AN ELECTRON MICROSCOPE STUDY OF THE CYTOLOGY OF THE PROTOZOAN EUPLOTES PATELLA*

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The hypotrich protozoa possess some of the most highly differentiated locomotor organelles and cytoplasmic fibrillar structures in all of the ciliates. Judging from the many observations reported in the literature over the past seven or more decades, the genus *Euplotes* has been studied more extensively than other hypotrichs. Taylor (43) summarizes the studies from 1883 to 1910, emphasizing especially the fibrillar systems in non-dividing organisms. Later studies of non-dividing organisms have been made by Yocom (48), Taylor (41, 42), von Gelei (16), and Turner (45); dividing organisms were studied by Turner (44), von Gelei (15), Hammond (17), and Hammond and Kofoid (18).

Initially, our interest was directed toward the ciliary arrays and relationships in the cirri and membranelles; however, a more thorough study of the general cytology of the non-dividing organism has been undertaken. The purpose of this report is, therefore, to describe cytological aspects of the numerous organelles in *Euplotes patella* in interphase organisms only, utilizing the present levels of electron microscope resolution and present concepts of image interpretation, and to correlate these observations with the light microscope literature of the past five decades and with recent observations of general cytology. Particular attention is given to the ciliary rootlet system, mitochondria, and macronuclear structures; some observations on the pellicle and cilia are reported elsewhere (33, 35, 36).

Material and Methods

After isolation from local soil, *Euplotes patella* was grown on a lettuce infusion inoculated with *Aerobacter aerogenes*. Pierson's taxonomic study (25) was the basis for classification.

In the preparation of sections for electron microscopy, methods were employed which did not involve centrifugation in any step, since previous experience with the preparation of protozoa indicated that pellicular structures may be damaged by such treatment. A sintered glass filter of medium porosity was used to concentrate the organisms from the culture fluid, and fixation as well as subsequent steps was carried out without transfer to another container.

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No suction was used in any step following fixation; changes of fluid were made by aspiration after the organisms had settled out of suspension. The fixation fluid was 1 per cent osmium tetroxide in 0.9 per cent sodium chloride with MacIlvaine's buffer (pH 7.4, 0.05 M), and the fixation time was 1 hour at 22°C. One 15 minute change of fluid of the same saline and buffer concentration as the fixation fluid was used as a wash, followed by 15 minute changes of 50 per cent, 75 per cent, and absolute ethyl alcohol. Infiltration was accomplished by three 30 minute changes of methacrylate monomer which consisted of 40 per cent ethyl methacrylate and 60 per cent *n*-butyl methacrylate. Polymerization was carried out by the use of both 1 per cent Luperco CDB (0.5 per cent 2,4-dichlorobenzoyl peroxide) and ultraviolet light at a temperature of 60°C.

Sections were cut at 0.025 μ, using either an International Rotary or a Porter-Blum microtome, and mounted on carbon membranes. Micrographs were taken at original magnifications of 3,000 to 20,000 with the RCA EMU-2A and EMU-3A electron microscopes, using the 100 kv. beam in the latter instrument.

Sections of the fixed preparations were also cut for staining and observation on the light microscope. One- and 2-μ sections were stained with Azure B and also with Feulgen reagent and fast green counterstain, without removing the methacrylate embedding medium.

OBSERVATIONS

Cirri and Membranelles.—In the low-power survey micrograph (Fig. 1) an oblique dorso-ventral section through the entire organism is shown that includes two cirri. As previously described (33, 35, 36), the eighteen cirri in *Euplotes patella* are composed of five to eight rows of cilia usually displaying hexagonal packing, with perfect or nearly perfect bilateral symmetry (Fig. 2). In thirty-three cirri surveyed, the number of cilia ranged from twenty-one to thirty-five with an average of twenty-seven. Cirri are usually seen in pellicular depressions with groupings of small vesicles near their bases; no sheath, membrane, or extraciliary matrix is present to unite the cilia, each of which has its own separate membrane (Fig. 3).

The membranelles are located ventrally along the anterior and left margins of the organism, and each consists of two or three rows of cilia numbering fifteen to twenty-five in each row; often the third row, if present, is much shorter than the other two and may have as few as four cilia. The distance between the centers of adjacent membranelles may be only 1 μ. No sheath or extraciliary membrane has been observed around the membranelles so that, as in the cirri, the cilia are in direct contact with the environmental fluid (Fig. 4, *ME*).

Cilia.—Many of the details of cilium structure have been described earlier (35) and will not be repeated here. However, certain salient features are worthy of review. It was shown that the structure of the ciliary shaft in *Euplotes* is typical of cilia elsewhere, showing two single central fibrils, nine peripheral double fibrils, and an outer membrane which is continuous with the outer pellicular membrane. It was further shown, however, that the ciliary membrane is double-layered and has numerous protrusions which may contribute to the functional unity of both cirri and membranelles (Fig. 3, *PC*). In the basal region, the central fibrils are present, but no connections with the rootlet

filaments (described below) have been observed, although the rootlet filaments are connected intimately with the peripheral fibrils.

In addition to this earlier description, an additional structural relationship in the basal region of the cilium should be described. Small granular structures measuring 65 to 90 mμ in diameter have been observed at the basal portions of the cilia of both cirri and membranelles. At the extreme basal ends of the peripheral ciliary fibrils, either several granules or a ring is present where the rootlet filaments join (Fig. 3); this is the level of the fiber plate described by Taylor (41). At a level about 170 mμ more distal (Taylor's basal plate), similar granules are seen; these granules along with rootlet filaments at the same levels undoubtedly account for the appearance of a plate in light microscope preparations.

Bristles.—In addition to the cilia in the cirri and membranelles, a few structures termed bristles are found at 3-μ intervals on the dorsal surface in several rows. Hammond (17) reports that they "are not rigid, nor are they vibratile, but . . . show slight movements which may be passive." As seen here, the appearance in cross-section (Fig. 5, *B*) is that of a typical cilium except that no rootlet fibrils have been observed to connect with the basal portions of the bristles. The bristle locations may be occupied either by single or paired cilia. Of the pairs, one is a typical cilium, while in the other the ciliary fibrils are poorly seen, and the size and shape may be slightly different (Fig. 5, *B*). As frequently described in the light microscope literature, each bristle (or pair of bristles) is located in a cone-shaped pellicular depression which is surrounded just inside the pellicle by six or more rod-shaped granules. The "granules" appear here as vacuoles consisting of a small amount of low density material surrounded by a single-layered membrane (Fig. 5, *CV*). In appearance they are identical to the vacuoles seen near the cilia of membranelles and cirri (Figs. 2, 3, and 4, *CV*); such vacuoles are typically located near most cilia in this organism and for this reason, the term "ciliary vacuole" has been adopted here.

Rootlet Filaments.—Most of the active cilia in *Euplotes* have rootlet filaments closely associated with their basal portions. These individual filaments may be grouped into bundles which constitute the fibrils described in light microscope observations by Yocom (48) and others. The individual filaments of these bundles have an average diameter of 21 mμ[1] and a central portion of low density (Fig. 3, *R* and Fig. 4, *FI*). No periodic structure has been observed either in the filaments or in the fibrils.

Individual filaments can be seen which are not in fibril bundles (Fig. 4, *MF* and *FA*) and which connect adjacent membranelles and adjacent cilia. Such connecting filaments also occur between cirri (Fig. 2, *FC*), and in a few cases between cirri and membranelles (not shown) and between cirri and the sub-

[1] One correction should be noted to the earlier publication (35): the proper diameter of the ciliary rootlet filaments is larger than previously given and properly should be 21 mμ.

pellicular filaments (Fig. 2, *FP*). The short filaments connecting adjacent cilia are at two different levels in relation to the basal portions of the cilia, one at the extreme proximal end and the other about 170 mμ more distal as described above. These interconnections are not usually by the most direct line between cilia, but rather almost at a tangent to the circle of peripheral fibrils (Fig. 4, *FA*). On a few occasions such filaments in cirri have been seen tangentially touching three or more cilia in much the same way.

