THE FINE STRUCTURE OF *GIARDIA MURIS*

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ABSTRACT

Giardia is a noninvasive intestinal zooflagellate. This electron microscope study demonstrates the fine structure of the trophozoite of *Giardia muris* in the lumen of the duodenum of the mouse as it appears after combined glutaraldehyde and acrolein fixation and osmium tetroxide postfixation. *Giardia muris* is of teardrop shape, rounded anteriorly, with a convex dorsal surface and a concave ventral one. The anterior two-thirds of the ventral surface is modified to form an adhesive disc. The adhesive disc is divided into 2 lobes whose medial surfaces form the median groove. The marginal grooves are the spaces between the lateral crests of the adhesive disc and a protruding portion of the peripheral cytoplasm. The organism has 2 nuclei, 1 dorsal to each lobe of the adhesive disc. Between the anterior poles of the nuclei, basal bodies give rise to 8 paired flagella. The median body, unique to *Giardia*, is situated between the posterior poles of the nuclei. The cytoplasm contains 300-A granules that resemble particulate glycogen, 150- to 200-A granules that resemble ribosomes, and fusiform clefts. The dorsal portion of the cell periphery is occupied by a linear array of flattened vacuoles, some of which contain clusters of dense particles. The ventrolateral cytoplasm is composed of regularly packed coarse and fine filaments which extend as a striated flange around the adhesive disc. The adhesive disc is composed of a layer of microtubules which are joined to the cytoplasm by regularly spaced fibrous ribbons. The plasma membrane covers the ventral and lateral surfaces of the disc. The median body consists of an oval aggregate of curved microtubules. Microtubules extend ventrally from the median body to lie alongside the caudal flagella. The intracytoplasmic portions of the caudal, lateral, and anterior flagella course considerable distances, accompanied by hollow filaments adjacent to their outer doublets. The intracytoplasmic portions of the anterior flagella are accompanied also by finely granular rodlike bodies. No structures identifiable as mitochondria, smooth endoplasmic reticulum, the Golgi complex, lysosomes, or axostyles are recognized.

INTRODUCTION

Giardia is a zooflagellate which inhabits the intestines of many vertebrates. Its trophozoite form was first described by Antoni van Leeuwenhoek in 1681 (19). From his lucid description of the parasite, zoologists of later generations were able to recognize the organism described, as *Giardia lamblia*. (The genus was later named for the 19th century French biologist, and the species after the Bohemian physician, Wilhelm Lambl.) Based upon different host specificity and minor structural variations, nearly 50 different species of *Giardia* recovered from the intestines of numerous vertebrates have been reported. As methods for the investigation of unicellular organisms improved, however, the multiplicity of species listed could be reduced to 2 morphologic groups. This simplifica-

tion was possible because the major cytologic variations among the species of *Giardia* were in their dimensions, and the size, incidence, and shape of the median body, a transient organelle peculiar to *Giardia*. The following general description of the fine structure of the trophozoite of *Giardia muris* is thus applicable to the genus as a whole, with only those parts devoted to the median body perhaps being peculiar to *Giardia muris*.

With the recognition of its widespread distribution, its association with certain intestinal diseases in man (30, 32), and the recent development of an *in vitro* culture method for *Giardia* (17), interest in this noninvasive, intestinal flagellate has greatly increased. There are several detailed light microscope studies of *Giardia* (9), but no comparable electron microscope description of the parasite has yet appeared. In 1954, Rossi-Espagnet and Piccardo (27) studied whole mounts of the organism with the electron microscope but were unable to extend descriptions beyond those previously based upon light microscopy. Cheissin (5) in 1964 described the fine structure of the sucking disc and the median bodies of *Lamblia duodenalis* from the rabbit, and Takano and Yardley (30) recently described *Giardia lamblia* found in human ileum biopsies, but inadequacy of preservation of the organism with osmium tetroxide prevented detailed interpretation of several aspects of its fine structure, particularly the microtubular component of the adhesive disc, the relationships between the microtubules of the median body and other organelles, and the striations of the peripheral ventral cytoplasm. The purpose of this study is to describe the fine structure of the trophozoite of *Giardia* in the lumen of the duodenum of the mouse as it appears after combined glutaraldehyde and acrolein fixation and osmium postfixation.

MATERIALS AND METHODS

The trophozoites of *Giardia muris* were located in the duodenum of mice of the AKD-2 strain. The protozoa were fixed in situ by removing the duodenum and immersing and mincing the tissue in cold fixative. The fixative employed was 6.5% glutaraldehyde and 2% acrolein (28) buffered to pH 7.45 with 0.1 M phosphate buffer. After 2-hr fixation, the tissues were washed for 2 hr in the buffer and then postfixed for 2 hr in cold 1.3% osmium tetroxide buffered to pH 7.4 with the 0.1 M phosphate. The tissues were then dehydrated in a graded series of cold alcohols, brought to room temperature in 100% ethanol, and dehydration was completed in propylene oxide. The embedding medium was Epon 812 (20). Sections were cut with glass or diamond knives on a Porter-Blum microtome. Sections displaying silver to gold interference colors were picked up on uncoated, or Formvar- and carbon-coated copper grids. Various electron microscopic stains, uranyl acetate, lead citrate, lead hydroxide, and lead tartrate, were used singly and in combination to increase contrast of the material. Sections were examined with an RCA EMU 3-F or a Siemens Elmiskop I. Sections for light microscopy were cut at 1 μ and stained with toluidine blue in borate or by the periodic acid–Schiff (PAS) reaction.

OBSERVATIONS

General Configuration and Structural Relationships

Giardia muris is a binucleate zooflagellate, 10 to 12 μ in length and 5 to 7 μ in width. It is rounded anteriorly and tapered posteriorly in a discoid form, with a convex dorsal surface and a concave ventral surface (Fig. 1). A bilobed adhesive disc occupies the anterior ⅔ of the ventral surface. One nucleus is located above each lobe of the disc (Fig. 3). The medial edges of the lobes of the adhesive disc form the ventral (median) groove and the outer edge forms the medial limit of the lateral (marginal) groove (Figs. 2, 15). The lateral limit of the marginal groove is formed by a striated portion of the cytoplasm above and lateral to the adhesive disc (Figs. 2, 15).

Giardia has 8 flagella, 2 of which emerge ventrally, 2 anterolaterally, 2 posterolaterally, and 2 caudally. Their basal bodies are near the midline,

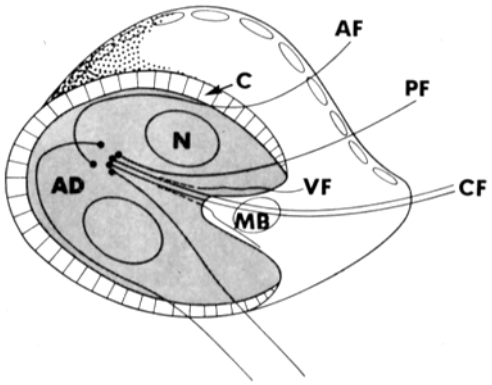


FIGURE 1 An idealized representation of the ventrolateral aspect of *Giardia muris* as seen by light microscopy. *AD*, adhesive disc. *C*, ventrolateral flange. *MB*, median body. *AF*, *CF*, *PF*, *VF*, anterior, caudal, posterior, and ventral flagella. *N*, nucleus.

