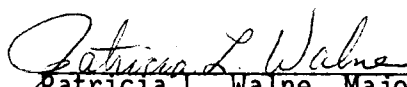
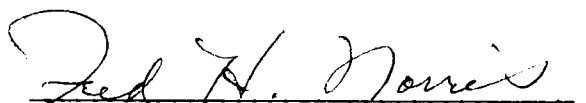


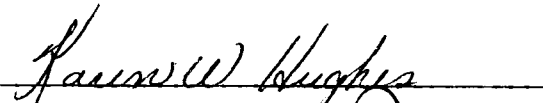
To the Graduate Council:

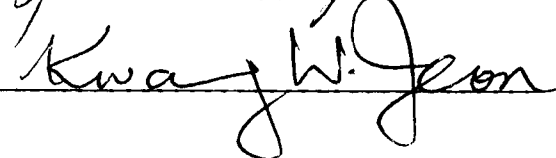
I am submitting herewith a dissertation written by Lula Lail Hilenski entitled "Ultrastructural and Experimental Investigations of the Euglenoid Flagellate, Peranema trichophorum (Ehrenberg) Stein, with Special Reference to Flagella, Mucocysts and Feeding Apparatus." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Botany.


Patricia L. Walne, Major Professor

We have read this dissertation
and recommend its acceptance:







Accepted for the Council:


Vice Chancellor
Graduate Studies and Research

ULTRASTRUCTURAL AND EXPERIMENTAL INVESTIGATIONS OF THE EUGLENOID
FLAGELLATE, PERANEMA TRICHOPHORUM (EHRENBERG) STEIN,
WITH SPECIAL REFERENCE TO FLAGELLA, MUCOCYSTS
AND FEEDING APPARATUS

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Lula Lail Hilenski
August 1980

3045019

ACKNOWLEDGMENTS

I am pleased to acknowledge and thank those people who have helped me through my graduate study and the completion of this dissertation. I am deeply grateful to my major professor and mentor, Dr. Patricia L. Walne, for her concern, support and encouragement. Her counsel both as a professional scientist and personal friend has been invaluable to me. I thank the other members of my doctoral graduate committee, Drs. Karen Hughes, Kwang Jeon and Fred Norris of The University of Tennessee, Knoxville, and Dr. Jytte Nilsson of the Institute of General Zoology, University of Copenhagen, Denmark, for helpful comments and careful reading of the manuscript. To Dr. Norris I am especially indebted for his personal interest, concern and kindness to me.

I am very appreciative of my predecessors in the laboratory, Drs. Maybelle Gomez, Russell Porcella and Larry West, who offered technical suggestions, training and personal time on electron microscopic techniques. I also thank Mr. Dick Williams (Supervisor, Life Sciences EM Facility, The University of Tennessee, Knoxville) for his infinite patience, good humor and excellent assistance in EM training. I express gratitude to Drs. Sandra Bell, Karen Hughes and Robert Miller for the opportunity to serve as a Graduate Teaching Assistant in their courses.

I extend a special thanks to Dr. David Hibberd of the Culture Centre of Algae and Protozoa, Cambridge, England, for his example of warm collegiality and inspired teaching in the 1977 summer course on non-green algal flagellates at Miami University, Oxford, Ohio. I

am also grateful to Dr. Alan Heilman and Mr. Frank Ralston for loyal friendship and helpful discussions.

I acknowledge the following financial assistance: Graduate Teaching Assistantship, Department of Botany, UTK; Hilton A. Smith Predoctoral Fellowship, UTK; Research Assistantship, Cotton Quality Laboratories, UTK; Research Associate, Department of Plant and Soil Science, UTK.

I also thank Ms. Wynne Browne and Ms. Ann Lacava for their technical assistance in the preparation of this manuscript.

I am deeply thankful for the lifelong love and support of my parents, Julius and Catherine Lail. My most loyal and enthusiastic supporter has been my best friend and mate, Ski, to whom I am most indebted for his love, understanding, humor and patience which have sustained me while I pursued this degree.

ABSTRACT

This investigation characterizes the ultrastructure and cytochemistry of specialized organelles involved in the basic biological phenomena of motility, secretion and ingestion in a colorless, phagotrophic, biflagellated euglenophyte, Peranema trichophorum. Particular attention is given to (1) the organization of the flagella, flagellar mastigonemes and flagellar roots; (2) the mucocysts, which are unique extrusive organelles; (3) the interrelationships among the organelles and lamellar systems of the feeding apparatus; and (4) the general cellular organization.

With respect to motility, the organism has a conspicuous anterior or leading flagellum and a trailing flagellum which is appressed to the ventral surface of the pellicle. Both short and long nontubular mastigonemes surround the anterior flagellum. Short mastigonemes form a bilateral array parallel to the long axis of the flagellum. Each array consists of tiers which longitudinally overlap each other by one-half of a tier length. Individual short mastigonemes in the tiers are spaced 25 nm apart and are linked by fine fibrils. Tufts of long mastigonemes emanate from spiral rows and curve anteriorly toward the distal end of the flagellum.

Two basal bodies, lying at an angle to each other, are associated with each flagellum at the base of the reservoir. Attached to the basal bodies or originating close to them are microtubular, fibrous and striated roots. Three microtubular roots extend from the basal bodies and continue anteriorly in the reservoir wall. A striated fiber connects microtubular roots to the basal bodies. At

least three striated roots, consisting of cross-striations with a periodicity of 30-38 nm, extend from the microtubular roots and the striated fiber to the rodorgan.

Concerning secretion, there are rows of ejectile organelles or mucocysts located beneath the pellicle. Immature mucocysts are tubular structures with amorphous contents. Mucocysts may protrude through the pellicle and discharge a ball of mucilaginous material or may themselves be ejected from the cell through the pellicular grooves. Ejected mucocysts show a uniform structure consisting of an inner tube with helical striations, an outer tube with a diamond-shaped pattern, and a dense middle band. Fine fibrils 8-9 nm in diameter emanate from mucocyst tips.

The ingestion or feeding apparatus consists of a prominent rodorgan, a system of complex lamellae and an expandable cytostomal sac, which together form an independent ingestion system lateral to the canal and reservoir. The rodorgan is anchored by lamellae which diverge into the double serrated marginal lamella and the articulating lamella, both of which arch over the rim of the cytostome. A row of microtubules extends along the lower plate of the articulating lamella. Striated roots connect the base of the rodorgan to the flagellar bases.

Typical euglenoid diagnostic features in Peranema include the pellicular striae, helical symmetry, dictyosomes, contractile vacuole and reservoir. Ruthenium red labeling, indicative of acid mucopolysaccharide constituents, is localized in the mucilage strands on the pellicle surface. Atypical of euglenoids are the stacked cristae in

some mitochondria and the absence of dense heterochromatin in the interphase nucleus. Endosymbiotic bacteria, rod-shaped structures 1 μm long and 0.3 μm wide, are abundant in the cytoplasm.

This investigation provides a framework for future considerations of phylogenetic affinities among euglenoids and other flagellated algae and protozoans and for biochemical and biophysical investigations of specialized organelles involved in motility, secretion and ingestion.

TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION.	1
II. MATERIALS AND METHODS	13
Culture Methods	13
Descriptive Methods	13
Analytical Methods	21
III. RESULTS	24
Cellular Organization	24
Flagellar Organization.	57
Mucocyst Structure and Development.	94
Phagocytosis.	107
Feeding Apparatus	112
IV. DISCUSSION	134
Flagellar Structure	134
Mucocysts	145
Feeding Apparatus	148
Diagnostic Euglenophycean Features.	150
V. SUMMARY AND CONCLUSIONS	157
Flagella, Flagellar Mastigonemes and Flagellar Roots.	157
Mucocysts	159
Feeding Apparatus	160
General Cellular Organization	162
LIST OF REFERENCES.	163
APPENDIX.	132
APPENDIX A. CULTURE MEDIUM	173
APPENDIX B. COMMON CHEMICALS USED IN ELECTRON MICROSCOPY	174
APPENDIX C. STAINED YEASTS FOR STUDIES OF PHAGOCYTOSIS	178
APPENDIX D. REPRESENTATIVE FIXATION PROCEDURE.	180
APPENDIX E. PREPARATIVE PROCEDURES AND STAINS FOR THICK SECTIONS	182
APPENDIX F. PROCEDURES FOR ULTRASTRUCTURAL CYTOCHEMISTRY	183
APPENDIX G. PREPARATIVE PROCEDURE FOR SCANNING ELECTRON MICROSCOPY	187
APPENDIX H. ISOLATION OF FLAGELLA AND MASTIGONEMES	188
VITA.	189

LIST OF PLATES

PLATE	PAGE
I. Anterior Structural Features.	25
II. Cell Surface Morphology	28
III. Anterior End Morphologies	30
IV. Posterior End Morphologies.	32
V. Ultrastructure and Cytochemistry of the Pellicle Complex	35
VI. Pellicle and Reservoir.	38
VII. Cell Cleavage	40
VIII. Arrays of Microtubules in Dividing Cell	42
IX. Contractile Vacuole Region	45
X. The Nucleus	47
XI. Mitochondria	50
XII. Dictyosomes and Dense Body.	52
XIII. Endosymbiotic Bacteria	55
XIV. Flagellar Structure	58
XV. Comparison of Anterior and Trailing Flagella.	60
XVI. Overview of Flagellar Mastigonemes.	63
XVII. Long Mastigonemes	65
XVIII. Fine Structure of Short Mastigoneme Ribbons	67
XIX. Shadow-Cast and Negative-Stain Preparations of Mastigonemes	70
XX. Detached Mastigoneme Ribbons.	72
XXI. Isolated Long Mastigonemes and Mastigoneme Ribbons. . .	74
XXII. Differences Between Anterior and Trailing Flagella. . .	77

PLATE	PAGE
XXIII. Model of Flagellar Mastigonemes	79
XXIV. Flagellar Bases and Basal Bodies.	82
XXV. Basal Bodies and Flagellar Roots.	86
XXVI. Striated Roots.	89
XXVII. Model of Flagellar Bases and Associated Roots	92
XXVIII. Early Stages of Mucocyst Development.	96
XXIX. Later Stages of Mucocyst Development.	98
XXX. Discharge of Mucilaginous Material from Mucocysts . . .	100
XXXI. Structure of Discharged Mucocysts	103
XXXII. Details of Mucocyst Fine Structure.	105
XXXIII. Food Vacuoles and Autophagosomes	108
XXXIV. Scanning Electron Microscopy of Phagocytosis.	110
XXXV. Structure of the Rodorgan	113
XXXVI. Longitudinal Lamellae	116
XXXVII. Canal and Cytostome	119
XXXVIII. Striated Fibril in Reservoir Wall	121
XXXIX. Cytostome-Associated Lamellae	123
XL. Articulating Lamellae and Outer Rod Connections	125
XLI. Double Serrated Marginal Lamella.	128
XLII. Model of Feeding Apparatus.	132

LIST OF ABBREVIATIONS

Measurements and Other Symbols

Au/Pd	gold/palladium
C	Centigrade
cm	centimeter (10^{-2} meter)
CPD	critical point drying
EDTA	ethylenediaminetetraacetic acid
EGTA	ethyleneglycol-bis (β -amino-ethyl ether) N, N' tetraacetic acid
FW	formula weight
kV	kilovolt
LD	light/dark
LM	light microscopy
μm	micrometer (10^{-6} meter)
nm	nanometer (10^{-9} meter)
OsO ₄	osmium tetroxide
psi	pounds per square inch
PTA	phosphotungstic acid
Pt/C	platinum/carbon
RR	ruthenium red
SEM	scanning electron microscopy
TEM	transmission electron microscopy
v/v	volume per volume
w/v	weight per volume

Organelles

AcV	accessory vacuole
AL	articulating lamella
AlV	alveolate vesicle
AX	axoneme
bb	basal body
Can	canal
CV	contractile vacuole
Cys	cytostome
D	dictyosome
DM	double serrated marginal lamella
ER	endoplasmic reticulum
F ₁	anterior flagellum
F ₂	trailing flagellum
IA	inner anchoring lamella
IB	inner dense body
IRo	inner rod
LL	longitudinal lamella
MR	microtubular root
Mu	mucocyst
N	nucleus
OA	outer anchoring lamella
OB	outer dense body
ORo	outer rod
PR	paraflagellar rod
Res	reservoir

Organelles (continued)

RO	rodorgan
SF1	striated fibril
SFr	striated fiber

CHAPTER I

INTRODUCTION

Unicellular organisms have historically served as important instruments for the study of basic biological phenomena, since nearly all life processes occur in these single cells. By virtue of its particular complement of specialized structures, the colorless unicell Peranema trichophorum, a phagotrophic, biflagellated euglenophyte, is especially well suited for investigations on motility, secretion and ingestion. To provide a framework for future biochemical and biophysical investigative approaches to these phenomena, the present study provides detailed ultrastructural and cytochemical characterizations of the flagella, the mucocysts, which are extrusive organelles, and the complex feeding apparatus consisting of a prominent rodorgan and cytostome. Historically, the features of motility and phagotrophy have served as focal points for research on the organism. A partial review of past research on these topics and on the taxonomy of Peranema is presented below and summarized in Table 1.

Although the first report of Peranema (Ehrenberg, 1838) described it as a ciliate, Trachelis trichophorus, Dujardin (1841) recognized it as a flagellate and established the genus Peranema to include highly metabolic euglenoids with a single long flagellum that moved only at the tip. Dujardin described three species: P. protracta, which he identified as Ehrenberg's (1838) ciliate, P. globulosa and P. virescens, an ill-defined greenish form. In 1878 Stein transferred

TABLE 1
A PARTIAL SUMMARY OF PERTINENT LITERATURE
ON THE BIOLOGY OF PERANEMA

Date	Author	Contribution
1838	Ehrenberg	First described organism as a ciliate, <u>Trachelis trichophorus</u>
1841	Dujardin	Recognized organism as a flagellate and established genus <u>Peranema</u> ; named organism <u>Peranema protracta</u>
1878	Stein	Placed species <u>trichophorum</u> in genus <u>Peranema</u> erected by Dujardin; made extensive investigation of organism; described single flagellum and single rodorgan
1910	Hartmann and Chagas	First to describe second flagellum in <u>Peranema</u> which they believed ended in a thickening at opening of reservoir
1924	Korschikow	Noted a nonmotile second flagellum adhering to periplast in <u>Peranema</u>
1928	Hall and Powell	Described <u>Peranema</u> as a colorless, uniflagellate organism 22-60 μ m in length, with food vacuoles that form at the base of the gullet and then pass to the posterior, except at early prophase when the cytoplasm is free of food vacuoles
1929	Hall	Used vital dyes to identify mitochondria and Golgi apparatus in <u>Peranema</u> ; mitochondria occur in spiral rows beneath periplast; Golgi apparatus are scattered throughout cytoplasm
1933	Lackey	Described two flagella and two rod-organs; also saw fibril from rod which extends in direction of blepharoplasts

TABLE 1 (Continued)

Date	Author	Contribution
1934	Hall	Confirmed existence of two flagella in <u>Peranema</u> ; observed rhizoplast which is visible in prophase and extends from granules on nuclear membrane to blepharoplasts
1936	Mast and Hawk	Found that sudden increase in light intensity produced a shock reaction in <u>Peranema</u>
1937	Shettles	Showed that <u>Peranema</u> responds to rapid increase in light intensity by a "shock" reaction; the flagellum is most sensitive to light
1942	Shortess	Found that rapid changes in temperature caused shock reactions in <u>Peranema</u> similar to those elicited by rapid changes in light intensity
1945	Pitelka	Confirmed by light microscopy the existence of a second flagellum in <u>Peranema</u> ; also reported that the <u>cytostome</u> and reservoir are two separate structures
1950	Chen	Observed that the feeding apparatus of <u>Peranema</u> is independent of the reservoir; that <u>Peranema</u> feeds on milk, living yeast, and certain unicellular algae, including species of <u>Euglena</u> ; that particulate food seems to be essential; that starch grains, oil-droplets and protein particles are engulfed and digested
1953	Storm and Hutner	Reported that <u>Peranema</u> exhibits complex nutritional requirements, including steroids and vitamins, and that lipid requirement might point to deep-seated adaptation to phagotrophy
1954	Huber-Pestalozzi	Investigated systematics and morphology of Euglenophyta

TABLE 1 (Continued)

Date	Author	Contribution
1955	Pitelka and Schooley	Reported that the anterior flagellum consists of (1) a fibrous axoneme; (2) a cross-striated sheath present as two easily separated ribbons; and (3) a border of mastigonemes
1958a	Leedale	Estimated from light-microscopic observations of mitosis that <u>Peranema</u> has 177 chromosomes
1958b	Leedale	Examined nuclear structure and division in 24 species of Euglenineae; reported always more than one endosome in interphase nucleus of <u>Peranema</u> ; endosome dumb-bell shaped during metaphase
1959	Leedale	Determined time and rate of mitosis for 40 species of Euglenineae; mitosis in colorless species, including <u>Peranema</u> , shows irregular periodicity independent of light
1959	Roth	Described general ultrastructure of <u>Peranema</u> , including details of rodorgan and flagella; also observed numerous endonuclear bacteria
1963	Christen	Discussed taxonomy of colorless euglenoids; erected genus <u>Pseudoperanema</u> to accommodate <u>Peranema</u> since biflagellate nature of organism was not in original description
1963	Archibald <u>et al.</u>	Identified the iodophobic reserve carbohydrate in <u>Peranema</u> as paramylon, a β (1 $\rightarrow$ 3) linked D-glucose residue which is insoluble in water
1963	Jahn <u>et al.</u>	Studied the locomotion of <u>Peranema</u> with high-speed microcinematography and described the flagellar beat
1963	Pitelka	Categorized and discussed fibrous structures of protozoa including rodorgan of <u>Peranema</u>

TABLE 1 (Continued)

Date	Author	Contribution
1963	Pringsheim	Described the formation of food vacuoles in <u>Peranema</u> using light microscopy
1964	Tamm	Investigated developmental events of <u>Peranema</u> flagellar replication and regeneration; found that during division, the anterior flagellum rapidly shortens at constant rate and disappears while simultaneously two new leading flagella elongate
1966	Allen <u>et al.</u>	Described improved techniques for maintaining cultures of <u>Peranema</u> ; they grew the organism on a milk-lecithin medium and in a nearly defined autoclavable biphasic agar medium fortified with fat-soluble antioxidants
1966	Mignot	Studied the general ultrastructure of <u>Peranema</u> and other Euglenineae
1967	Leedale	Described the phylogeny and diagnostic characteristics of the genus <u>Peranema</u> ; observed that well-fed cells were packed with paramylon, thus obscuring much of the cell structure; advised starving cells before examining their cytology
1970	Curds and Cockburn	Found that <u>Peranema</u> was one of the four most frequently seen flagellates in effluent samples from used-water treatment plants; also found organism in activated sludge plants when organic loading was high
1973	Sleigh	Described <u>Peranema</u> as an active but unspecialized feeder; cytostome lies beside the flagellar invagination and is supported by two skeletal rods, whose main structural component is a bundle of microtubular fibrils; for food capture rods may be protruded and withdrawn

TABLE 1 (Continued)

Date	Author	Contribution
1974	Nisbet	Constructed a model for the feeding apparatus of <u>Peranema</u> from electron micrographs; proposed that cyto-stome, which lies close to the internal opening of the canal, can be moved forward during feeding, closing the passage to the reservoir and displacing the flagella; proposed a working hypothesis for movement of the feeding apparatus
1977	Nisbet	Described flagellar regeneration in <u>Peranema</u>
1978	Leedale	Discussed euglenoid phylogeny; proposed that in phagotrophic line, ingestion organelle of <u>Peranema</u> arose from <u>Menoidium</u> -type canal skeletal system
1979	Hänel	Investigated systematics, morphology and ecology of 54 colorless flagellates in sewage treatment plants; differentiated <u>Peranema cuneatum</u> from <u>P. trichophorum</u>

P. globulosa to the genus Heteronema and listed P. trichophorum (Dujardin's P. protracta) as the single species.

In contrast to other early light microscopists who observed only one flagellum in Peranema (Stein, 1878; Hall and Powell, 1928), Hartmann and Chagas (1910) were the first to describe a second flagellum which they believed ended at the opening to the reservoir. Korschikow (1924) also made a brief note of a second, nonmotile flagellum that adheres to the periplast. Korschikow's description was later confirmed and elaborated on by Lackey (1933), Hall (1934) and Pitelka (1945), all three of whom recognized that the second flagellum, appressed to the ventral surface of the cell, is difficult to observe with light microscopy and had been overlooked in the earlier studies.

The original generic description of P. trichophorum as a monoflagellate has created difficulty among taxonomists. Christen (1963) established the genus Pseudoperanema to accommodate the biflagellate P. trichophorum. Leedale (1967), however, disagreed with Christen's reclassification and stated that rules of biological nomenclature provide that the original description and not the name of the species be changed; therefore, the species Peranema trichophorum is valid. Leedale also noted that Peranema-like species which genuinely have only one emergent flagellum should be placed in the genus Peranemopsis, established in 1940 by Lackey (Lackey, 1940).

Inclusion into the genus Peranema is proposed by Pitelka (1945) for members of the genus Heteronema, whose type species H. marina remains a "nomen nudum," and for the species Peranema cuneatum, which is differentiated from P. trichophorum on the basis of cell

shape and characteristics of the periplast (Hänel, 1979). Although there are probably several freshwater species of Peranema besides the type species, P. trichophorum (Leedale, 1967), their status remains unresolved due to a lack of substantive documentation and descriptions.

Some of the earliest studies focused on the response of Peranema to changes in light intensity (Mast and Hawk, 1936; Shettles, 1937) and temperature (Shortess, 1942), both stimuli producing a shock reaction manifested by reorientation of the anterior end of the organism. By means of high speed microcinematography, Jahn et al. (1963) found that during gliding movement in Peranema, the flagellar beat consists of a horizontal bending of the distal third of the flagellum, resulting in a 90° deflection of the tip. The beat is similar to that of a cilium.

Studies on the anterior flagellum have centered on its replication and regeneration (Tamm, 1964; Nisbet, 1977) and on its fine structure (Chen, 1950; Pitelka and Schooley, 1955; Roth, 1959). The "sheath" surrounding the anterior flagellum has been of particular interest. Chen observed that the sheath consists of numerous fibrils radiating from the axis, giving the flagellum a "test-tube brush" appearance. Treatment of unfixed specimens with chemicals that disrupt fibrillar proteins resulted in separation of the anterior flagellum into three fibrils, whose structure could not be resolved with the techniques available at that time (Chen, 1950). Pitelka and Schooley (1955) were the first to demonstrate that the sheath surrounding the anterior flagellum is composed of two "hemi-cylinders" which, when dried, appear as flat, striated bands. In addition to

the striated sheath, a border of nontubular mastigonemes up to 4 μm in length appears on both sides of the flagellum.

While the anterior flagellum has been implicated as a receptor for mechanical, chemical (Chen, 1950) and light (Sleigh, 1973) stimuli, its role in these responses remains speculative. Evidence that the swollen basal region of the anterior flagellum serves as a photoreceptor for an adjacent pigmented stigma (Sleigh, 1973) is unsubstantiated, since this is the only report of an eyespot in Peranema.