Motorium.—The motorium is a structure first described in *Epidinium* (*Diplodinium*) *ecaudatum* by Sharp (40), and later in *Euplotes eurystomus* by Yocom (48); its presence has been disputed by Turner (45). It was described by Yocom as a bilobed body about 8 μ long lying close to the right anterior corner of the triangular cytostome and at which rootlet fibrils from membranelles and cirri converged. On a few occasions in this study, a close grouping of filaments has been observed in the region of the cytostome (Fig. 7, *M*). In this micrograph, the filaments are shown to be grouped immediately inside the gullet edge and to be extending both from the gullet wall and from the membranelles. In other micrographs, filaments have been seen entering from a third direction; hence this is a region of convergence of filaments from several directions. These filaments show no elaboration of structure or change in diameter in this region, but have the same diameter and appearance as the rootlet filaments. It is difficult to assign dimensions to the motorium region, but no section has been observed measuring longer than 4 μ or wider than 2 μ. For these reasons, it seems permissible to conclude that this is the motorium of the light microscopists.

Pellicle.—This term is used here to denote only the outermost membranes of the organism. Two such membranes are present; the outer one is termed the pellicular membrane (Fig. 4, *PM*), while the inner one is termed the cytoplasmic membrane (Fig. 4, *CM*). Both were previously demonstrated (35) to be double-layered membranes in which the thickness of the two layers and the distance between the layers are approximately the same. The pellicular membrane, which is continuous with the ciliary membrane (Fig. 3, *PM*) and has the same structure, has an over-all thickness of 9 mμ and is characterized by many small protuberances (Figs. 3 and 5, *PC*). The cytoplasmic membrane lacks such irregularities, has a thickness of 12 mμ, and is usually closely applied to the pellicular membrane (Fig. 4) unless the latter has been disrupted in preparation (Fig. 5). At the points of emergence of cilia, this membrane appears to be discontinuous, usually ending at an enlargement close to the cilium (Fig. 3, *CM*).

Subpellicular Filaments.—In very close relationship to the inner surface of the cytoplasmic membrane, there is a system of filaments running predominantly in the antero-posterior direction (Fig. 5, *AF*). In some sections, single filaments may be seen in cross-section (Fig. 4, *FS*), but in others there are

several filaments grouped together. These filaments average 22 mμ in diameter; this figure is determined from rather few measurements, since the plane of the section seldom allows careful determination of size. The distance between filaments or groups of filaments is quite variable, ranging from 50 to 100 mμ. Another series of filaments, somewhat closer together, is also present just below the pellicle, perpendicular to the first series, but still parallel to the pellicle (Fig. 5, *TF*). When they are adequately resolved, filaments appear similar in structure to the rootlet filaments that have a low density central portion and a higher density outer portion.

Micronucleus.—Throughout this entire study, only six or seven micronuclei have been seen. The oval shape usually observed is attributed to distortion arising from sectioning, since a nearly spherical shape 2.5 to 3 μ in diameter is reported in the literature of light microscopy. In one section (Fig. 6, *F*) filaments similar in size and appearance to the rootlet filaments described above have been observed in very close association with the membrane of the micronucleus.

All of the micronuclei seen have shown the same internal appearance, which is assumed to be that of the non-dividing nucleus. The nucleoplasm appears spongy because of a dense material in a less dense matrix, all of which is surrounded by a double-layered membrane. Each layer of the membrane is 7 mμ thick, with a separation of 7 mμ (Fig. 8). Suggestions of annuli and membrane discontinuities have been observed, but no three-dimensional reconstructions have been possible.

Macronucleus.—In the survey micrograph (Fig. 1, *M*), a considerable portion of the long, C-shaped macronucleus is shown; it contains dense bodies embedded in a homogeneous matrix of lower density bounded by a membrane. In the light microscope, Azure B staining of 2 μ sections has shown that these dense bodies all contain nucleic acids; Feulgen staining both in this study and in that of Faure-Fremiet, Rouiller, and Gauchery (10) has further shown that most of the bodies are Feulgen-positive, though a few stain with the fast green counterstain. Therefore, the most numerous bodies contain deoxyribose nucleic acid, while the others contain ribose nucleic acid and protein; the latter are thus considered to be nucleoli. In the electron microscope, two types of bodies can also be distinguished; the most numerous ones are denser and more compact than the others, although little other structural difference can be seen even at higher magnifications. The less compact bodies are the nucleoli; however, they fail to show the granular appearance which has been described in metazoan nucleoli by Bernhard *et al.* (3).

The macronuclear envelope is double-layered (each layer is 7 mμ thick, with a separation of 7 mμ) and has many nuclear bodies applied closely to the inner layer (Fig. 8). The membrane is further characterized by annuli (Fig. 9, *A*), which have an outside diameter of 70 mμ and an inside diameter of 40 mμ.

The macronuclear reorganization bands have been observed in several sections during this study and were mentioned briefly in an earlier report (34). Each band is composed of a solution plane and a reconstruction plane, which together are approximately equal in width to the diameter of the macronucleus (Fig. 10). The macronuclear membrane is intact during the changes taking place at this point in the nucleoplasm. The solution plane is composed of granules and filamentous structures measuring up to 40 mμ in diameter. The reconstruction plane appears as a homogeneous zone with no structure evident except for a few darker, small regions or granules; however, since it has not been viewed in optimally thin sections, the resolution obtained has not allowed such careful observation as was possible for the solution plane. In the Feulgen-stained, thicker sections, the solution plane appears strongly Feulgen-positive as do the chromatin granules, while the reconstruction plane shows a diffuse, much lighter Feulgen-positive reaction.

Mitochondria.—The mitochondria often appear to be more concentrated near the pellicle than in the endoplasm (Fig. 1). The general shape is that of either a sphere or ellipsoid, with numerous slightly distorting irregularities of the edge which make it difficult to observe its relationship to the tubules. The internal structure consists almost completely of rather uniform sized tubules which appear to be surrounded by or embedded in a material of lesser density (Fig. 11). The tubules are randomly oriented with the exception that two to four cross-sections often appear side by side. The tubule wall is clearly defined from the material of lesser density, which seems to be present in some portions of a given mitochondrion but absent in others.

Microbodies.—Numerous sections of round or oval structures measuring 250 to 350 mμ in diameter have been observed in the cytoplasm. They are characterized by a single-layered membrane which surrounds a nearly homogeneous inner material; they do not contain *typical* tubules or cristae, but have an internal density which is similar to that of the mitochondrial matrix (Figs. 4, 5, and 13, *A*). They are very close to or in contact with the mitochondrial surface in about two out of three sections and occur with a frequency of about one for each fifteen mitochondria.

Food Vacuoles.—Numerous food vacuoles may be seen filled with bacteria which appear ranging from densely packed (Fig. 1, *F*) to less well packed (Fig. 12). The diameters may be as large as 13 μ with a spherical shape, or smaller in size and more irregular. The bacteria characteristically contain a central granule in a central space, around which smaller granules are arranged in a concentric ring, all in a less dense, homogeneous matrix. The bacterial cell membrane is also apparent, and in the sections observed seems to be intact. The outer limit of the vacuole has a continuous single-layered membrane measuring 14 mμ thick. Similar membranes surround small groups or even individual bacteria; as a result, complex membranes are frequently formed by apposition, but no true double-layered membranes have been observed.

Contractile Vacuoles.—The individual vacuoles comprising the contractile vacuole system in *Euplotes* have been seen on numerous occasions. They appear to be quite small. The wall is a simple, single-layered membrane, and the vacuole contents are structureless and lacking in inclusions (Fig. 1, *V*). Little information from these sections can be added to descriptions by King (20) and Taylor (42).

Cytoplasmic Rods.—A large number of rod or ribbon-shaped structures have been observed concentrated close to the gullet region on non-ciliated surfaces (Fig. 13, *CR*). These rods are usually oriented perpendicular to the cell membranes, and are 50 to 70 mμ in diameter and up to 2.5 μ in length. Attempts to demonstrate internal structure have failed even in thinner sections, so that the appearance is that of a homogeneous solid cylinder with no apparent membrane. Frequently the rods show a tendency to become diffuse and less dense. They are often oriented perpendicular to the pellicle and may be associated with indentations of the pellicular membranes.

Cytoplasmic Granules.—Dense granules with diameters up to 160 mμ are found scattered uniformly throughout the cytoplasm (Figs. 1 and 2). At higher magnifications they appear quite dense. Their appearance is somewhat variable, either compact or somewhat diffuse, with or without sharply defined edges, but always with an inner granular structure if observed in thin sections (Figs. 4 and 8, *GG*).

Cytoplasmic Ground Substance.—The cytoplasm also contains granular and fibrillar structures all of which are 8 mμ in diameter or smaller (Figs. 3, 8, and 11). In some cases, quite dense granules measuring about 6 mμ have been observed in contrast with material of lesser density in which they are embedded or attached (Fig. 11).