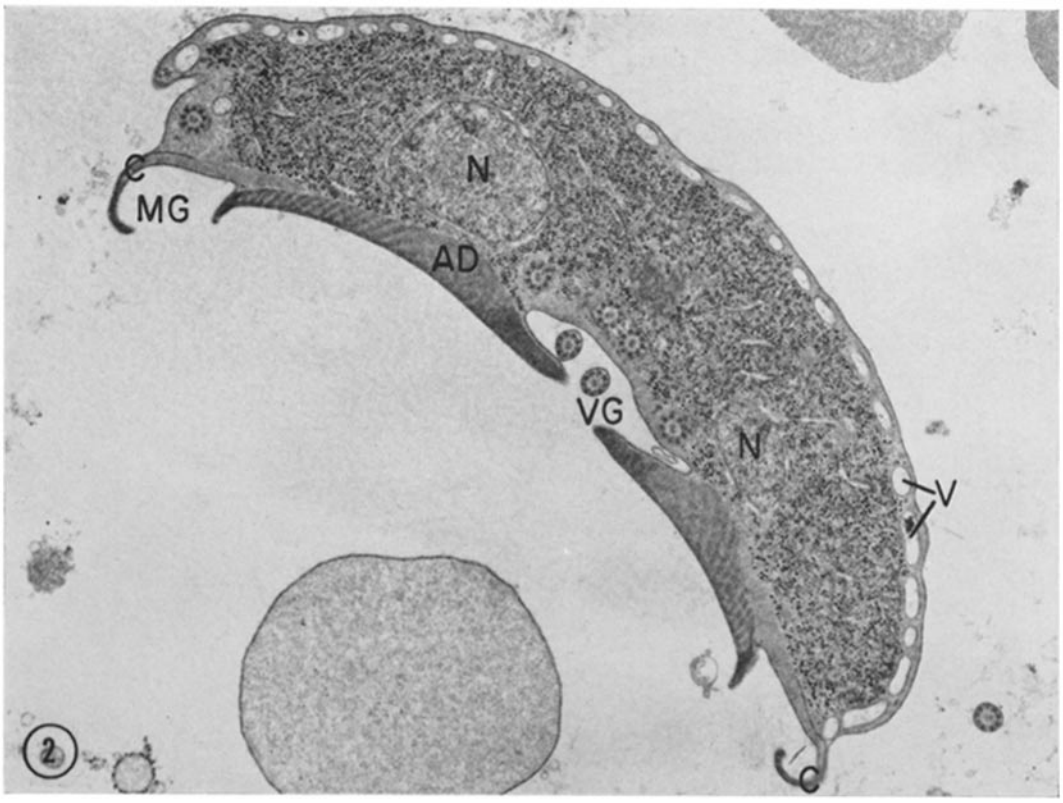


FIGURE 2 A transverse section through *Giardia muris*, showing its discoid shape, general topography, and cytoplasmic structures. The marginal groove (*MG*) is the space between the striated rim of cytoplasm (*C*) and the lateral ridge of the adhesive disc (*AD*). The ventral groove (*VG*) is the space between the medial lips of the 2 lobes of the adhesive disc. One nucleus (*N*) is over each lobe of the disc. The central area of cytoplasm contains granules and clefts, whereas the dorsal portion of the cytoplasm is agranular and is occupied by vacuoles (*V*). The flagella are cut in cross-section. × 15,500.

anteroventral to the nuclei (Fig. 4), so that the intracytoplasmic portions of the anterior, caudal, and lateral flagella subsequently course considerable distances (Fig. 1).

The median body is located dorsal to the posterior poles of the nuclei. It consists of an oval aggregate of curved microtubules, some of which extend ventrally along side the intracytoplasmic portions of the caudal flagella (Figs. 12, 13).

Peripheral to the areas occupied by the median body, basal bodies, and flagella, the central area of cytoplasm contains 2 types of granules embedded in a matrix of moderate density. The larger of the granules conform in structure and staining characteristics to particulate glycogen, and the smaller, to ribosomes. Scattered in varying numbers throughout the granular cytoplasm are fusiform clefts which vary in length and width (Fig. 14). The peripheral cytoplasm is agranular. Ellipsoid vacuoles occupy most of the cell periphery that is not occupied by the adhesive disc.

No structures identifiable as mitochondria, smooth endoplasmic reticulum, the Golgi complex, lysosomes, pigment bodies, or axostyles are recognized.

Nuclei

The 2 nuclei together occupy approximately ¼ the length and ⅔ the width of the organism. Each nuclear envelope enclosing a 300- to 600-A perinuclear cistern, the outer membrane of which is partially studded with ribosomes. The nuclear envelope is interrupted at irregular intervals with circular nuclear pores, traversed by thin diaphragms.

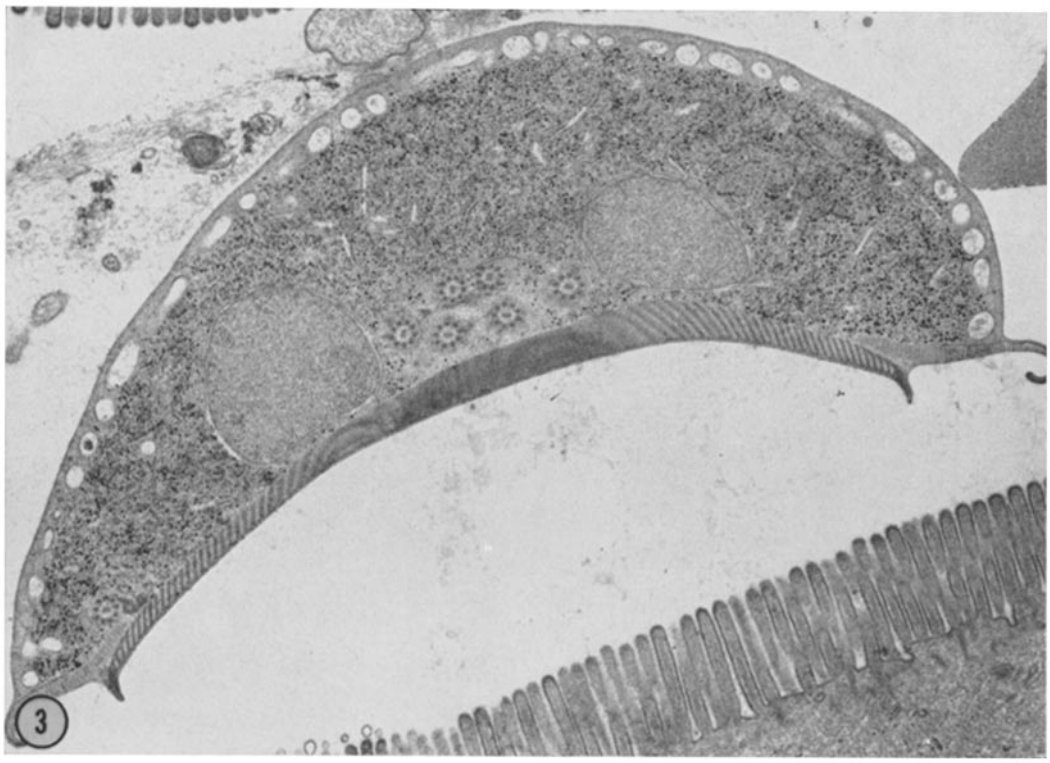


FIGURE 3 A transverse section through *Giardia muris* farther anterior than in Fig. 2, through the region in which the lobes of the adhesive disc are confluent. The spatial relationship between this organism and the microvilli of the duodenal epithelial cells is typical. × 17,000.