Accurate descriptions of the feeding apparatus are difficult to obtain with light microscopy due to the violent euglenoid movement of Peranema during ingestion. Initial accounts described the reservoir as a "gullet" for passage of food into the organism (Hall and Powell, 1928; Lackey, 1933; Hall, 1934). Later studies reported two openings, an anterior one leading to the reservoir and a posterior, more-ventral opening leading to a cytostome which serves as the conduit for food into the organism (Pitelka, 1945; Chen, 1950). More recent observations with electron microscopy (Nisbet, 1974) depicted one common opening leading to both the reservoir and cytostome. In a model of the feeding apparatus based on ultrastructural observations, Nisbet postulated that the reservoir is laterally displaced during ingestion by the ordorgan as food enters the canal and is passed to an expanding cytostome.

Peranema is the only phagotrophic euglenoid species that has been successfully cultivated. It can be grown on a wide variety of undefined media, including beef-suet infusion with bacteria (Hall and Powell, 1928; Mast and Hawk, 1936), lettuce infusion with bacteria

(Roth, 1959), balanced salt solution with rice (Shettles, 1937) and Chalkley's medium with yeasts (Nisbet, 1974). In a thorough investigation of cultivation and growth of Peranema, Chen (1950) successfully established axenic cultures, with greatest cell densities (1.7×10^{-4} cells/ml) occurring on milk medium with calcium sulfate.

The voracious phagotrophy of Peranema is well documented (Hall and Powell, 1928; Pitelka, 1945; Chen, 1950; Roth, 1959; Pringsheim, 1963; Allen et al., 1966; Leedale, 1967; Sleight, 1973; Nisbet, 1974). The organism is an unspecialized feeder consuming both digestible and indigestible materials, including polystyrene latex beads (Allen et al., 1966) and other flagellates and algae (Chen, 1950) such as whole Euglena cells (Leedale, 1967). Peranema also feeds on detritus (Sleight, 1973) and is one of the four most common flagellates found in sewage treatment plants (Curds and Cockburn, 1970; Hänel, 1979).

That Peranema has a deep-seated adaptation to phagotrophy is suggested by the complex nutritional requirements of the organism. Storm and Hutner (1953) found that Peranema requires vitamins, steroids and lipids in the growth medium. A minimal, partially defined medium containing soy lecithin was reported by Allen et al. (1966). In contrast to Chen (1950) who claimed that Peranema needs particulate food, Allen et al. found no absolute requirement for particulates, although growth was enhanced with the addition of methyl cellulose to the cultures.

In common with other euglenoids, the reserve carbohydrate in Peranema is paramylon, a β 1-3 linked D-glucose residue (Archibald et al., 1963). Paramylon granules are abundant in well-fed cells but are lacking in starved cultures (Chen, 1950; Leedale, 1967).

The present study was undertaken in part to provide basic information on the ultrastructural details of the flagellar apparatus and flagellar mastigonemes. In his review of flagellar appendages, Bouck (1972) defined mastigonemes as "any essentially filamentous appendages that are clearly distinct from the flagellar membrane or its amorphous coverings." A description of the nontubular mastigoneme units in the flagella of Euglena (Bouck et al., 1978) provided the impetus for examination of mastigonemes of the anterior flagellum of Peranema. A major portion of the present study is thus directed to constructing a comprehensive diagram of mastigonemal organization to serve as a model for future developmental and experimental studies on mastigonemes.

Since the fine structure of the flagellar apparatus, particularly the flagellar roots, is an important phylogenetic indicator in algal flagellates (Moestrup, 1978), attention is also focused on the flagellar root system of Peranema, with special reference to the previously undescribed striated roots. The information presented in this study may prove pertinent in future considerations of phylogenetic affinities, not only among the euglenoids but also in relation to other classes of flagellated algae and protozoans.

The studies of Mignot (1966) and Nisbet (1974) provided the framework within which the complex interrelationships among the ingestion organelles could be confirmed in the present investigation. Future studies on the feeding apparatus might seek to resolve questions concerning its function and chemical composition, information which is essential to understanding the feeding mechanism.

Since there have been few ultrastructural studies of Peranema, investigations were made both of basic structural features of the cell and of heretofore-undescribed structures such as the mucocysts. In summary, the primary objectives of this study were (1) to describe basic cell structure; (2) to describe in detail the flagellar root system and to develop a model for flagellar mastigonemal organization; (3) to characterize the mucocysts, a unique type of extrusion organelle; (4) to extend previous studies on the feeding apparatus and confirm the interrelationships among the ingestion organelles.

CHAPTER II

MATERIALS AND METHODS

I. CULTURE METHODS

Culture Conditions

Cultures of Peranema trichophorum Stein (No. 1260/1) were obtained from The Culture Centre of Algae and Protozoa, Cambridge, England. Axenic cultures were maintained on an undefined medium containing milk and calcium sulfate (Chen, 1950) (Appendix A) in 250-ml flasks containing 150 ml of medium, or in 15-ml test tubes containing 8 ml of medium. The cultures were grown at 20° C on a 16:8 LD cycle with fluorescent illumination of ca. 1000-2000 lux. A partially defined medium (Allen et al., 1966) and a medium containing Chalkley's medium with dried baker's yeast (Nisbet, 1974) were tried, but cell densities were not as great as with the milk medium.

II. DESCRIPTIVE METHODS

Light Microscopy (LM)

Observational modes. For routine examination, cells were observed in the living condition or were fixed either in 0.5% glutaraldehyde buffered with 0.05 M sodium cacodylate or in Parducz fixative (Parducz, 1967) (Appendix B). Fixation in glutaraldehyde generally caused elongate cells to become spherical. This problem was solved in part by drawing a drop of fixative underneath the coverslip instead of fixing cells in bulk in a test tube.

Bright-field, dark-field, phase-contrast, polarized light and Nomarski differential-interference-contrast optics were employed for viewing the cells with one of the following microscope systems: (1) Zeiss WL Research Microscope, equipped with phase-contrast and Nomarski differential-interference-contrast optics, a Zeiss photographic system and a Leica M4 dark box M35, or an Olympus PM-10-A 35 mm photographic system; (2) Wild M-20 binocular microscope, equipped with fluorescence and bright-field optics and a Nikon Microflex AFM photographic apparatus and dark box M35 S; (3) Olympus VANOX Universal Research Microscope, equipped with an oblique illumination system and Olympus PM-10-A 35 mm photographic system; and (4) Leitz Wetzlar SM-POL polarizing microscope. Photographic data were recorded on Kodak Panatomic X film (ASA 32) utilizing a green filter and on Kodak Ektachrome film (ASA 160) using appropriate color-balancing filters.

Phagotrophy. Cultures of Peranema were exposed to the following materials: carmine particles; polystyrene latex beads, diameter 5.7 μm (Polysciences, Inc.); polystyrene latex beads, diameters 1.3 μm and 0.36 μm (Dow Chemical Co.); polyvinyl toluene latex beads, diameter 1.9 μm (Dow Chemical Co.); heat-killed yeasts stained with neutral red (Jensen and Bainton, 1973) (Appendix C); Euglena acus; spores of the fungi Boletus griseus (spore size 14 μm X 4 μm) and Tylopilus subflavidus (spore size 14 μm X 7 μm). Phagotrophy of these materials by Peranema was observed with the aid of bright-field, phase-contrast and Nomarski differential-interference-contrast optics.

Ingestion of Euglena acus by Peranema was recorded with a Series 500 Cinephotomicrographic Apparatus (Sage Instruments), equipped with a Bolex H16 M camera and a normal-speed motor. The microscopes employed with the apparatus were a Wild M-11 microscope, equipped with bright-field and phase-contrast optics, and a Zeiss WL Research Microscope, equipped with phase-contrast and Nomarski differential-interference-contrast optics. The film used was Kodak 16 mm Plus-X Reversal (100-ft rolls).

Transmission Electron Microscopy (TEM)

Thin sections. Cells were harvested by centrifugation at 400 X g for 3 minutes and concentrated to a loose pellet. Fixation protocols included the following: (1) unbuffered 2% osmium tetroxide (OsO_4) in double-distilled water for 3 hours at room temperature; (2) 2% OsO_4 buffered in 0.05 M sodium cacodylate, pH 6.8, for 3 hours at room temperature; (3) 1.5% glutaraldehyde buffered in 0.05 M sodium cacodylate in 0.2 M sucrose, pH 6.8, for 1 hour at room temperature, and post-fixed in 2% OsO_4 buffered in 0.05 M sodium cacodylate for 1 hour at room temperature; (4) 2% glutaraldehyde buffered in 0.05 M sodium cacodylate, pH 7.2, for 1 hour at room temperature, and post-fixed in 2% OsO_4 buffered in 0.05 M sodium cacodylate for 3 hours at room temperature; (5) 1% buffered OsO_4 (Paramecium fixative, Jurand and Selman, 1969, Appendix B); and (6) simultaneous 1% OsO_4 and 1% glutaraldehyde buffered in cold 0.05 M sodium cacodylate for 30 minutes on ice, and post-fixed in cold 2% OsO_4 buffered in 0.1 M sodium cacodylate, pH 7.0, for 3 hours on ice (Franke et al., 1969). The simultaneous OsO_4 -glutaraldehyde

fixative was superior to the other fixatives for preserving general ultrastructural detail and was used for most of the routine preparations (see Appendix D for representative fixation procedure).

Following fixation in any of the above-mentioned solutions, cells were rinsed three times in 0.1 M sodium cacodylate buffer and were dehydrated through a graded acetone series and two changes of 100% propylene oxide. Subsequent infiltration and embedding were effected in Spurr's (1969) low-viscosity resin (Appendix B), followed by polymerization in a 60° C oven for 12-48 hours.

Ultrathin sections were obtained with either a Porter-Blum MT-1 ultramicrotome, equipped with glass knives made with an LKB Glass Knife Maker, or with a Sorvall MT-2 ultramicrotome, equipped with a DuPont diamond knife. Sections were mounted on uncoated precleaned copper grids of various mesh sizes. Sections were post-stained in one of the following: (1) 1% potassium permanganate (Bray and Wagenaar, 1978) for 1-2 minutes, followed by 0.2% aqueous lead citrate for 15 minutes; (2) uranyl acetate saturated in 50% methanol (Hayat, 1970) for 20 minutes, followed by Reynolds lead citrate (Reynolds, 1963) for 5 minutes; and (3) uranyl acetate saturated in double-distilled water for 20 minutes, followed by Reynolds lead citrate for 10 minutes (see Appendix B for stain formulae).

Specimens were examined in a Zeiss EM 9S-2 transmission electron microscope operated at 60 kV. Electron micrographs were recorded on DuPont Graphic Arts film.

Negative staining. Negative staining was used to visualize flagellar mastigonemes and isolated mucocysts. Cells were concentrated to a loose pellet by centrifugation at 400 X g for 3 minutes and washed three times with double-distilled water. A drop of OsO_4 -fixed or unfixed cells was placed on a carbon/Formvar-coated copper grid and air-dried. To facilitate drying, the grid was placed overnight in a Petri dish containing Drierite (anhydrous calcium sulfate, Hammond Drierite Co.). An alternative method of fixation involved pipetting living cells onto coated grids and exposing the cells to osmium vapor before air-drying. Whole-cell specimens on the grids were then stained with either 0.5% aqueous uranyl acetate or 2% aqueous phosphotungstic acid (PTA), pH 7.0. Isolated flagella and mastigonemes were pipetted onto coated grids, allowed to settle for 1-2 minutes, then stained with 1% aqueous uranyl acetate.

Specimens were examined and micrographs recorded as described previously for thin sections.

Shadow casting. The study of flagellar structure and mastigonemal organization was facilitated with shadow casting. A drop of OsO_4 -fixed or unfixed cells was allowed to air-dry onto carbon/Formvar-coated grids. Flagella of unfixed specimens were partially fragmented by air-drying; the axonemal components and the mastigonemes were therefore more readily observed in these preparations. Specimens were shadowed by evaporation with one of the following: (1) 50% platinum-50% carbon rods at an angle of 16° ; (2) 0.02-gram chromium chips at an angle of 14° ; (3) 5 cm of gold-palladium (60/40) wire at an angle of 9° or 11° (yielded ca. 20-nm coating of specimen). The

metals were shadowed in a vacuum of ca. 6×10^{-5} Torr in a Denton Vacuum Evaporator (Model DV 515).

Thick sections. The quality of some of the fixation and embedding procedures was determined by examining thick sections of epoxy-embedded cells with bright-field optics. Thick sections (.05-1.0 μm) were cut with glass knives on an MT-1 Porter-Blum ultramicrotome. Sections were picked up with the sharpened tip of a dissecting needle and were floated onto a drop of distilled water on a glass slide pretreated with a gelatin-chromium potassium sulfate solution (Appendix E). The slide was heated (2-3 minutes) on an Eberbach slide warming tray until the sections were dry. A drop of methylene blue, Azure II or crystal violet (Appendix E) was placed on the adhered sections, and the slide was returned to the warming tray for ca. 1 minute. The sections were washed for ca. 10 seconds with distilled water, mounted with a coverslip and Permount, and examined under bright-field optics.

Phagotrophy. Several attempts were made to examine phagocytosis with transmission electron microscopy. Cells were fed the following materials: neutral-red-stained yeasts for 20 and 40 minutes; Boletus griseus spores for 2.5 hours; polystyrene latex beads (diameter of 5.7 μm) for 45 minutes; and cells of Euglena acus for 24 hours. After these intervals, cells were fixed and processed for transmission electron microscopy as described in Appendix D.

Ruthenium Red (RR) staining for acid mucopolysaccharides. Cells were concentrated to a loose pellet by centrifugation at 400 X g for

3 minutes, washed once in distilled water and fixed in 2% glutaraldehyde buffered in 0.05 M sodium cacodylate with 0.1% RR (ICN Pharmaceuticals, Inc.), pH 7.2, for 1 hour at room temperature (Appendix F). After three rinses in 0.05 M sodium cacodylate, cells were post-fixed in 2% OsO_4 buffered in 0.05 M sodium cacodylate with 0.1% RR, pH 7.2. Dehydration was effected in an acetone series and three changes of 100% propylene oxide, followed by infiltration and embedding in Spurr's low-viscosity resin. Sectioning, post-staining and examination were as described for thin sections.

Acid phosphatase. Cells concentrated to a loose pellet by centrifugation at 400 X g for 3 minutes were fixed in an equal volume of 2% glutaraldehyde buffered in 0.1 M sodium cacodylate, pH 7.0, for 1 hour at room temperature. After sequential washings in 0.05 M sodium cacodylate followed by 0.05 M sodium acetate buffer, pH 5.0, samples were incubated for 1 or 5 hours in one of the following media: (1) Gomori reaction medium (Gomori, 1952); (2) Gomori reaction medium plus substrate; and (3) Gomori reaction medium plus sodium fluoride inhibitor but without substrate (Appendix F). Incubation temperature was maintained at 37° C in a water bath. Following incubation, cells were washed in distilled water and saturated with 0.02% ammonium sulfide for 3 minutes. After four rinses in distilled water, cells were post-fixed overnight in 1% OsO_4 buffered in 0.05 M sodium cacodylate and subsequently were processed for thin sectioning and transmission electron microscopy as described in Appendix D.

Scanning Electron Microscopy (SEM)

Cells were concentrated to a loose pellet by centrifugation at 400 X g for 3 minutes, washed three times with distilled water, and fixed with one of the following: (1) 2% unbuffered aqueous OsO_4 for 15 minutes; (2) Parducz (1967) fixative for 15 minutes; or (3) 1% unbuffered OsO_4 for 15 minutes, followed by 1% glutaraldehyde buffered in 0.1 M sodium cacodylate, pH 5.6, for 30 minutes. Another preparative technique involved the treatment of cells with 2% glusulase (a snail gut mucilage enzyme, Endo Laboratories, Inc.) at room temperature either for 1 hour before or after fixation in 2% OsO_4 . A drop of fixed cells was transferred to a 12-mm round glass coverslip coated with polylysine (Mazia et al., 1975) (Appendix G). The coverslip was placed in the bottom of a Petri dish where subsequent washing and dehydration were carried out on cells attached to the coverslip glass. An attempt was made to fix cells directly with OsO_4 while they were attached to the coverslip, but the cells did not adhere after fixation.

After dehydration of cells in a graded acetone series, the coverslip was transferred directly to a 13-mm coverslip holder (Bomar Co.) immersed in 100% acetone. The holder was placed in an acetone-filled specimen chamber of a Bomar SPC-900/EX Drying Apparatus. Acetone was subsequently replaced by liquid CO_2 and critical point drying was carried out at ca. 31° C and 1072 psi. The glass coverslip was mounted on an aluminum SEM pedestal with Neolube (Huron Industries, Inc.) and left overnight in a vacuum desiccator at room temperature.

Specimens were coated with ca. 30 nm gold or gold/palladium (60/40) on a rotating stage in a Denton Vacuum Evaporator Model DV 515 at ca. 6×10^{-5} Torr and were examined in an ETEC Autoscan scanning electron microscope operated at 20 kV. Photographic data were recorded on Polaroid types 55 and 105 positive/negative films.

III. ANALYTICAL METHODS

Isolation of Flagellar Mastigonemes

Cells were concentrated to a loose pellet by low centrifugal force at 400 X g for 3 minutes to avoid premature deflagellation and washed once with double-distilled water. The loose pellet was then resuspended in ca. 2 ml of cold double-distilled water and immediately agitated for 2 minutes at top speed in a Super-Mixer (Lab-Line Instruments, Inc.) to deflagellate the cells (Bouck *et al.*, 1978) (Appendix H). Whole cells were removed from the suspension by centrifugation at 400 X g for 3 minutes. The supernatant containing the flagella was recentrifuged for 30 minutes at 17,500 rpm (37,000 X g) in a Beckman Model J-21C centrifuge, yielding a white pellet containing isolated flagella and mastigonemes. Aliquots from the pellet were placed on carbon/Formvar-coated grids, negatively stained with 1% aqueous uranyl acetate, and examined with transmission electron microscopy.

Isolated flagella were resuspended in the anionic detergents Sarkosyl NL-30 (sodium lauryl sarcosinate, ICN Pharmaceuticals, Inc.) at 1% (v/v) in distilled water or Sarkosyl NL-100 (Alrose Chemical Co.) at 1.5% (w/v) in distilled water. After ca. 18 hours, the

flagella in detergent solution were centrifuged at 40,000 rpm (192,000 X g) for 30 minutes in a SW 50.1 rotor in a Beckman L3-50 ultracentrifuge. Samples of the pellet were placed on carbon/Formvar-coated grids, negatively stained with 1% aqueous uranyl acetate and examined by transmission electron microscopy.

Colchicine Treatments

Several studies were undertaken to investigate the effects of the plant alkaloid colchicine on phagocytosis and motility in Peranema. Cells were subjected to concentrations of 1.5×10^{-6} M, 2.5×10^{-5} M, 2.5×10^{-3} M, 5×10^{-3} M and 1×10^{-2} M for up to 102 hours, during which time cellular motility and phagocytosis of stained yeast cells by Peranema were observed at hourly intervals for 4 hours, then at daily intervals, with bright-field and phase-contrast optics.

Another study involved investigation of the ultrastructural effects of colchicine on intracellular microtubules. Cells were kept in 0.01 M colchicine for 4 days with fresh colchicine added daily. Cells were viable and motile at the end of this period and were subsequently fixed and processed for transmission electron microscopy as described in Appendix D.

EDTA Treatments

Attempts were made to study the effects of reduced concentrations of extracellular calcium on the striated flagellar roots, structures which are presumptive contractile organelles. High concentrations of calcium in the milk medium were diluted by daily washings and maintenance of cells in double-distilled water. After

4 days, cells were fixed and processed for transmission electron microscopy (Appendix D).

In an alternative procedure to the above, cells were washed with the chelating agent ethylenedinitrilotetraacetic acid (EDTA disodium salt) at 0.01 M, pH 6.5, and maintained in distilled water overnight. Calcium in the form of calcium sulfate (0.1% in distilled water) was added to control cells. Other controls consisted of cells that had been washed in distilled water only. The three samples were fixed and processed for transmission electron microscopy as described in Appendix D.

CHAPTER III

RESULTS

I. CELLULAR ORGANIZATION

Overview of Prominent Cellular Features

Cells of Peranema trichophorum range from 60-80 μm in length and 10-20 μm in width. During gliding movement, the body is elongate with tapering anterior end (Plate I, Figure 1), and the anterior flagellum is extended with only its distal end in motion. The trailing flagellum, seldom seen with light microscopy, is appressed to the ventral surface of the cell and serves as an anchor to the substratum.

Scattered in the cytoplasm of well-fed cells are abundant ovoid paramylon granules which generally obscure observation of cellular internal structure with the light microscope (Plate I, Figure 1). Starved cells (Plate I, Figure 2) lack granules, suggesting that paramylon, a β 1:3-linked glucan, has been utilized as a reserve food source.

The pyriform reservoir, situated in the anterior quarter of the body, is connected with the outside by a short canal. The two flagella originate at the base of the reservoir (Plate I, Figures 3 and 4) and emerge from the cell through the canal opening. Adjacent to the reservoir is the rodorgan, an ingestion organelle composed of two parallel rods which move as a unit during feeding (Plate I, Figures 3 and 5). A contractile vacuole is latero-posterior to and contiguous with the reservoir (Plate I, Figures 6-8).

Plate I. Anterior structural features.

- Figure 1. Cell grown in milk medium has abundant ovoid paramylon granules that are usually absent from anterior quarter of body. Anterior flagellum (arrow) is conspicuous; appressed, trailing flagellum, seldom seen with light microscopy, is not shown. Elongate body shape is typical of cells in swimming or gliding position. Fixed with glutaraldehyde. Bright-field optics, LM. X 1400.
- Figure 2. Cell starved for 6 days lacks paramylon granules. Fixed with glutaraldehyde. Bright-field optics, LM. X 1100.
- Figure 3. The two shafts of the rodorgan (arrows) and the insertion of the two flagella at reservoir base (arrow heads) are evident. Fixed with Parducz fixative. Nomarski optics, LM. X 4000.
- Figure 4. Thick section showing canal opening and insertion (arrow) of one flagellum in cytoplasm near expanded portion of reservoir. Densely stained ovoid structures are paramylon granules. Fixed with glutaraldehyde/OsO₄. 1- μ m thick section. Methylene blue stain. Bright-field optics, LM. X 2700.
- Figure 5. Thick section shows rodorgan (arrow) in cytoplasm adjacent to reservoir containing a flagellar shaft. Fixed with glutaraldehyde/OsO₄. 1- μ m thick section. Methylene blue stain. Bright-field optics, LM. X 2700.
- Figure 6. Latero-posterior to the reservoir is the contractile vacuole (arrow). Note the thick anterior flagellum and the prominent rodorgan. Fixed with Parducz fixative. Nomarski optics, LM. X 2500.
- Figure 7. Thick section showing juxtaposition of contractile vacuole and reservoir. Also shown are the rodorgan and the anterior flagellum. Fixed with glutaraldehyde/OsO₄. 1- μ m thick section. Methylene blue stain. Bright-field optics, LM. X 2700.
- Figure 8. Thin section showing reservoir and latero-posterior contractile vacuole separated by a narrow cytoplasmic bridge. Large electron-lucent vacuoles and profiles of the two flagella are seen in oblique section. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 4000.