Bacteria.—In early sections of an unidentified species of *Euplotes*, numerous bacteria were observed in the cytoplasm. In contrast to bacteria in the food vacuoles, these had no surrounding membrane except the bacterial cell membrane; otherwise their appearance was similar to that of bacteria in the food vacuoles (Fig. 12). No such bacteria have been observed in this clone of *Euplotes patella.*

DISCUSSION

Ciliary Structure.—The basal portion of the *Euplotes* cilium seems to be typical of protozoan cilia, as indicated by the studies of Sedar and Porter (39), Pitelka (26), and Randall (29), except that the two central fibrils are present only in *Euplotes.*[2] In most protozoan cilia, the basal portions are composed only of extensions of the peripheral ciliary fibrils with little or no elaboration of structure. This is in contrast to the non-protozoan cilia described by Fawcett

[2] It should be noted that the terminology of Randall is at variance with that of other workers, while the structures shown are quite similar, *e. g.* the portions of peripheral ciliary fibrils in the basal region of the cilium are termed rootlets.

and Porter (11), Rhodin and Dalhamn (32), and Bradfield (4, 5), which show more complex structures in the basal portions.

The granules observed here in *Euplotes* are not the structures for which Lwoff (22) has used the term "kinetosome", for they are much too small. The concept presented by Sedar and Porter (39), "that the basal body or kinetosome is tube-shaped and that therefore the terms 'basal corpuscle or granule' are descriptively inappropriate," is worthy of special note. It is apparent from their study as well as this one that the "basal body" is not a separate structural part of the cilium, but that it is composed only of extensions of the same fibrils present in the ciliary shaft. Therefore, it appears that this region of the protozoan cilium should properly be referred to as a "basal portion" rather than a "basal body" or "kinetosome." These observations make Lwoff's concept of kinetosome duplication (22) more difficult to understand and interpret; although ciliary duplication in the protozoa might be thus accomplished, the formation of organelles such as trichocysts from the basal portions of cilia is more obscure. In reality, we have had little new evidence for many years for modes of formation of cilia. The example observed by Sedar and Porter which was presented as a stage in the development of a new cilium is probably an example of certain surface regions of *Paramecium* which have two typical cilia in each pellicular depression. In the development of the connecting cilium of the retinal rods in the mouse, De Robertis (6) described the region where the cilium is first seen as having numerous mitochondria, a circumscribed region of Golgi material, and some dense particles; no application to ciliary formation was possible, however.

Neuromotor System.—One of the early descriptions of the rootlet system in *Euplotes* was given by Yocom (48), who supported Engelmann's hypothesis (9) of a nerve-like function for the rootlet filaments. Running anteriorly from each of the five anal cirri, Yocom described fibrils which fused, resulting in a single fibril which extends anteriorly to the motorium. He also described a membranelle fibril connecting the motorium to a lattice-work structure in the anterior lip, as well as fibrils extending in several directions from certain cirri. Taylor (41) showed that each of the cirri has a "fibre plate" in addition to the more distal "basal plate," and that the rootlet fibrils are connected predominantly at the level of the fiber plate in the cirri. He also described three creeping and six swimming motions. From microdissection experiments in which he cut several rootlet fibrils, he concluded that the rootlets were neither supporting nor contractile, but functioned in the coordination of movements. Hammond (17) saw additional fibrils interconnecting the cirri; he also studied the cirri during asexual reproduction and demonstrated that the old cirri were resorbed and two new sets formed *de novo*.

Because the present study elaborates on the extent of fibrillar interconnection of structures in *Euplotes*, the following more complete list is given of structures which are now known to be directly interconnected by fibrils; for each,

the previous observer, if any, is designated. (1) Cirri and motorium (48); (2) membranelles and motorium (48); (3) Cirrus and adjacent cirrus (48, 17); (4) Membranelle and adjacent membranelle; (5) Cirrus and membranelle directly; (6) Cirrus and subpellicular filaments; (7) Motorium and subpellicular filaments; (8) Cilium and adjacent cilium; (9) Region of micronucleus with unknown structures.

We must be careful to note that the above listing does not imply that every cirrus is connected directly with every other cirrus or every membranelle directly with every cirrus and so forth, but indicates only that in some cases such direct interconnections do exist.

The complexity of this as well as other ciliary rootlet systems has stimulated experimenters since the middle of the nineteenth century to theorize and experiment with concepts of function. It is our contention that although rootlets may undoubtedly serve several functions, if we consider the many known variations, the evidence supports the view that a major function is the coordination of ciliary beat. Evidence has been summarized by the author (36) which indicates furthermore that the intracellular fibrils and filaments in rootlet systems may function as electrical conductors in a way similar to the conduction in nerve fibers.

The concept that rootlets function in ciliary coordination raises a major question for such protozoa as *Euplotes*: what structure constitutes the sensory portion of this neuromotor system? Three possibilities exist. The bristle cilia were earlier termed "sensory bristles"; however, such a function seems unlikely, since they have no rootlet fibrils or filaments according to present observations. The external fibrillar system described from silver techniques by Turner (45) was also said to serve a sensory function; however, no such system of fibrils has been observed in this study. It is possible that silver uniformly deposited on or in the membrane and that pellicular ridges and depressions were then mistaken for fibrils.[3] It is possible that the subpellicular filaments described here have a sensory role, since they are very similar both in size and appearance to the rootlet filaments, and since filaments connect them with both cirri and the motorium. Although the pellicle is quite rigid in *Euplotes*, and it is probable that these fibrils contribute at least some rigidity, it does not seem amiss to suggest a possible dual function of both sensory perception and structural bracing.

The proximity of rootlet filaments to the micronucleus presents a further

[3] This evidence raises some doubts with respect to other structures demonstrated by silver techniques. Silverline systems could in some cases be demonstrations of surface structure analogous to shadowcasting effects and silica-type surface replicas as viewed in the electron microscope. Taylor (43) has earlier expressed such doubts, and Ehret and Powers (8) recently have presented phase microscopic evidence disputing the existence of the "outer fibrillar complex" of *Paramecium*, which had been demonstrated by silver staining of dried organisms, but the wet methods of von Gelei (14) must be similarly considered, since the *Euplotes* external fibrillar system was also demonstrated in this way.

question, for it is difficult to ascribe a role in the functional system to such a structural relationship. According to Kudo (21), a flagellar rhizoplast connects the blepharoplast and the nucleus in the mastigamoebidae, and, in the polymonad parasites of the termite intestine, each of the several nuclei is associated with a blepharoplast from which a flagellum extends. Manton (23) stresses a similar association of nucleus with rootlet fibrils in several algae, but offers no functional explanation.

Vacuoles.—Three types of vacuolar structures exist in *Euplotes*: the food vacuoles, the contractile vacuoles, and the vacuoles associated with the cilia. The latter two appear rather simple structurally. Each has a single-layered membrane and shows little substructure either in the membrane or in the internal portion. Both are rather small in all sections observed (Figs. 1 to 5), which is in agreement with the descriptions by Yocom (48) and Hammond (17) of the ciliary vesicles, and by Taylor (42) of the contractile vacuole system.

The function of the ciliary vacuoles remains obscure. According to the account of Kudo (21), Rees was the first to see them and believed them to be contractile vacuoles, but this was disputed by Griffin, who used microchemical tests to show a possible role in the storage of reserve fatty acids.

Mitochondria.—The internal structure of the protozoan mitochondrion was first observed in sections of *Paramecium caudatum* by Ornstein and Pollister (24), but no interpretation of structure was proposed. Powers, Ehret, and Roth (27), in a study of *Paramecium aurelia* and *Paramecium bursaria*, interpreted the mitochondrial structure as composed of osmiophilic tubules surrounded by less dense material, with lumina that are infrequently continuous with the cytoplasm; no mitochondrial membrane was observed. A similar interpretation was presented by Powers, Ehret, Roth, and Minick (28) for mitochondria in *Euplotes patella*. The interpretation by Sedar and Porter (39) in *Paramecium multimicronucleatum* was that a double-layered membrane is present, and that the lumina of the tubules (microvilli) are continuous with the space separating the membrane layers. In *Paramecium caudatum*, Wohlfarth-Bottermann (46) demonstrated that the tubules were present not only after fixation with osmium tetroxide, but also after fixation in formalin or Champy's solution, and that the tubules remained after fixation with osmium tetroxide at pH 3.0 though the matrix material disappeared. The mitochondrial structure observed here in *Euplotes* consists of numerous tubules, which usually appear in a surrounding matrix of lesser density. The relationship of the tubules to the outer edge of the mitochondrion is more difficult to interpret here than in *Paramecium*, since the edge is characterized by many small irregularities. In a few cases, two parallel membrane-like structures may be seen, but they are visible for very short distances and are the exception rather than the typical case (Fig. 11, *D*). When visible, these parallel structures are separated by a distance smaller than the diameter of the tubular lumen.