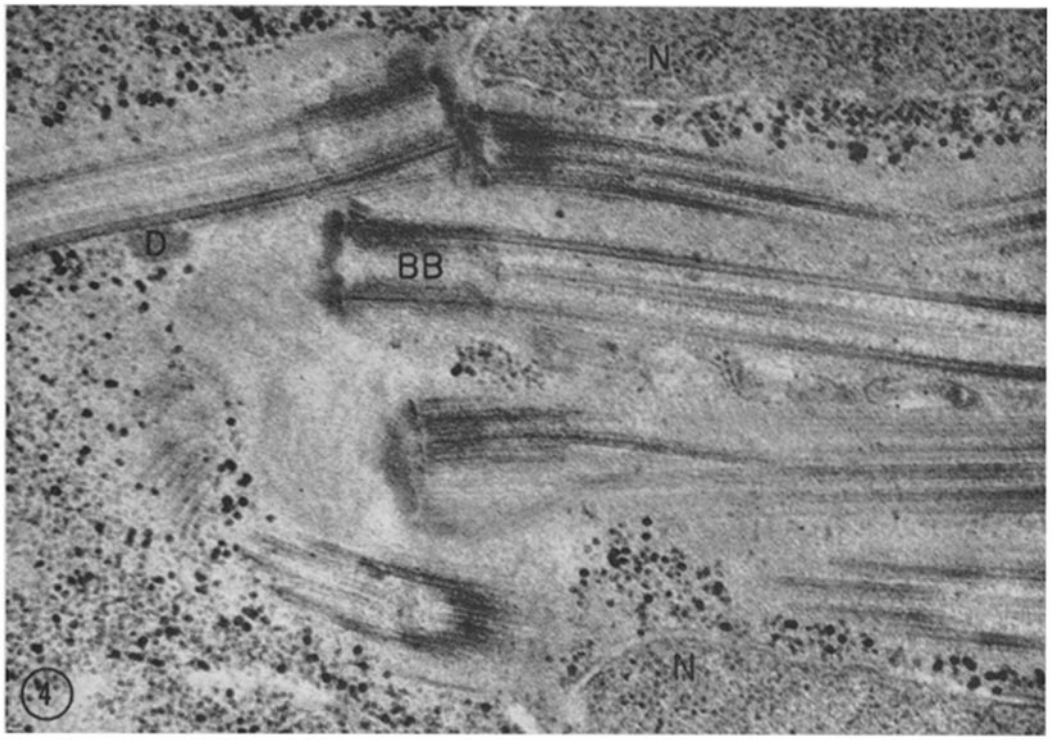
The karyoplasm of the interphase nucleus consists of a homogeneous nuclear sap and uniformly scattered ribosomes (Figs. 3, 14). Dense aggregates of heterochromatin are only rarely observed; when present, they abut on the nuclear membrane. Chromosomes and nucleoli have not been observed.

Basal Bodies and Flagella

The basal bodies are located near the midline between the anterior poles of the nuclei. They are oriented parallel to the long axis of the organism. Although the basal bodies are in a small area, no more than 4 have been seen in one section and only

FIGURE 4 The basal bodies (*BB*) are located between the anterior poles of the nuclei (*N*). An anterior flagellum has a dense, finely granular substance (*D*) adjacent to its medial surface. × 56,000.

FIGURES 5 to 7 A row of microtubules (*mt*) is disposed in the form of an "S" as seen in cross-section through the region of the 6 central paired flagellar shafts, dividing the group into 3 units on each side of the midline. More posteriorly, over the median groove, the microtubules form isolated rows both dorsal and ventral to the caudal flagella (*C*); (also see Figs. 2, 13). Other microtubules, occurring singly or in groups of 2 or 3, are found along the early intracytoplasmic courses of the caudal and ventral (*V*) flagella. *L* denotes the posterolateral flagella. Fig. 5, × 15,000. Fig. 6, × 53,000. Fig. 7, × 49,000.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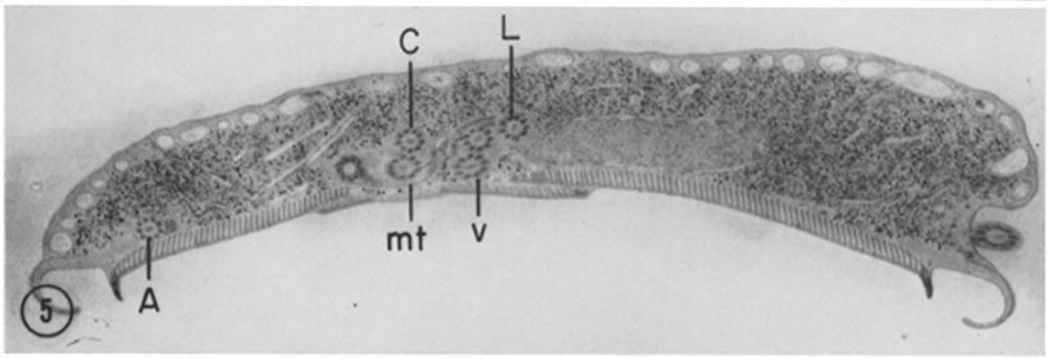
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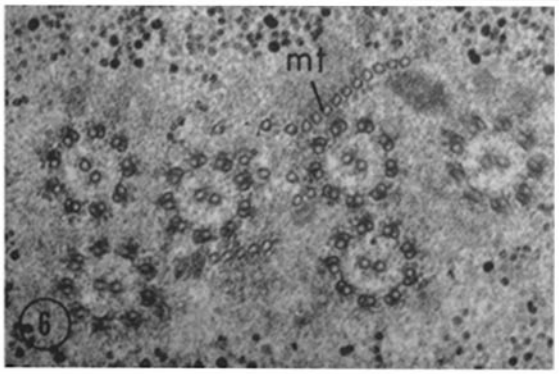
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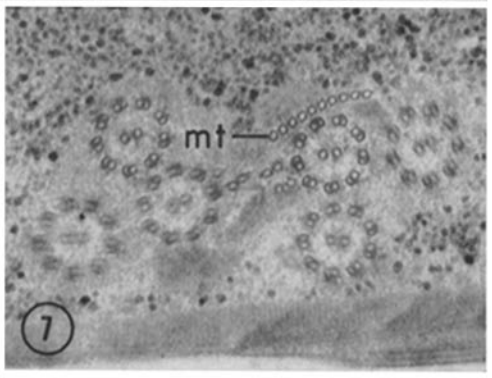
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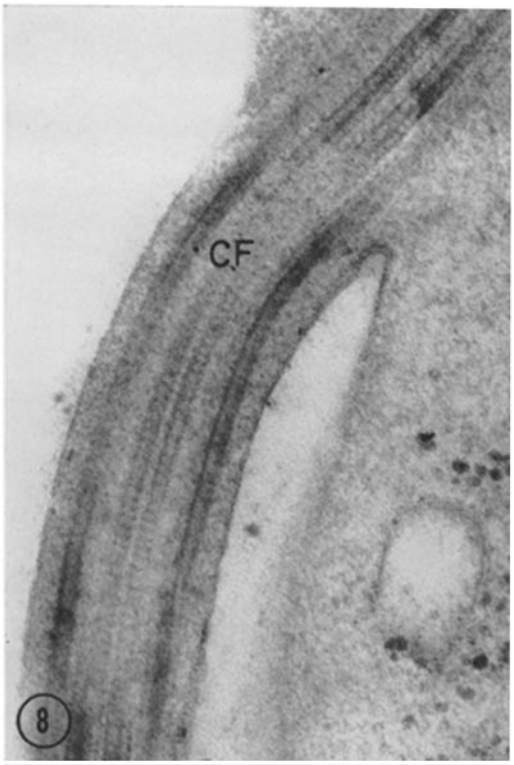
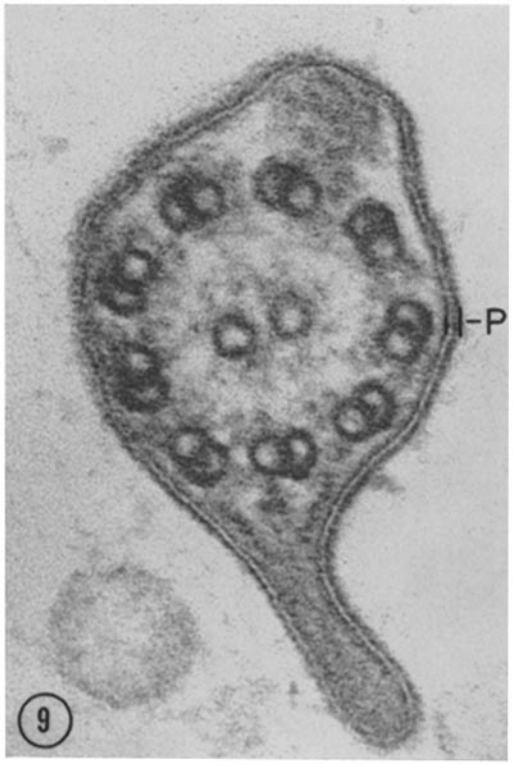
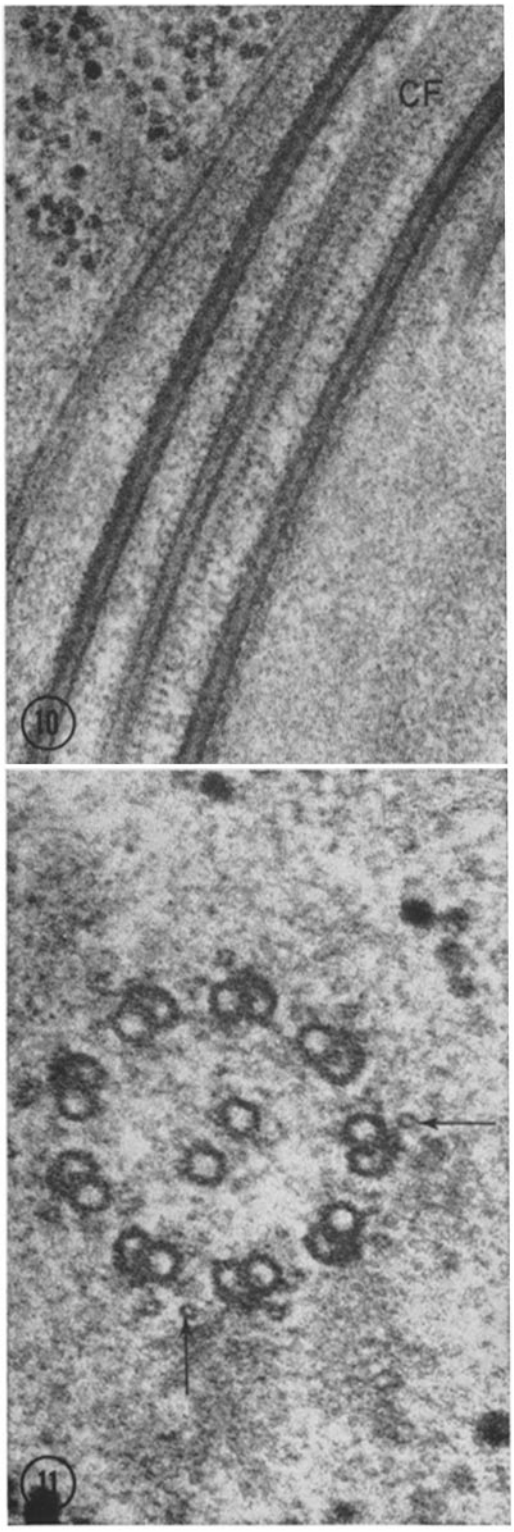