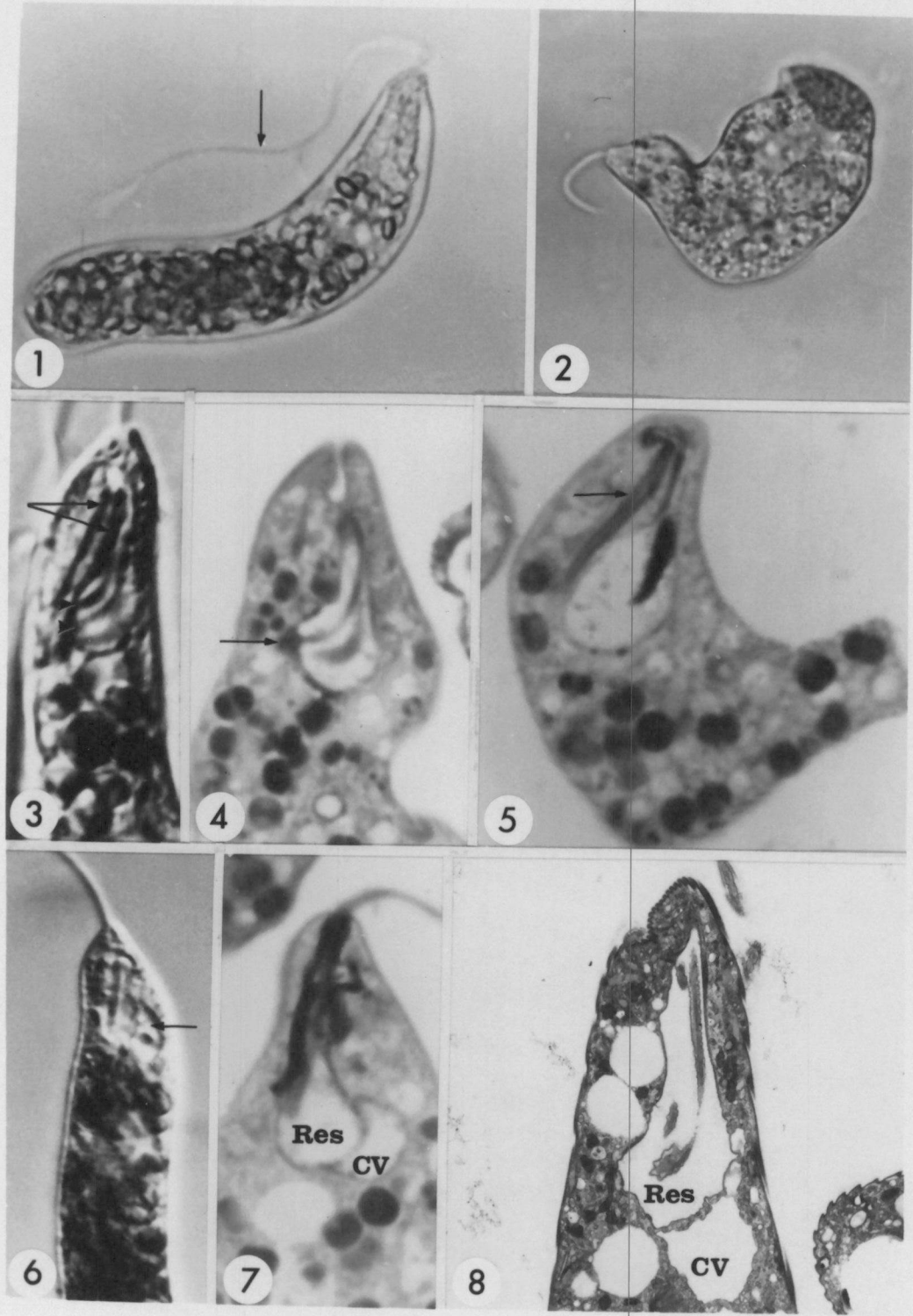


Plate I.

Cell Surface Morphology and Symmetry

At the cell surface the pellicle is helically striated and covered with thin strands of mucilage (Plate II, Figure 9). Mastigonemes form a reticular network around each of the two flagella. The trailing flagellum lies within a groove on the ventral surface and extends posteriorly ca. two-thirds the length of the body. Body shape is highly protean due to the flexible pellicle; stationary and/or feeding cells undergo violent euglenoid movement resulting in gross distortions of body form (Plate II, Figures 10 and 11).

Both the anterior and the trailing flagella emanate from a subapical invagination or canal which is often obscured by mucilage (Plate III, Figure 12). The canal is highly extensible, changing in size and shape from a narrow slit (Plate III, Figure 13) to a circular opening (Plate III, Figure 14) to a funnel-shaped channel (Plate III, Figure 15). Pellicular striae become narrower as they curve toward the canal opening and continue into the interior of the canal (Plate III, Figure 14).

Although the posterior end of the cell is generally round (Plate IV, Figure 16), a multiplicity of shapes is found due to the elasticity of the pellicle. Some common modifications include a hook (Plate IV, Figure 17), spine (Plate IV, Figure 18) or spiral (Plate IV, Figure 19). The hooked posterior end is often found in cells from starved or older cultures. As the pellicular striae terminate posteriorly, the number of striae is reduced by the tucking of discontinuous striae under continuous ones (Plate IV, Figures 16, 18 and 19) until only four or five striae remain (Plate IV, Figures 18 and 19). The undertucking phenomenon can readily be

Plate II. Cell surface morphology.

- Figure 9. The typical body shape of the organism in gliding position is elongate with slightly tapering anterior end. Dimensions of the cell shown in ventral view are ca. 60 μm long X 12 μm wide. Emerging from anterior canal opening are the anterior or leading flagellum and the trailing flagellum which lies in a ventral groove in the pellicle and extends posteriorly ca. two-thirds the length of the organism. Flagella and cell surface, differentiated into pellicular striae, are covered with thin strands of mucilage. Fixed with OsO_4 /glutaraldehyde. CPD. SEM. X 3000.
- Figure 10. Pellicular flexibility allows for euglenoid movement or "metaboly" and results in various cell shapes, including the helical configuration shown here. Pellicular striae are visible in regions of the cell surface devoid of mucilage (arrow). Fixed with OsO_4 /glutaraldehyde. CPD. SEM. X 2700.
- Figure 11. Cell surface undulations and contortions are particularly evident during feeding. A yeast cell adheres to mastigonemes of anterior flagellum. Fixed with OsO_4 . CPD. SEM. X 2700.

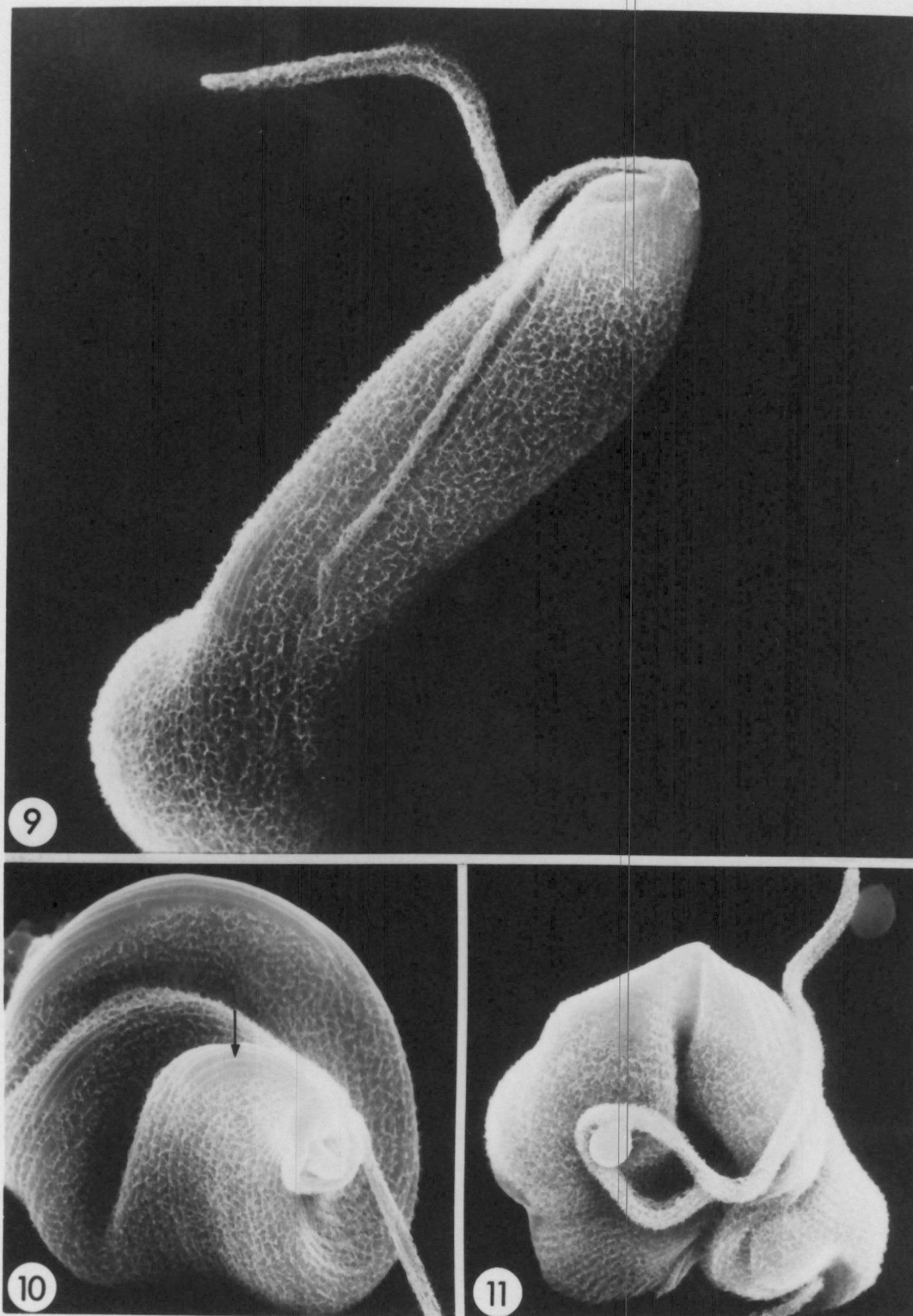


Plate II.

Plate III. Anterior end morphologies.

- Figure 12. Two flagella emerge from anterior invagination or canal. Trailing flagellum curves posteriorly and lies in a groove in the pellicular surface. Mucilage obscures point of emergence of anterior flagellum. Fixed with OsO_4 /glutaraldehyde. CPD. SEM. X 6700.
- Figure 13. The disparity in size of the two flagella is evident in this anterior view of a narrow canal opening. Smaller trailing flagellum is detached from groove (arrow). Fixed with OsO_4 /glutaraldehyde. CPD. SEM. X 4300.
- Figure 14. Pellicular striae curve into circular opening of the canal. Although this cell was treated with glucuronidase for 1 hour prior to fixation, some mucilage strands (arrow) are still evident. Portion of rodorgan is visible below canal orifice. Fixed with OsO_4 . CPD. SEM. X 7100.
- Figure 15. Expanded canal region showing anterior flagellum with mucilage strands. Fixed with OsO_4 /glutaraldehyde. CPD. SEM. X 6800.

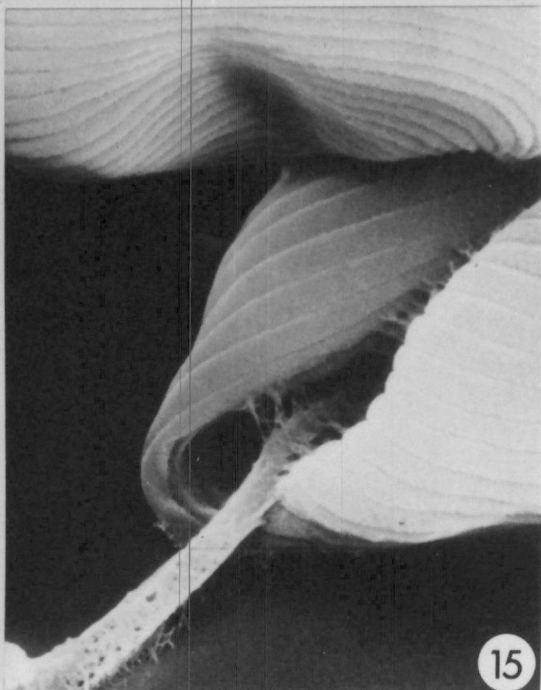
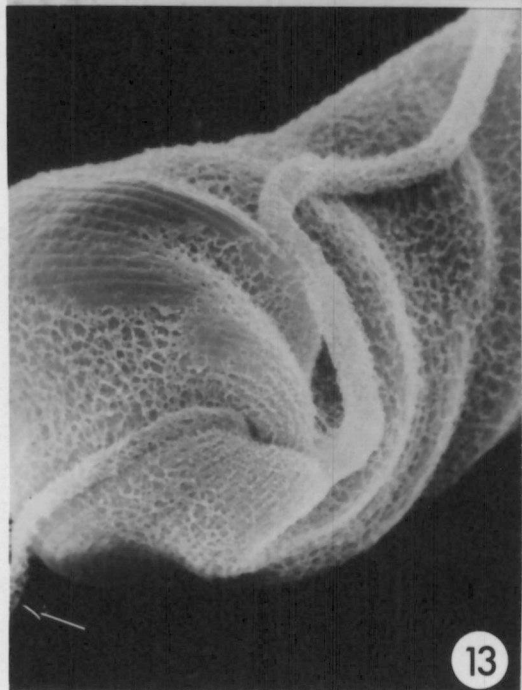
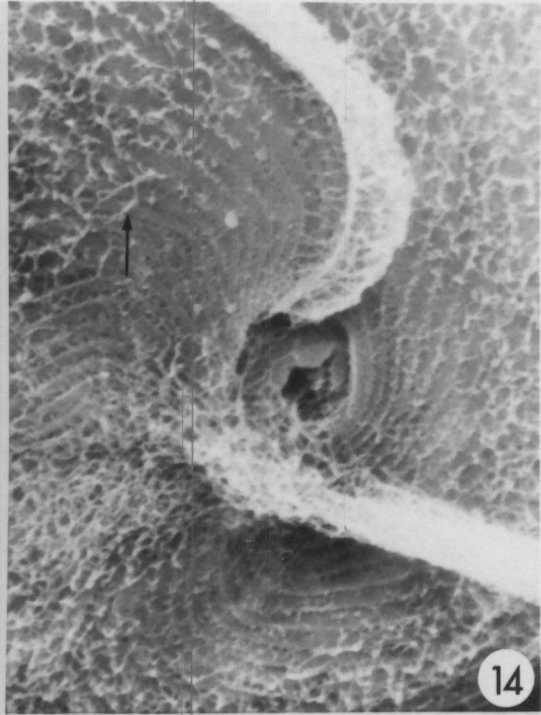
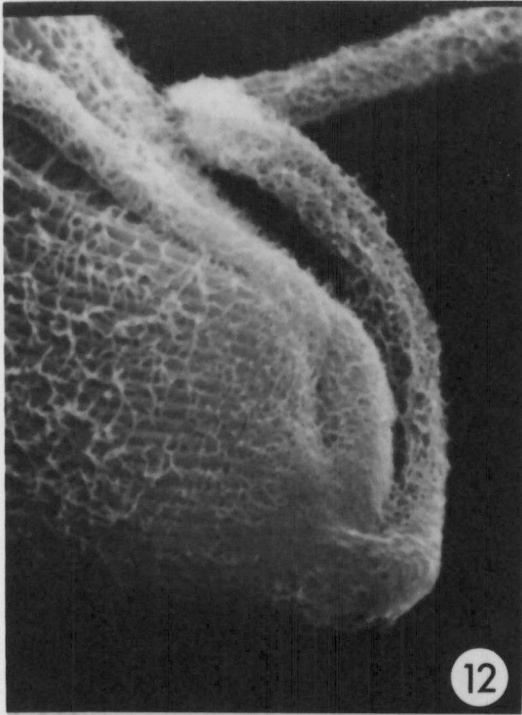


Plate III.

Plate IV. Posterior end morphologies.

- Figure 16. Rounded posterior end of cell with pellicular striae curved inward into an invagination. Reduction in number of striae occurs when one stria (discontinuous=d) is tucked under the ridge of a continuous (=c) stria. Fixed with OsO_4 . CPD. SEM. X 7400.
- Figure 17. Posterior end of cell extended laterally into a hook. This configuration is most often found in starved or older cultures. Fixed with OsO_4 /glutaraldehyde. CPD. SEM. X 3400.
- Figure 18. Posterior end may be modified into a tail spine of four or five striae. Glusulase treatment removed surface mucilage, making pellicular striae readily observable. CPD. SEM. X 8400.
- Figure 19. Posterior striae whorled into a left-handed (S) helix. Undertucking of striae is pronounced along left side of micrograph (arrows). CPD. SEM. X 12,600.

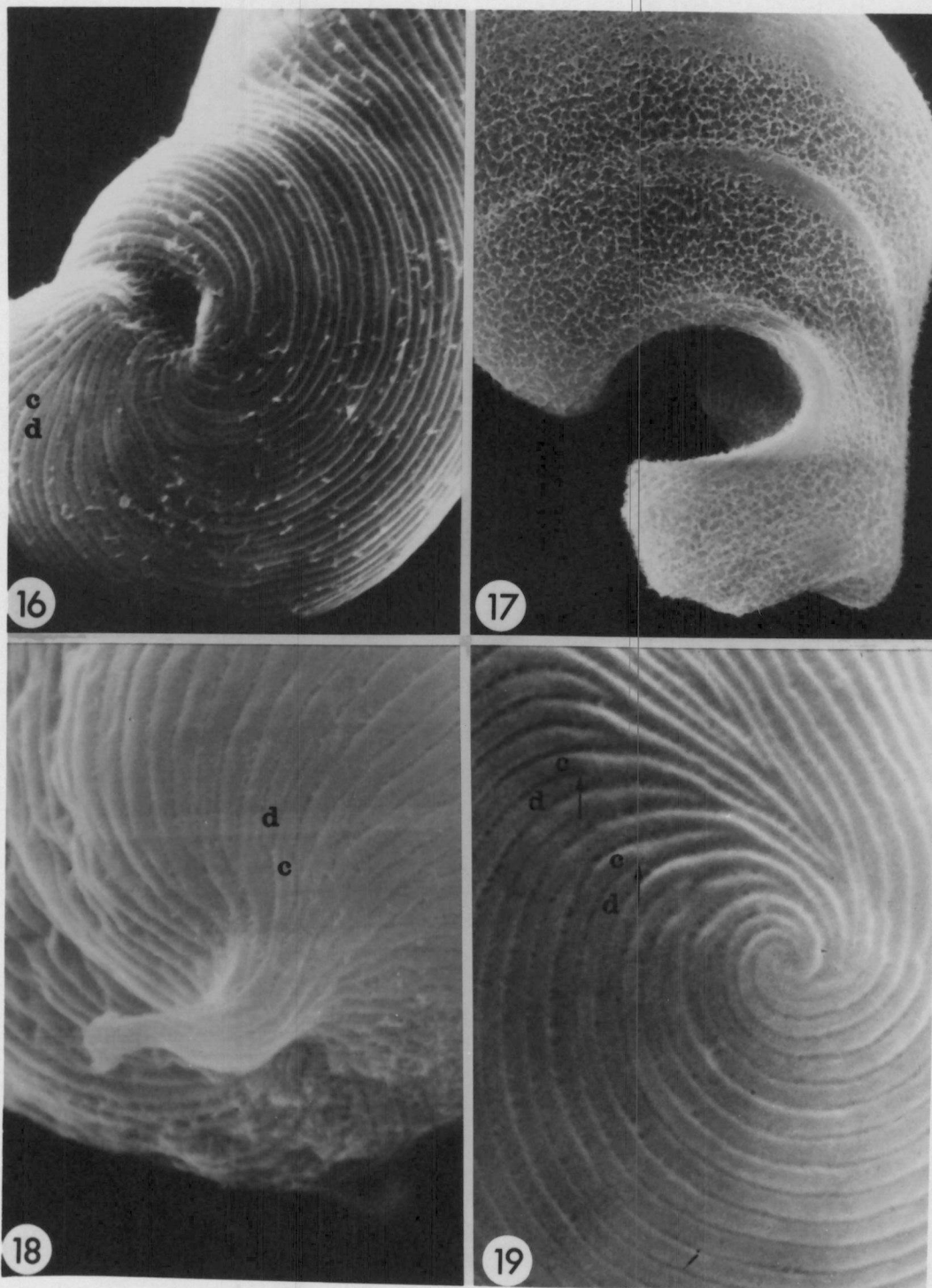


Plate IV.

observed in cells treated with glusulase (a snail gut enzyme) that removes mucilage from the pellicle (Plate IV, Figure 18). Helical symmetry of the organism is readily apparent in surface view (Plate IV, Figure 19) and in thick sections (Plate V, Figure 20) which show the striae whorled into a left-handed or S-helix (counterclockwise as seen from the posterior end of the cell).

Pellicle Fine Structure

Thin sections of the internal organization of the pellicle show that the helical striae consist of alternating ridges and grooves (Plate V, Figures 21 and 22). Associated with each stria are (1) tubular cisternae of endoplasmic reticulum in which acid phosphatase activity is localized (Plate V, Figure 21) and (2) regularly arranged microtubules, two on the ridge and four on one side of the groove (Plate V, Figure 22). Pellicular material appears in thin sections as electron-dense intracellular strips, each 24 nm wide, that lie immediately beneath a 9.6 nm tripartite plasmalemma (Plate V, Figure 22). External to the plasmalemma are parallel rows of small mucilage projections ca. 30 nm long (Plate V, Figure 22) and mucilage strands (Plate V, Figure 23) 200-600 nm long. Tangential sections of the pellicle reveal a striated pattern of ca. 19 nm periodicity (Plate V, Figure 24).

Cytochemistry of Cell Surface Components

Ruthenium red, which binds selectively to acid mucopolysaccharides on the cell surface and forms, in combination with OsO_4 , electron-dense complexes (Luft, 1971), is localized in the mucilage strands (Plate V, Figure 25) and at the grooves between the pellicle ridges

Plate V. Ultrastructure and cytochemistry of the pellicle complex.

- Figure 20. Thick section showing helical arrangement of pellicular striae and densely stained paramylon granules. Fixed with glutaraldehyde/OsO₄. 1- μ m thick section. Methylene blue stain. Bright-field optics, LM. X 2700.
- Figure 21. Acid phosphatase activity is localized in tubular elements of subpellicular endoplasmic reticulum. Fixed with glutaraldehyde/OsO₄. TEM thin section. X 49,900.
- Figure 22. Transverse section through pellicular stria showing characteristic ridge and groove. Regularly arranged microtubules are associated with each stria, two on the ridge and four on one side of the groove. The pellicle itself is 25 nm thick, composed of layer of dense material underlying the 9.6 nm plasmalemma. External to the plasmalemma are rows of small mucilage projections ca. 30 nm in length (arrow) and mucilage strands. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 93,000.
- Figure 23. Oblique section of pellicle showing mucilage strands (ca. 170 nm in length) extending from the cell surface. Tubular elements of endoplasmic reticulum underlie the pellicle. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 108,500.
- Figure 24. Tangential section of pellicle showing striated pattern with an average periodicity of ca. 19 nm. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 53,400.
- Figure 25. Ruthenium red labeling (arrow) is localized in the mucilage strands that extend 500-600 nm from the cell surface. Fixed with glutaraldehyde/OsO₄. TEM thin section. X 27,400.