Microbodies.—Rhodin (31) in studying the proximal convoluted tubule of the rat kidney, described structures which were surrounded by a single-layered membrane and which had a ground substance of the same opacity as the stroma of the mitochondria; he used the term "microbodies" to designate them. He looked for intermediate stages between microbodies and mitochondria, but observed none. Rouiller and Bernhard (37) reported similar, though denser structures in liver cells following starvation-refeeding, partial hepatectomy, and carbon tetrachloride poisoning; they give evidence which strongly suggests ". . . that the microbodies can be precursors of mitochondria." Structures similar to those observed by Rhodin are present in micrographs of *Paramecium caudatum* by Wohlfarth-Bottermann (47), who attempted to present a sequence of stages in mitochondrial secretion and regeneration. His sequence does not seem justified from the evidence given, but the microbody-like structure is presented as a stage in "regeneration"; the author does not acknowledge the term "microbody," however.

In addition to the problem of mitochondrial formation, the method of mitochondrial-cytoplasmic interchange is one of the basic problems in cytoplasmic events. The concept presented by Wohlfarth-Bottermann whereby mitochondria rupture is only one of several possible methods. In addition, either temporary or permanent pores in the mitochondrial membrane may serve this purpose. Still another possibility is a method similar to the pinocytosis of cell membranes; material could exude in to the cytoplasm, causing the formation of a microbody-vesicle which is later detached from the mitochondrion. This latter concept could explain the increased member of microbodies present at the stages of cellular activity studied by Rouiller and Bernhard. In view of the lack of evidence, none of these possibilities can be decisively included or excluded as a method of mitochondrial-cytoplasmic interchange, although it does seem appropriate now functionally to associate microbodies and mitochondria.

Macronucleus.—The macronuclear membrane has been shown to have annular structures similar to those demonstrated in a variety of oocyte nuclear membranes by Afzelius (1), Bairati and Lehmann (2), Gall (12), Harris and James (19), Rebhun (30), and others, though the outside diameters of the annuli usually have been 80 to 120 mμ rather than the smaller size observed here. Gall reports that the annuli "appear to be composed of eight or ten smaller masses arranged in a circle," and Rebhun further reports that "these granules appear to be hollow vesicles." In this regard, it has not been possible to relate positionally the annuli and the macronuclear granules here, but it may be of importance that the macronuclear granules are often applied directly to the membrane. In regard to this relationship, Ehret and Powers (7) have shown from phase microscope observations of unfixed squash preparations that material may be extruded from the macronucleus in *Paramecium bursaria* during conjugation.

The chromatin of the macronucleus shows no organized pattern of the type demonstrated in the macronuclear chromatin bodies of *Tokophrya infusionum* by Rudzinska and Porter (38).

In the light microscope descriptions of Yocom (48) and Turner (45), it is stated that the first evidence of approaching division in *Euplotes eurystomus* is the appearance at the tips of the macronucleus of two bands which separately traverse each half, meet in the middle, and disappear, leaving the chromatin in a "greatly altered condition."[4] Each band is composed of a forward or proximal solution plane and a distal reconstruction plane, which together are approximately equal in length to the diameter of the macronucleus. Turner, who used several nuclear stains including the Feulgen reaction, reported that the solution plane stained deeply, while the reconstruction plane was unstained and structureless; the reorganized nuclear portions always stained more deeply than the middle portions. The reconstruction plane in reality is faintly Feulgen-positive when viewed in 2-μ sections.

The two zones of the reconstitution bands do not seem to show gradual changes across the zones, but rather specific levels or interfaces of structural change. Three such levels can be recognized in the two-band arrangement. The first is a change from the original chromatin granule to a network of filaments whose diameters are about $\frac{1}{10}$ those of the original granules, but with no loss in stainability. The second is a change to an even smaller structural level in which little or nothing can be resolved, and in which there is some change in staining. After these two steps which are apparently breakdown procedures, the third step is a reformation of bodies resembling the original in size and structure, but, as Turner states, differing in their staining properties.

A recent electron microscope study by Faure-Fremiet, Rouiller, and Gauchery (10) of the reorganization bands in *Euplotes eurystomus* gave no positive conclusion regarding the occurrence of endomitosis; structures similar to those described were observed here. Gall (13), however, has shown by the use of labelled thymidine that synthesis of deoxyribose nucleic acid takes place in the reorganization band. For this reason, the reorganization band should be related to interphase activity and not to mitotic events. Apparently, DNA replication cannot take place in the granular state of the macronucleus.

Cytoplasmic Components.—Two cytoplasmic components have been observed which are worthy of comment. The granules scattered randomly throughout the cytoplasm have not been observed previously to the author's knowledge. They are present in considerable numbers in all sections, so that great numbers must be present in a single organism. The cytoplasmic rods which are usually present in only one localized area are of interest for a similar reason. However,

[4] The implication that the reorganization phenomenon is an integral part of mitosis is perhaps improper, since Taylor (41) has shown that mutilation of *Euplotes* results in similar macronuclear reorganization, and since Fauré-Fremiet, Rouiller, and Gauchery (10) have shown that the phenomenon may last as long as forty per cent of the time of the mitotic cycle.

little can be suggested with respect to the function of these structures, since they are too small to be observed by cytochemical techniques.

Typical ergastoplasm, endoplasmic reticulum, and Golgi bodies have been conspicuous by their absence in sections of *Euplotes*.

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SUMMARY

1. Structurally the "sensory bristles" in *Euplotes patella* are typical cilia, but no ciliary rootlets connect their bases.

2. The "neuromotor fibrils" are composed of filaments 21 mμ in diameter. At the point of junction of the filaments with the peripheral ciliary fibrils a granular structure 65 to 90 mμ in diameter is seen which has dense central and peripheral zones separated by a less dense layer. Information on the interconnection of organelles is expanded.

3. A system of subpellicular fibrils is described. The external fibrillar system described by others could not be found.

4. The motorium is shown to be a mass of intertwining rootlet filaments.

5. The micronucleus is shown to have a spongy, dense material in a less dense material, all of which is surrounded by a double-layered membrane.

6. The double-layered macronuclear membrane contains annuli whose outside diameter is 70 mμ; the macronuclear bodies are sometimes closely applied to the membrane. In the macronuclear reorganization bands, the solution plane is a fine network, while the reconstruction plane is devoid of structure at the level of resolution observed.

7. The mitochondria are composed of tubules, only occasionally oriented, usually embedded in a surrounding material of lower density.

8. Microbodies whose diameters are 250 to 350 mμ are frequently observed in close association with mitochondrial surfaces.

9. The food vacuoles, contractile vacuoles, and ciliary vacuoles are bounded by single-layered membranes. In the food vacuoles, the bacteria are surrounded by membranes individually or in small groups.

10. Cytoplasmic rods localized in the oral region, and cytoplasmic granules dispersed at random, are described. No typical ergastoplasm, endoplasmic reticulum, or Golgi material was observed.