members of 3 pairs of basal bodies have been observed. The finding of 3, rather than the expected 4 pairs of basal bodies could be due to the sampling problem inherent in the study of ultrathin sections, or to the stage of maturation of the trophozoites studied; however, it is in keeping with previous detailed light microscopic studies employing staining selective for basal bodies and flagella (9). The distribution of the basal bodies and initial parts of the flagella which course anteroposteriorly is consistent. As shown in Figs. 5 to 7, half of the 6 central paired flagella are on each side of the midline. One pair is the most dorsal and central of the group (caudal flagella), another pair is just ventral to it (ventral flagella), and a third pair is lateral to these (posterolateral flagella). The basal bodies of the anterior flagella are further anterior, and at the level depicted, the shafts of the anterior flagella are at the lateral margins of the organism. The intracytoplasmic portions of the caudal flagella run parallel above the median groove and emerge at the posterior end of the trophozoite. The ventral flagella emerge through the median groove. The posterolateral flagella curve close to the medial surfaces of the nuclei and emerge laterally in the caudal third of the organism. The anterior flagella course anteromedially, cross in the midline, curve posteriorly beneath the striated extension of the cytoplasm, and emerge laterally at the level of the middle of the nuclei. The emerging axial filament complexes are enclosed by a 500-A layer of cytoplasm and the plasmalemma (Fig. 8).

The fine structure of the basal bodies and flagella is much the same as in other protozoa and metazoan cells (7, 10, 11, 29) and will not be described in detail here. The tubular central pair of fibrils of the flagellum are composed of 12 to 14 filamentous subunits. The peripheral doublets are composed of similar subunits. Each peripheral doublet appears as an incomplete cylinder fused to a complete one. In longitudinal sections, the central pair of microtubules of the flagella have a regular periodicity of about 100 A, produced by thin linear densities extending from the tubules as radial "spokes" (Figs. 8, 10). Cross-sections of the shafts of the flagella reveal hollow fibers, less than 90 A

FIGURES 8 and 9 Longitudinal (Fig. 8) and transverse (Fig. 9) sections through an emergent flagellum, showing the periodicity of the central fibrils (*CF*) in the former and the thin rim of cytoplasm and plasmalemma (*P*) around the axial filament complex in the latter. Fig. 8, × 96,000; Fig. 9, × 220,000.



in diameter, peripheral and parallel to the outer fibers of the axial filament complex (Fig. 11). In appropriate views, 1 or 2 fibers can be seen peripheral to each outer doublet.

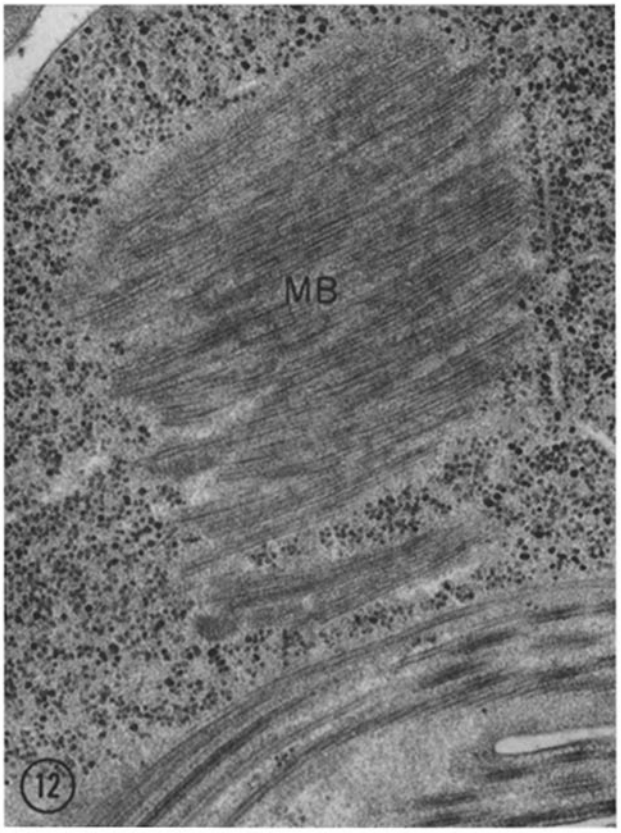
The axial filament complex of the intracytoplasmic portion of the flagellum is not enclosed by a membrane, but is surrounded by a rather homogeneous cytoplasmic ground substance similar to the interfibrillar matrix of the shaft of the flagellum. Each anterior flagellum is accompanied throughout its long intracytoplasmic course by a finely granular dense rod which is irregular in shape. This dense structure marks the medial side of each anterior flagellum. The intracytoplasmic portions of the caudal flagella are accompanied by a row of microtubules which are continuous, presumably, with the ventral extension of the median body.

