FOOD VACUOLE MEMBRANE GROWTH WITH MICROTUBULE-ASSOCIATED MEMBRANE TRANSPORT IN PARAMECIUM

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ABSTRACT

Evidence from a morphological study of the oral apparatus of Paramecium caudatum using electron microscope techniques have shown the existence of an elaborate structural system which is apparently designed to recycle digestive-vacuole membrane. Disk-shaped vesicles are filtered out of the cytoplasm by a group of microtubular ribbons. The vesicles, after being transported to the cytostome-cytopharynx region in association with these ribbons, accumulate next to the cytopharynx before they become fused with the cytopharyngeal membrane. This fusion allows the nascent food vacuole to grow and increase its membrane surface area. The morphology of this cytostome-cytopharynx region is described in detail and illustrated with a three-dimensional drawing of a portion of this region and a clay sculpture of the oral apparatus of Paramecium. Evidence from the literature for the transformation of food vacuole membrane into disk-shaped vesicles both from condensing food vacuoles in the endoplasm and from egested food vacuoles at the cytoproct is presented. This transformation would complete a system of digestive vacuole membrane recycling.

Some physiological processes within cells require a large amount of “new” membrane within a relatively short time. The origin of this new membrane is a question which often is not easy to answer. Does the new membrane come from preexisting membrane, is there a large pool of readily available membrane subunits, or is the cell able to make new membrane subunits fast enough to keep pace with its obvious need for new membrane material? On the other hand, the question of what eventually happens to all this membrane is not always easy to solve either. In order to approach a solution to these problems it is particularly advantageous to choose for study an organism or tissue having a system in which large quantities of membranes are needed quickly and an organism which is easy to grow and easy to manipulate in the laboratory.

Food vacuole formation in heterotrophic, free-living protozoans entails a rapid expansion of the oral surface area of the cell if the cell is to maintain its former size and shape. In the ciliated protozoan Paramecium a specialized area of membrane capable of undergoing rapid expansion is located on the posterior, dorsal surface of the buccal apparatus. Placing cells in a solution of Congo red after a period of starvation stimulates the formation of food vacuoles from this zone. As many as 16 food vacuoles have been seen to form in the span of 10 min in such cells. This means that around 500 μm² of new membrane surface area must be formed for...
each new food vacuole having a diameter of 12 \mu m, and this must come either from preexisting membrane subunits or from a flow of membrane into this zone from the surrounding buccal cavity plasma membrane.

Recent reports on peritrich ciliates (23), on the hypotrich ciliate Euploides (20), and on an apotome ciliate (5) have suggested that at least some of this membrane comes from membrane-limited disk-shaped vesicles which accumulate on the cytoplasmic surface of this food vacuole-forming region and which subsequently fuse with the membrane of the developing food vacuole. Similar disks are also known to be present near the cytostome of Paramecium (8, 19, 26, 28, 33) and have been reported to fuse with the plasma membrane in this area (28, 33). None of the published pictures of supposed membrane fusion, however, have been unequivocal.

The results of an electron microscope study of the oral region of Paramecium caudatum are reported here. This study serves two objectives; first, to clarify morphological details of the oral region of Paramecium and, second, to use this new information in an effort to further identify the role of the disks as well as their associated microtubules and to identify the source of new food vacuole membrane. The thesis will be developed in this paper that the disks are being moved to this specific region of the buccal cavity in association with microtubules where they fuse with the cell membrane, thereby providing "new" membrane for the developing food vacuole. Evidence for a recycling of digestive vacuole membrane will then be discussed.

The terminology used in this paper for the parts of the buccal apparatus of Paramecium will conform to the definitions of Corliss (7). In addition, the terminology used for more recently described structures, e.g., filamentous reticulum and cytopharyngeal ribbons, will conform to those used and described by Pitelka (26).

MATERIAL AND METHODS

Electron Microscopy

Cultures of P. caudatum were grown at room temperature in hay infusion medium. Cells were fixed individually for electron microscopy and handled one at a time before sectioning as previously described (3). Cells were sectioned either longitudinally or transversely after attaching each cell to the sectioning block in the precise orientation desired. Complete sets of serial sections were obtained and were mounted on Formvar-supported large-hole grids. The grids were kept in chronological order so that the exact position of each section within the entire series could be determined. Sections were cut with the use of a diamond knife and Sorvall MT-II ultramicrotome. Uranyl acetate followed by lead citrate were used as stains. A Hitachi HU-11A electron microscope operated at 75 kV was used during the study.

Some of the cells used here were the same cells as those used in a previous study (3).

Freeze-Fracture

Cells were fixed 15 min in 0.5% glutaraldehyde in 0.05 M cacodylate buffer, were subjected to a 2-h treatment in 25% glycerol, and were frozen in liquid mono-iodo-fluoromethane (Ucon 22 of Union Carbide Corp., New York). Fracturing was done at −100°C in a Denton freeze-etch unit (Denton Vacuum Inc., Cherry Hill, N. J.). The fractured surfaces were coated at an angle of 45° with carbon-platinum and then at 90° with carbon. The replicas were cleaned, mounted on grids, and examined with the electron microscope.

