feeding ciliates. This will be sought by careful ultrastructural observations of food vacuole formation and digestion in *Euplotes*, with electron-dense tracers included to identify food vacuoles of a given age throughout the digestive cycle.

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NOTE ADDED IN PROOF

The full report by McKanna on membrane recycling in feeding peritrichs has now appeared (*J. Cell Sci.* (1973) **13**, 663–686). In a report recently brought to my attention (*Monitore Zool. Ital.* [N.S.] (1971) **5**, 65–80), Nobili & Rosati Raffaelli described 'electron-dense bodies' (disk-type structures) in the oral cytoplasm of 5 species of hypotrichs, including *Euplotes*. Their micrographs clearly show some of these bodies in continuity with the cytopharyngeal surface membrane, releasing 'extrapellicular fibrils' (extracellular lamellae of my report) into the buccal cavity. Also, Allen (*J. Cell. Biol.* (1973) **59**, 6*a*) has recently provided evidence for membrane recycling accompanying food vacuole turnover in *Paramecium*.

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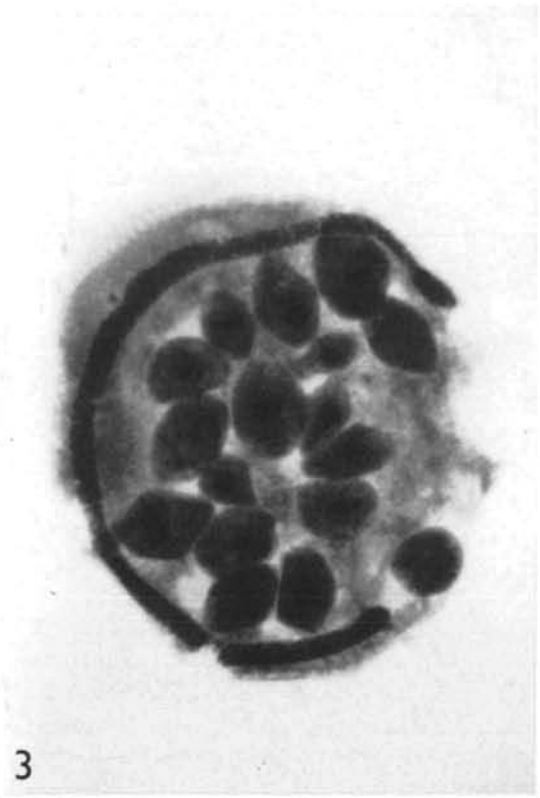
Fig. 3. Acetocarmine preparation of a *Euplotes* allowed to feed on *Tetrahymena* for 5 min (feeding experiment 1). This cell contains 17 ingested *Tetrahymena*. × 370.

Fig. 4. Whole-mount of a starved *Euplotes* prepared by the osmium-pyrogallol procedure. The collection of osmiophilic pharyngeal disks at the base of the gullet is clearly illustrated (arrow). Nomarski interference contrast. × 570.

Fig. 5. Enlarged view of the pharyngeal disks from the same cell as in Fig. 3. Some individual disks can be seen (arrows). Bright field. × 2100.

Fig. 6. Pharyngeal disks (arrow) as seen in a living *Euplotes* with Nomarski interference contrast optics. Since this represents an optical section, the actual volume occupied by the disks is much more extensive. × 2100.



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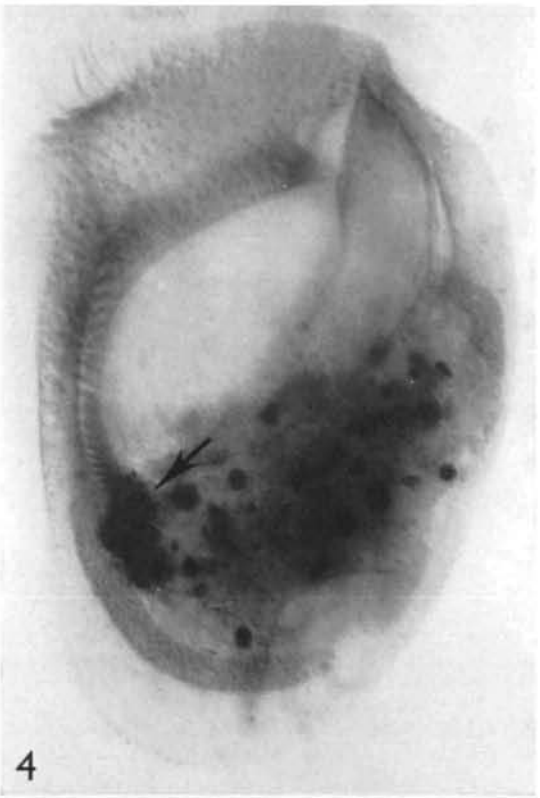