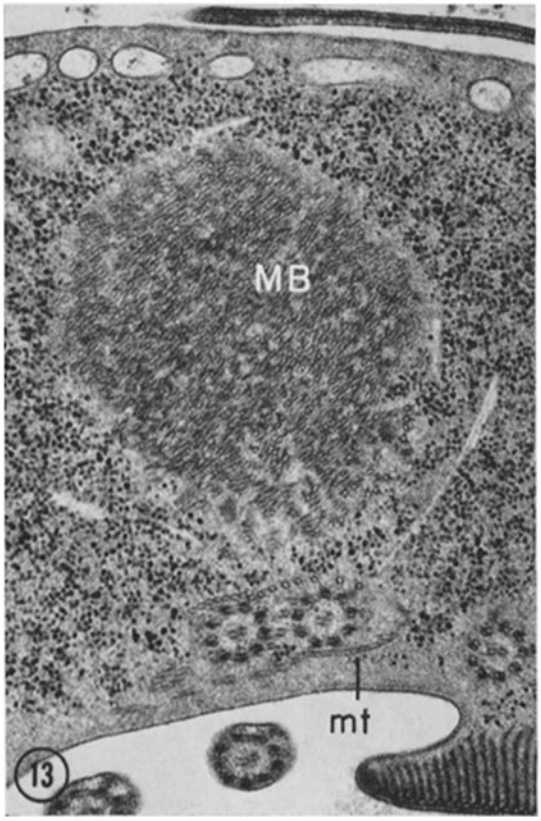
Median Body

The median body, peculiar to *Giardia*, is absent in many of the trophozoites examined. It lies in the middle of the organism dorsal to the shafts of the caudal flagella. The body is not limited by a membrane. About 2 μ in diameter, it is crescentic in shape as seen in mounts of whole cells, and generally ovoid as seen in sections (Figs. 12, 13). The microtubules that form the median body do not have origin or insertion into any other structure. Within the body, the microtubules are randomly arranged, but the ventral extensions of groups of 6 to 14 microtubules are in regular rows. These groups of tubules that continue along the shafts of the caudal flagella secondarily provide a row of microtubules subjacent to that portion of the plasmalemma of the ventral surface not modified to form the adhesive disc. The microtubules do not appear to have direct continuity with the tubules of the flagella, but if they have free ends, their distal terminations cannot be distinguished from sites where they simply curve out of the plane of section.

The microtubules which make up the median body are 250 A in diameter and have a dense outer wall, composed of 12 to 14 40-A subunits, and a

FIGURES 10 and 11 Longitudinal (Fig. 10) and transverse (Fig. 11) sections through the intracytoplasmic shaft of a flagellum. The periodicity along the central fibers (*CF*) is evident in the longitudinal section, and hollow filaments (arrows) are seen peripheral to the outer doublets in the cross-section. Fig. 10, × 100,000; Fig. 11, × 220,000.





FIGURES 12 and 13 The median body (*MB*) as it is seen in sections parallel (Fig. 12) and perpendicular (Fig. 13) to the long axis of its component microtubules. Between the microtubules in the median body and surrounding their ventral extensions (*mt*), the cytoplasmic ground substance is homogeneous and of low electron opacity. Fig. 12, × 39,000; Fig. 13, × 37,000.

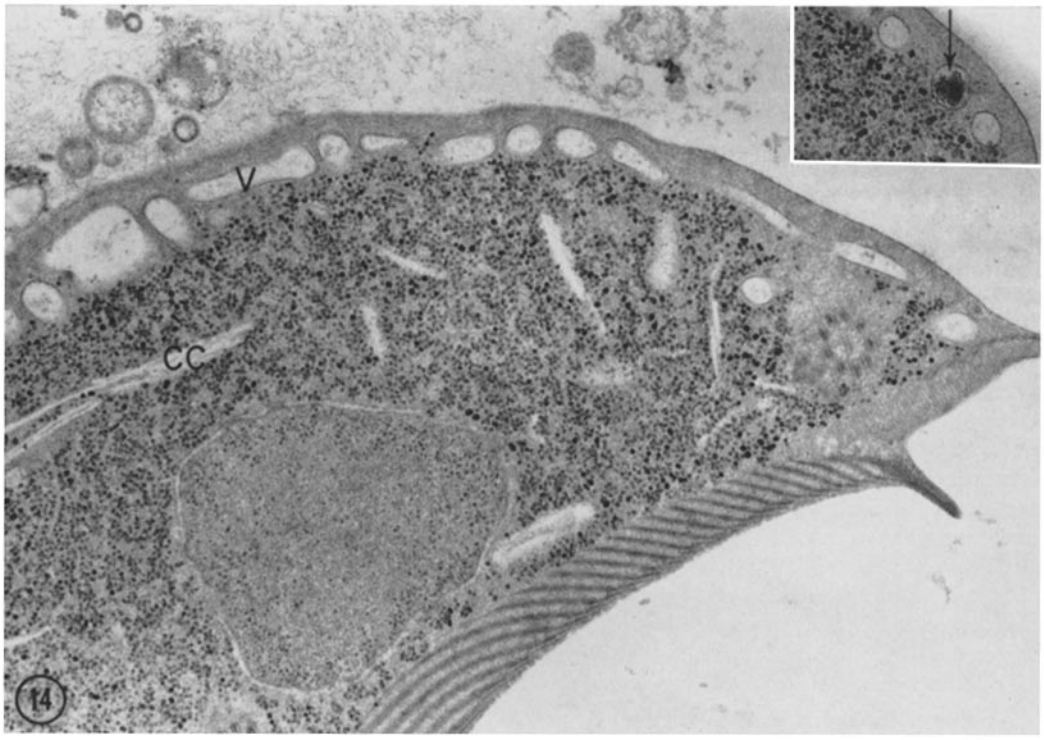


FIGURE 14 Nonmembrane limited fusiform clefts (*CC*) occur in the cytoplasm, with ribosomes and glycogen particles intermingled along their margins. The clefts vary in length, width, and orientation, but have a uniform shape. Occasionally, such cleftlike discontinuities of the cytoplasm have 1 or 2 lines of increased density near their centers, coincident with their long axes. The vacuoles (*V*) in this section are clear, but about 10% of them contain dense granules (arrow, insert) that are distinctly different from the granules in the cytoplasm. × 30,000.

light center. In longitudinal sections, with the fixation and staining employed here, they do not have the periodicity described for microtubules of other tissues (12) nor the periodicity of the axial fibers of the flagella. The ventral microtubules do, however, have occasional lateral extensions, with dimensions similar to those of the subfibrils within their walls, that join adjacent microtubules.

Cytoplasmic Granules

The cytoplasm of *Giardia* is densely packed with 2 types of granules. The sparser granules of the two, 300 A in diameter, are very dense, ovoid, and smooth in contour. They are usually scattered at random, but occasionally are clustered in rosettes and rarely in linear array. The tightly packed subunits of the granules stain intensely after short exposure to lead salts (26) and are presumed to be glycogen.