OBSERVATIONS

Figure Orientations

Except for Figs. 1, 3–5, and 17–22, all figures are oriented with the more dorsal surface toward the top. In addition, longitudinal sections (Figs. 2, 8–11, 13–16) have the more posterior end to the right, and cross-sectioned figures (Figs. 6, 7, 12) have the right side to the observer's right, so that one is observing the buccal apparatus from the posterior toward the anterior end. Figs. 3–5 have the anterior end toward the top, and the cell's right is to the observer's right, so that the cell is viewed from the dorsal toward the ventral surface.

Buccal Cavity

The general features of the oral region of Paramecium observed in this study are the same as
FIGURE 1  Pictures of a clay model, made approximately to scale, of the space occupied by the buccal cavity. The anterior end is to the top of each picture. The details of the pellicle covering the buccal cavity are sculptured into the surface of the model. The observer is viewing the buccal cavity as though from inside the cell, i.e., the cell has been stripped away leaving only the buccal cavity intact. Fig. 1 a shows the left side with its ventral edge to the left and dorsal edge to the right of the figure. v indicates the pattern of pellicular ridges (lines) and cilia (round dots) in the vestibule. The quadrulus (q) and dorsal (dp) and ventral (vp) peniculi are each composed of four rows of cilia. The region ventral to the peniculi consists of a smooth surface characterized by the alveolar sacs (not shown) and an underlying filamentous reticulum (fr) but no cilia. The filamentous reticulum is also continuous over the peniculi, quadrulus, and ribbed wall but this was not shown in the model. The ventral peniculus ends at about the cross-sectional level of the posterior end of the vestibule. The two ventral ciliary rows of the dorsal peniculus end just posterior to the above, whereas the two dorsal rows end near the posterior end of the buccal cavity. Fig. 1 b shows the dorsal surface. The four ciliary rows of the quadrulus (q), which are widely spaced at the anterior end of the buccal cavity, converge in the middle of the buccal cavity and then, as a group, angle over to the left side and pass down the left flank of the buccal cavity and finally traverse its posterior end. The cytostome-cytopharynx complex (cy) encircles the buccal cavity's posterior dorsal surface, surrounding its developing food vacuole (fv). Along the left side of this region, next to the quadrulus, are the cytopharyngeal ribbons (cr); their origins are indicated here by short lines bordering the left and posterior margins of a developing food vacuole. The right side of the food vacuole is bordered by a ridge or lip (rl) which runs between the quadrulus and the ribbed wall (rw) in the anterior part of the buccal cavity. Fig. 23 is a detailed drawing of what a segment of the left cytostomal lip, such as that indicated by the box, would be like in three dimensions. Fig. 1 c is the right side of the buccal cavity. This is distinguished by the nonciliated ribbed wall (rw) bordered dorsally by the ridge of the right cytostomal lip (rl) and ventrally by the endoral membrane (e), shown here as a row of cilia separating the ribbed wall from the vestibule (v). Fig. 1 d shows the buccal cavity lying on its left dorsal surface so that the vestibular opening (vo) can be seen and also the anarchic field (af) of basal bodies from which a new buccal apparatus is organized during cell division.
A segment of the left wall of the buccal cavity that has been freeze-fractured. The viewer is seeing a segment of the same surface from inside the cell as seen in Fig. 1a. The dorsal surface is to the top of the picture and the posterior direction is to the right. The posterior ends of the ventral peniculus (vp) and the two ventral rows of the dorsal peniculus (dp) are present. The normal somatic pellicular architecture of the vestibule (v) can also be seen. A short segment of the quadrulus (q) passes down the left buccal cavity wall to close the gap between itself and the dorsal peniculus. The space within the posterior end of the buccal cavity and the nascent food vacuole is to the right and contains many fractured cilia (c). A short segment of the sawtooth-contoured left lip of the cytostome is indicated as ll. ps, parasomal sacs. x 7,000.

Those previously described (10, 17, 19, 26, 28). The new information which will be presented here emphasizes the food vacuole-forming region, known as the cytostome-cytopharynx complex, and to a lesser extent, the ribbed wall. A clay model based on serial sections of the buccal cavity space and vestibular space was sculptured to facilitate the understanding of the oral region. Four aspects of this model are presented (Figs. 1a–d) and should be referred to during the following descriptions. The observer views the model as though from inside the cell.