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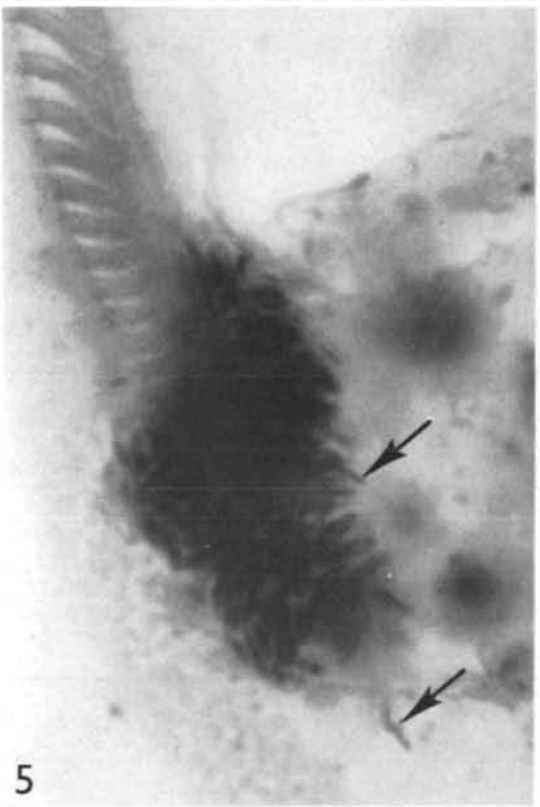

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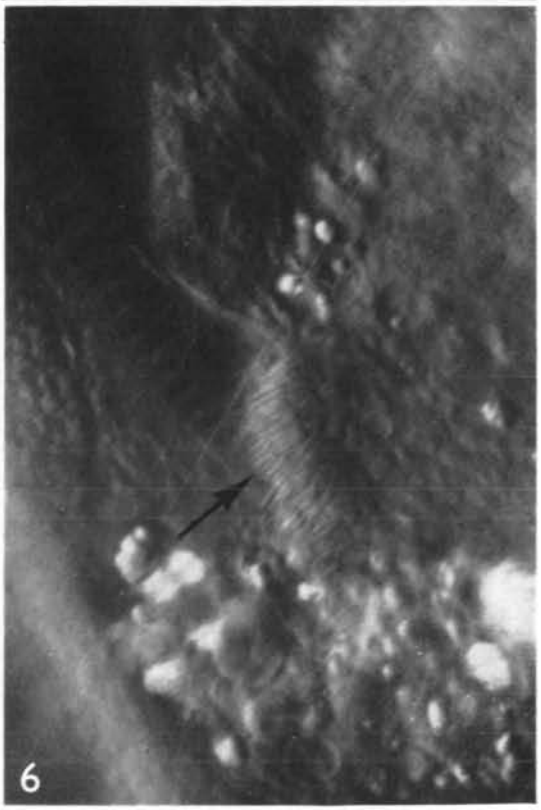