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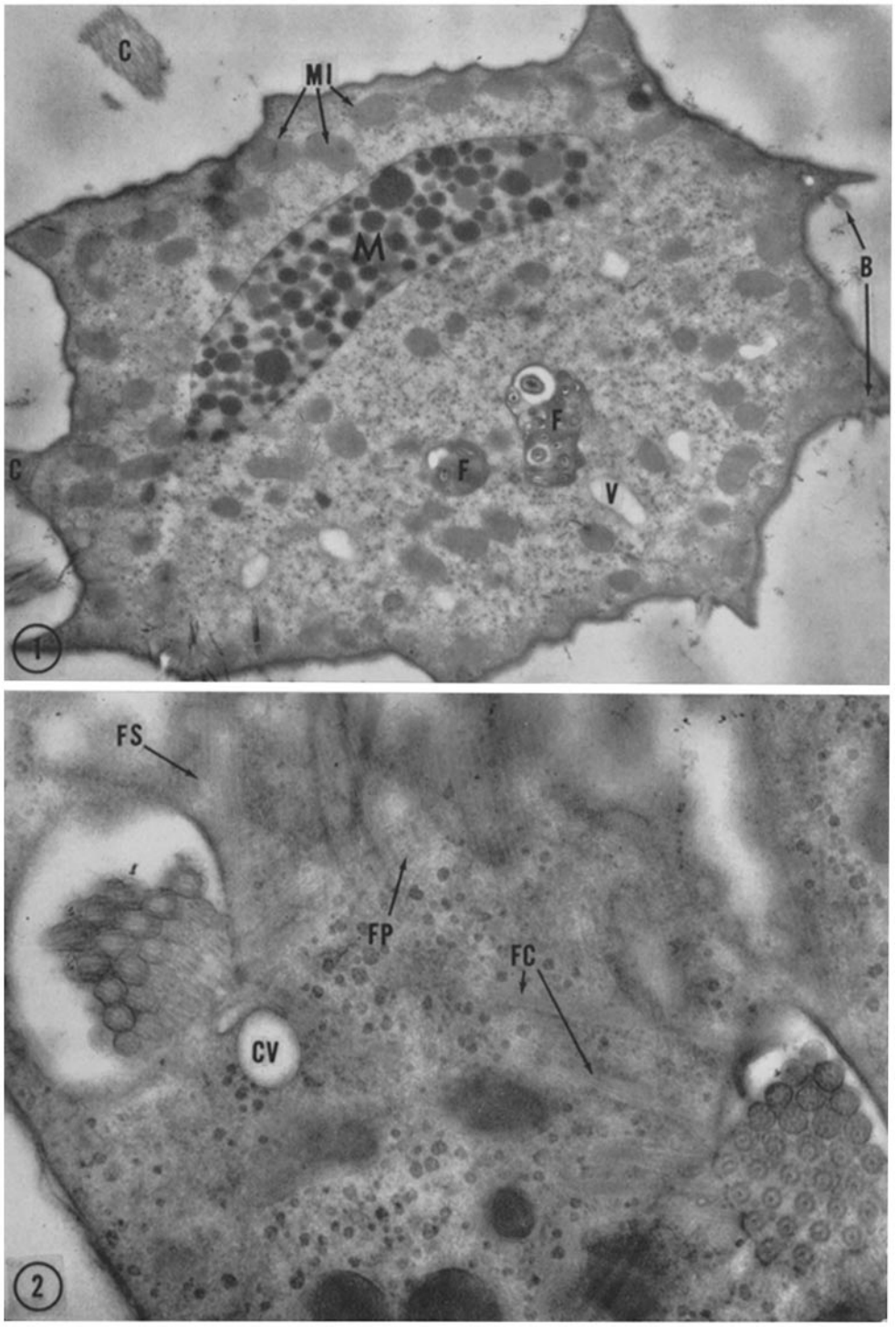
EXPLANATION OF PLATES

PLATE 299

FIG. 1. A survey micrograph of *Euplotes patella*, showing the dorsal surface on the right and the ventral surface on the left. Three bristle cilia (*B*) are shown close to the pellicular ridges. A large portion of the long, C-shaped macronucleus (*M*) is included along with sections of cirri (*C*), food vacuoles (*F*), contractile vacuoles (*V*), and mitochondria (*MI*). Many cytoplasmic granules are shown randomly dispersed in the cytoplasm of the organism. × 4100.

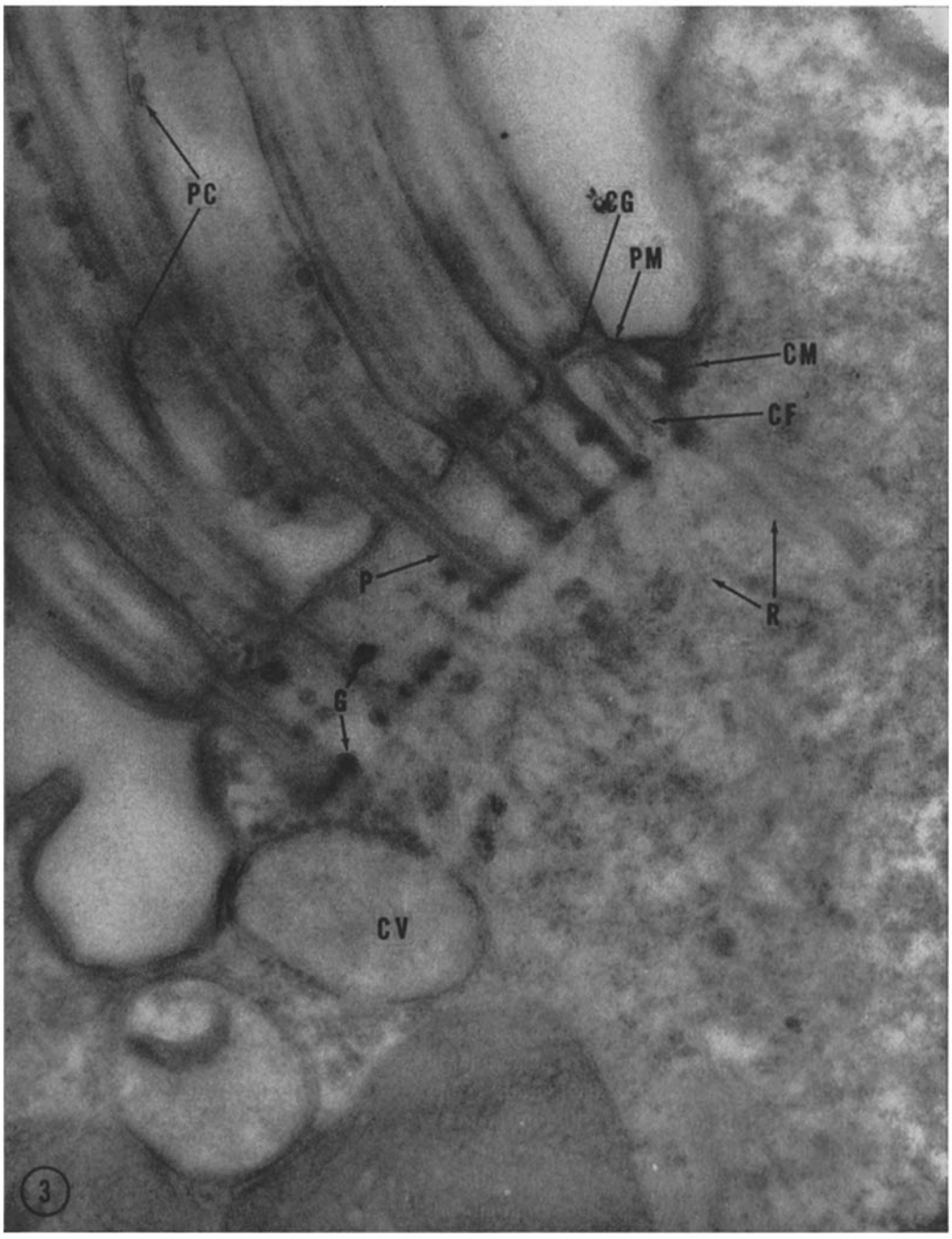
FIG. 2. Two cirri sectioned so as to include the pellicle between them. Filaments (*FC*) are shown connecting the cirri, while other filaments (*FP*) are shown connecting the left cirrus with the subpellicular filaments (*FS*). A ciliary vacuole (*CV*) is also shown. Notice the typical cirrus structure shown by the right cirrus, which has been cut in cross-section. There is a right-left symmetry, except for the absence of two cilia, which are perhaps out of the plane of the section. Central fibrils are present in the cilia in both the shaft and basal portions. Numerous cytoplasmic granules of varying sizes are shown also. × 13,000.



(Roth: Cytology of *Euplotes patella*)

PLATE 300

FIG. 3. A longitudinal section of the cilia of one cirrus. The peripheral ciliary fibrils (*P*) proceed directly into the basal region of the cilium, where they join with the rootlet filaments (*R*). Small granules (*G*) may be seen at this point, as well as at slightly more distal points; they have dense outer and inner zones separated by a less dense zone. The central ciliary fibrils (*CF*) are shown in the basal region of the cilium; a granule (*CG*) is associated with the central ciliary fibrils. The ciliary membrane is continuous with the pellicular membrane (*PM*); the cytoplasmic membrane appears to be discontinuous at the points of emergence of cilia, and ends at an enlargement (*CM*). Protrusions of the ciliary membrane (*PC*), and ciliary vacuoles (*CV*) are shown. × 45,000.