The two peniculi, ventral and dorsal, curve over the left surface of the buccal cavity from their anterior ends at the buccal overture (the opening between the vestibule and the buccal cavity). The four ciliary rows of the ventral peniculus end in a staggered manner in the vicinity of, but dorsal to, the posterior end of the buccal overture (Figs. 1a, 2), while the dorsal peniculus extends beyond this zone. The latter’s ventral two rows of cilia end a short distance beyond the terminus of the ventral peniculus (Figs. 2, 22), while its dorsal two rows continue on to the end of the buccal cavity in close association with the quadrulus. The quadrulus also begins anteriorly at the buccal overture. It passes over the anterior half of the buccal cavity along the dorsal wall. Its four rows of cilia are not closely associated with each other in this part of the buccal cavity. At the posterior end of the oral opening RICHARD D. ALLEN Membrane Growth and Transport in Paramecium 907
these four rows angle across the dorsal surface from right to left and converge together along the right side of the dorsal peniculus (Figs. 1 b, 2, 3). From here they spiral in close association with the two ciliary rows down the left side and over the posterior end of the buccal cavity where they end. They stop a short way beyond the end of the two dorsal ciliary rows of the dorsal peniculus. The endoral membrane is composed of a single row of cilia, two rows of basal bodies, along the posterior two-thirds of the right side of the buccal overture (Fig. 1 c). However, the groove in which this membrane is placed and the nonciliated basal bodies continue to the anterior end of the buccal overture. The four ciliary complexes described above are the only cilia found within the buccal cavity proper; however, the funnel-shaped vestibule leading to the buccal overture has cilia arranged in the same pattern as the cell’s surface (Fig. 2). The only exception to this is a field of basal bodies on the vestibular side of the endoral membrane which makes up the anarchic field that will eventually give rise to the membranelles of a new oral region after cell division (Fig. 1 d) (17, 34).

The ribbed wall covers the right side of the buccal cavity (Fig. 1 c). Along its dorsal margin the ribbed wall borders the quadrulus from the anterior margin of the buccal overture to a point about halfway to the posterior end of the buccal cavity where the cytostome begins (Fig. 3). From this point it borders the cytostome to its posterior end. In the anterior half of the buccal cavity the ribbed wall is bordered on its ventral side by the endoral membrane and in the posterior half by a nonciliated region which is characterized only by the three membranes of the pellicle and an underlying filamentous reticulum. The ribs of the ribbed wall, of which there are approximately 40 or 50, begin near the endoral membrane and curve upward and posteriorly along the right wall of the buccal cavity. Microtubules are not found within these ribs except at their extreme dorsal ends. The ribs end dorsally adjacent to a ridge or lip, which runs most of the length of the buccal cavity and separates the ribbed wall from the quadrulus in the anterior part of the buccal cavity, and which borders the right edge of the cytostome in the posterior part of the buccal cavity (Figs. 3–5). In oblique section, this lip is characterized by several bands of microtubules, in groups of two to four microtubules per band, which lie parallel to and

Figure 3 The dorsal surface of the buccal cavity is shown in the same orientation as Fig. 1 b. Three rows of the quadrulus (q) are seen at the top (anterior end) of the micrograph as they begin to angle across the buccal cavity (bc) surface. A section through a developing food vacuole (fv) occupies the lower part of the figure. The anterior apex of the cytostome-cytopharynx complex (bold arrow) is bordered on the left by the cytopharyngeal ribbons and on the right by the right cytostomal lip which bears groups of microtubules, two to four per group. The pouches at the dorsal ends of the alveolar sacs (a) of the ribbed wall line the right side of the anterior extension of this lip. Pouches from the alveolar sacs that surround the basal bodies of the quadrulus (thin arrows) also border the left side of the anterior extension of this lip. The row of cytopharyngeal ribbons (cr), after passing out of the section plane near the anterior apex of the food vacuole, reappears at the posterior end of the buccal cavity next to a group of postoral (po) fibers which underlie the right cytostomal lip. Postoral fibers are also present at the top of the figure. The cytostomal cord (cc) is cut at four places along its length. × 12,000.

Figure 4 A section serial to that in Fig. 3 showing the anterior part of the buccal cavity at higher magnification. The dorsal ends of the ribbed wall’s alveolar sacs (a) end next to the right side of the ridge that separates the quadrulus from the ribbed wall (line between brackets). Two small bands of microtubules begin along the ends of each of these sacs and extend posteriorly within the ridge for an unknown distance. Postoral microtubule bundles (po) underlie the ribs of the ribbed wall. A section through the cytostomal cord (cc) is at the top of the micrograph and near it are the blind pouches, arising from the alveolar sacs (arrows) which line the left side of the ridge. × 20,000.

Figure 5 A section serial to that in Fig. 3, showing the posterior end of the buccal cavity at higher magnification. The microtubular ribbons (below bracket) of the left lip meet the right cytostomal lip (rl) in this area. A section of the cord (cc) bordering the end of the ribs is present next to bundles of postoral (po) fibers. Several pairs of microtubules lying under the membrane on the right side of the single membrane-limited region identify the cytopharynx on this side of the nascent food vacuole. × 20,000.
within the lip. The alveolar sacs, parallel to and between the ribs, end in a series of blind pouches next to the right side of this lip (Figs. 4, 6). Small groups of microtubules, two to four in a group, are observed to arise along the sides of these alveolar pouches (Fig. 6) and from there they extend posteriorly in the right cytostomal lip (Fig. 4). At the posterior end of this right lip, pairs of these microtubules extend beyond the buccal cavity following the margin of the developing food vacuole (Fig. 7). In addition, bundles of hexagonally packed microtubules (postoral fibers) underlie the ribbed wall (Figs. 3-5). These microtubules may originate in the filamentous reticulum which coats the endoplasmic surface of the ribs. They pass posteriorly, the number of microtubules per bundle increasing as they go, following the contour of the ribs. Eventually, they extend far beyond the buccal cavity (Fig. 7) and end in the endoplasm, some near the posterior end of the cell. These bundles underlie the right lip of the developing food vacuole in close proximity to the microtubules within the right lip of the cytostome (Fig. 5).