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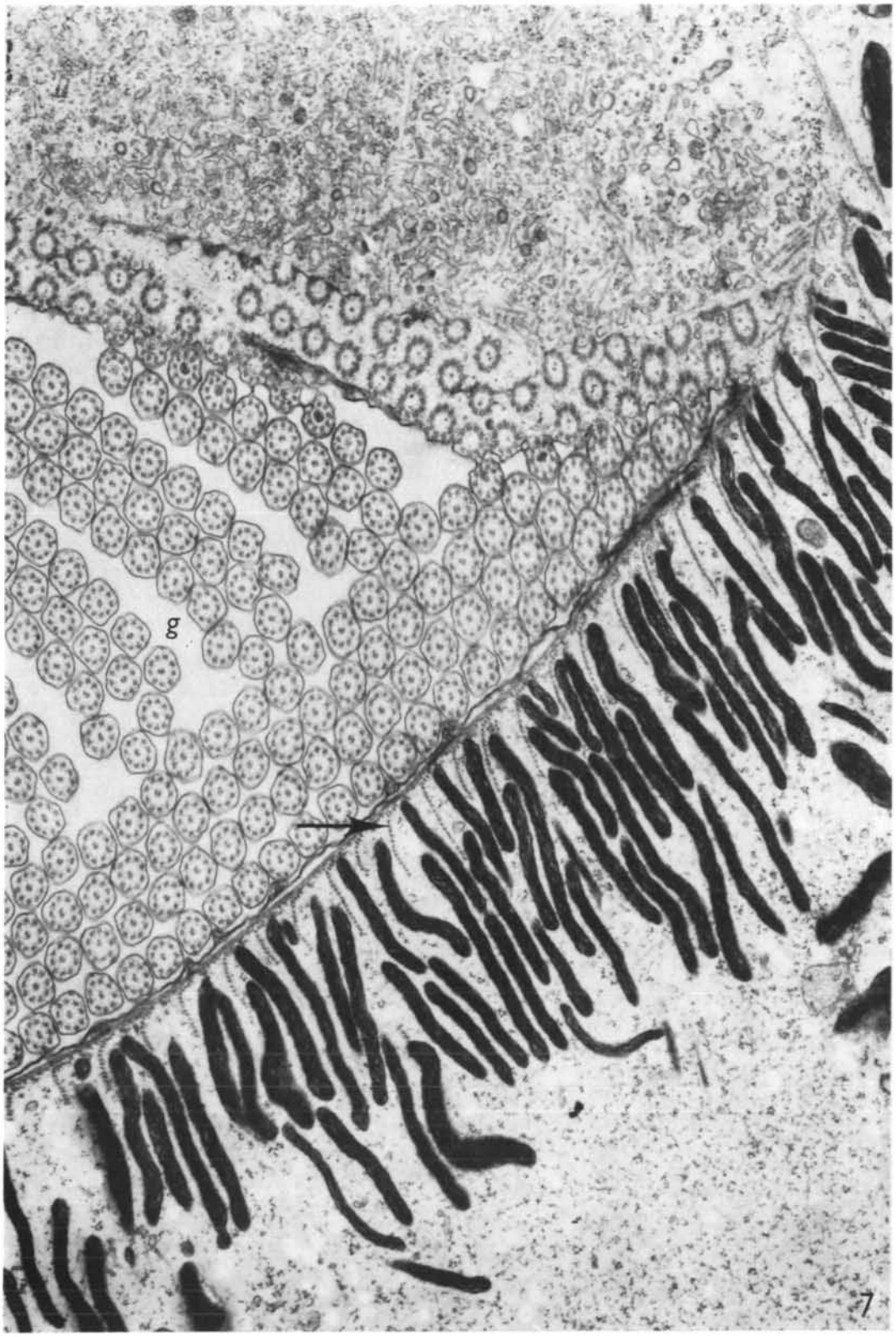
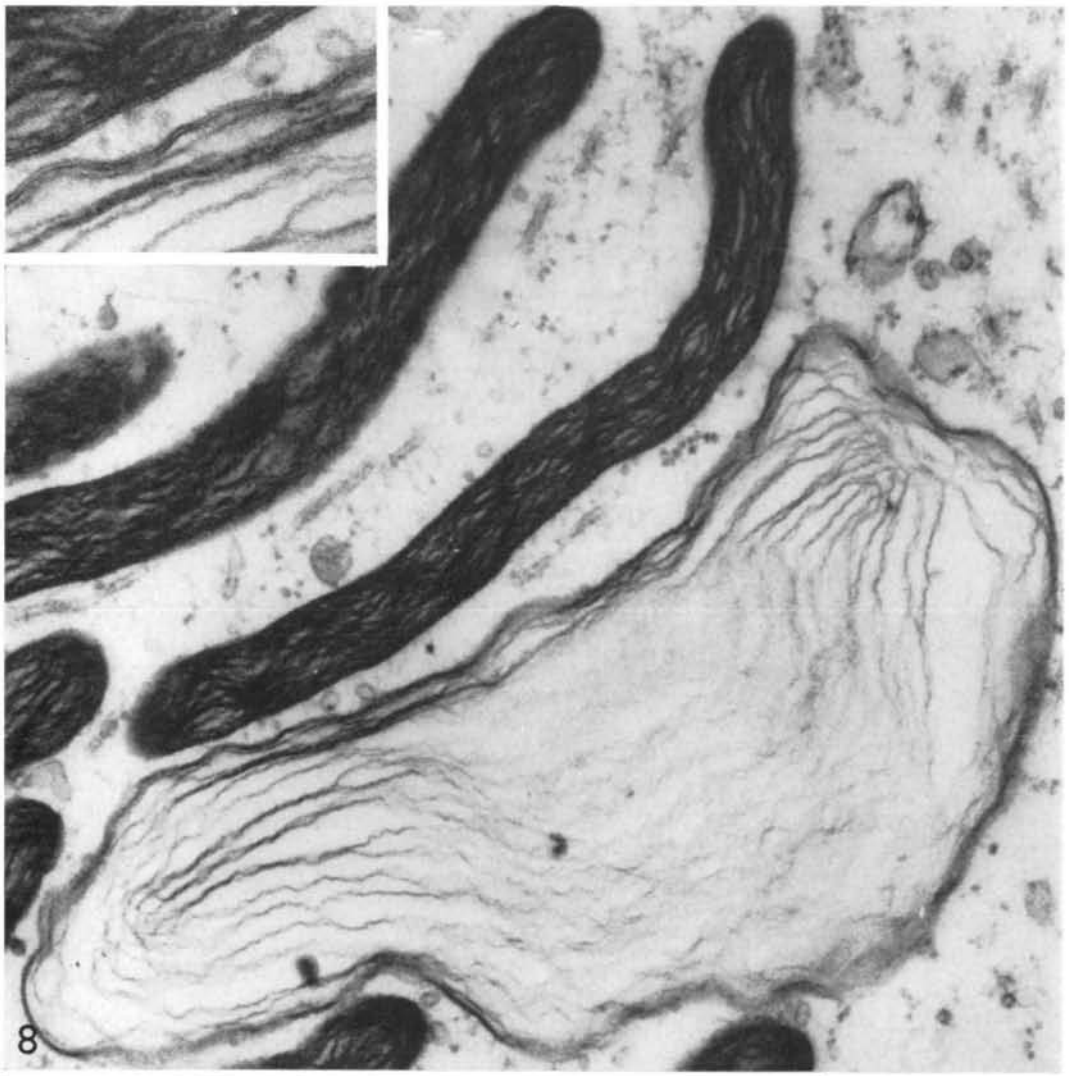
Fig. 7. Electron micrograph showing a number of electron-dense pharyngeal disks arranged in the cytoplasm just beneath the surface membrane near the base of the gullet (*g*). A number of membranellar cilia are seen in cross-section within the gullet cavity. Note the rows of microtubules (arrow) separating adjacent disks. × 22000.