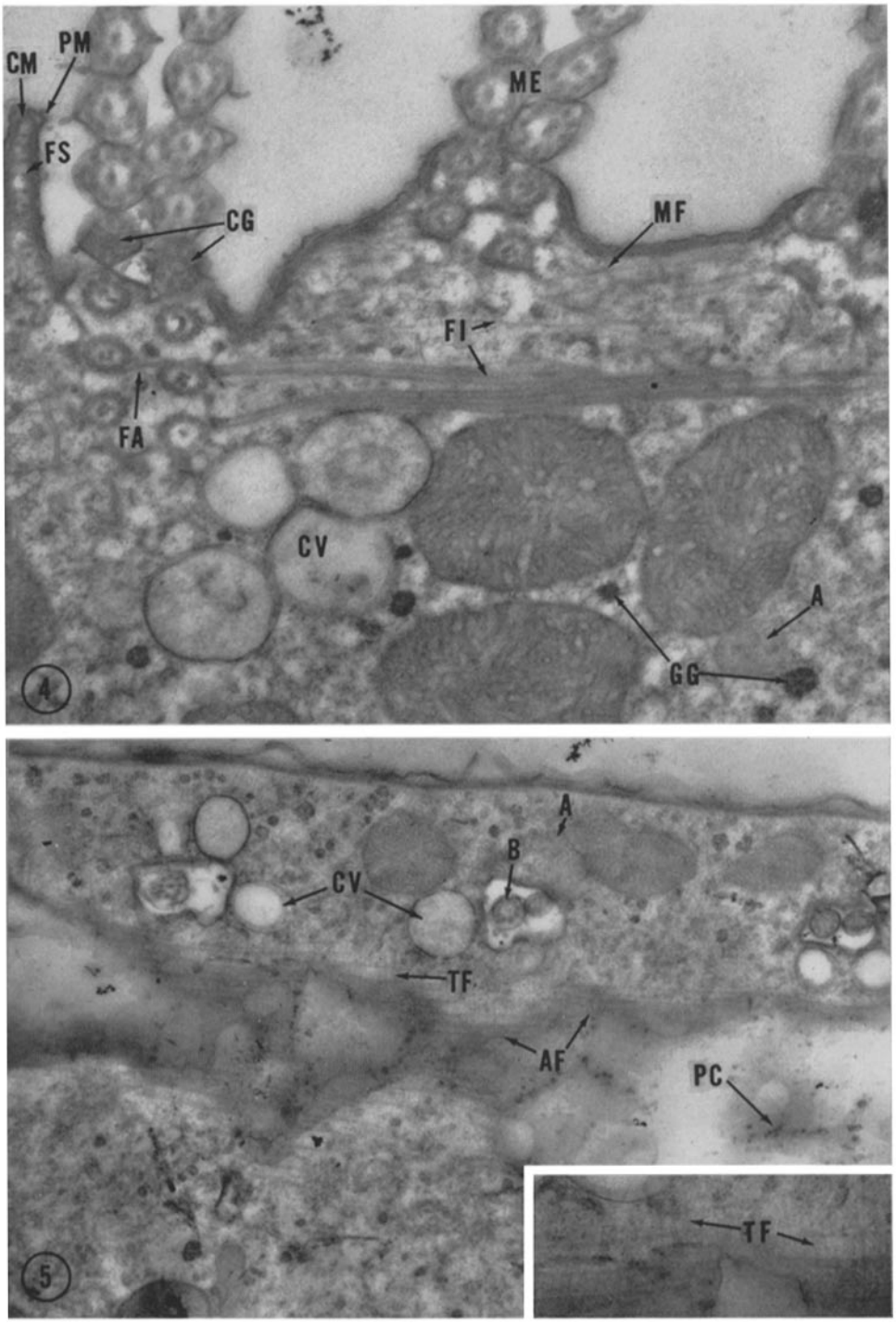


(Roth: Cytology of *Euplotes patella*)

PLATE 301

FIG. 4. Portions of three membranelles (*ME*) are shown with associated filaments, Filaments interconnecting adjacent membranelles (*MF*) are shown, as well as filaments (*FI*) connecting a membranelle with the motorium or with membranelles farther away. Filaments connecting adjacent cilia (*FA*) are visible, as well as a cross-section of cilia at the level of the granule (*CG*) of the central fibrils (see also Fig. 3). The pellicular membrane (*PM*), cytoplasmic membrane (*CM*), and filaments of the subpellicular fibril system (*FS*) are seen. Deeper in the cytoplasm are sections of three mitochondria, some cytoplasmic granules (*GG*), and a microbody (*A*). × 32,000.

FIG. 5. A section nearly parallel to the dorsal surface. Bristle cilia (*B*) are shown to have the typical ciliary structure and to be located in pellicular depressions, with ciliary vesicles (*CV*) surrounding them. Antero-posteriorly directed filaments (*AF*) of the subpellicular fibril system are nearer the pellicle than are transverse filaments (*TF*) of the same system. The many protrusions (*PC*) of the pellicular membrane appear as circles. A microbody (*A*) is also seen in typical close association with a mitochondrion. × 15,000. Inset, × 27,000.