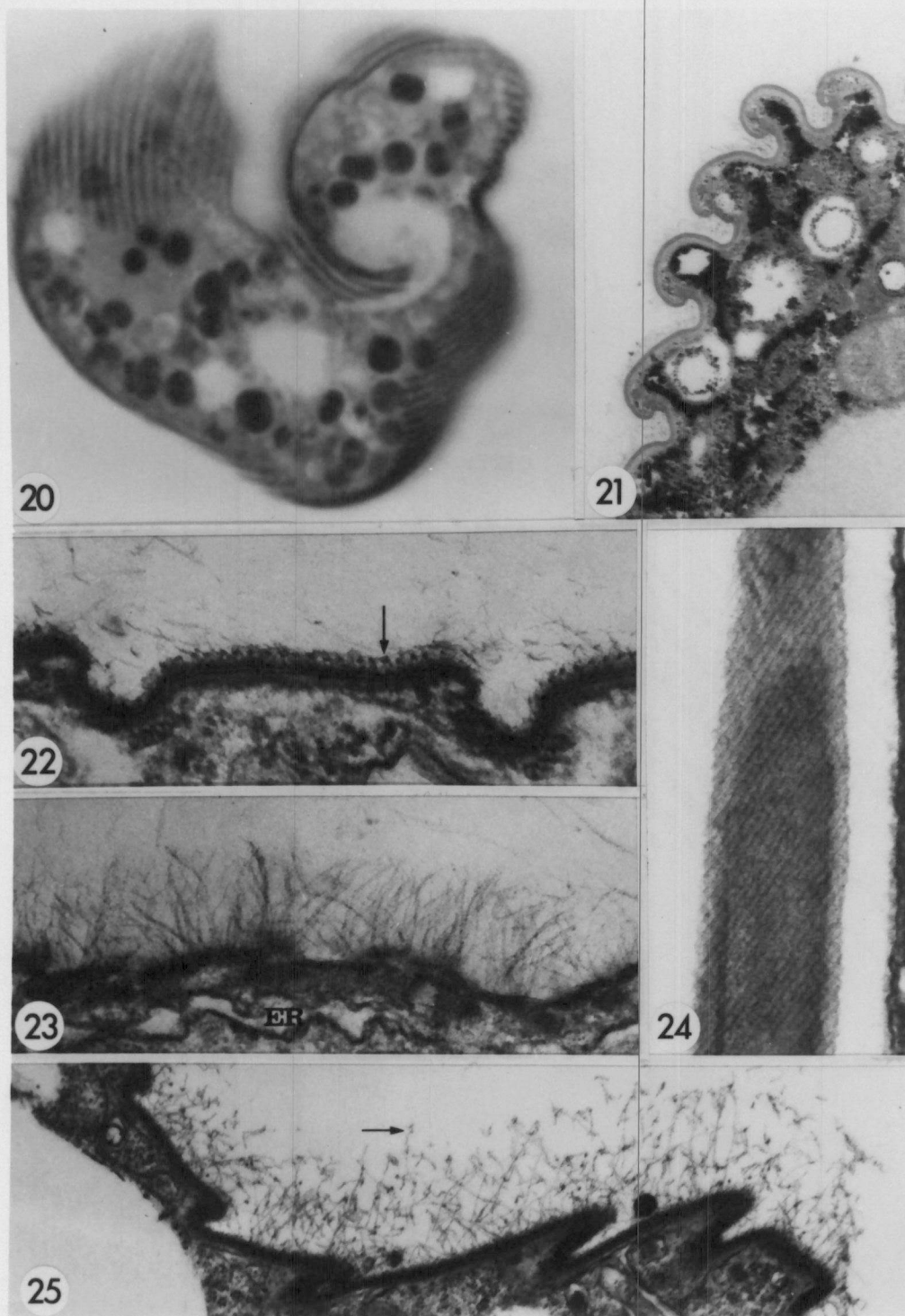


Plate V.

(Plate VI, Figure 26). The reaction product often appears as a ball of mucilage (Plate VI, Figure 27) that projects toward the posterior vortex of the cell.

Heavy labeling with ruthenium red is also visualized within the reservoir (Plate VI, Figure 28) and on the mastigonemes of the anterior flagellum outside the reservoir (Plate VI, Figure 29). Comparisons with untreated cells (Plate VI, Figures 30 and 31) show that the precipitate is localized in mucilage strands in the reservoir, on flagellar mastigonemes, in peri-reservoir vesicles, and in sub-pellicular mucus bodies, which are extensions of the endoplasmic reticulum and which open to the outside at the pellicle articulations (Plate VI, Figure 28).

Cell Cleavage and Pellicle Duplication

During cell cleavage, the anterior flagella of the two daughter cells beat continuously, and there is violent euglenoid movement (Plate VII, Figures 32 and 33) that makes observation of the division process difficult. Cleavage begins at the anterior end and progresses posteriorly following the helix of the pellicle until the two daughter cells are joined only by a narrow connection at their posterior ends.

Characteristic cytoplasmic indicators of cell cleavage include the appearance of immature pellicular striae in the groove between mature ridges (Plate VII, Figures 34 and 35) and the association of microtubules at the base of the canal in an area where the cytoplasm is homogeneous (Plate VII, Figure 34). Sections at different levels of the same dividing cell (Plate VIII, Figures 36-39) and of a different cell (Plate VIII, Figure 40) show that the microtubules are

Plate VI. Pellicle and reservoir.

- Figure 26. Ruthenium red labeling (arrow) is localized to the posterior side of the notch of each pellicular stria and to mucilage strands on the cell surface. Fixed with glutaraldehyde/OsO₄. TEM thin section. X 31,700.
- Figure 27. Oblique section of pellicle showing ruthenium red labeling of extracellular mucilage strands and of a ball of mucilage at pellicular groove. Fixed with glutaraldehyde/OsO₄. TEM thin section. X 51,700.
- Figure 28. Transverse section of proximal portion of reservoir showing ruthenium red precipitate within the reservoir localized in flagellar mastigonemes, mucilage strands, peri-reservoir vesicles (arrow), and subpellicular mucus bodies (arrow head). Fixed with glutaraldehyde/OsO₄. TEM thin section. X 15,900.
- Figure 29. Ruthenium red precipitate is visualized in the long and short mastigonemes of the anterior flagellum outside the reservoir. Fixed with glutaraldehyde/OsO₄. TEM thin section. X 29,000.
- Figure 30. Oblique section of reservoir of untreated cell (cf. Figure 28) showing long mastigonemes emanating from flagella. Note the microtubular root extending from the base along the reservoir wall (arrow). Fixed with glutaraldehyde/OsO₄, then OsO₄. TEM thin section. X 17,700.
- Figure 31. Transverse section of reservoir showing three groups of single rows of microtubules immediately beneath the reservoir membrane (arrows). Fixed with glutaraldehyde/OsO₄, then OsO₄. TEM thin section. X 29,000.

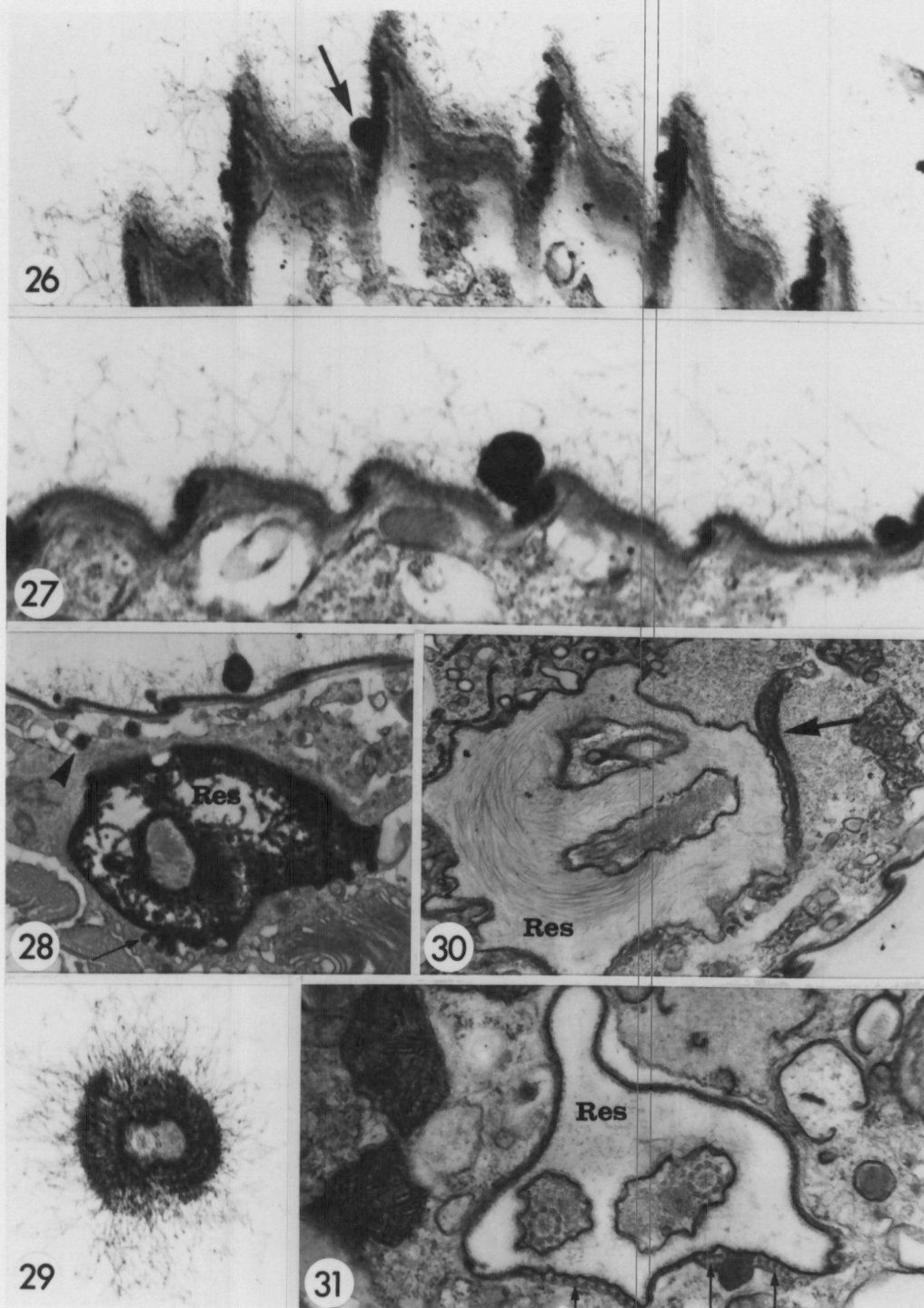


Plate VI.

Plate VII. Cell cleavage.

- Figure 32. Light micrograph of dividing cell showing both daughter cells with flagella. Fixed with glutaraldehyde. Bright-field optics, LM. X 1100.
- Figure 33. Scanning electron micrograph of dividing cell. Note the contortions of the body shape due to euglenoid movement. Fixed with OsO_4 . CPD. SEM. X 2500.
- Figure 34. At the ultrastructural level, two indicators of cleavage are (1) minute daughter pellicular complexes between mature ones and (2) microtubular arrays on one side of the reservoir near the base of the canal (arrow). Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 6300.
- Figure 35. Newly formed pellicular complexes have two microtubules (arrow), typical of mature ridges. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 16,200.

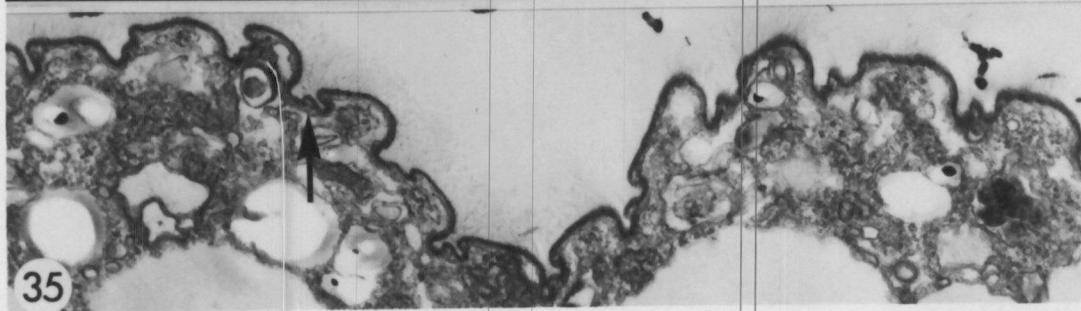
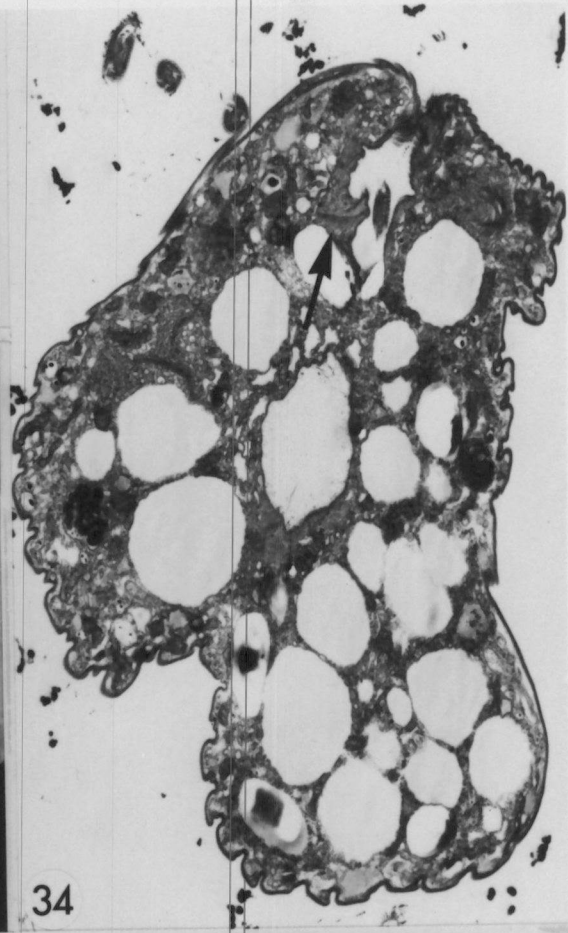
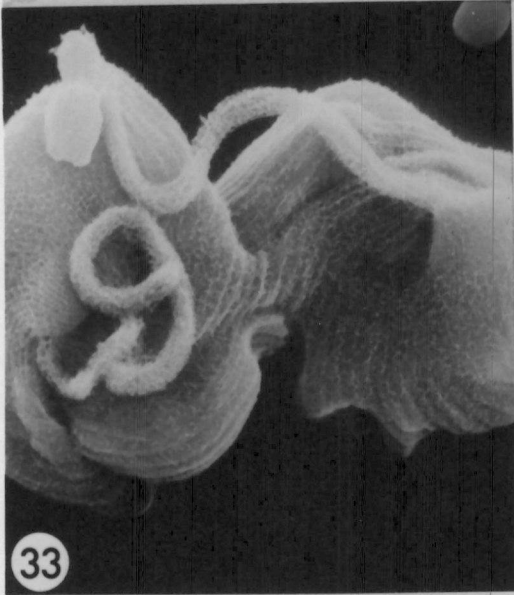
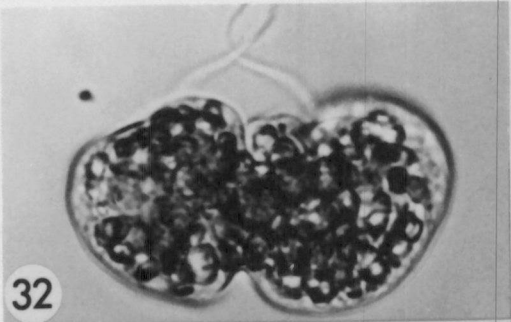


Plate VII.

Plate VIII. Arrays of microtubules in dividing cell.

- Figure 36. Microtubular arrays along one side of anterior end of reservoir near base of the canal. The cytoplasm in this area is devoid of vesicles and ribosomes. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 30,100.
- Figure 37. At a different level in the same dividing cell (cf. Figure 36), microtubules are grouped into three arrays, two shown in oblique and one in transverse section (arrows). Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 51,600.
- Figure 38. Oblique section of helical bands of microtubules in homogeneous cytoplasm. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 89,500.
- Figure 39. Transverse section of cross-linked (arrow) microtubules at base of canal and in canal ridges and grooves. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 120,500.
- Figure 40. Area of homogeneous cytoplasm from different dividing cell than shown in Figures 36-39, showing three arrays of microtubules (arrows). Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 32,800.

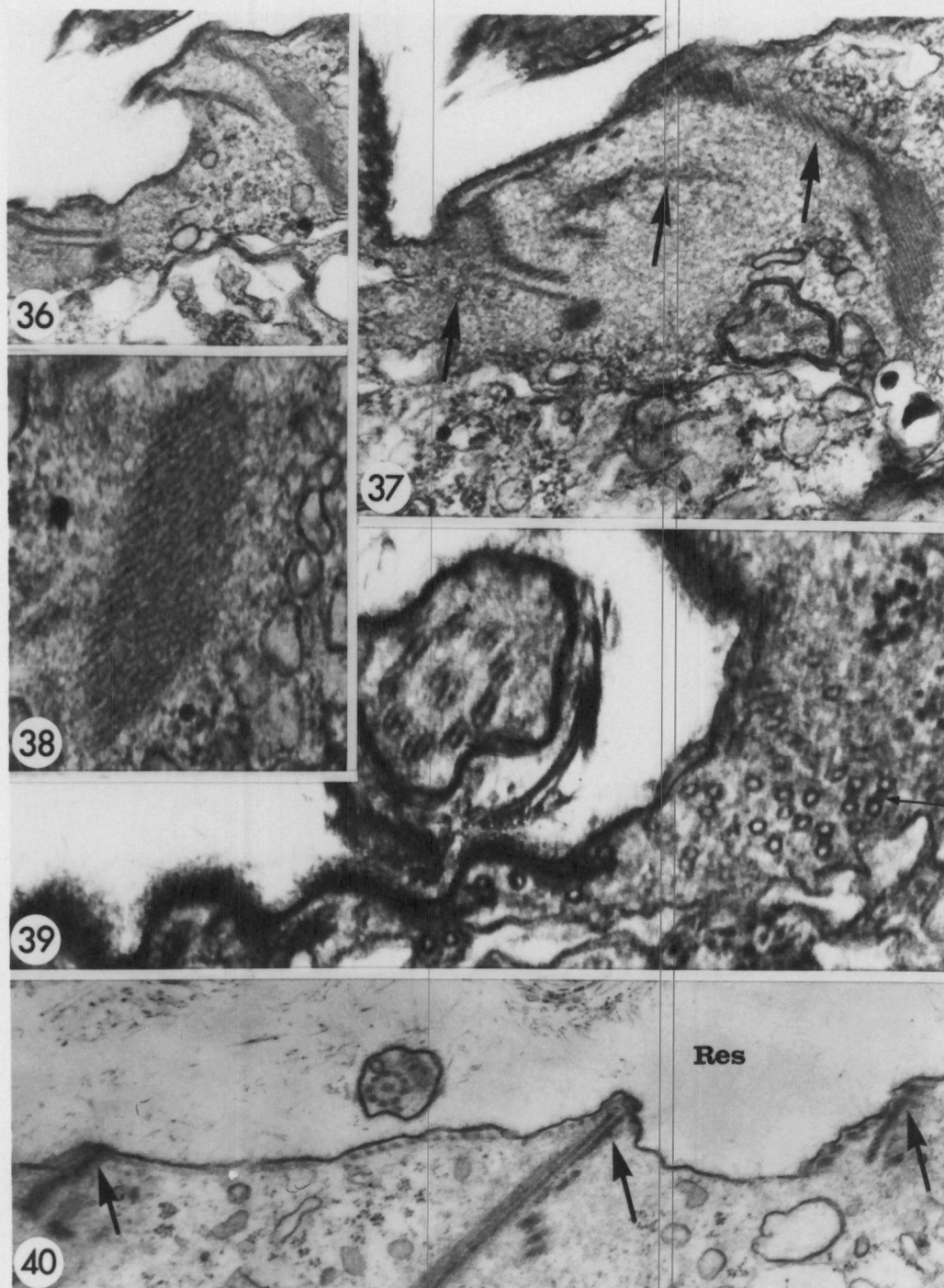


Plate VIII.

aggregated in at least three parallel arrays that extend through the cytoplasm to the reservoir wall.' Paired microtubules are evident near the base of the canal (Plate VIII, Figure 39).

Contractile Vacuole

The contractile vacuole is a single-membrane-bounded spherical cavity latero-posterior to the reservoir (Plate IX, Figure 41). In the cytoplasm near the vacuole are profiles of accessory vacuoles (Plate IX, Figure 42), numerous types of vesicles including the distinctive alveolate vesicles which may fuse with the reservoir membrane (Plate IX, Figure 43) or with accessory vacuoles (Plate IX, Figure 44), and vacuoles containing presumptive mucilage strands (Plate IX, Figure 44). Occasionally vesiculate cytoplasmic strands extend into the interior of the contractile vacuole (Plate IX, Figure 45).

Nucleus

The nucleus contains coarsely granular nucleoplasm and large electron-dense nucleoli (endosomes) (Plate X, Figure 46) which are often irregularly shaped and contain regions of low electron density (Plate X, Figures 47-49). The electron-microscopic image of the nucleoplasm varies with different methods of fixation. Heterochromatic areas are evident in cells fixed sequentially with glutaraldehyde followed by OsO_4 (Plate X, Figure 46) but are not evident in cells fixed simultaneously with glutaraldehyde and OsO_4 (Plate X, Figure 50). Dense filamentous structures identified in other euglenoids as permanently condensed chromosomes were never observed. The nuclear envelope

Plate IX. Contractile vacuole region.

- Figure 41. The contractile vacuole is a single-membrane-bounded cavity ca. 2 μm in diameter surrounded by numerous accessory vacuoles and alveolate vesicles. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 13,200.
- Figure 42. Irregularly shaped accessory vacuoles are separated from the reservoir by thin cytoplasmic strand (arrow). Fixed with OsO_4 buffered with sodium cacodylate. TEM thin section. X 15,000.
- Figure 43. Transverse section of alveolate vesicles shows spine-like projections radiating from surface. Membrane of one alveolate vesicle adjacent to reservoir membrane, which is sectioned obliquely (arrow). Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 53,200.
- Figure 44. Peri-reservoir vacuole containing fibrous material similar to mucilage strands lining the reservoir wall. Note juxtaposition of alveolate vesicles with accessory vacuoles (arrows). Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 36,600.
- Figure 45. Vesiculate cytoplasmic projections extend into the interior of the contractile vacuole. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 36,600.