g

Figs. 8, 9. Pharyngeal disks at higher magnification showing their lamellar contents, which are particularly clear in those disks fixed in a swollen condition. The inset in Fig. 8 shows the clear trilaminar unit membrane limiting the disks, and the thinner profiles of the internal lamellar layers. In Fig. 9, some of the lamellar layers are aggregated at circular foci (arrows). Fig. 8, ×77500; inset, ×148000; Fig. 9, ×44500.



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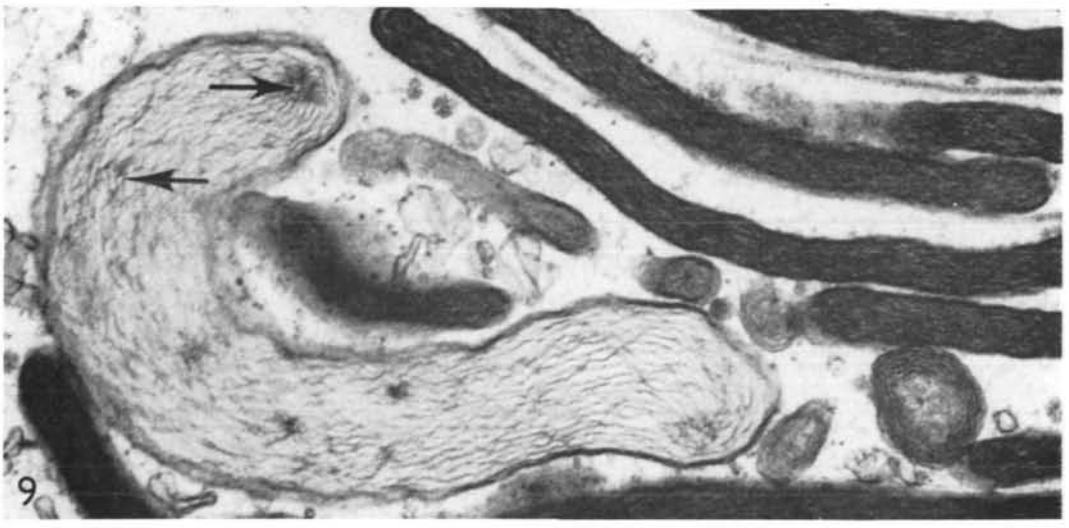

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Fig. 10. The apparent fusion of a pharyngeal disk (arrow) with the surface membrane of the gullet is illustrated in this electron micrograph; the disk contents, exposed to the external medium, have begun to swell. The clump of lamellar material free in the gullet may have been ejected from a disk recently fused with the surface. Some of the lamellar material is loosely packed on the external surface of the cell membrane, with a higher degree of ordering evident over crests in the surface (lower part of the micrograph, shown enlarged in the inset). × 33 500; inset, × 65 500.

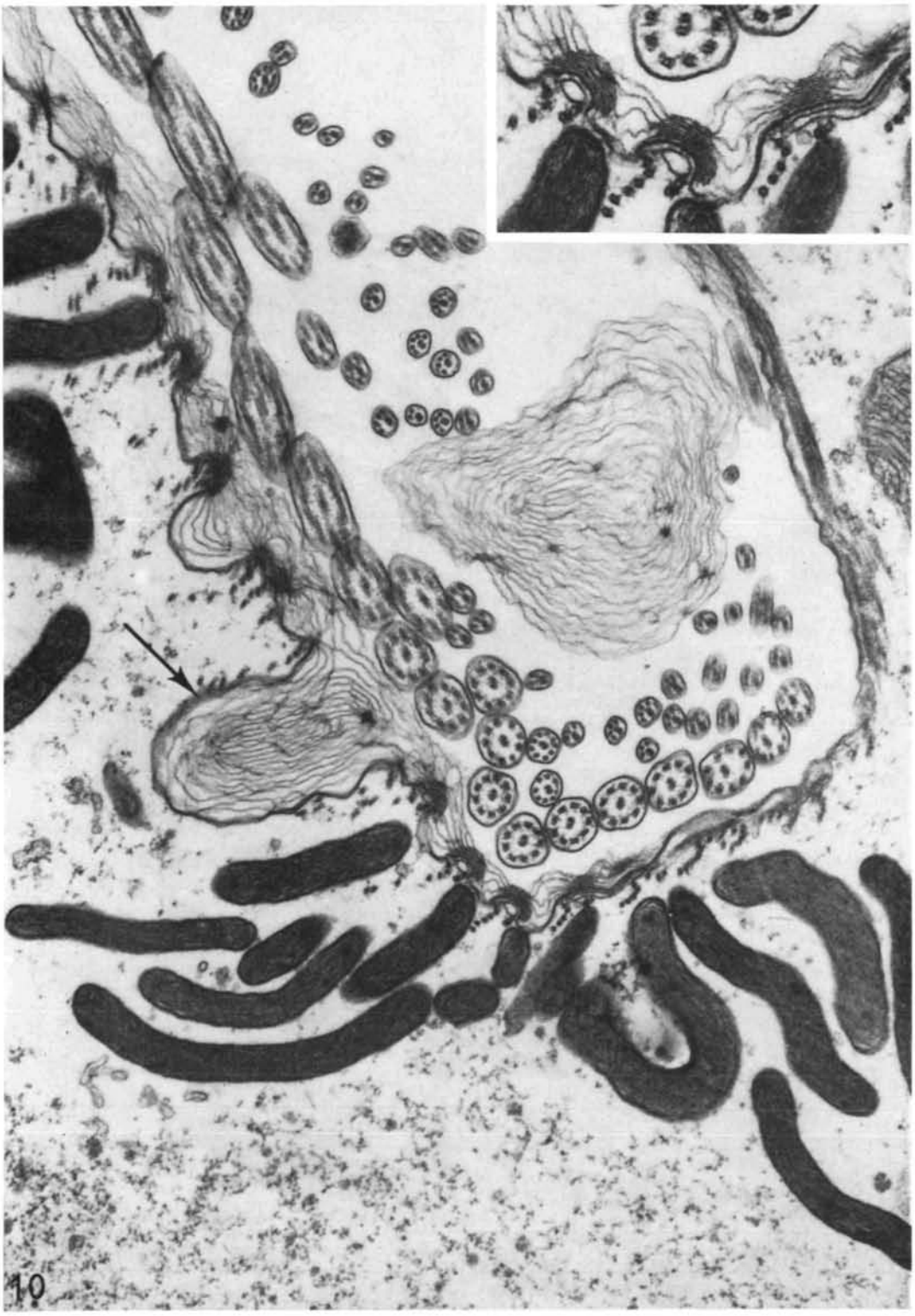
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Fig. 11. A cross-section near the terminus of the gullet (*g*), showing a whorl of pharyngeal disks and thick layers of lamellar material on the outer surface of the plasma membrane. Numerous circular foci are seen among the loosely packed extracellular layers. × 22000.