The cytostome-cytopharynx complex comprises the posterior dorsal surface of the buccal cavity. It differs from the remainder of the buccal cavity since it lacks an underlying alveolar system, i.e., this region is lined by only a single membrane. Its anterior extremity forms an acute angle between the ribbed wall on one side and the quadrulus on the other (Fig. 3) at the same level along the longitudinal axis as the posterior end of the vestibule. However, the single membrane-lined region does extend anteriorly along the dorsal surface of the buccal cavity in the form of the narrow lip or ridge which separates the anterior half of the ribbed wall from the quadrulus (Fig. 6).

Posterior to this acute angle, the cytostome-cytopharynx complex broadens out over the dorsal posterior part of the buccal cavity and is rounded off at its posterior end (Fig. 3), thus taking on a shape approximating the silhouette of an elongated teardrop. The left edge of this region, beginning at the apex of the acute angle and bordering the right edge of the quadrulus, is lined with the ends of microtubular ribbons (Figs. 7-10). Because of its importance in the formation of new food vacuoles this region will be described in detail.

**Left Margin of the Cytostome**

The left margin of the cytostome has a particularly complex organization. Along much of this margin the pellicle bends sharply from the dorsal surface to form the left border of the buccal cavity (Fig. 1b). Along the dorsal margin of this bend are rows of microtubular or cytopharyngeal ribbons beginning at the bend and extending toward the nascent food vacuole. These microtubular bands, each consisting of 10-12 microtubules all arranged in one plane, lie against the membrane along one of their edges (Figs. 9, 11, 20); thus, each band stands away from the membrane rather than lying flat against it. These bands are placed at intervals of 0.25 μm along this lip. Since the lip is approximately 15 μm long there are about 60 ribbons all together. Pitelka (26) felt that these ribbons probably arose from the right row of basal bodies of the quadrulus. On first observation this certainly seems to be true, and yet on studying this area

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**Figure 6** The dorsal surface of a cross-sectioned buccal cavity (bc) at some point along its anterior half. The single membrane-limited ridge, the extension of the cytopharynx into this region, separates the alveolar sacs (a) of the quadrulus (q) to the left from those of the ribbed wall (rw) to the right. Pairs of microtubules line this single membrane to which they may be bridged. Prominent bridges (arrows) occur between the lateral groups of microtubules and the alveolar sacs on both sides of this ridge. This section is probably near the origins of these microtubules. A blind pouch at the end of the alveolar sac of one rib is marked by an asterisk. × 60,000.

**Figure 7** The posterior tip of the buccal cavity in transverse section. The cilia of the quadrulus fill the cavity. The last two units of the dorsal row of cilia of the dorsal peniculus (dp) appear at the bottom of the figure. Cytopharyngeal ribbon (cr) profiles are found above and below the cytostomal cord (cc) and in an area to the left of the cord. Disk-shaped vesicles are associated with all these profiles. The profiles to the left are probably of ribbons which extend ventrally toward the cytoproct. A portion of the nascent food vacuole (fv) membrane is found dorsally. Pairs of microtubules, probably continuous with those in the right cytostomal lip, are found along one bend of this membrane (bracket). Hexagonally packed microtubular bundles (postoral fibers) are found to the right of the buccal cavity and food vacuole (po). × 8,000.
carefully the electron micrographs do not support this conclusion. Rather, at least some of the microtubules have their origins in the edge of the filamentous reticulum along the right side of the quadrulus (Figs. 8, 12). The filamentous reticulum at this point has the same highly organized structure as it has under the ventral edge of the ribbed wall. The ends of the microtubules of the bands are embedded in the electron-opaque nodes of the reticulum. Opaque material next to one band is

**FIGURE 8** The left cytostomal lip bears rows of cytopharyngeal ribbons (cr) which originate in the edge of the filamentous reticulum (fr). These ribbons curve over the cytostomal cord (cc) as they proceed into the endoplasm. Blind fingerlike evaginations of the alveolar sac system (ar) extent up to the cord between the ribbons. Membrane-limited disks also accumulate between the ribbons. The postciliary microtubules (pm), arising from the right row of basal bodies of the quadrulus, pass into a wedge-shaped zone between the ends of the ribbons and the pellicular membranes but are not continuous with the microtubules of the ribbons. × 25,000.