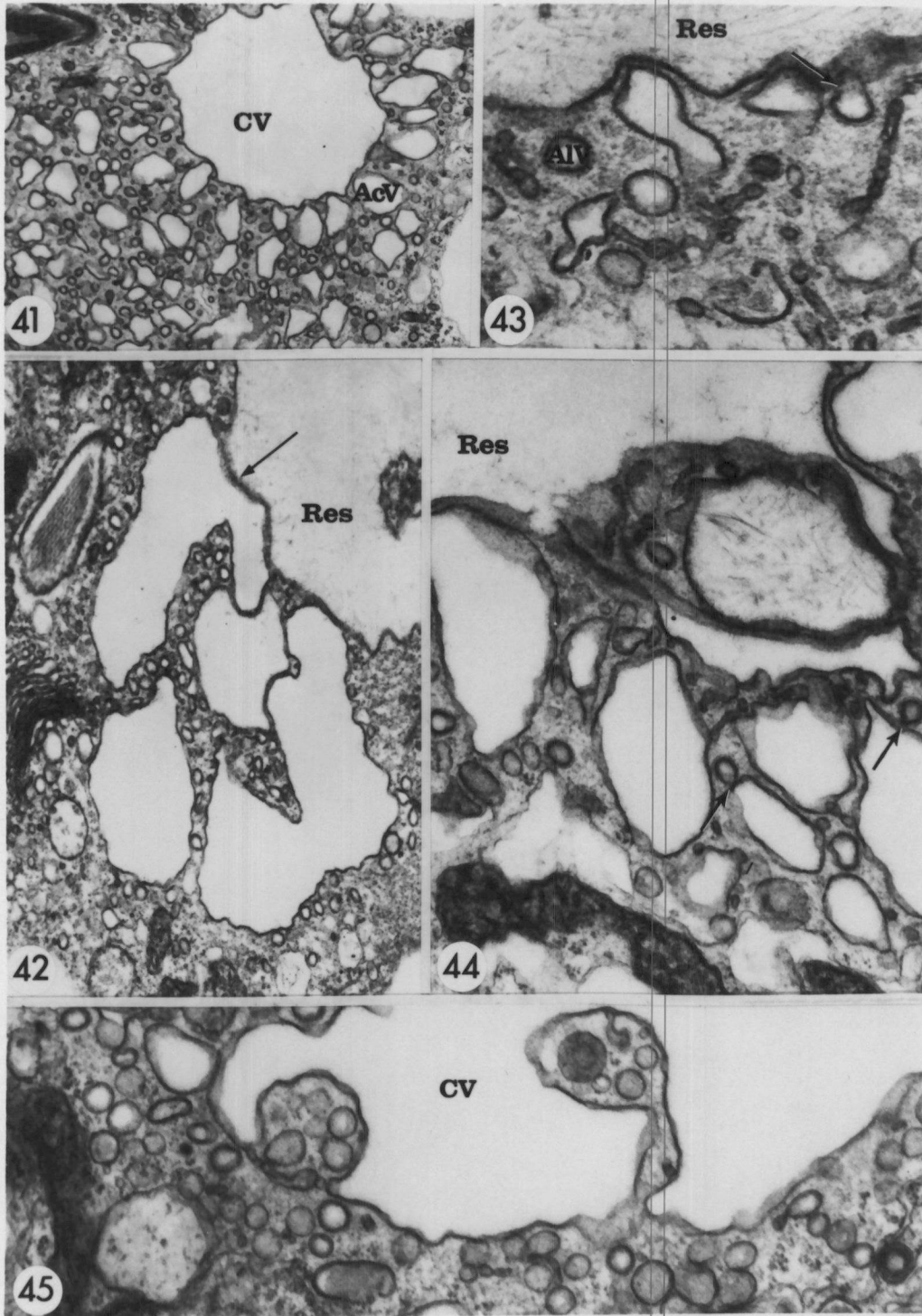


Plate IX.

Plate X. The nucleus.

- Figure 46. Nucleoplasm appears as coarsely granular material in a less-dense matrix, with scattered areas of chromatin. Large, perhaps multiple endosomes (nucleoli) show regions of low electron density. Fixed with glutaraldehyde/OsO₄. TEM thin section. X 19,700.
- Figure 47. Irregularly shaped endosome with invaginations of nucleoplasm (arrow) and a thread-like extension encircling an adjacent area of nucleoplasm. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 14,500.
- Figure 48. Thread-like extensions may connect two endosomes (or two lobes of the same endosome). Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 14,500.
- Figure 49. The endosomal extension may terminate in a knob-like tip of low electron density. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 11,800.
- Figure 50. Nucleus with large endosomes, one of which lies adjacent to the nuclear envelope. Note absence of small areas of high electron density as shown in Figure 46. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 16,100.
- Figure 51. The nuclear envelope consists of two "unit" membranes separated by a peri-nuclear space and traversed at intervals by pores. In transverse section, the pores contain dense septa, on either side of which are zones of intermediate electron density. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 115,400.
- Figure 52. In surface view, the nuclear pores are circular with a diameter of ca. 78 nm; small spine-like projections radiate outward from the circumference. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 115,400.

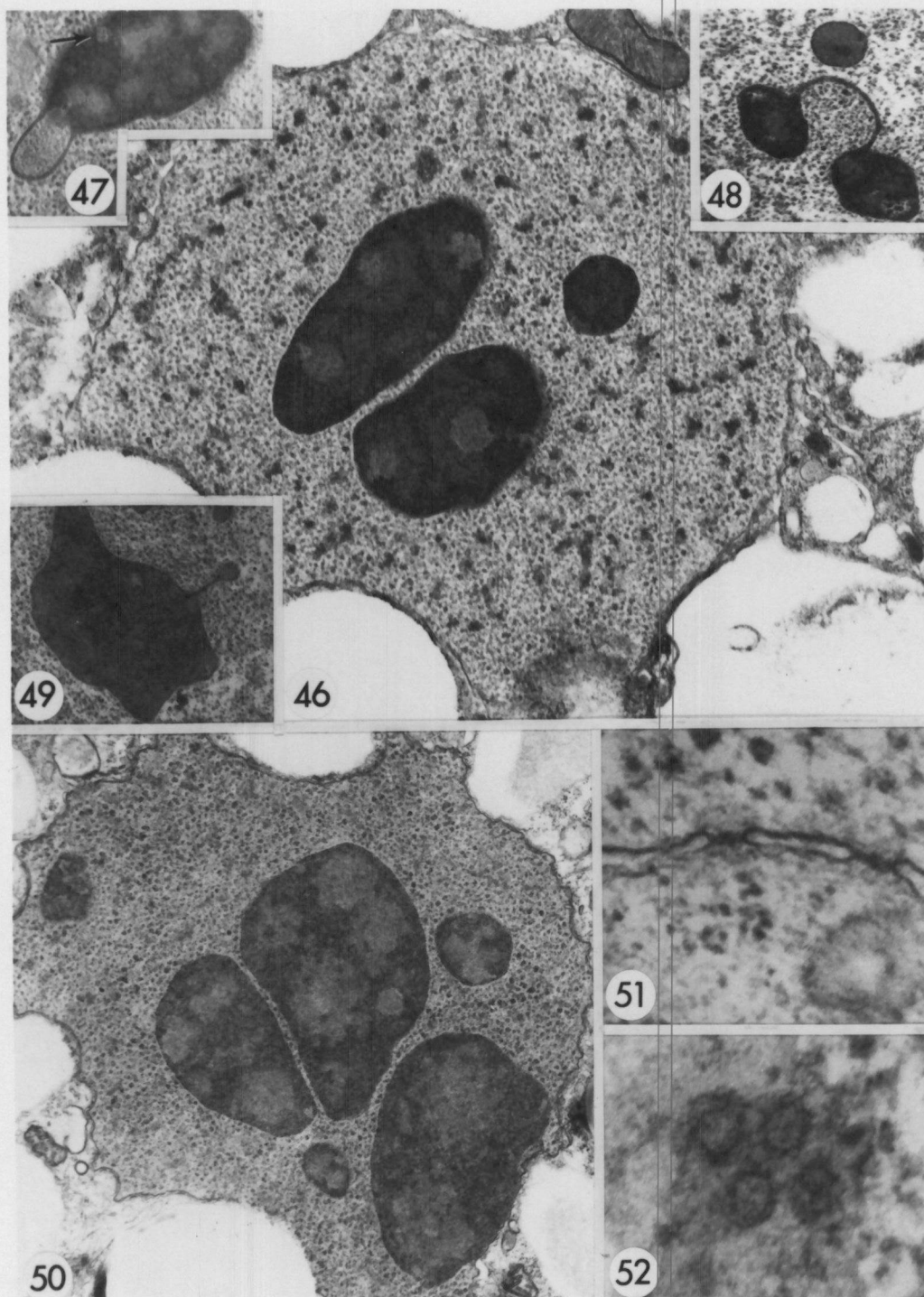


Plate X.

is a double membrane with randomly distributed pores that are traversed by dense septa (Plate X, Figure 51). In surface view the pores are circular with a diameter of 78 nm (Plate X, Figure 52). Small spine-like projections radiate from the circumference of the pores.

Mitochondria

Mitochondria are typically elongate (Plate XI, Figure 53), but diverse shapes are common. The mitochondrial surface is irregular due to undulations of the outer membrane. The inner membrane is infolded and forms flattened, sac-like cristae which usually are randomly aligned (Plate XI, Figure 53) but may be ordered into stacks (Plate XI, Figures 54 and 55). Occasionally lacunae (Plate XI, Figure 56) or matrix-filled cavities (Plate XI, Figure 54) occur in the interior of the organelle.

Dictyosomes

Dictyosomes, typical of those found in other euglenoids, are stacks of 20-25 curved or flattened cisternae, some of which contain dense regions (Plate XII, Figure 57). Wide intercisternal spaces are often found within the stacks. Polyribosomes and profiles of rough endoplasmic reticulum occur near the forming face, and vacuoles and small vesicles accumulate near the maturing face. Several dictyosomes are usually positioned near the contractile vacuole (Plate XII, Figure 58).

Cytochemical localization of acid phosphatase activity shows electron-dense lead sulfide reaction product within dictyosome

Plate XI. Mitochondria.

- Figure 53. Mitochondria are typically elongate but diverse shapes are common, including the bilobed mitochondrion shown here. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 30,100.
- Figure 54. Elongate mitochondrion with area of stacked cristae and matrix-filled cavity. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 53,800.
- Figure 55. Mitochondrion in which stacked cristae are aligned parallel to the long axis of the organelle. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 46,800.
- Figure 56. Mitochondria with lacunae or cavities in the interior of the organelle. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 19,700.

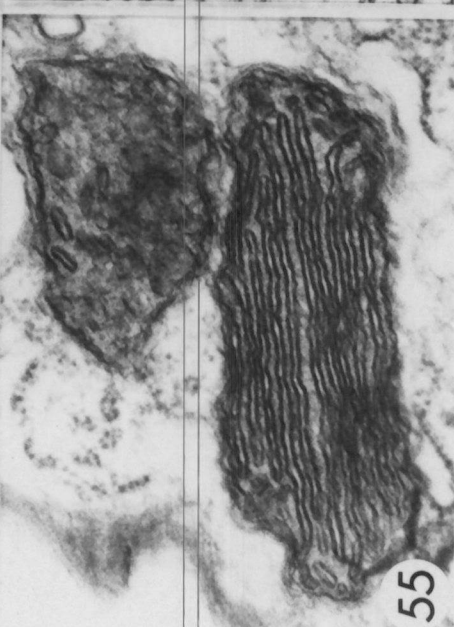
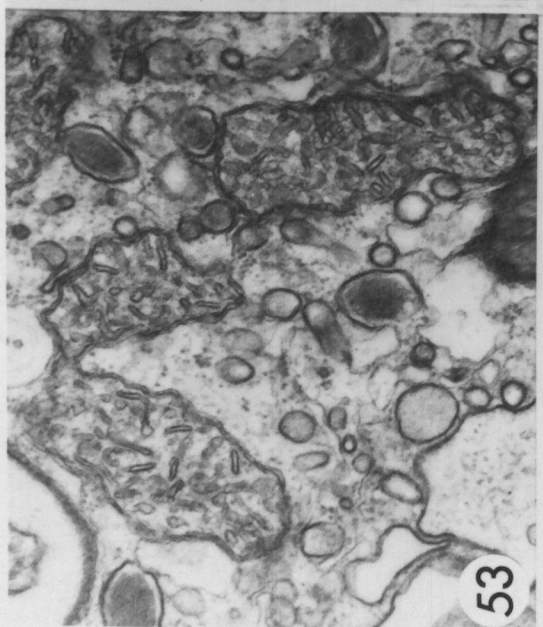
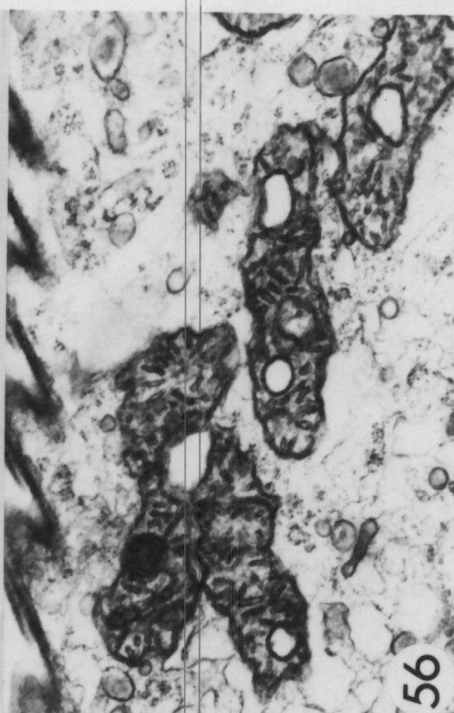


Plate XI.

Plate XII. Dictyosomes and dense body.

- Figure 57. Dictyosomes typically consist of a stack of 20-25 curved or flattened cisternae. Elements of rough endoplasmic reticulum and clusters of free ribosomes are often in close association with the forming face of the dictyosome. Vacuoles and small vesicles accumulate at maturing face. Note the wide inter-cisternal spaces and the areas of increased electron densities in some of the cisternae. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 38,200.
- Figure 58. Dictyosomes are often adjacent to the contractile vacuole. In contrast to the dictyosome shown in Figure 57, the two shown here have fewer cisternae (ca. 15) and dilated intercisternal spaces. The cisternae are more distended toward the forming face. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 19,700.
- Figure 59. After treatment of cells with Gomori reaction medium plus substrate, labeling of acid phosphatase is visualized in dictyosome cisternae. Fixed with glutaraldehyde/ OsO_4 . TEM thin section. X 33,300.
- Figure 60. Control cell incubated in Gomori reaction medium plus inhibitor, with dictyosome showing no acid phosphatase labeling. Fixed with glutaraldehyde/ OsO_4 . TEM thin section. X 41,900.
- Figure 61. Electron-dense body lying free in cytoplasmic matrix, a narrow zone of granular material surrounding the periphery. Note the tracts of low electron density and a cytoplasmic invagination within the body. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 94,700.
- Figure 62. Dense asymmetrically lobed body measuring ca. 1 μm in length. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 51,600.
- Figure 63. Electron-dense body in different cell (cf. Figure 62) showing similar lobed shape. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 106,800.

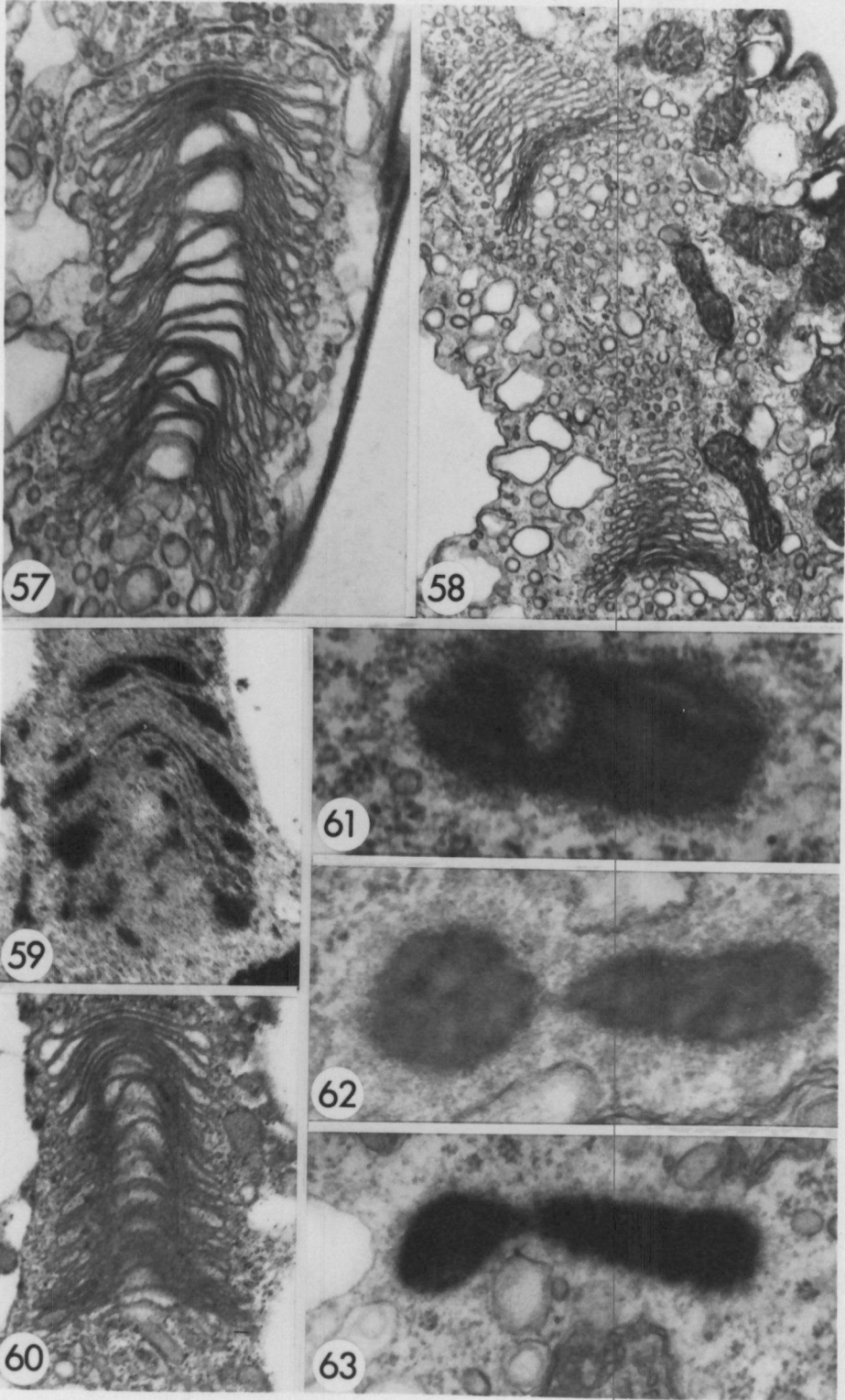


Plate XII.

cisternae, particularly in the dilated cisternal margins (Plate XII, Figure 59). Control cells show no reaction product (Plate XII, Figure 60).

Dense Bodies

Elongate or asymmetrically lobed dense bodies ca. 1 μm long (Plate XII, Figures 61-63) are observed in the cytoplasm of a few cells. Tracts of low electron density and cytoplasmic invaginations may occur within the electron-dense matrix. No membranes are associated with either the periphery or the interior of the body; only a narrow zone of dense granular material surrounds the structure, which remains unidentified.

Endosymbiotic Bacteria

Rod-shaped intracellular particles comparable in size and appearance to bacilloid bacteria are randomly distributed throughout the cytoplasm (Plate XIII, Figures 64-68) and, in one case, within the nucleus (Plate XIII, Figure 69). Cytoplasmic bacteria are encircled by a transparent zone or capsule and are bounded by a cell envelope ca. 20 nm thick that is composed of two distinct unit membranes (Plate XIII, Figures 64 and 65). Internally the bacteria contain dense granules ca. 10 nm in diameter, vacuole-like regions and areas of low density within which are fibrillar elements (Plate XIII, Figure 65). The bacteria appear to divide by binary fission (Plate XIII, Figures 66-68). There is no evidence that bacterial division is synchronous with host cell division.

Plate XIII. Endosymbiotic bacteria.

- Figure 64. Longitudinal section of bacilloid bacterium ca. $1\text{ }\mu\text{m}$ long X $0.3\text{ }\mu\text{m}$ wide within a vesicular element of the endoplasmic reticulum. Bacterial matrix is homogeneous and contains presumptive bacterial ribosomes. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 72,300.
- Figure 65. Transverse section of two bacterial endosymbionts. Note delicate fibrils in presumptive nuclear area (arrow). Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 87,800.
- Figure 66. Longitudinal section of bacterium in presumptive early stage of binary fission showing slight infurrowing. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 44,600.
- Figure 67. Presumptive dividing bacterium (different cell from that shown in Figure 66) assumes hour-glass shape. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 51,700.
- Figure 68. Presumptive final stage of binary fission shows nearly complete separation of walls of young cells. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X, 44,800.
- Figure 69. Portion of nucleus of Peranema showing presumptive endonuclear bacterium. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 32,800.

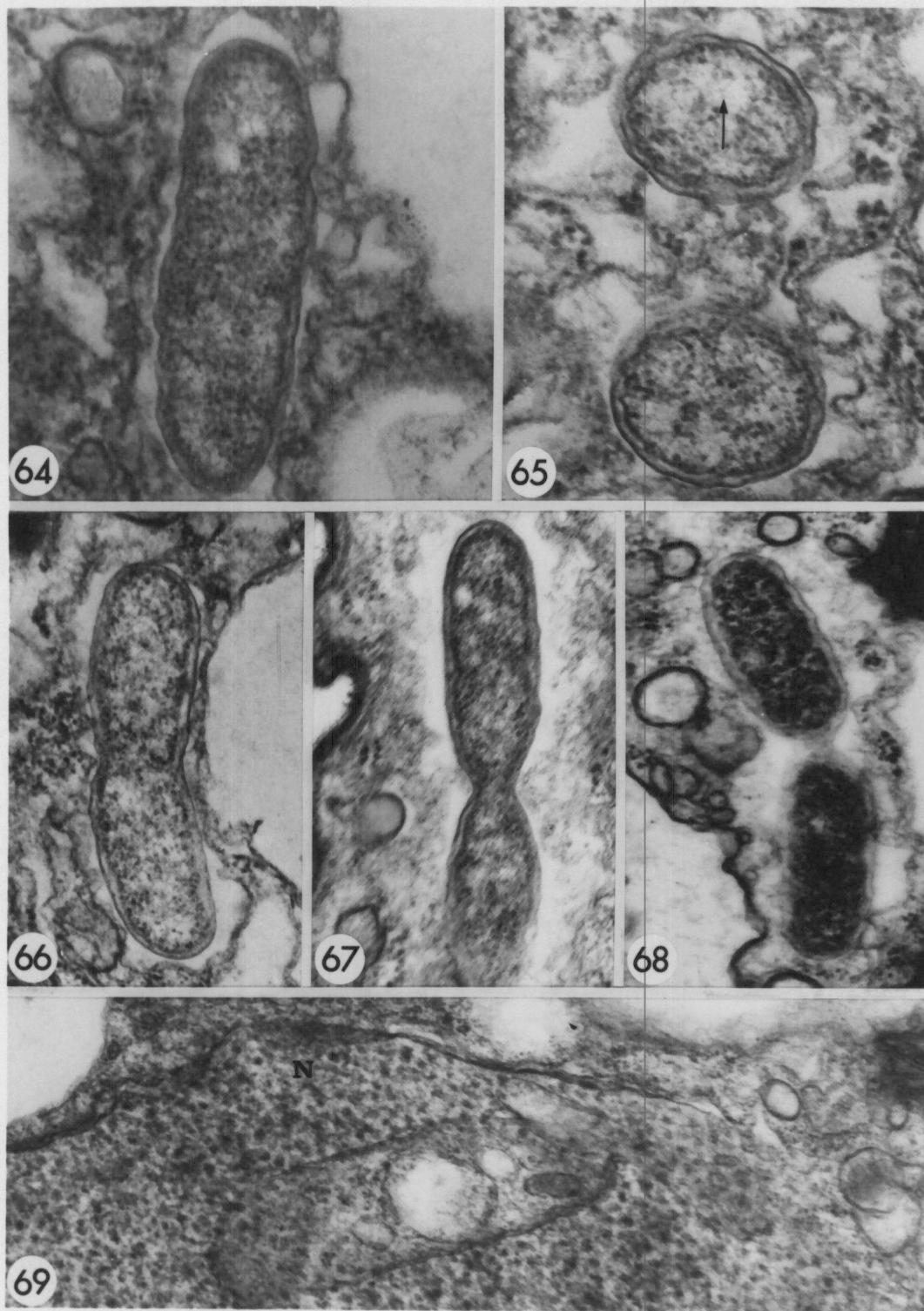


Plate XIII.