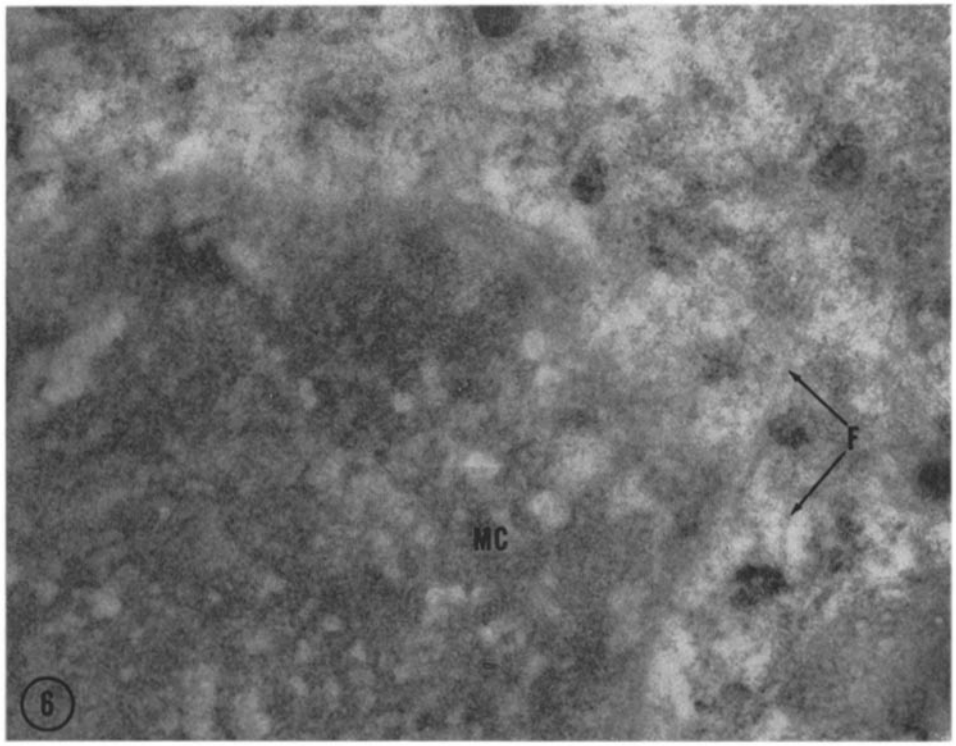


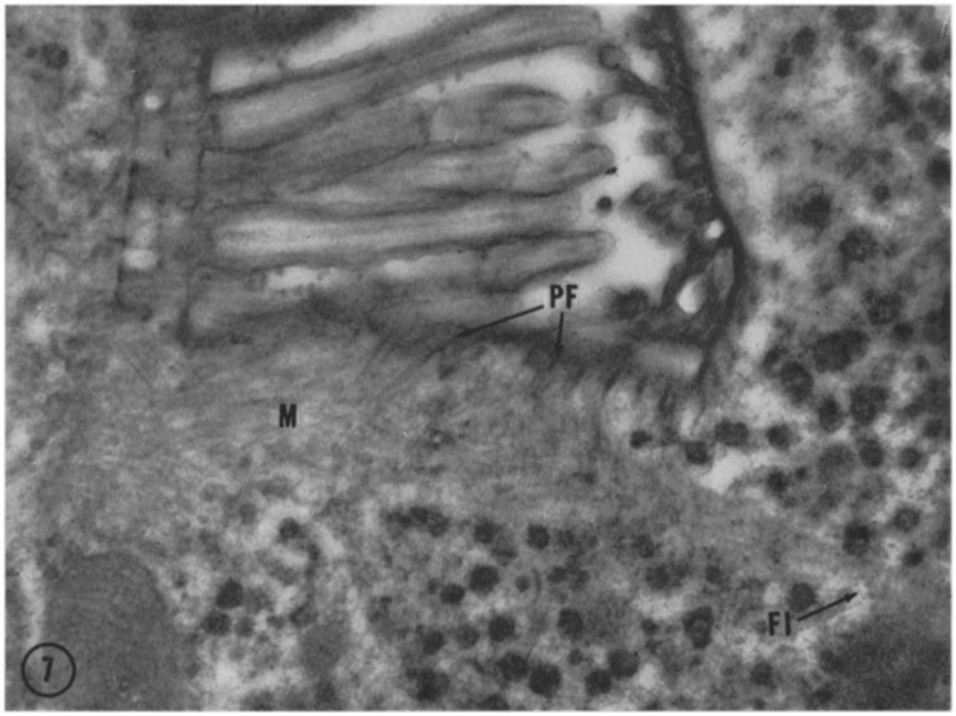
(Roth: Cytology of *Euplotes patella*)

PLATE 302

FIG. 6. Filaments (*F*) in the region of the micronucleus (*MC*). These filaments are similar in size and appearance to the rootlet filaments. It is probable that they are a part of the neuromotor system, although it is not known what structures they may connect with the micronucleus. × 48,000.

FIG. 7. Rootlet filaments (*FI*) from the membranelles as they enter the region of the motorium (*M*). (The striated appearance is due to cutting many parallel filaments obliquely, not to striation of the filaments.) The motorium is closely applied to the pellicle of the anterior oral region and filaments (*PF*) are seen extending directly from it to the pellicle. The motorium is a region of closely intertwining filaments, of which a portion (*M*) (measuring about 1 by 3 μ) appears in this section. Rootlet filaments from cirri also enter this region, but are not included in this section. × 32,000.

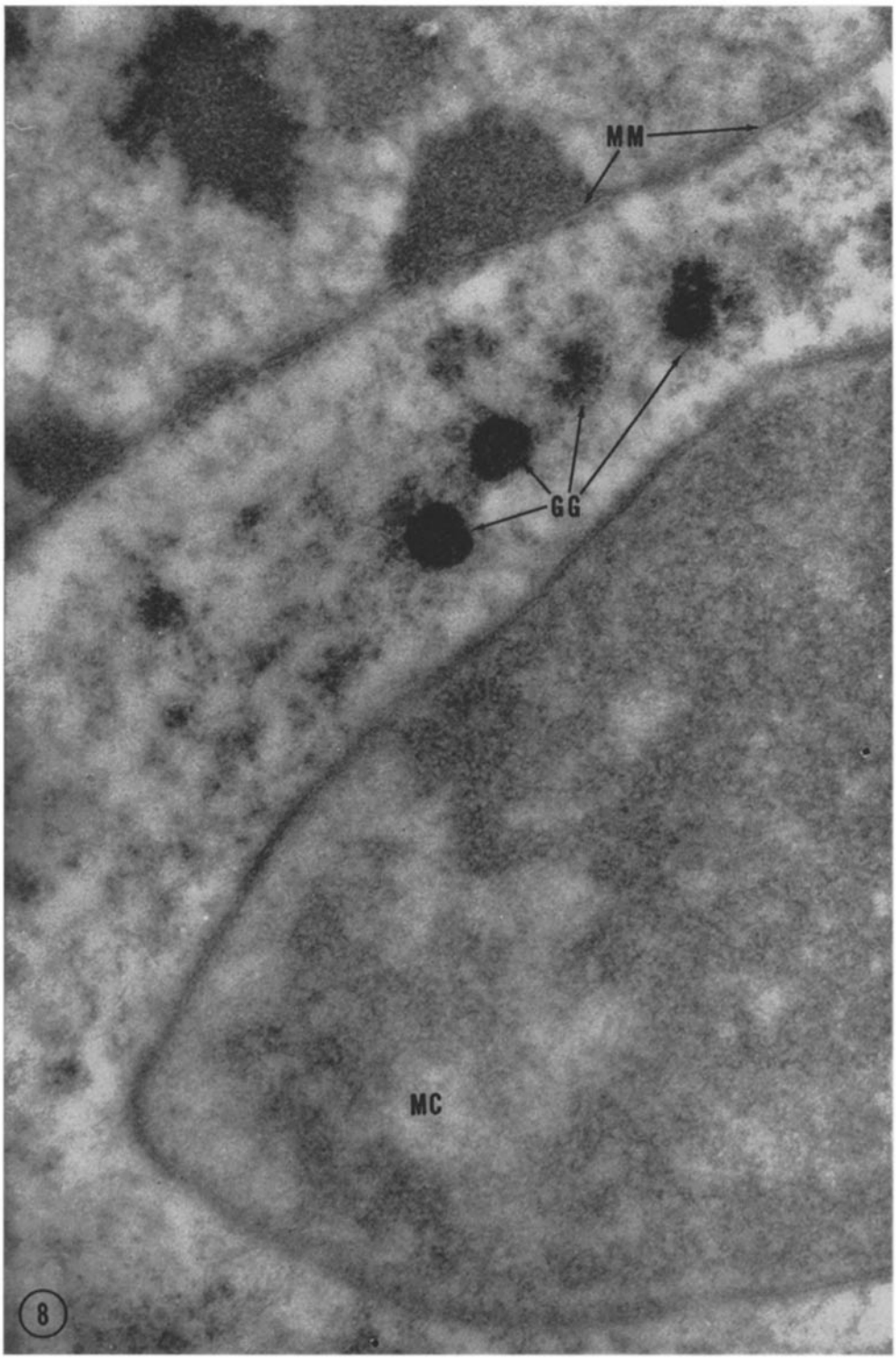




(Roth: Cytology of *Euplotes patella*)

PLATE 303

FIG. 8. Portions of the macronucleus and the micronucleus. The micronucleus (*MC*) is shown at the lower right with its double-layered membrane and with an internal appearance of dense, spongy material in a matrix of lesser density. At the upper left is a very small portion of the macronucleus, which shows its double-layered membrane and the closeness with which the macronuclear bodies are applied to the membrane (*MM*). Several cytoplasmic granules (*CG*) are shown between the two nuclei and illustrate the range of appearance which they may present. The ground substance of the cytoplasm is shown here. × 69,000.