**FIGURE 9** The microtubular ribbons are oriented normal to the single membrane-limited cytopharynx. The cytostomal cord lies over the edges of these ribbons and thereby forms "boxes" into which disks accumulate. The disks are also oriented normal to the cytopharyngeal membrane within these boxes. Pairs of microtubules are found next to the membrane (arrows) to the right of each larger microtubular band. The cilia of the quadrulus which extend through the cytostome opening appear to be oriented parallel to the ribbons since both are sectioned transversely. × 40,000.
linked to opaque material of adjacent bands by thin filaments crossing the 0.25-μm space between the bands. The right radial (postciliary) bands of microtubules from the basal bodies of the right edge of the quadrulus curve up behind the ends of the cytopharyngeal ribbons (Figs. 8, 12, 16) but are not continuous with them. Rather, they are relatively short and end in the narrow wedge-shaped region between the ends of the ribbons and the pellicle.

A series of boxlike regions are thus formed along the left cytostomal lip (Fig. 9). The floor of each box is composed of the single membrane covering the cytopharynx. The two sides are adjacent microtubular bands while the end is closed off by the fibrillar reticulum (Fig. 8). The opening closest to the developing food vacuole remains clear. Finally, the roof is covered by a bundle of microfibrils that will be called the cytostomal cord (Figs. 7–9). This cord lies over the ends of the microtubular ribbons and extends uninterrupted along the full length of the left edge of the cytopharynx following the contour of this edge. Beyond the anterior pointed end of the cytostome the cord continues along the right edge of the quadrulus (Figs. 3, 4). In one cell the cord could be followed all the way to the buccal orverture. At the posterior end of the buccal cavity the cord may end abruptly, as was observed in one cell, or it may continue on around the tip of the buccal cavity and proceed for some distance anteriorly over the dorsal ends of the ribs of the ribbed wall (Fig. 5). The cord may be held in position by electron-opaque material similar to that of the filamentous reticulum. In any case, the cord is invariably found in this location adjacent to the ends of the microtubular ribbons.

The microtubular ribbons themselves appear to be held in position next to the cytopharyngeal membrane by specialized microfilaments bearing many arms. These microfilaments link the distal microtubule of each ribbon to this membrane. This filament can be seen in both longitudinal (Fig. 13) and cross section (Figs. 11, 20). In cross section, it appears as an electron-opaque dot between the microtubular band and the membrane. The arms which project from this filament at intervals of 140 Å are best seen in longitudinal section. This filament extends for only a short distance, probably no more than 1 μm, along the microtubular band. At the end of the filament the bands are no longer attached to the membrane but curve abruptly away from the membrane and extend off into the cytoplasm, some for many micrometers. Often, the bands curve back over the cord forming a “U” curve (Fig. 14). Some of the bands, probably those in the anterior region, pass dorsally and lie close to food vacuoles which at times lie above and to the left of the buccal cavity. Bands in the posterior portion of the buccal cavity (Fig. 7) bend over the cord and extend ventrally and posteriorly ending near the cytopyroct. The actual terminus of these bands has not been located with certainty but it is most probable that they end in the endoplasm.

In addition to the ribbons of 10–12 microtubules, a pair of microtubules (one pair for each large band) also arises at about the same level as the bands (Fig. 11). Each pair runs parallel to the more posterior or counterclockwise side of the large bands (Figs. 9, 13) and remains close to the cytopharyngeal membrane. These pairs of microtubules are not attached by arm-bearing filaments to the cytopharyngeal membrane but may be attached directly to this membrane by bridges. They do not seem to arise from the filamentous reticulum as do the large bands but appear to abut the alveolar sac system at their ends (Fig. 11). The length of these small bands has not been determined but they are relatively short and are no longer seen along the larger bands beyond the point where these larger bands curve away from the membrane. Rather, they continue close to the membrane of the developing food vacuole for a short distance, about 1 μm, beyond the point that the larger bands curve away (Fig. 15).

The membranes in the area of the left edge of the cytostome-cytopharynx complex also show some specializations. The alveolar sac system extends beyond the right edge of the quadrulus and ends along a line marked by the beginning of the microtubular ribbons. Along this terminal line are found blind fingerlike continuations, some 0.5 μm long, of the alveolar sac system which extend between the bands of microtubules (Fig. 8). These projections angle away from the plasma membrane and end near the cytostomal cord (Fig. 16). In fact, in some cases the cord appears to surround the tips of these projections. Electron-opaque material resembling the nodes of the filamentous reticulum seems to connect the tips of these evaginations to the cord (Fig. 16). Evaginations also arise from the alveolar sac along the right side of the quadrulus in the anterior half of the buccal
FIGURE 10 A section of the same cell and same area as Fig. 8 but a few sections ventral to it. This shows the edge of the nascent food vacuole (fv) which is in continuity with the left lip of the cytostome-cytopharynx complex. Microtubular ribbons (cr) are present as are the cord (cc), disks, and an evagination of the alveolar sac (arrow) which is angling up toward the cord. × 20,000.