II. FLAGELLAR ORGANIZATION

Structure of Anterior and Trailing Flagella

The two flagella extend independently from the base of the reservoir and are identical in appearance at this level (Plate XIV, Figure 70). At the point of its insertion into the cytoplasm at the base of the reservoir, the diameter of each flagellum increases and the central doublet of the axoneme is absent, resulting in a swollen, hollow base with 9+0 axonemal arrangement (Plate XIV, Figures 71 and 72). At this level one flagellum contains striated material (Plate XIV, Figure 72) adjacent to the paraflagellar rod, which manifests helical striations of 28 nm periodicity and electron-dense material along the helical striae (Plate XIV, Figures 73 and 74). The paraflagellar rod is evident in both flagella in distal sections within the reservoir (Plate XIV, Figures 73-75) but is not evident in the portion of the trailing flagellum that emerges from the reservoir and lies in the pellicular groove (Plate XIV, Figure 76).

Outside the reservoir, the two flagella differ in thickness, a difference particularly evident in scanning electron micrographs (Plate XV, Figures 77 and 78) in which the diameter of the trailing flagellum is about half that of the anterior flagellum. Thin sections reveal that the greater total diameter of the anterior flagellum is due to (1) long nontubular mastigonemes, (2) two lateral short mastigoneme ribbons and (3) a paraflagellar rod (Plate XV, Figure 79). In contrast, the distal portion of the trailing flagellum

Plate XIV. Flagellar structure.

- Figure 70. Oblique section of the two flagella at the area of insertion into base of reservoir. Paraflagellar rod evident in both flagella at this level. Fixed with OsO_4 buffered with sodium cacodylate. TEM thin section. X 22,000.
- Figure 71. At flagellar base, the two central microtubules of the axoneme terminate, and the space between the outer doublets widens, resulting in a swollen, hollow structure. Flagellar membrane is continuous with the membrane of the reservoir. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 37,000.
- Figure 72. Transverse section of flagellar base showing axoneme devoid of central microtubules. Paraflagellar material to one side of axoneme consists of a dense rod with adjacent striated material. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 53,400.
- Figure 73. Oblique section of paraflagellar rod shows helical striations with periodicity of 28 nm. Numerous nontubular mastigonemes extend from flagellar surface. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 53,400.
- Figure 74. Tangential section of paraflagellar rod of anterior flagellum shows accumulations of dense material along the helical striae. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 106,800.
- Figure 75. Transverse sections of two flagella within reservoir reveal their similar diameters and paraflagellar rods. Fixed with unbuffered OsO_4 . TEM thin section. X 42,500.
- Figure 76. Transverse section shows 13 subpellicular microtubules and trailing flagellum that lacks a discernible paraflagellar rod and mastigoneme ribbons, resulting in a smaller diameter. Long mastigonemes curve away from pellicle surface. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 108,500.

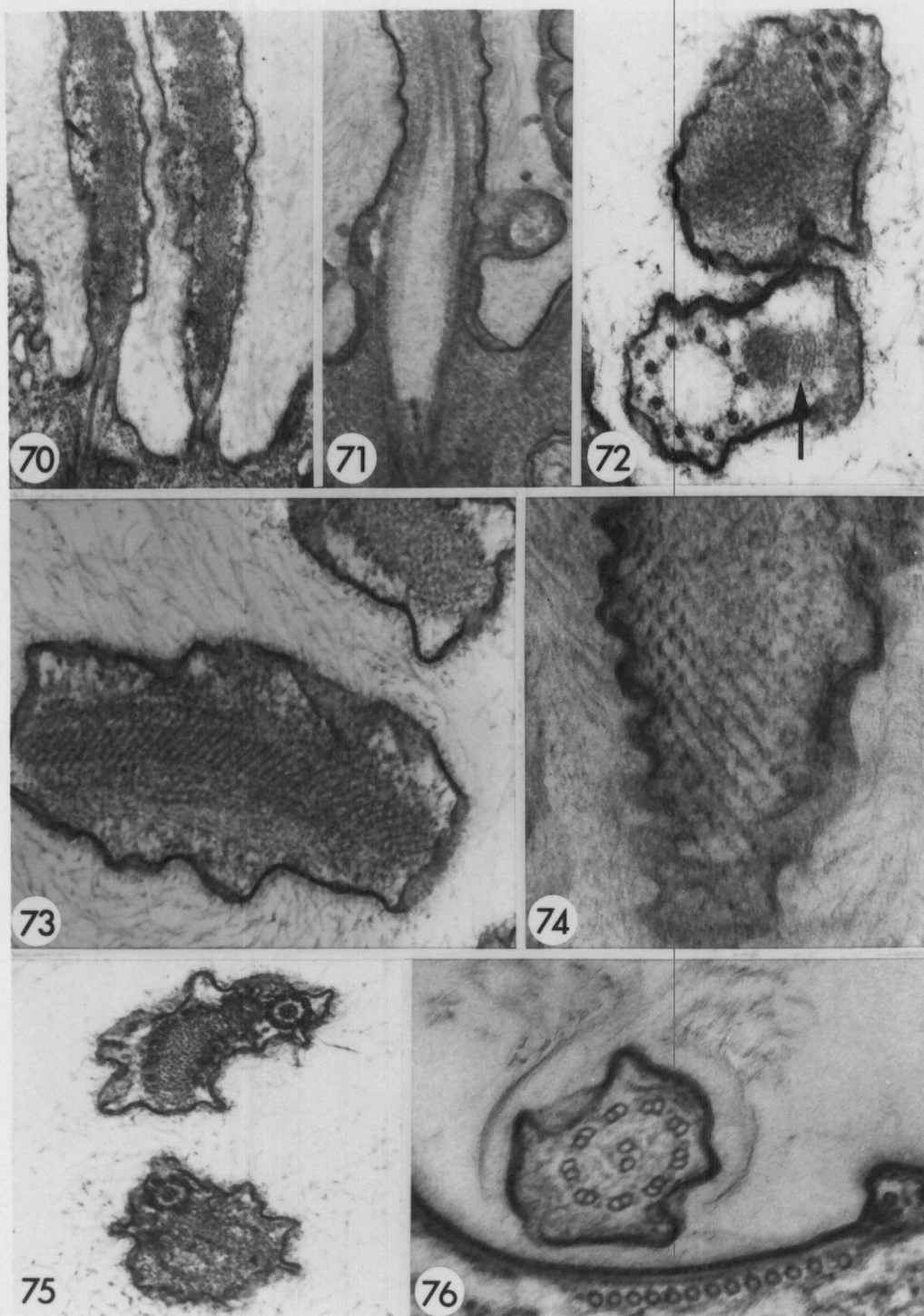


Plate XIV.

Plate XV. Comparison of anterior and trailing flagella.

- Figure 77. Diameter of the trailing flagellum (arrow) is ca. one-half that of the anterior flagellum. Fixed with OsO_4 . CPD. SEM. X 3400.
- Figure 78. Anterior flagellum is thick due to surrounding mastigonemes (arrow). That the mastigonemes appear to be wrapped compactly around the flagellum is an artifact of preparation. Fixed with OsO_4 . CPD. SEM. X 14,700.
- Figure 79. Transverse thin section shows thickness of anterior flagellum due in part to internal paraflagellar rod and to two lateral ribbons of nontubular mastigonemes external to the flagellar membrane. Thin strands connect outer doublets of flagellar microtubules to the central doublet (arrow). Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 96,400.
- Figure 80. Transverse section of trailing flagellum shows its position within pellicular groove, subtended by eight microtubules. Long mastigonemes emanate from the flagellum and curve away from cell surface; mastigoneme ribbons are absent. Mucilaginous material lies between the pellicle and the flagellar membrane. Undefined electron-dense material lies within flagellum (arrow). Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 96,400.

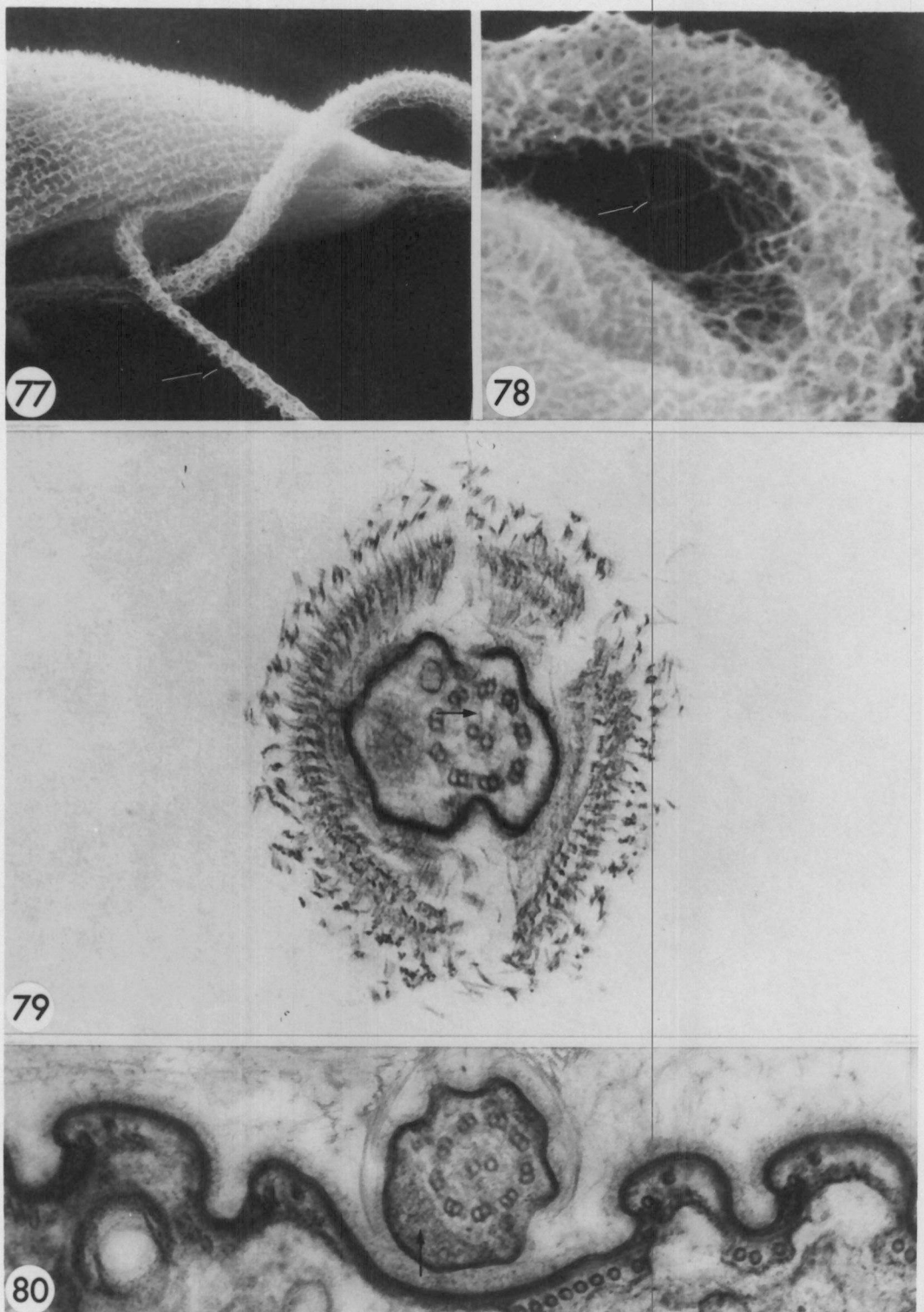


Plate XV.

lacks the rod and lateral ribbons and has fewer mastigonemes (Plate XV, Figure 80).

Flagellar Mastigonemes

Overview of flagellar appendages. The surface of the anterior flagellum is covered with hair-like appendages that give it a "test-tube brush" appearance (Plate XVI, Figure 81). The appendages, or nontubular mastigonemes, are of two distinct types: (1) long mastigonemes that extend 0.8-2.8 μm from the flagellum (Plate XVI, Figure 82) and (2) shorter mastigonemes which are part of two lateral arrays or ribbons parallel and external to the flagellar membrane (Plate XVI, Figures 81-83).

Long mastigonemes. Tufts of long mastigonemes, ranked in tiers (Plate XVII, Figure 84), radiate from the shaft and then curve anteriorly toward the tip of the flagellum (Plate XVII, Figures 85 and 86). In some sections, mastigonemes appear to emanate from axonemal microtubules (Plate XVII, Figure 86). In all transverse sections of the long mastigoneme tiers, they have been observed only on the side of the axoneme opposite the paraflagellar rod, suggesting that they are arranged in a unilateral array.

Short mastigoneme ribbons. Short mastigonemes form a bilateral array around the anterior flagellum (Plate XV, Figure 79; Plate XVI, Figure 82; Plate XVII, Figure 86). Each ribbon lies parallel to the long axis of the flagellum (Plate XVIII, Figures 87 and 88) and consists of lateral tiers which are sharply delimited laterally and

Plate XVI. Overview of flagellar mastigonemes.

- Figure 81. Oblique section of anterior flagellum shows "test-tube brush" appearance with electron-dense core corresponding to flagellar axis. Short mastigoneme ribbon is network of regularly spaced components both perpendicular (vertical arrows) and parallel (horizontal arrows) to long axis of flagellum. Extending centrifugally from ribbon border are randomly arranged long mastigonemes. Fixed with glutaraldehyde/OsO₄. TEM thin section. X 36,000.
- Figure 82. Transverse section of anterior flagellum showing two types of nontubular mastigonemes; short mastigonemes are arranged in two laterally aligned ribbons; long mastigonemes curve toward right. Short mastigoneme ribbons with regularly spaced filaments perpendicular to the long axis of the flagellum are external to the flagellar membrane. Long mastigonemes arise from side of flagellum opposite paraflagellar rod. Mucilage strands from pellicle surface are shown at left side of micrograph. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 99,900.
- Figure 83. Oblique view of anterior flagellum as it emerges from the canal shows portions of short mastigoneme ribbons, long mastigonemes and helical striae of paraflagellar rod. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 72,300.

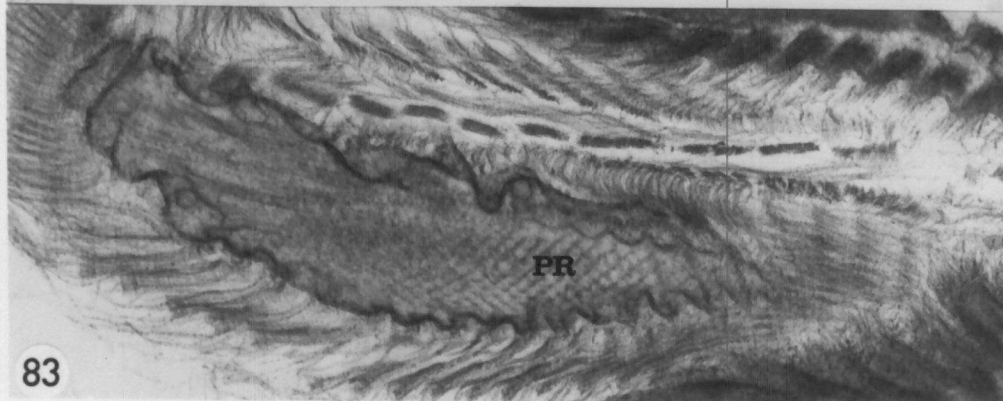
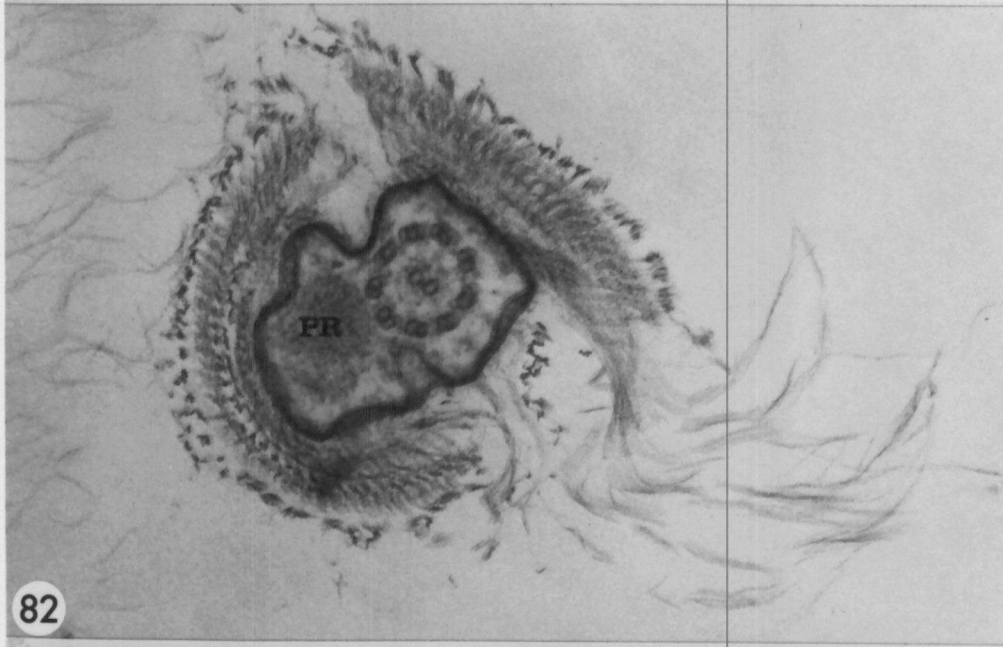
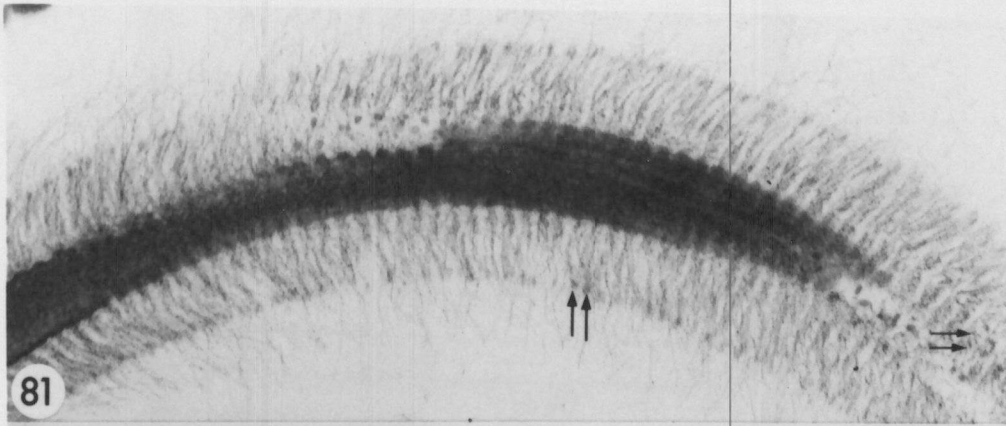


Plate XVI.

Plate XVII. Long mastigonemes.

- Figure 84. Tangential section of anterior flagellum showing long mastigonemes radiating from rows perpendicular to the long axis of flagellum. Mastigonemes emanate from small tufts (arrows) and curve anteriorly toward the tip of the flagellum. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 105,000.
- Figure 85. Tangential view of anterior flagellum shows three rows of long mastigonemes (arrows). Mastigonemes radiate outwardly, then curve abruptly toward distal end of flagellum. Long mastigonemes overlies short mastigoneme ribbons, seen in tangential section. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 75,800.
- Figure 86. Transverse section of flagellum showing long mastigonemes extending from side of flagellum opposite paraflagellar rod and curving toward the right. Dense mastigonemes traverse flagellar membrane and connect with one of the axonemal doublet microtubules (arrow). Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 75,800.

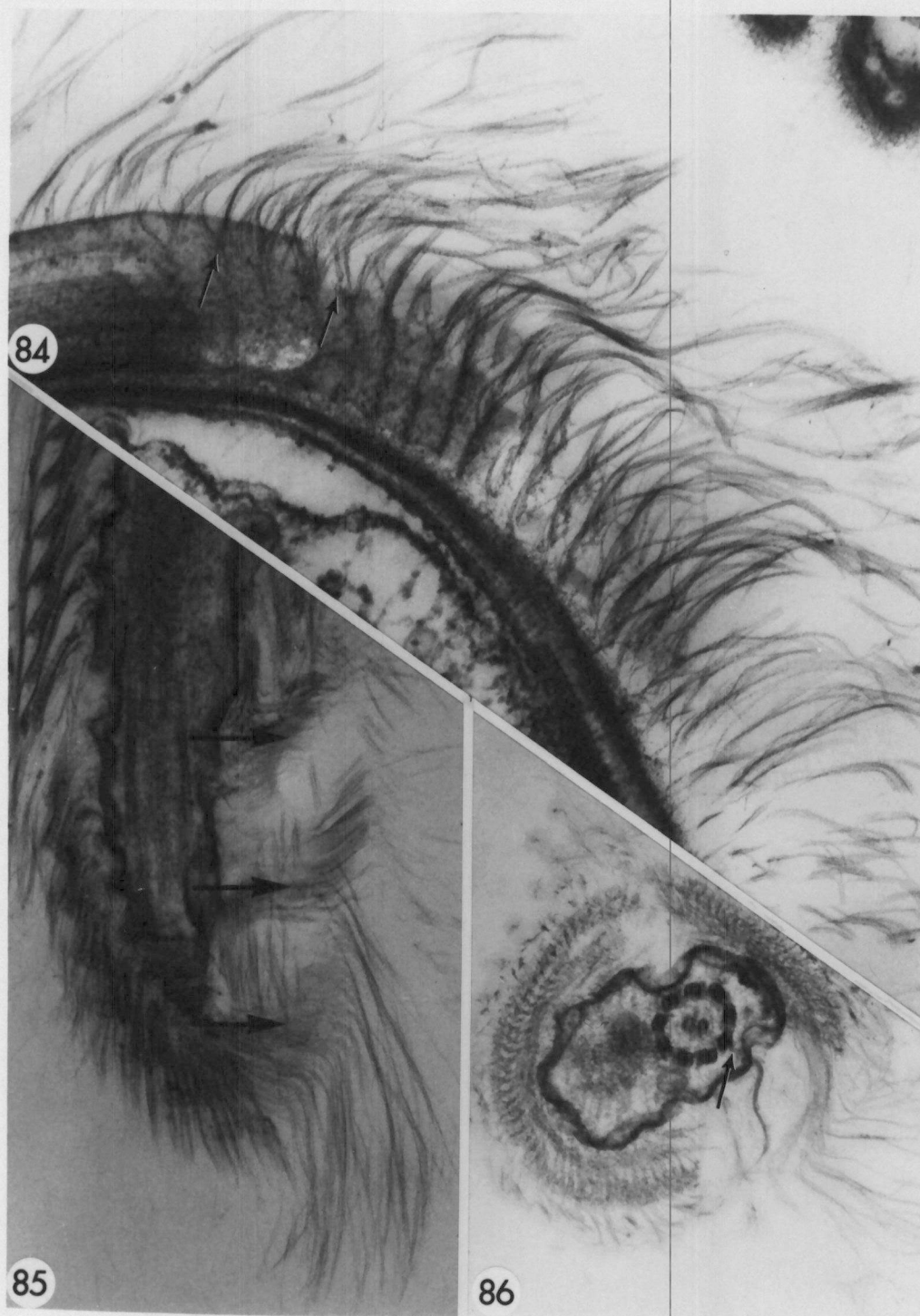


Plate XVII.