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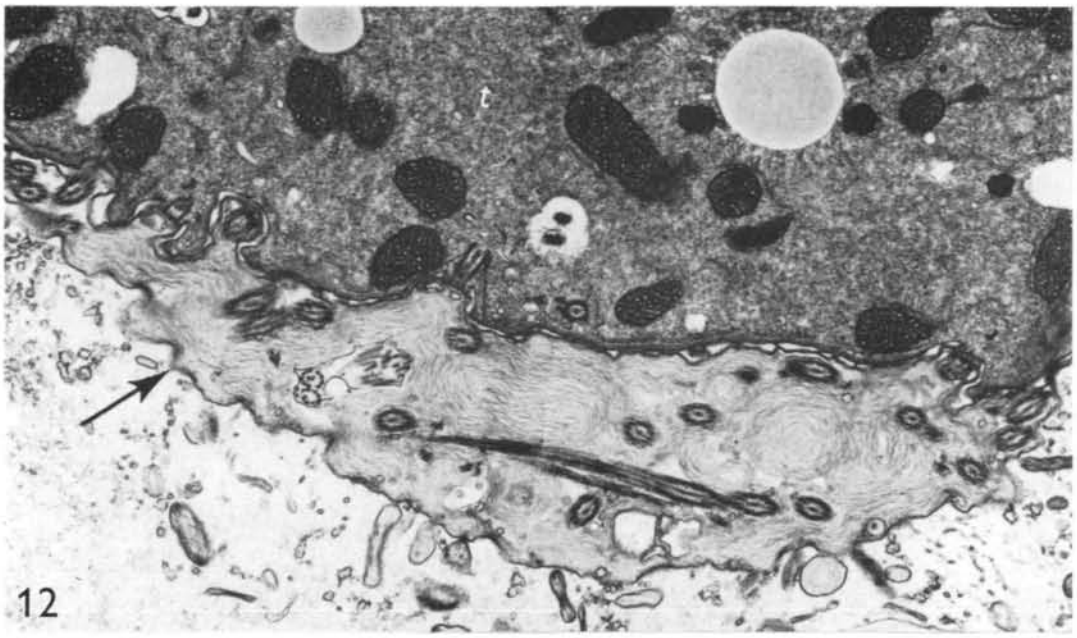
Fig. 12. A portion of a food vacuole in a *Euplotes* fed with *Tetrahymena* 8 min before fixation. Between the food vacuole membrane (arrow) and the enclosed *Tetrahymena* (*t*), layers of lamellar material can be seen in which are embedded *Tetrahymena* cilia. Glutaraldehyde-osmium tetroxide combination fixation. × 13000.