FIGURE 11 The cytopharyngeal ribbons oriented normal to the membrane appear to be attached to the membrane indirectly by a filament. This filament, in cross section, appears as a dot (arrows) with arms. The adjacent pairs of microtubules are attached to this membrane directly by bridges. The origin of these pairs must be against the edge of the alveolar sac (a), since the pair at the right lies within an indentation of the edge of this sac system. × 50,000.

FIGURE 12 The microtubules of the ribbons do not all start at the same plane. In this figure, two to six microtubules per ribbon (arrows) can be seen enmeshed in the filamentous reticulum at their origins. The obliquely sectioned bands of microtubules between the ribbons and the pellicle are the postciliary bands (pm) from the quadrulus. × 50,000.

FIGURE 13 An enlargement of the central area of Fig. 10. The arms of the arm-bearing filament lying between the distal microtubule of a ribbon and the cytopharyngeal membrane are best seen in longitudinal section. The arms are located at intervals of 14 nm. These ribbons do not follow the membrane very far; however, the adjacent pairs (arrows) continue close to the membrane beyond the point that the ribbons curve away. × 50,000.
cavity (Figs. 3, 4) and are associated with the extension of the cord into this region.

One of the most striking features of this left lip of the cytostome is the large accumulation of uniquely shaped membrane-limited disks along the full length of the lip (Figs. 7–10, 15, 22). These disks vary in diameter from 0.2 to 0.5 μm and also vary in thickness. The thickness, however, appears to depend on the conditions of fixation of the cells. In some cells the disks are extremely flat (Figs. 8, 22), all 40 nm thick, while in other cells many are swollen (Figs. 7, 9, 21), and in the most extreme situation they are spherical. The membranes of the disks are similar in appearance and width, 90 Å, to that of the plasma membrane. They have a three-ply structure and the leaflet closest to the lumen of the disk is more electron opaque and thicker than the cytoplasmic leaflet (Fig. 17). The darker leaflet is coated on its luminal surface by a 90-Å thick, medium electron-opaque material. This material sometimes has a beaded appearance when viewed in thin sections (Fig. 17). The plasma membrane is asymmetric in the same manner as the disks and also has a 90-Å coat on its outer surface. The disks also appear to be beaded after freeze-fracturing (Fig. 18). However, these beads are presumably found within the membranes of the disks since freeze-fracturing typically cleaves a membrane along its midline (6, 25). The beads are most prominent on the B face of the membrane, i.e., on the inside surface of the more electron-opaque leaflet of this membrane. The lumen of the disks is electron translucent; a few electron-opaque granules are occasionally present.

The disks are often found to be specifically associated with the cytopharyngeal microtubular bands. Many lie flat against the bands at a distance of about 40 nm from the microtubules. Frequently they are aligned in single file along a single band (Fig. 19). The gap between the disk and the microtubules contains a finely filamentous material but electron-opaque bridges such as those that have been reported in nerve axons between microtubules and synaptic vesicles (31) or between microtubules and other membranes (see reference 13) have not been regularly detected.2

The disks are particularly numerous along the microtubular ribbons next to the cytopharyngeal membrane (Figs. 7, 9, 10, 15). Thus in this region they lie within the “boxes” under the cytostomal cord and above the cytopharyngeal membrane. The disks are oriented with their edges closest to the membrane (Fig. 9). Also in this zone the membranes of some disks can be seen to be continuous with the cytopharyngeal membrane (Figs. 20, 21). In these cases the edges of the disks have fused with the underlying plasma membrane and their lumens are now open to the lumen of the cytopharynx.

The discoidal vesicles also appear to be able to attach to other microtubular systems in the cytoplasm such as those within the right lip of the cytostome and even postciliary microtubules (Fig. 6). However, these occasional associations appear to be more random and no large accumulation of vesicles occurs at the right lip and no vesicles have been observed to be continuous with the cytopharyngeal membrane in this region.

The single membrane of the cytostome-cytopharynx complex has a different topography along its left edge, i.e., next to the ribbons, than over most of its surface (Fig. 22). In this zone the membrane contour shows sharp angles, whereas, in the remainder of the developing food vacuole, the membrane is relatively smooth or, if folded, the topography of the folds is more smoothly curved.

DISCUSSION
Cytopharynx

The new information which this morphological study contributes leads to a more concise definition of the cytopharynx of Paramecium. The right lip of the cytostome extends from the posterior dorsal end of the buccal cavity, past the anterior tip of the cytostome, and on to the anterior end of the buccal cavity as a single membrane-limited ridge. Within this lip microtubules, in small groups of two to four, pass posteriorly from their origins near the dorsal ends of the alveolar sacs which cover the ribbed wall. In the anterior half of the buccal cavity microtubules also enter the ridge from the margins of the alveolar system bordering the left side of the ridge. Pairs of these microtubules extend beyond the posterior end of the buccal cavity and border a portion of the nascent food vacuole membrane. At the left lip of the cytostome a series of bundles, each composed of 10–12 microtubules, is interspersed with pairs of microtubules. These pairs arise next to the ends of alveolar sacs and pass close to the membrane of the nascent food vacuole. Based on the definition

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given by Corliss (7) it thus seems correct to refer to this single membrane-limited area subtended by periodically arranged and closely apposed microtubular pairs as the cytopharynx of *P. caudatum*. This area is found on both the right and left sides of the cytostomal opening and continues as a ridge which passes anteriorly along the dorsal surface of the buccal cavity.