Plate XVIII. Fine structure of short mastigoneme ribbons.

- Figure 87. One of the short mastigoneme ribbons is seen in surface view toward the top and in progressively more centripetal portions toward the bottom of this micrograph. Each ribbon lies parallel to the long axis of the flagellum and consists of overlapping tiers of short mastigonemes. The tiers are sharply delimited laterally and are regularly spaced at distances of 70-80 nm. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 45,700.
- Figure 88. Tiers in the ribbons emanate from transverse striations (arrows); long mastigonemes extend beyond the ribbon margins. Helical striae of paraflagellar rod are evident. Fixed with Paramedium fixative. TEM thin section. X 68,900.
- Figure 89. Oblique section of short mastigoneme ribbon showing portion of flagellar axis. Tiers are arranged in lateral rows and overlap longitudinally by one-half of a tier length. Note the presence of numerous vesicles of varying electron densities. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 62,000.
- Figure 90. Surface view of short mastigoneme ribbon shows that each tier is composed of laterally aligned, regularly spaced components that are parallel to the long axis of the flagellum (arrow). Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 58,500.
- Figure 91. More centripetal section of ribbon than shown in Figure 90 shows that the laterally aligned short mastigonemes are spaced at distances of 25 nm and are interconnected by fine fibrillar elements (arrow). Fixed with Paramedium fixative. TEM thin section. X 103,300.

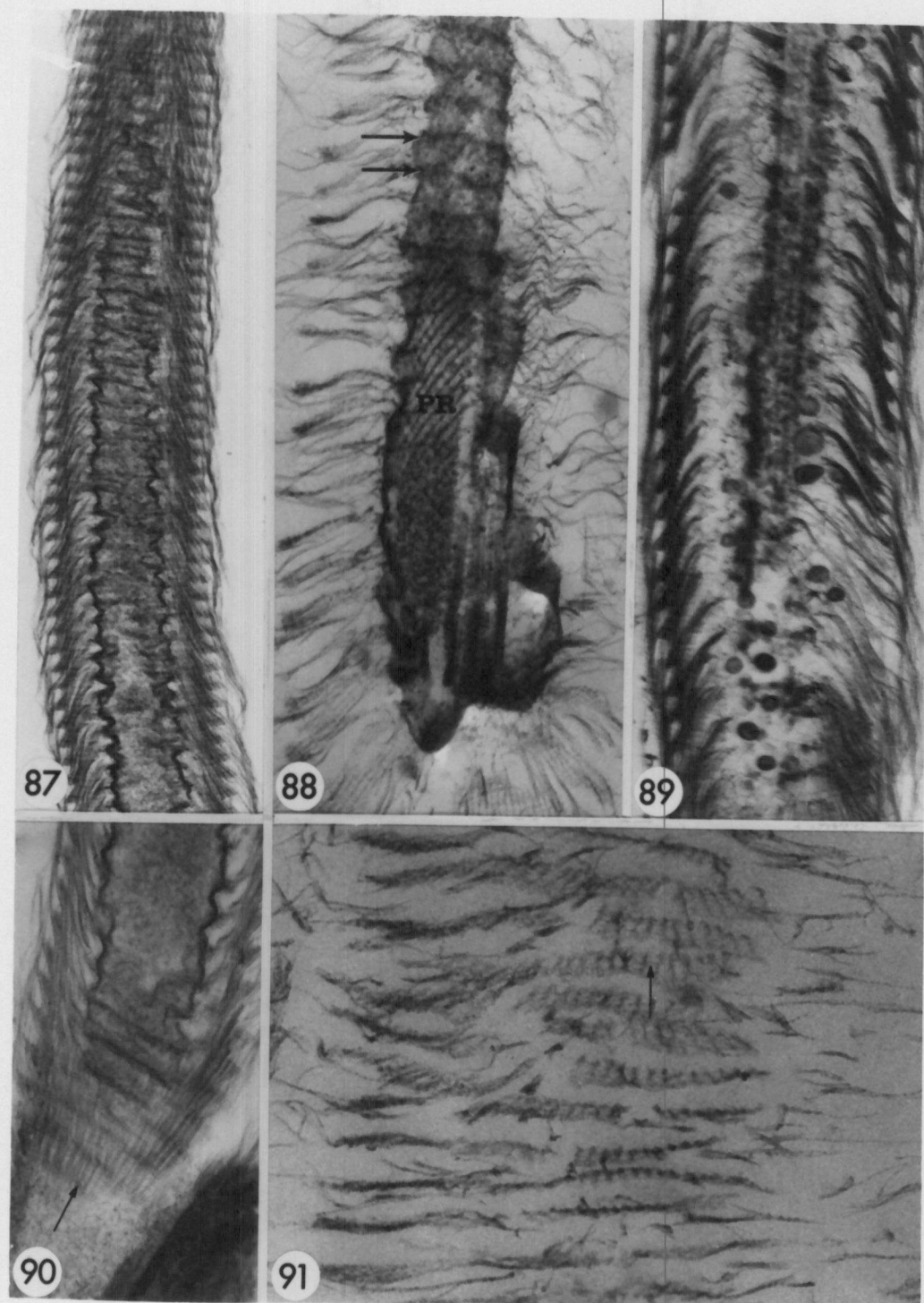


Plate XVIII.

which overlap each other longitudinally by one-half of a tier length (Plate XVIII, Figure 89). The tiers emanate from transverse striations and are regularly spaced at distances of 70-80 nm. Individual mastigonemes parallel to the long axis of the flagellum are evident in surface view (Plate XVIII, Figure 90) and in more centripetal sections of the tiers (Plate XVIII, Figure 91). These short mastigonemes are spaced at intervals of 25 nm and are linked by fine transverse fibrils.

Intact and Dissociated Flagella and Mastigonemes

Both shadow-cast (Plate XIX, Figure 92) and negative-stain (Plate XIX, Figure 93) preparations of intact anterior flagella show four distinct regions: (1) opaque flagellar axis; (2) short mastigoneme ribbons which appear as "felt" material; (3) medium-length mastigonemes, 0.8-1.3 μm long, and (4) long mastigonemes, 2.2-2.8 μm long. Short mastigoneme ribbons are easily dissociated from the flagellar shaft upon air-drying and appear as cross-striated bands with a periodicity of 80 nm (Plate XIX, Figures 94 and 95).

Details of ribbon structure can be more readily seen in isolated, dissociated flagella (Plate XX, Figures 96 and 97; Plate XXI, Figure 98). In these preparations, it is evident that the cross-striated appearance of the ribbons is due to overlapping of individual tiers of mastigonemes. Each tier is fan-shaped (Plate XX, Figure 96) and consists of laterally aligned short mastigonemes, 0.3 μm long, which terminate in a rim of material at the broad base of the fan. The laterally aligned mastigonemes and the basal rims account for the vertical and horizontal striae, respectively. The structural

Plate XIX. Shadow-cast and negative-stain preparations of mastigonemes.

- Figure 92. Shadow-cast preparation of intact anterior flagellum showing opaque flagellar axis surrounded by amorphous "felt" and long mastigonemes. Mastigonemes of uniform medium length (ca. $1\ \mu\text{m}$) appear on both sides of the flagellum but are more evident to the left of the axis (arrow). Small tufts of long mastigonemes occur periodically along the axis (arrow heads). Fixed with OsO_4 . TEM. Shadow-cast with Pt/C at 16° . X 16,100.
- Figure 93. Negative-stain preparation of intact anterior flagellum showing regions corresponding to those seen in shadow-cast preparation (cf. Figure 92): (1) opaque axis; (2) "felt"; (3) medium length mastigonemes ($0.8\ \mu\text{m}$); and (4) long mastigonemes ($2.2\ \mu\text{m}$). The "felt" material consists of a mat of short mastigonemes. Some long mastigonemes are intertwined and surround a small electron-dense sphere. Unfixed. TEM. Negative-stain with 1% aqueous uranyl acetate. X 25,300.
- Figure 94. Short mastigoneme ribbons dissociate from flagellar axis upon air-drying. Shadow-cast preparations reveal cross-striations with a periodicity of ca. 80 nm. Long mastigonemes are also detached from the flagellum (the alignment pattern of the long mastigonemes is an artifact of drying). Axonemal microtubules are seen within the axis as longitudinal fibers. Unfixed. TEM. Shadow-cast with Au/Pd at 11° . X 18,500.
- Figure 95. Negative-stain preparation of air-dried flagellum showing the two short mastigoneme ribbons detached from the axis. Unfixed. TEM. Negative stain with 1% aqueous uranyl acetate. X 14,500.

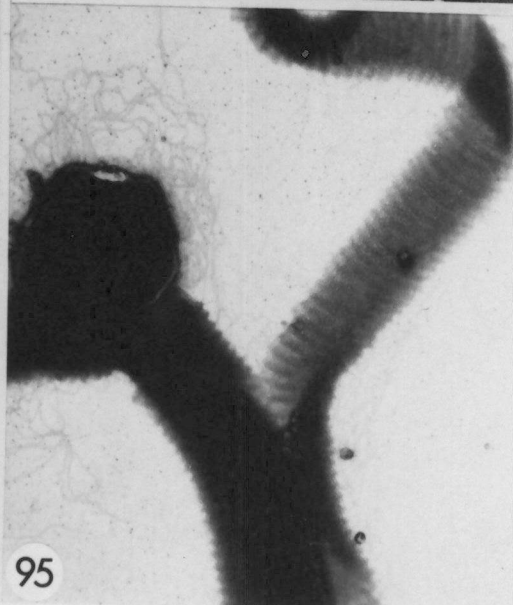
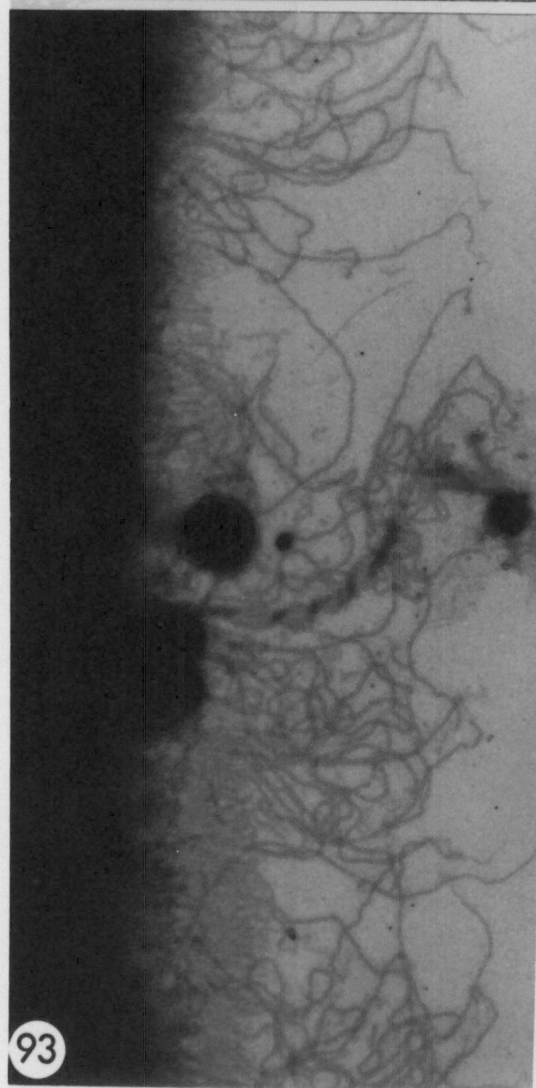
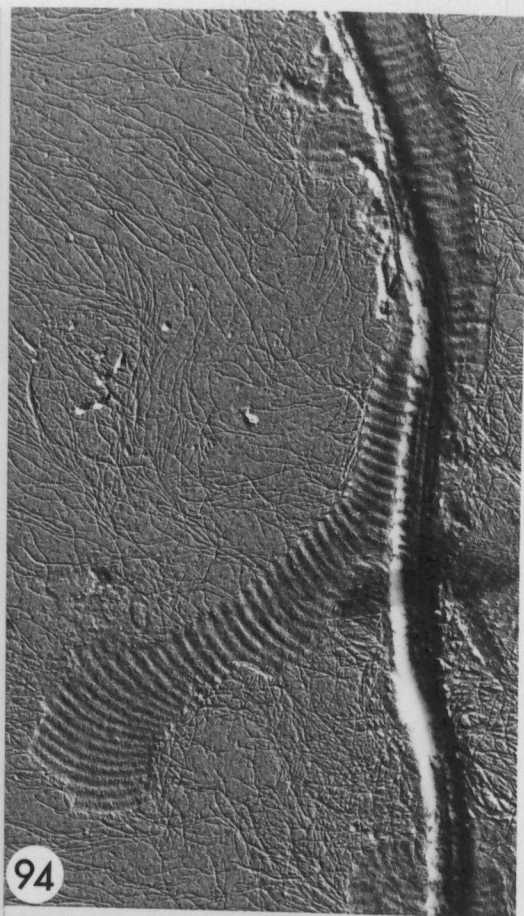
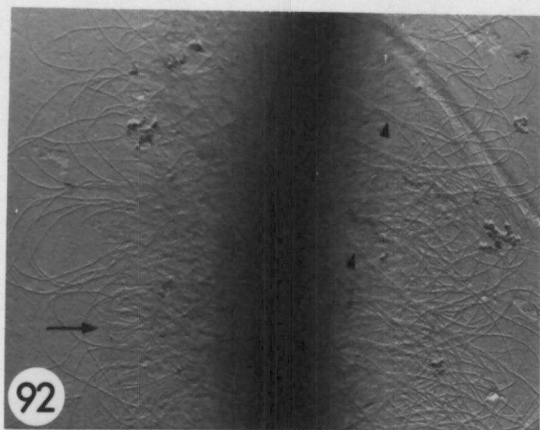


Plate XIX.

Plate XX. Detached mastigoneme ribbons.

- Figure 96. Partially dissociated ribbon reveals individual short mastigonemes in the cross striae. Fragments of the flagellar axis are shown to the right. The insert (X 23,800) shows an isolated tier ca. 250 nm long which is partially overlapped by a second tier to the left. Fixed with OsO_4 . TEM. Shadow-cast with Au/Pd at 11° . X 17,600.
- Figure 97. Negative-stain preparation of isolated flagella showing long mastigonemes randomly scattered on carbon/Formvar-coated grid, and a short mastigoneme ribbon which dissociated from the flagellum as a unit, the individual mastigonemes remaining intact. Unfixed. TEM. Negative-stain with 1% aqueous uranyl acetate. X 15,000.

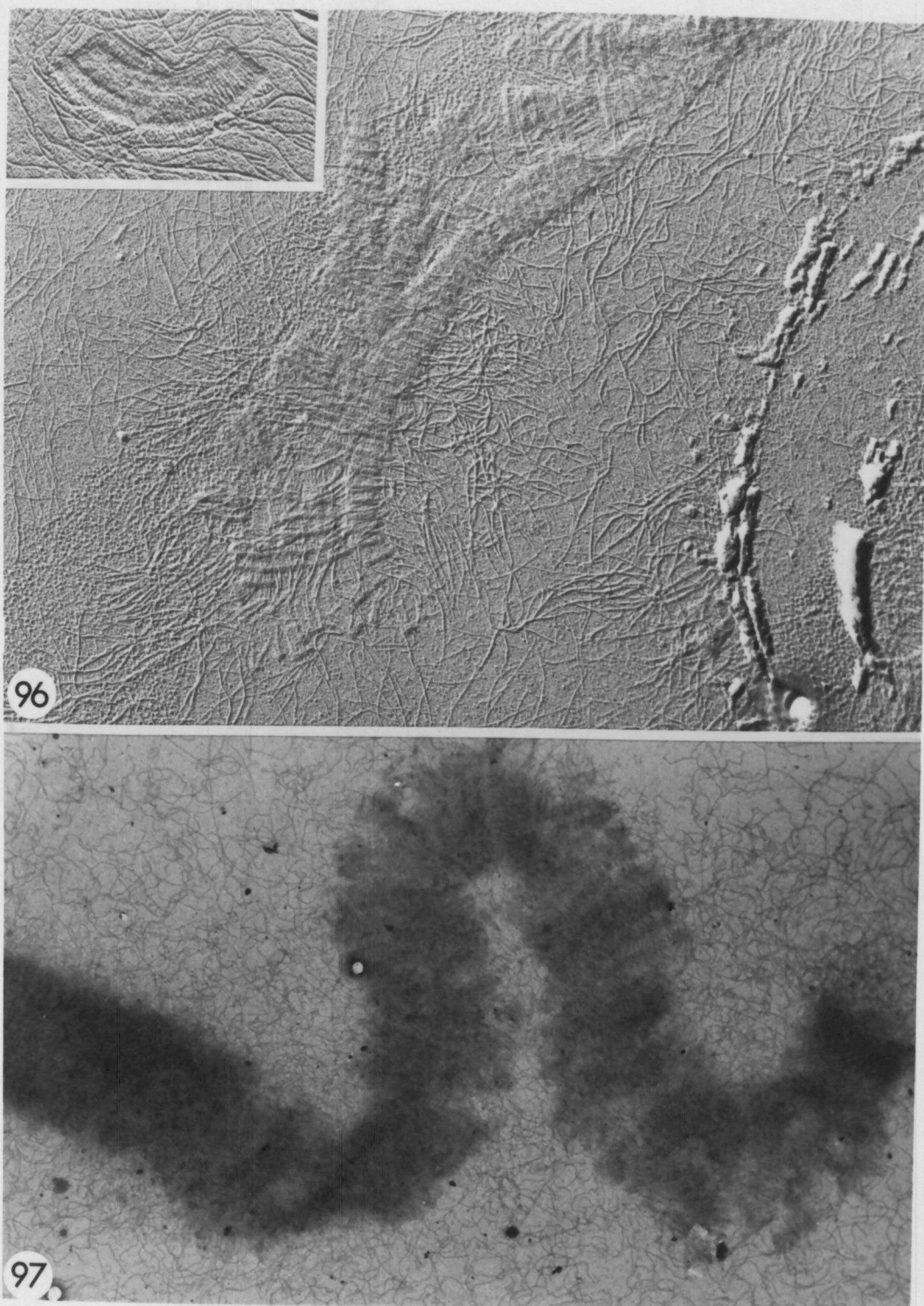


Plate XX.

Plate XXI. Isolated long mastigonemes and mastigoneme ribbons.

Figure 98. Negative-stain preparation of partially dissociated short mastigoneme ribbon. Note areas of short mastigonemes and loops (arrows). Unfixed. TEM. Negative-stain with 1% aqueous uranyl acetate. X 23,700.

Figure 99. Isolated long mastigonemes exposed to the anionic detergent, Sarkosyl. One mastigoneme measures 1.4 μm in length (arrow). Note terminal narrowing of long mastigonemes and the relative size of the short mastigonemes dissociated from ribbon (arrow heads). Unfixed. TEM. Negative-stain with 1% aqueous uranyl acetate. X 63,700.

Figure 100. Long mastigonemes of dismembered flagella are often associated with lumps or strips of material. Unfixed. TEM. Negative-stain with 1% aqueous uranyl acetate. X 19,900.

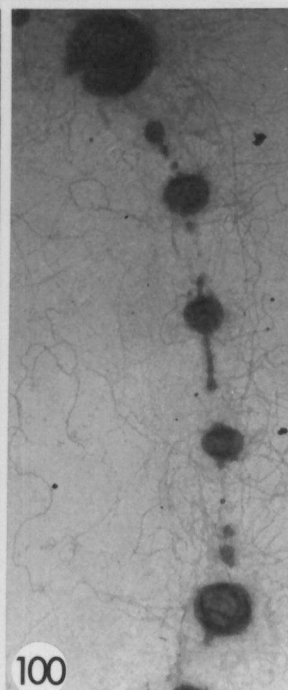
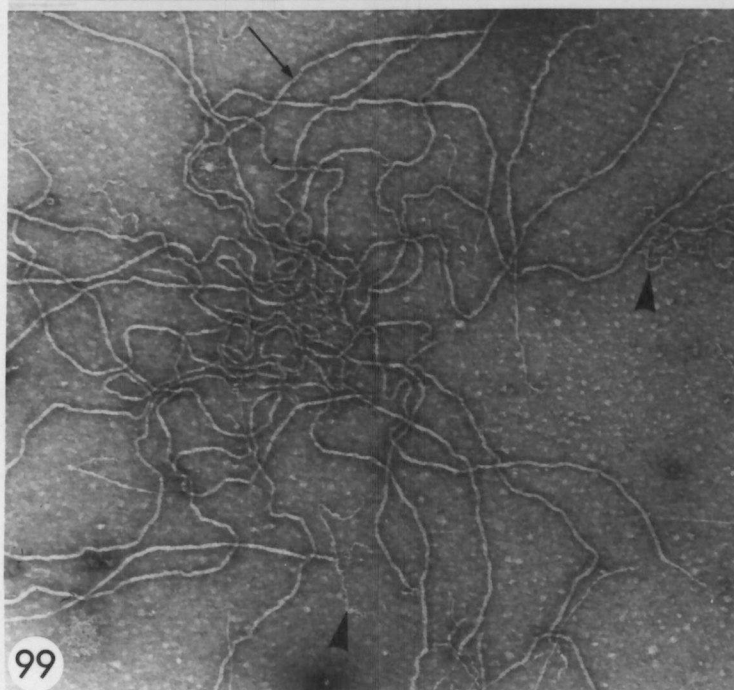
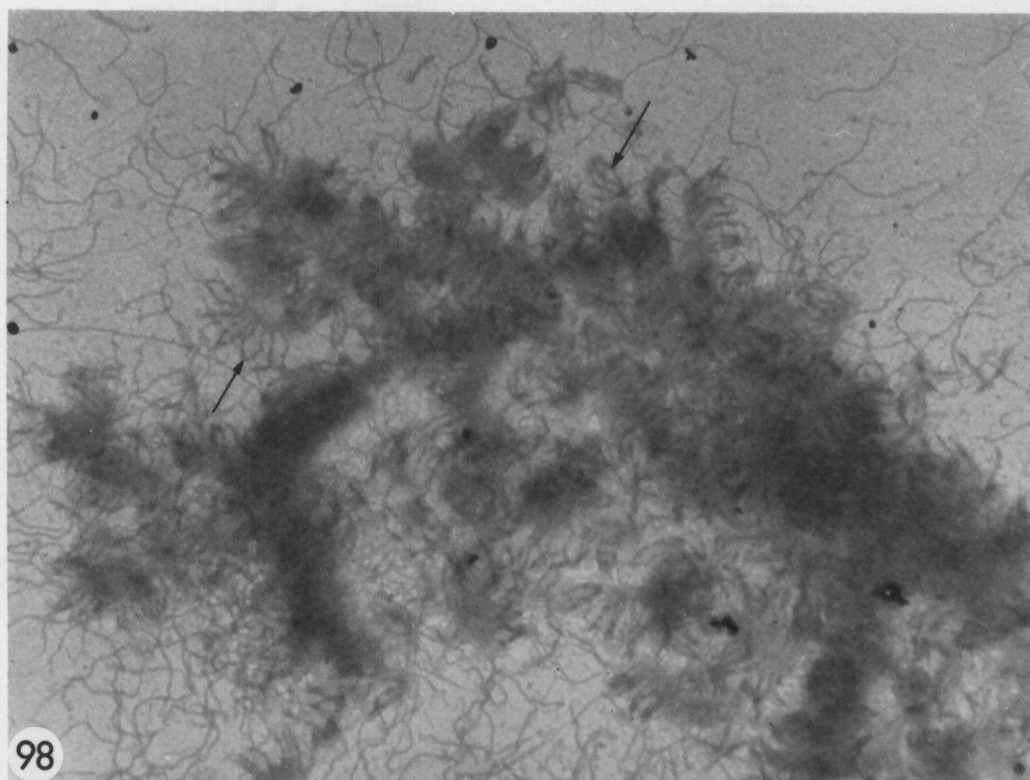


Plate XXI.

integrity of the ribbon remains intact in preparations of isolated flagella (Plate XX, Figure 97). Although no exact orientation of individual mastigonemes can be discerned within intact ribbons, there are areas in partially dissociated ribbons that appear to contain loops (Plate XXI, Figure 98).