The more numerous granules, 150 to 200 A in size, are believed to be ribosomes. They are for the most part scattered randomly throughout the cytoplasm, but may occasionally be seen in uniform parallel rows, separated by a 200- to 300-A gap, comprising short segments of rough-surfaced endoplasmic reticulum.

Vacuoles

The only organelles, other than the nuclear envelopes, that are composed of unit membranes are the ovoid vacuoles that are aligned beneath the dorsal plasmalemma, beneath the ventral plasmalemma posterior to, and between, the lobes of the adhesive disc. The width of the membrane that limits a vacuole is identical to that of the plasmalemma, but no continuity between the two has been observed.

The vacuoles have spherical or elongate profiles. When elongate, the long axis of the flattened vacuoles is parallel to the plane of the overlying cell

membrane (Figs. 2, 3 and 5). The vacuoles are 100 mμ in width and between 100 and 400 mμ in length. The interior of the vacuoles is of uniformly low density and is devoid of internal membranes. About 10% of the vacuoles contain clusters of very dense particles embedded in a matrix of moderate density (Fig. 14). The particles in the vacuoles are distinctly different from either of the categories of granules in the cytoplasm.

Adhesive Disc

The adhesive disc consists of a single layer of microtubules each having a thin fibrous band extending dorsally from its wall (Figs. 15, 18). The row of microtubules is situated about 200 A deep to the ventral cell membrane, which has a smooth course over the disc's structural subunits. The uniformly spaced microtubules, 250 to 300 A in diameter, are generally parallel to the long axis of the cell, but those at the lateral edges curve anteriorly and the central tubules arch dorsally. Slender filamentous bridges connect adjacent microtubules.

The thin ribbonlike structures projecting dorsally from the microtubules have a horizontal, 100-A periodic substructure (Figs. 15, 16 and 18). The ribbons are in parallel array and extend inward to the outer margin of the endoplasm. The 200-A thick ribbons are seen edge-on in sections in which the microtubules are cut in cross-section. In sections in which the microtubules are cut longitudinally, a vertical, close cross-banding of the ribbons is seen along the length of the tubules. At the lateral and posterior margins of the discs and in the area of fusion between the 2 lobes, the edges of the ribbons are vertical, but in the middle of each lobe, the edges of the fibrous ribbons are curved.

The arrangement of the structural components of the adhesive disc does not change in the formation of the lips of the median groove. The disc merely tapers bluntly at its margins (Figs. 2 and 13). Laterally, however, the disc forms a dense crest. Frequently, the entire crest portion appears as a dense fusion of the fibrous bands, bounded by the plasmalemma (Figs. 15, 18).

The junction between the adhesive disc and the endoplasm is demarcated by a narrow margin of clear cytoplasm. An even thinner band of clear cytoplasm also separates the disc from the ventrolateral striated flange. At the junction of the disc and the endoplasm, the fibers appear extremely dense and slightly thickened.

Ventrolateral Flange

The ventrolateral flange is dorsal and lateral to the lobes of the disc. It extends peripherally from the finely granular rodlike structures medial to the anterior flagella. Half of the length of the striated portion protrudes beyond the body of the organism. From its thickest portion above the lateral crest of the adhesive disc, it tapers to a thin, curving free edge (Figs. 15, 18). The flexibility of this structure is evident from its variety of positions in different specimens and occasionally by its different contour on opposite sides of the same organism.

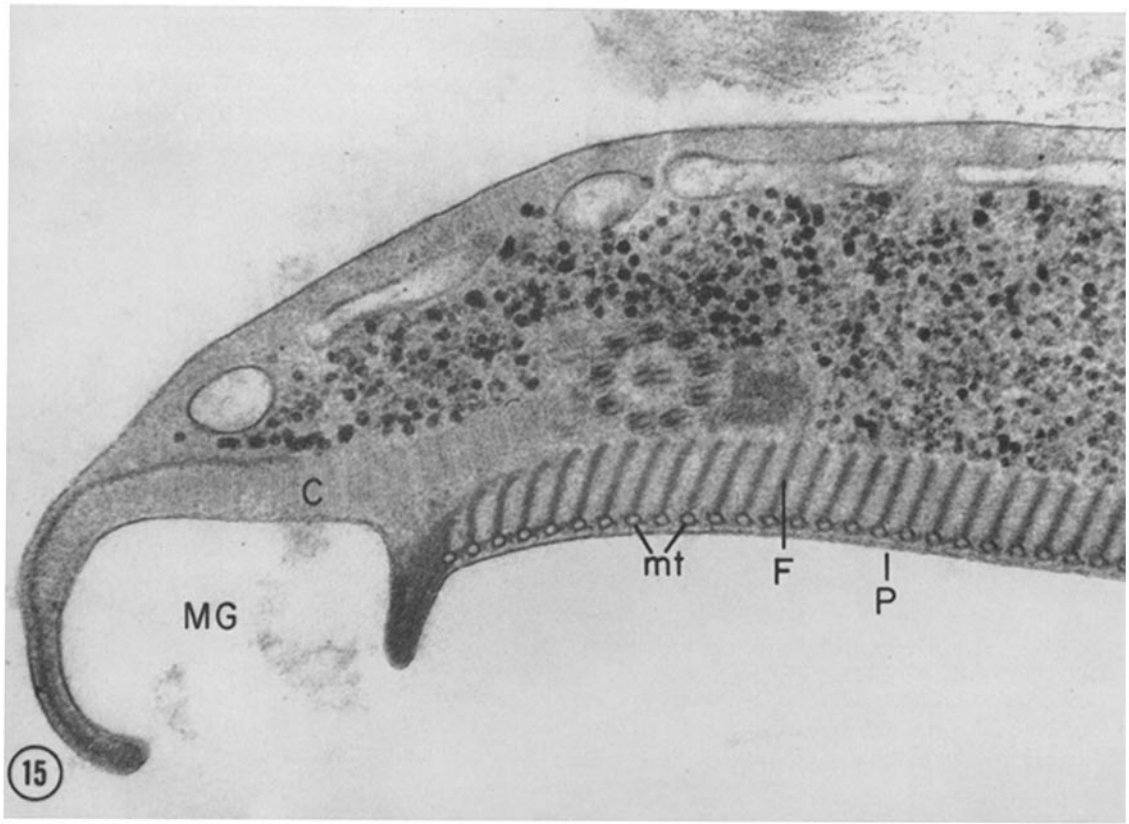
It is composed of alternating light bands and fine dark lines of varying periodicity. In favorable sections every other dark line is seen as a continuous density, whereas the striations between the continuous lines appear punctate. In a plane of section such as in Fig. 19, the nearly continuous striations are 200 A apart, with thicker punctate densities bisecting the intervening spaces. The dorsal terminations of the striations are accentuated by an increase in density (Fig. 18).