**Left Lip of the Cytostome: Specialized for the Transport, Exocytosis, and Growth of Membranes**

The morphology of the left lip of the cytostome suggests that this region is specialized for sequestering preformed membrane material (i.e., disk-shaped vesicles) and for insuring that these vesicles contact the cytopharyngeal membrane in the proper orientation needed to allow them to fuse with this membrane thus providing additional surface area to the growing food vacuole. Fig. 23 is a drawing of a segment of this left lip and should be referred to in this discussion.

The cytopharyngeal ribbons arise at regular intervals along this lip and pass along the cytopharyngeal membrane from their points of origin for a short distance. They soon leave the membrane and fan out into the endoplasm; some extend above the buccal cavity, some pass to the left of the buccal cavity, and some pass ventrally and posteriorly towards the cytoproct. The function of these ribbons extending into the endoplasm appears to entrap and to sequester disk-shaped vesicles and to guide or move these vesicles to the oral region. Two morphological observations seem to support this postulated function. First, bands of microtubules of cytopharyngeal origin frequently have disk-shaped vesicles aligned in single file along one side of the band. Second, the greatest number of such vesicles are found next to the left lip of the cytostome in close association with these ribbons. Based on serial sections it is observed that the density of the population of disks decreases with increasing distance from the cytostome.

Where do the disks come from? These disks probably arise from food vacuole membrane, both during the process of food vacuole condensation (12, 23) and from endocytosis of the old food vacuole membrane at the cytoproct (3). Vesicles are known to arise by pinocytosis from circulating food vacuoles in a number of ciliates (9, 11, 12, 18, 23, 29, 33). These vesicles, of various initial shapes, may eventually be transformed in *Paramecium* to the special disk shape. In addition, we have shown (3) that the food vacuole membrane at the cytoproct is engulfed by endocytosis. The resulting tubular fragments may then be transformed into disks and moved again to the cytostome.

At the left lip of the cytostome the vesicles fuse with the cytopharyngeal membrane. Assuming that this membrane can flow within its own plane, as seems to be the case for other membranes (24, 30), the disks after fusion would flatten and become integrated into the plane of the growing food vacuole membrane, thereby increasing its surface area. The function of the arm-bearing filaments that attach the ribbons to the cytopharyngeal membrane may be to restrict the sidewise flow of the membranes of the disks and thus to allow this membrane to flow toward the...
food vacuole. The presence of such a filament linking microtubules to membranes seems to be unique although its 140-Å periodicity is nearly the same as that originally reported for the arms on the outer fibers of cilia (14) and more recently in *Chlamydomonas* flagella (16). Here it was also observed that the arms are readily detached during preparations for negative staining. Tucker (32) reports that arms having a 110-Å periodicity occur on a microtubular band, the lamella, in the cytopharyngeal basket of the ciliate *Phascolodon*. Also, an arm-bearing flange projects from the inner end of each lamella. Tucker has reviewed the occurrence of arms on lamellae in other ciliates (see also reference 4) and suggests they may play a role in moving materials, including membranes, along microtubules during feeding (32). It is possible that the arm-bearing filaments along the cytopharyngeal ribbons in *Paramecium* may be equivalent to the arms on the lamellae of other ciliates and, in a ratchetlike action, they may propel the cytopharyngeal membrane toward the growing food vacuole as new membrane is added between the ribbons.

The angular appearance of the food vacuole membrane next to the left lip of the cytostome is another observation supporting the view that the disks are indeed fusing with the cytopharyngeal membrane. Such an angular appearance of membranes is found also in the plasma membrane covering the luminal surface of the mammalian ureter (15) and urinary bladder epithelium (21, 27, 31), where it may result from the rapid fusion of fusiform vesicles with the epithelial plasma membrane (15, 21, 27).

The boxlike regions formed at the ends of the cytopharyngeal ribbons may contribute to the overall function of this system. As the vesicles are moved along the ribbons to the cytostome, they become trapped within these boxes. The orientation of the vesicle edge next to the membrane is also maintained within these boxes. Thus a readily available pool of preformed membrane is waiting to be added to the food vacuole membrane when new membrane is required.

The function of the cytostomal cord which lies over the ends of all of these cytopharyngeal ribbons, and passes to the anterior end of the buccal cavity, can only be guessed at. A contractile function is suggested by its microfibrillar structure, which closely resembles the structure of the contractile myonemes of the peritrichs (1, 2), and its specific association with regularly spaced evaginations of the alveolar sac, bringing to mind the myoneme association with the endoplasmic reticulum and also with the alveolar sacs via linkage complexes. A contraction might either initiate the release of the filled food vacuole from the cytopharynx, or it might force the underlying disks into the cytopharyngeal membrane bringing about the fusion of these two membranes. On the other hand, the cytostomal cord also resembles the presumably noncontractile infraciliary lattice of *Paramecium* in its texture, but the cord does not branch as does the lattice. The possibility remains, however, that it is an unbranched segment of this lattice, and that it has a structural function only.