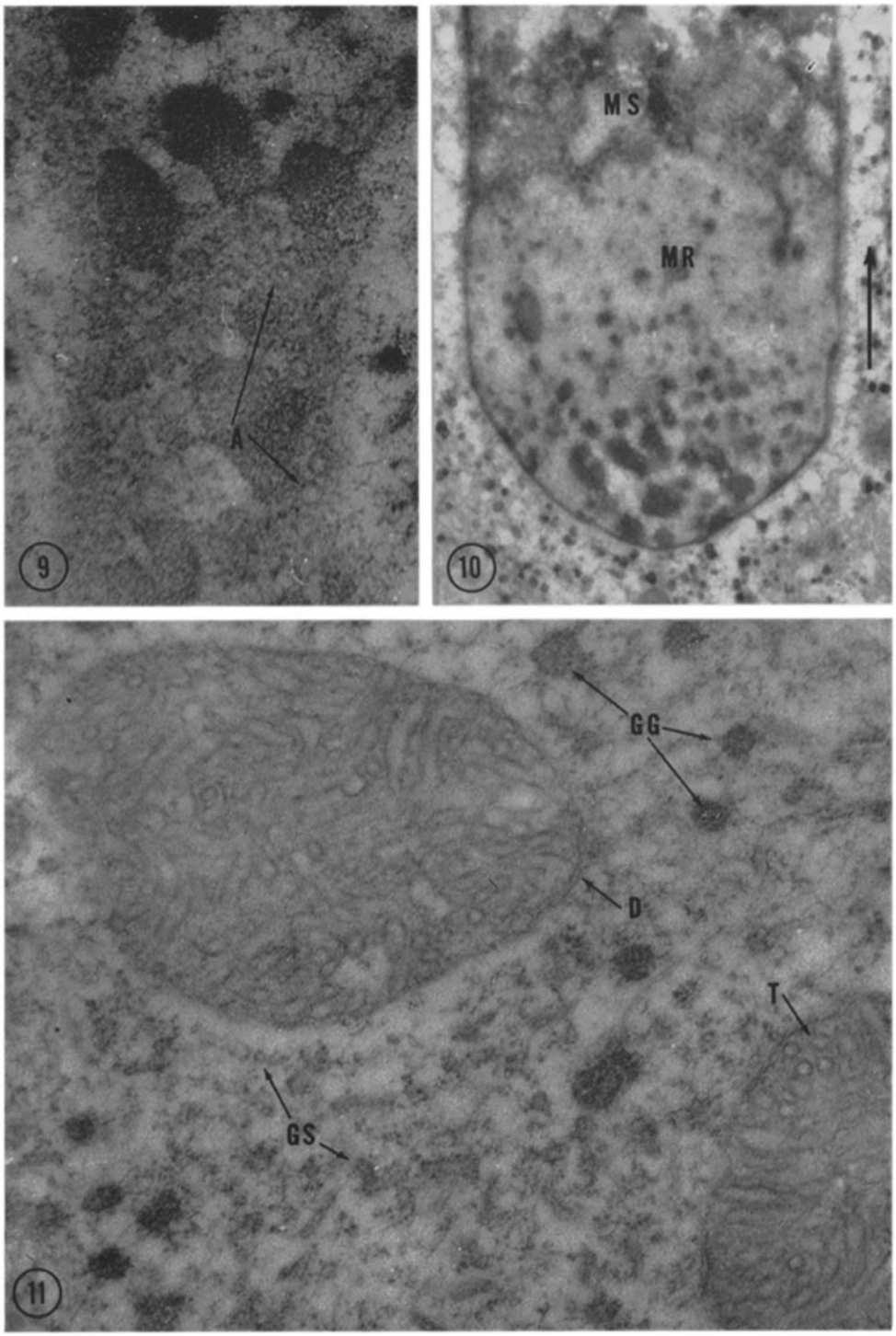
(Roth: Cytology of *Euplotes patella*)

PLATE 304

FIG. 9. A section across the surface of the macronucleus, showing the membrane annuli (*A*). × 48,000.

FIG. 10. The macronuclear reorganization band. The solution plane (*MS*) is composed of small granules, while the reconstruction plane (*MR*) is virtually without structure. The arrow indicates the direction in which the reorganization band was moving along the macronucleus. × 14,000.

FIG. 11. Sections of two mitochondria which are composed of numerous internal tubules (*T*), which frequently appear in groups of two to four indicating some parallel orientation. Material is seen surrounding the tubules (*T*) with the lumen of the tubule of lesser density and the tubule wall more dense; however, this is not always the case. Two parallel structures (*D*) are visible, but may be followed only for short distances, due to the irregularity of the mitochondrial surface. Notice the numerous, small, dense granules (*GS*) throughout the cytoplasm in addition to the larger, cytoplasmic granules (*GG*) described earlier. × 53,000.

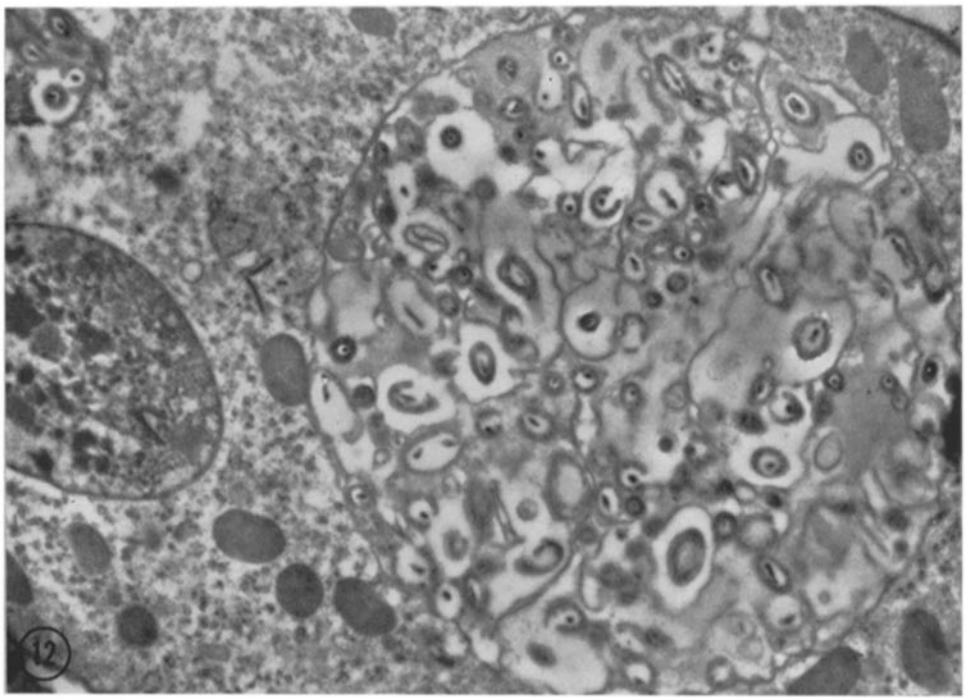


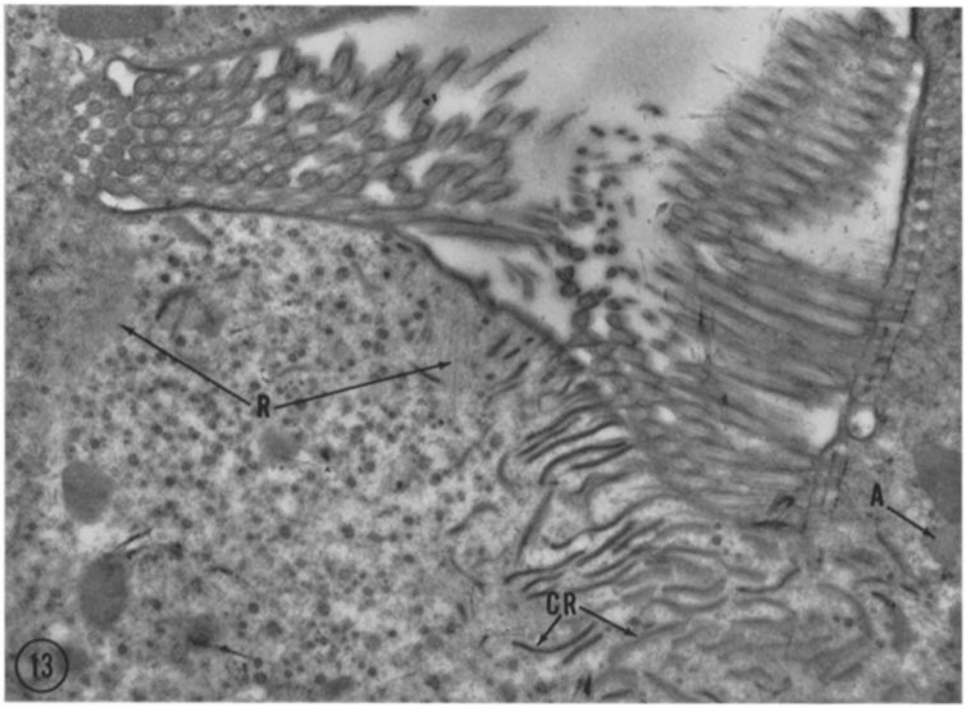
(Roth: Cytology of *Euplotes patella*)

PLATE 305

FIG. 12. A food vacuole in which bacteria, either singly or in groups, are surrounded by intravacuolar membranes. On the left edge is a section through the solution plane of a reorganization band of the macronucleus. × 7,000.

FIG. 13. A survey micrograph of the oral region. The cytoplasmic rods (*CR*), usually localized in one region only, present either a diffuse or a discrete and well defined appearance. Several groups of rootlet filaments (*R*) and a microbody (*A*) are shown also. The cilia at the right are from one membranelle and illustrate the two levels of granules associated with the basal portions of the cilia. × 12,000.





(Roth: Cytology of *Euplotes patella*)