DISCUSSION

Median Body

The median body, the vacuoles, and the cytoplasmic clefts in *Giardia* are apparently unique

FIGURES 15 to 17 The adhesive disc (*AD*) is composed of a layer of microtubules (*mt*) joined to the endoplasm by fibrous ribbons (*F*). Tangential sections through the adhesive disc show numerous patterns as a result of the obliquity of the section, the number of disc subunits included, and the portions of the arcs of the disc in the section. Sections that do not include the microtubules give the impression that the disc is composed of parallel ridges. Both cross- and longitudinal sections, however, reveal that the plasmalemma (*P*) covers the entire microtubular ventral surface of the disc without indentations. This architectural arrangement is similar to that of certain trypanosomes (2, 3, 24). *C*, ventrolateral flange. Fig. 15, × 67,000. Fig. 16, × 27,500. Fig. 17, × 22,500.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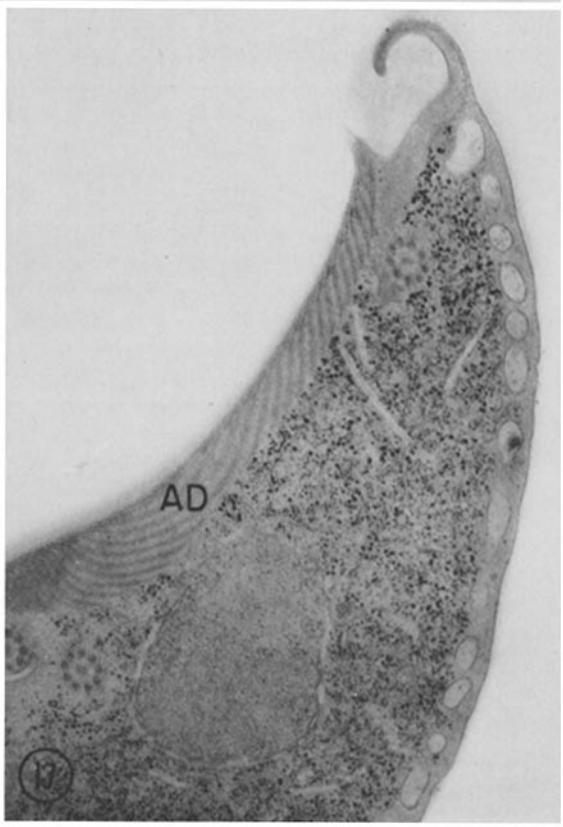
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among protozoa whose fine structure has been described to date. The occurrence of the median body and its general configuration were recognized by light microscopy as early as 1881 (9). Employing electron microscopy, Cheissin in 1964 (5) recognized that the median body consisted of packets of slightly curved "double membranes." These profiles are now commonly referred to as microtubules (18). The microtubules of the median body are similar to those of the contractile axostyle prominent in other zooflagellates (12, 24), but the irregular incidence of the median body, the organization of the microtubules which compose it, and the relationships of microtubules with other organelles make the median body distinct.

Unlike the axostyle of other zooflagellates which is a constant structure, the median body is present in only about half of the organisms observed. Filice stated that the median body is not observed in encysted organisms (9), and according to Cheissin's observations median bodies disappear prior to encystment. Unlike axostyle microtubules which are in rows of marked regularity, the microtubule distribution in the median body is random. The axostyle is ribbonlike; the median body is egg-shaped. A more important distinction between the axostyle and the median body is that the former is attached to the nuclear envelope or an intermediate striated rod (12, 24, 31), while the microtubules of the latter have only been observed to approach the basal bodies, the shafts of the caudal flagella, and the ventral plasmalemma, without having firm attachment to any of these. The periodicity of the microtubules that form the axostyle closely resembles the periodicity of the flagellar fibrils. A similar periodicity may not be present in the microtubules of the median body. In view of these differences in incidence, shape, internal organization, microtubular structure, and attachments, the axostyle and the median body do not appear analogous.

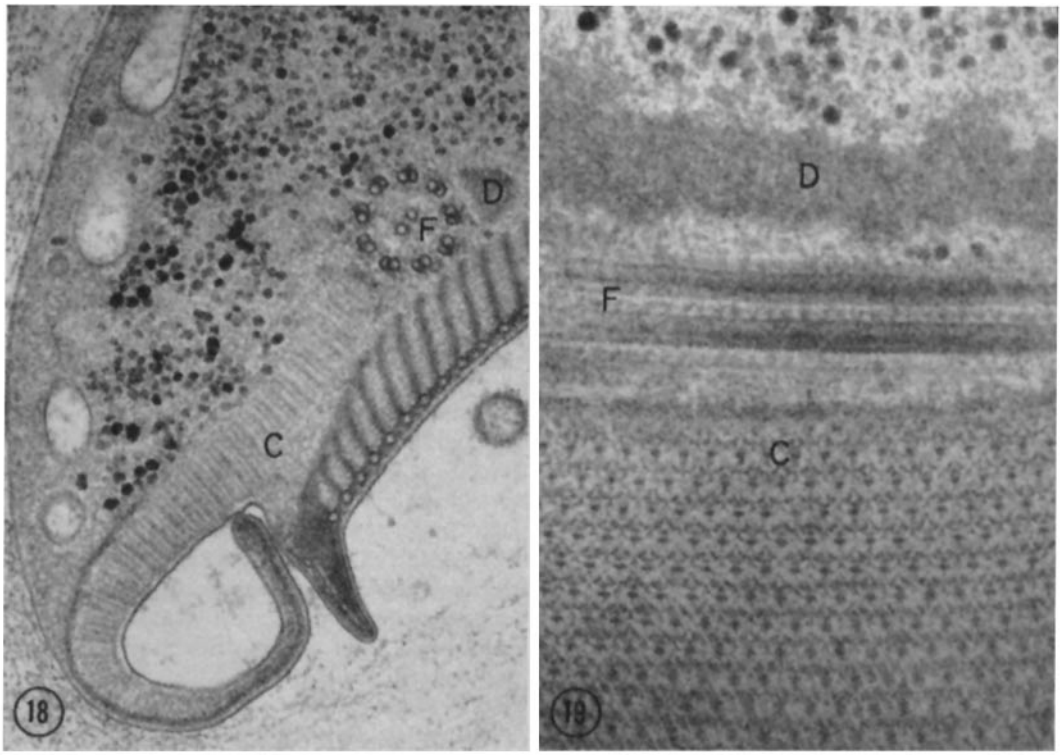
The apparent annular configuration of the median body, the absence of periodicity of its microtubules, and the lack of attachment to another organelle make it doubtful that the microtubules show contractility, and consequently their importance as an instigating force in cytoplasmic flow is unlikely. The median body's ventral extension of microtubules, however, might accomplish this by propagating motion from the caudal flagella with which these microtubules are so intimately associated. The structure of the median body and its ventral extension of microtubules, though, suggest that the microtubules may act primarily as structurally supportive elements. The microtubules form a stabile unit in a posteromedial location which balances the thick anterior portion of the disc. From this location, the microtubules extend to the ground substance around the basal bodies and the shafts of the caudal flagella to which they may lend support. The microtubules continue on, to buttress the ventral portions of the organism that are devoid of the adhesive disc, where they perhaps complement the microtubules of the disc in maintaining the discoid shape of the organism. During encystment, motility diminishes, the organism is shielded by a rigid encasement, and the microtubules of the median body disappear. A similar buttressing function may hold true for microtubules in the numerous other cells in which they have been observed (2, 8, 14, 18).