**Membrane Recycling: A Model**

The specialized morphology of the left cytostomal lip may thus be a link in an elaborate system designed to recycle food vacuole membranes in *Paramecium*. Membrane in the shape of disks is added to the cytostome-cytopharynx complex with the aid of the structures of the left lip of the
Figure 19 Cytopharyngeal ribbons may be found in the cytoplasm at some distance from the cytopharynx. Disks are frequently aligned along these ribbons in linear fashion. They are separated from the microtubules by a constant space of 40 nm. This is occupied by an amorphous material from which ribosomes and glycogen particles are excluded. \( \times 40,000 \).

Figure 20 The edges of disk-shaped vesicles fuse with the cytopharyngeal membrane so that the two membranes become continuous (arrows). Cross sections of ribbons and filaments attaching the ribbons to the membrane are present. cc, cytostomal cord; a, alveolar sac; fr, filamentous reticulum. \( \times 50,000 \).

Figure 21 Another micrograph of disks that are continuous with the cytopharyngeal membrane (arrows). cc, cytostomal cord; pm, postciliary microtubules. \( \times 35,000 \).
Figure 22. The growing edge of the food vacuole membrane has an angular contour (arrows). Many disks are present on either side of the zone where the nascent food vacuole joins the buccal cavity (bc). The dorsal two rows of the dorsal peniculus (dp) can be seen extending beyond the ends of the two ventral rows. A mature digestive vacuole (fv) is present in the cytoplasm to the left. × 15,000.

cytostome. The food vacuole is thereby permitted to expand, and the fully expanded food vacuole is released from the buccal apparatus. As the food vacuole circulates through the endoplasm, membrane is removed from the food vacuole by pinocytosis and is eventually transformed into disks. Finally, at the cytoproct the remainder of the food vacuole membrane is transformed by endocytosis into small tubules and then into disks. The ribbons of microtubules extending out into the endoplasm from the left cytostomal lip filter these disks from the endoplasm by attaching to the disks. The disks are then moved back to the cytostome in association with the microtubular ribbons. Fusion occurs between the disks and the cytopharyngeal membrane at the left lip and the cycle begins again.

Admittedly, the evidence presented in this paper for such a recycling process is entirely morphological and circumstantial. However, *Paramecium* is not the only ciliate which may have such a recycling mechanism. Kloetzel (20) has described membrane rods (later shown to be disks by McKanna [22]) in *Euplotes* which appear to fuse with the cytopharyngeal membrane. Cells which were first fasted and then refed showed a depletion in rod numbers. McKanna (22) has also proposed a recycling of membranes in the food vacuole system of the peritrichs and reports an accumulation of disk-shaped vesicles next to the cytopharynx after starvation and a dramatic depletion of these vesicles after rapid feeding. He also reviews the published evidence for a possible recycling system in suctorians. Bradbury (5) sees the possibility of a similar membrane recycling system operating in an apostome ciliate. Thus, the combined evidence for such a membrane-recycling system in a wide range of ciliates, together with the morphological evidences in *Paramecium* of vesicles specifically aligned along microtubules, or accumulated at the cytopharynx and continuous with the cytopharyngeal membrane, and of vesicle formation from egested food vacuoles at the cytoproct (3) presents, in my opinion, a strong case for the recycling model. Also, evidence to be published separately shows that the vesicles are firmly attached to the cytopharyngeal microtubules in *Paramecium*. A firm attachment would be required if the microtubules are involved.
FIGURE 23  A three-dimensional drawing of a part of the left lip of the cytostome, including the pellicle around three rows of the quadrulus (q) with its parasomal sacs (ps) and a segment of the membrane limiting the nascent food vacuole (fv). This portion is equivalent to the region in Fig. 1 b enclosed by a box (Fig. 1 b should be turned end for end to have the same orientation). Cytopharyngeal ribbons (cr) originate in the filamentous reticulum (fr) pass under the cord (cc), curve back sharply, and pass out into the endoplasm. Membrane-limited disks (d) are aligned along these ribbons and accumulate within the boxes under the cord. Here they fuse (arrow) with the membrane of the cytopharynx. It is assumed that this membrane can then flow toward the food vacuole and become an integral part of the food vacuole membrane. a, alveolar sac evaginations; bc buccal cavity.

more than passively, in vesicle movement. Finally, Weidenbach and Thompson (35) have recently shown that the food vacuole membranes of the ciliate Tetrahymena are biochemically distinct in their lipid content from other cell membranes, including the plasma membrane. This suggests that a unique subfraction of membrane exists in ciliates which is specialized to delimit digestive vacuoles. This special membrane thus appears to be able to fuse with the plasma membrane without becoming a permanent part of this membrane, but, rather, undergoing endocytosis or phagocytosis to be recycled by the cell.

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