Fig. 13. This structure probably represents a terminal food vacuole from which almost all digestible material has gone. Note the similarity between the lamellar contents of the food vacuole and those of the nearby pharyngeal disks. × 47000.



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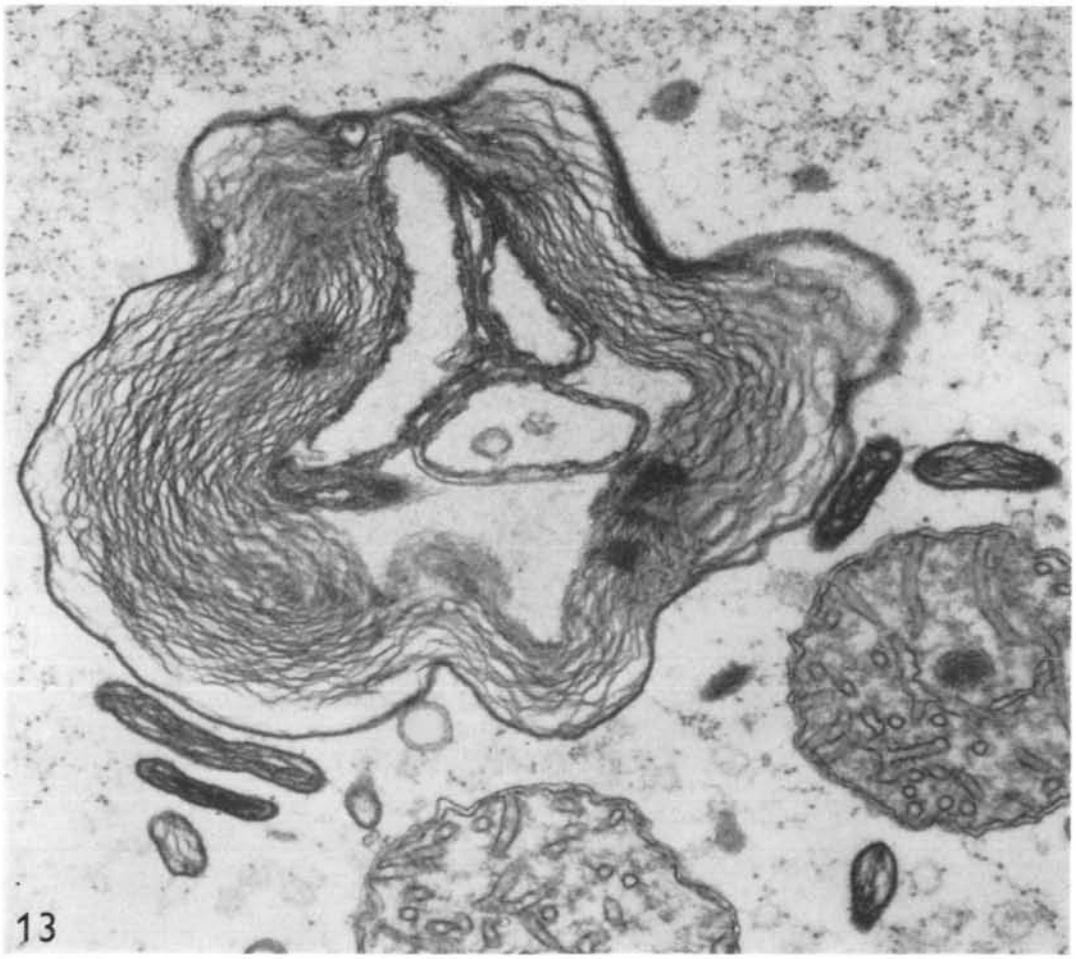

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