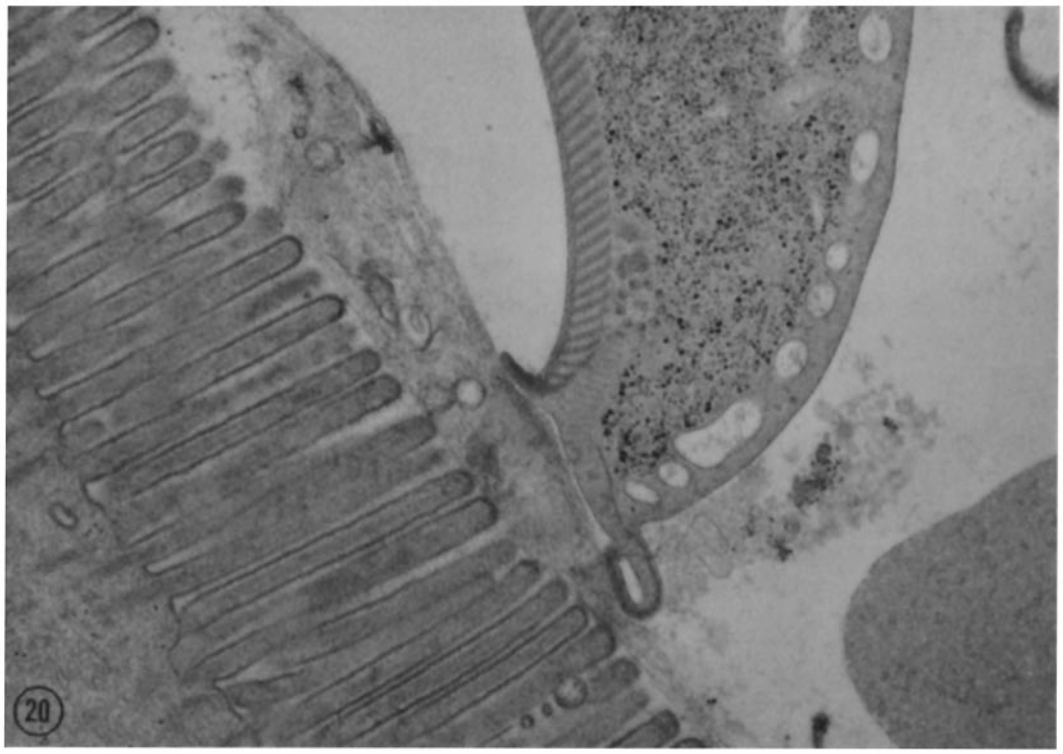
Vacuoles

The vacuoles at the periphery of the cell bear a superficial resemblance to mitochondria and kinetoplasts (11, 24) but they have sufficient differences to distinguish them from these structures. Although at low magnifications the shape, size, and presence of granules in the vacuoles are suggestive of mitochondria, this resemblance is quite superficial. In *Giardia*, the vacuoles are com-

FIGURES 18 and 19 Coronal (Fig. 18) and longitudinal (Fig. 19) sections through the periphery of *Giardia muris* showing the topographical relationship between the ventrolateral striated cytoplasm (*C*), the anterior flagellum (*F*), and the finely granular density (*D*) medial to that flagellum. The thick and thin striations of the peripheral cytoplasm are evident in Fig. 18. As shown in Fig. 19, the thick striations are composed of rows of loosely spaced dense filaments, while the thin striations are composed of nearly contiguous fine filaments in zig-zag array. Fig. 18, × 57,000. Fig. 19, × 114,000.

FIGURE 20 The periphery of *Giardia muris* in contact with the mucous stream covering the microvilli of a duodenal epithelial cell. It appears that the peripheral flange of striated cytoplasm is the grasping organelle of the ventral surface. × 33,000.


D
F
C
18
D
F
C
19


20

posed of only a single unit membrane surrounding the internal matrix, without infoldings of that membrane. Like mitochondria, the vacuoles do contain granules, but these granules are smaller than the mitochondrial matrix granules and are of different configuration. Unlike the elementary particles of mitochondria, the granules are not membrane-associated and are not in an ordered array. The vacuoles probably do not contain respiratory enzymes, but cytochemical and biochemical studies will be required to substantiate this.

The vacuoles differ from kinetoplasts in location, internal structure, and limiting membrane. Kinetoplasts are closely associated with the basal bodies, have an internal component of anteroposteriorly directed fibers, and, like mitochondria, they are limited by 2 membranes (24, 25, 31). None of these features apply to the vacuoles.

Mucocysts of other protozoa are rows of globular elements beneath the pellicle that discharge gelatinous or mucoid substances (24, 31). Like the ectoplasmic vacuoles, muciferous bodies are enclosed in a single unit membrane, are arranged peripherally, and often contain dense material. There is reason, therefore, to consider these as analogous structures, and, indeed, the location of the vacuoles is consistent with a secretory function, perhaps the secretion of the cyst wall. When the organism encysts, the encasement is formed over the dorsal surface first and then completed over the ventral surface as *Giardia* curls dorsoventrally into a spherical shape (9). The sequence of cyst formation, therefore, correlates well with the population density of the ectoplasmic vacuoles. Observations of the fine structure of the vacuoles during encystment will have to be made in order to substantiate their possible role in cyst formation.

Cytoplasmic Clefts and Granules

The fusiform electron-lucent areas of the endoplasm have not been described before, although they are also present in osmium tetroxide–fixed specimens and do not appear to be artifacts of fixation. The clefts are somewhat similar in architecture to the spindle-shaped bodies (trichocysts) found in holotrichous ciliates (24, 31), but their endoplasmic location in *Giardia* and their lack of internal periodicity characteristic of trichocyst material make it unlikely that the clefts and trichocysts are analogous.

The clefts have some similarity to the rodlike bodies which contain cytochromes in Gram-negative bacteria that reduce tellurium (16). Because of the existence in nature of such a morphologic unit which contains respiratory enzymes, a similar function for the clefts in *Giardia* should be considered. One might presume that respiratory enzymes should be present in this flagellate, but the absence of mitochondria is not without precedent. *Trypanosoma equiperdum* (3) and *Entamoeba* (6, 22) are examples of motile parasites without typical mitochondria. The fine structure of the clefts suggests that they may be inclusions. Crystals polymerized to various degrees, but of consistent internal structure, would result in the same shape. If extracted during specimen preparation, such crystals could account for the above observations.

The ribosomes constitute part of the scanty, short profiles of ergastoplasm. The absence of a recognizable protein secretory product and the absence of a Golgi apparatus suggest that the protein synthesized is used endogenously. The dearth of membrane-associated ribosomes compared to free ribosomes or polysomes further supports the view that the free ribosomes manufacture protein primarily for use by the cell.

The large granules are presumably a form of carbohydrate storage particle that closely resembles glycogen, but the possibility that they are amylose or amylopectin (4, 13) or a form of volutin granule (13, 23) cannot be excluded by cytology alone. These large granules are most likely responsible for the diffuse PAS-positive reaction in the cytoplasm of fixed organisms and convert iodine to a dark color in freshly obtained parasites. The observation that a large number of carbohydrate granules exist in the cytoplasm in the absence of smooth endoplasmic reticulum is not incompatible with the suggestion that the granules are glycogen. Even in certain metazoan cells such as brown adipose tissue and the glycogen body of the chick, glycogen is abundant despite a paucity of cytoplasmic membranes (8).

Structures of the Ventral Surface

The fine structure and periodicity of the peripheral rim of striated cytoplasm approximates the appearance of paramyosin. Tangential sections unmask the superimposed dot pattern of symmetry which simulates the hexagonal pattern found in paramyosin "fibrils" and crystals prepared by various extraction methods (15). Whether or not this protein is paramyosin is beyond the scope of this study, but it is suggested that this portion of the

organism is composed of a contractile protein which imparts a wide range of movement to the organelle. This extension of regularly packed filaments appears to be the grasping structure of the ventral surface (Fig. 20). Although different in function, it appears structurally related to the costae (1, 24) or paraxial rods (21) of other flagellates.

Adhesive disc or sucking disc is a poor term for the modified ventral surface of this organism. The disc appears to be a rigid structure composed of supportive, rather than contractile, elements which maintain a fixed shape. It is on this pontoonlike structure that the dorsal portion of the protozoan rests; the disc itself is not responsible for attachment to the substrate. Since it has no modifications for intake or transport of particles or fluid and is devoid of organelles, it is probably not directly active in the physiologic maintenance of the cell. In this regard, a term such as *striated disc* seems preferable until further knowledge of the function of this ventral structure is available.

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