Detached long mastigonemes appear to be flexible, non-rigid structures with narrow distal tips (Plate XXI, Figure 99). Long mastigonemes usually dissociate individually from isolated flagella (Plate XXI, Figure 99), but at times they appear to be associated with lumps or strips of flagellar material (Plate XXI, Figure 100), suggesting that the mastigonemes extend through the flagellar membrane and attach to intraflagellar structures.

Differences between the anterior and trailing flagella can readily be seen in the shadow-cast preparation shown in Figure 101 (Plate XXII). The trailing flagellum lacks mastigoneme ribbons and arrays of long mastigonemes; mastigonemes of medium length, however, occur on both sides (Plate XXII, Figure 101) and extend to its tip (Plate XXII, Figure 102). In contrast, the tip of the anterior flagellum is differentiated into a slender extension with a knob-like terminus from which medium-length mastigonemes radiate (Plate XXII, Figures 103 and 104).

Model of Flagellar Mastigonemes

Evidence from thin sections and shadow-cast and negative-stain preparations of intact and dissociated flagella can be integrated into a comprehensive model of the flagellar surface (Plate XXIII, Figure 105). Two short mastigoneme ribbons form a bilateral array parallel

Plate XXII. Differences between anterior and trailing flagella.

- Figure 101. Shadow-cast preparation of anterior flagellum shown at right and trailing flagellum at left. Diameters of electron-dense axes of two flagella are similar, but the trailing flagellum lacks the "felt" (short mastigoneme ribbons) and the arrays of long mastigonemes. Mastigonemes of medium length (ca. $0.9\ \mu\text{m}$) occur on both sides of the trailing flagellum. Fixed with OsO_4 . TEM. Shadow-cast with Pt/C at 16° . X 12,600.
- Figure 102. Mastigonemes extend to tip of trailing flagellum. Fixed with OsO_4 vapor. TEM. Shadow-cast with Au/Pd at 11° . X 11,200.
- Figure 103. Distal end of anterior flagellum tapers to slender extension with a knob-like terminus. Unfixed. TEM. Negative-stain with 0.5% aqueous uranyl acetate. X 33,300.
- Figure 104. Tufts of medium-length mastigonemes radiate from knob-like tip of anterior flagellum. Short mastigoneme ribbons terminate before reaching the extended tip. Fixed with OsO_4 vapor. TEM. Shadow-cast with Au/Pd at 11° . X 28,500.

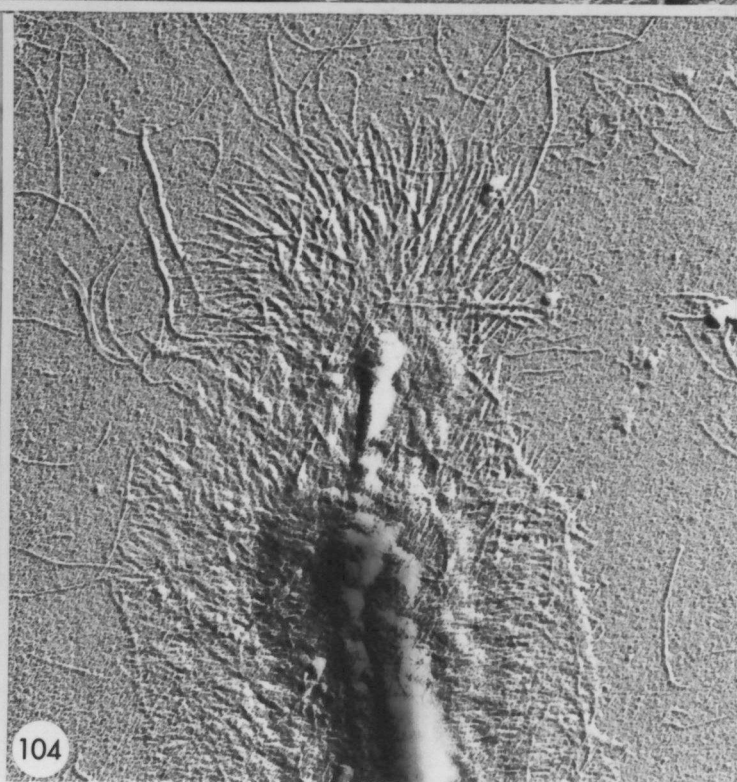
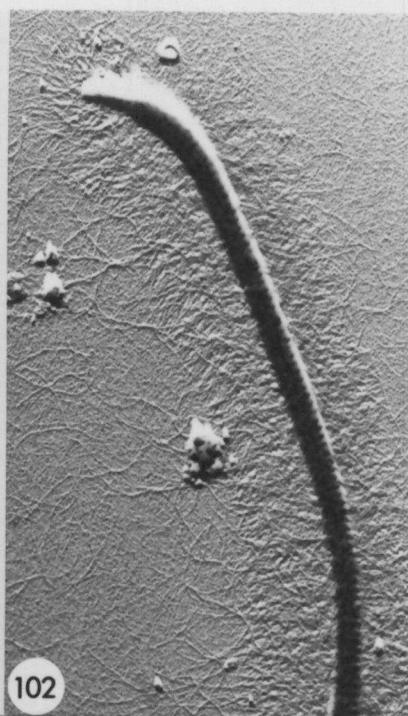
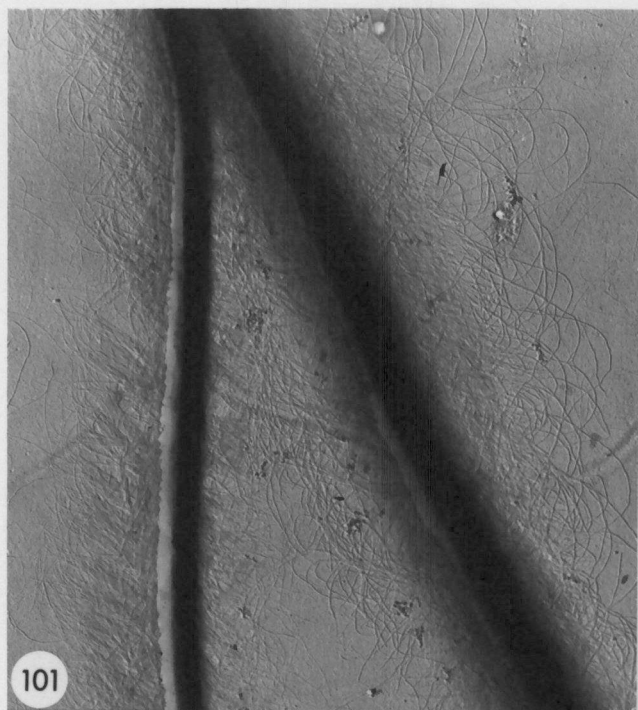


Plate XXII.

Plate XXIII. Model of flagellar mastigonemes.

- Figure 105. Diagram illustrating possible arrangement of short and long nontubular mastigonemes on flagellar surface. Short mastigonemes form two lateral ribbons parallel to the long axis of the flagellum. Each ribbon consists of individual tiers, ca. 250 nm long, which overlap in the longitudinal direction by one-half of a tier length (three overlapping tiers are shown in each ribbon). Short mastigonemes (arrow) in the tiers are spaced ca. 25 nm apart. Tufts of long mastigonemes (0.8-2.2 μm in length), arranged in a spiral configuration, curve toward the distal end of the flagellum (toward bottom of diagram).

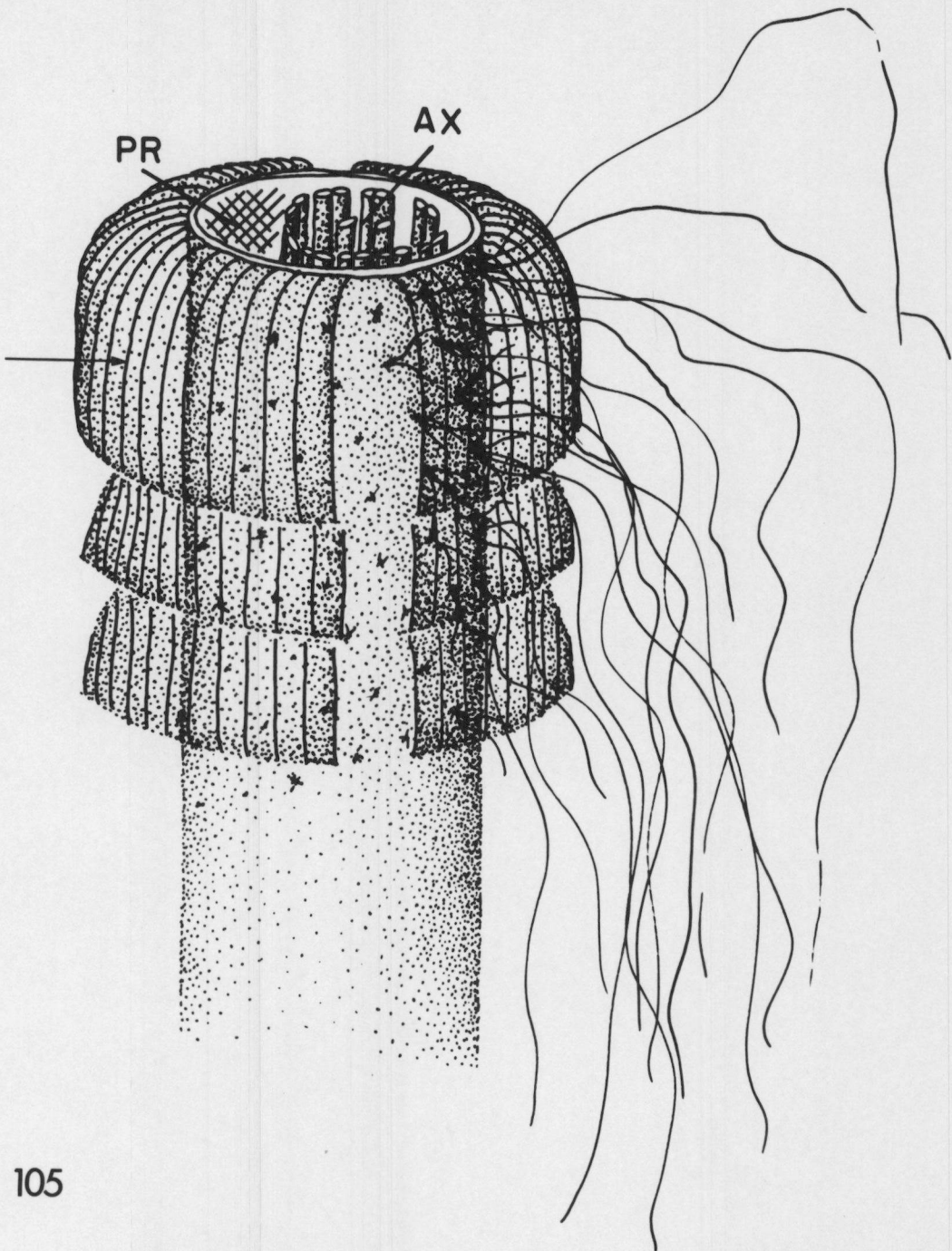


Plate XXIII.

to the long axis of the flagellum. Each ribbon consists of overlapping fan-shaped tiers of short mastigonemes spaced 25 nm apart and inter-linked by fine fibrils. Tufts of long mastigonemes emanate from spiral rows and curve anteriorly toward the distal end of the flagellum. Although shadow-cast preparations show a bilateral array of long mastigonemes, evidence from thin sections suggests that the mastigonemes emanate from intraflagellar structures, possibly axonemal microtubules, on the side of the flagellum opposite the paraflagellar rod.

Flagellar Bases and Basal Bodies

Transition zone. The structure of the flagellar bases and basal bodies in the transition zone is typical of euglenoids. (The transition zone is defined as that area between the flagellar axoneme with the "9+2" appearance and the basal body with the "pinwheel" appearance. Distal sections of the zone are near the flagellar axoneme; proximal sections of the zone approach the basal body [Leedale, 1967]). In distal sections, the central doublet of the axoneme is absent, and the nine outer doublets of the axoneme are connected by a narrow ring of dense material (Plate XXIV, Figure 106). As the microtubules of the peripheral ring enter the cytoplasm, electron-dense arms radiate at an angle of 60-70° from each doublet (Plate XXIV, Figures 107 and 108) and terminate in amorphous dense areas that are part of a ring that surrounds the arms and shaft. In more proximal sections, dense flagellar roots extend from the shaft (Plate XXIV, Figure 109), and the nine outer doublets give way

Plate XXIV. Flagellar bases and basal bodies.

- Figure 106. Transverse section of flagellar base in distal portion of transition zone. The nine outer doublets are connected by a narrow ring of electron-dense material. Fixed with unbuffered OsO_4 . TEM thin section. X 82,700.
- Figure 107. As the flagellar shaft enters into the cytoplasm at the base of the reservoir, electron-dense arms radiate from the nine outer doublets. In the center of the shaft are several dense granules. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 82,700.
- Figure 108. Each radiating arm is inclined at an angle of $60-70^\circ$ with a tangent to the circumference of dense material connecting the doublets. The arms terminate in dense amorphous areas that extend laterally to form a ring around the radiating arms and shaft. Fixed with unbuffered OsO_4 . TEM thin section. X 82,700.
- Figure 109. Proximal section through the transition zone showing flagellar roots extending from the shaft. Note one set of three fibers in the circumference of the shaft (arrow). Fixed with unbuffered OsO_4 . TEM thin section. X 101,600.
- Figure 110. Transverse section of basal body showing pinwheel arrangement of triplet fibers. Subfiber A, the innermost subfiber in the triplet, has two short diverging arms, one directed radially inward and connecting with a central ring of dense material, and the other connecting with the junction between subfibers B and C of the adjacent triplet. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 182,500.
- Figure 111. Longitudinal section of basal body shows hollow cylinder. Distal end of cylinder is open; proximal end lies near electron-dense granules and contains a small central structure corresponding to the opaque center of the basal body shown in Figure 110. Note the adjacent microtubular root seen in oblique section. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 58,500.

Plate XXIV. (Continued)

Figure 112. Cytoplasm at reservoir base is delimited by elements of endoplasmic reticulum and is finely granular and devoid of vesicles and ribosomes. In addition to the basal bodies of the flagella (bb_1 and bb_2) are two other basal bodies, one of which (bb_2)² is shown at an angle to bb_2). Two microtubular roots emanate from bb_1 , one eight-stranded root (MR_1) extending toward the reservoir membrane and the other seven-stranded root (MR_2) proceeding toward bb_2 . A third four-stranded root (MR_3) extends from bb_2 toward the peripheral cytoplasm. A striated fiber connects bb_2 and MR_2 . Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 44,600.

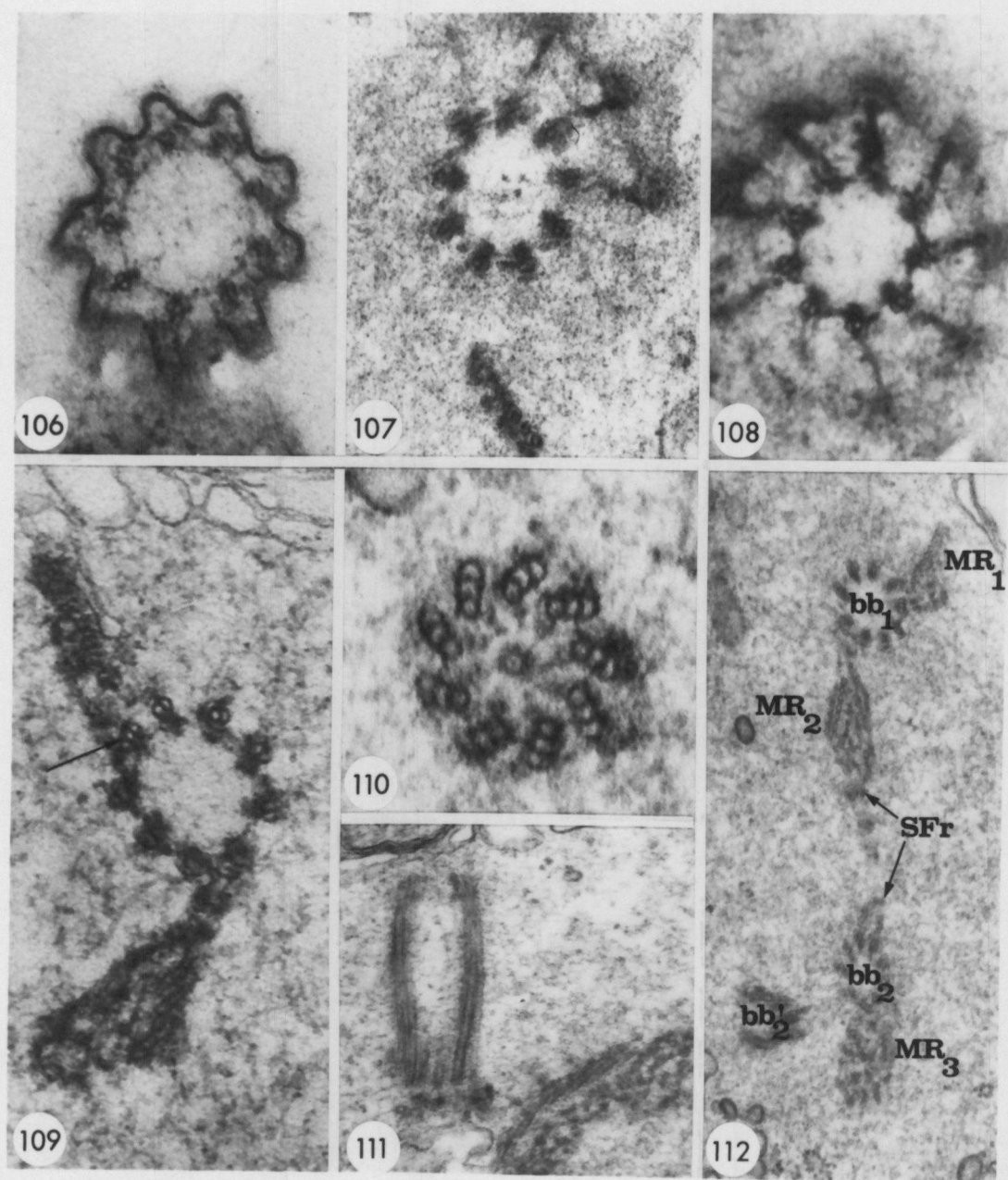


Plate XXIV.

to a pattern of nine skewed triplets, the typical "pinwheel" basal body (Plate XXIV, Figures 110 and 111).

Microtubular roots. Two pairs of basal bodies lie at the base of the reservoir in an area of homogeneous cytoplasm delimited by endoplasmic reticulum (Plate XXIV, Figure 112). At angles to the flagellar basal bodies (bb_1 and bb_2) are two additional basal bodies (bb'_1 and bb'_2). Associated with the flagellar basal bodies are three microtubular roots embedded in electron-dense matrices. Plate XXIV, Figure 112 shows transverse sections of eight-stranded (MR_1) and seven-stranded (MR_2) microtubular roots that extend from opposite sides of bb_1 and a four-stranded root (MR_3) that proceeds from bb_2 into the surrounding cytoplasm. Three groups of microtubules are found in the "left" wall of the reservoir (Plate VI, Figure 31, page 38) and will be referred to in the discussion on flagellar roots (page 141). A striated fiber composed of electron-dense bands of varying thickness extends between MR_2 and bb_2 (Plate XXIV, Figure 112; Plate XXV, Figure 113). In addition to flagellar-root microtubules, other microtubules radiate in all directions from the basal body region, some of them passing near the nucleus (Plate XXV, Figure 114).

Striated roots. There are several striated roots associated with the basal body region. They appear to emanate from various structures, including the striated fiber (Plate XXV, Figure 113) and the microtubular roots (Plate XXV, Figures 115-118). However, fine fibrils that extend from the microtubular roots to the rodorgan are sometimes seen in a position usually occupied by the striated roots (Plate XXV, Figure 116). The fine connections between one of

Plate XXV. Basal bodies and flagellar roots.

- Figure 113. Oblique section of flagellar roots and striated fiber associated with the two pairs of basal bodies. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 27,400.
- Figure 114. In the cytoplasm at the base of the reservoir is an amorphous mass of dense material from which microtubules radiate in all directions. Some of the microtubules pass near the nucleus. Fixed with OsO_4 buffered with sodium cacodylate. TEM thin section. X 36,000.
- Figure 115. Longitudinal section of basal portion of flagellum showing two lateral striated roots. Microtubules in longitudinal aspect are seen on the left (arrow). Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 51,000.
- Figure 116. Coarse electron-dense granules associated with proximal end of basal body and its roots. Fine fibrils (arrow) extend from the granular material to the rodorgan. Fixed with Paramecium fixative. TEM thin section. X 36,600.
- Figure 117. Striated root connects with proximal end of microtubular root which extends along left side of reservoir. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 50,500.
- Figure 118. Oblique section of basal body (arrow) showing connection of striated root to coarsely granular material associated with proximal end of basal body. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 67,200.

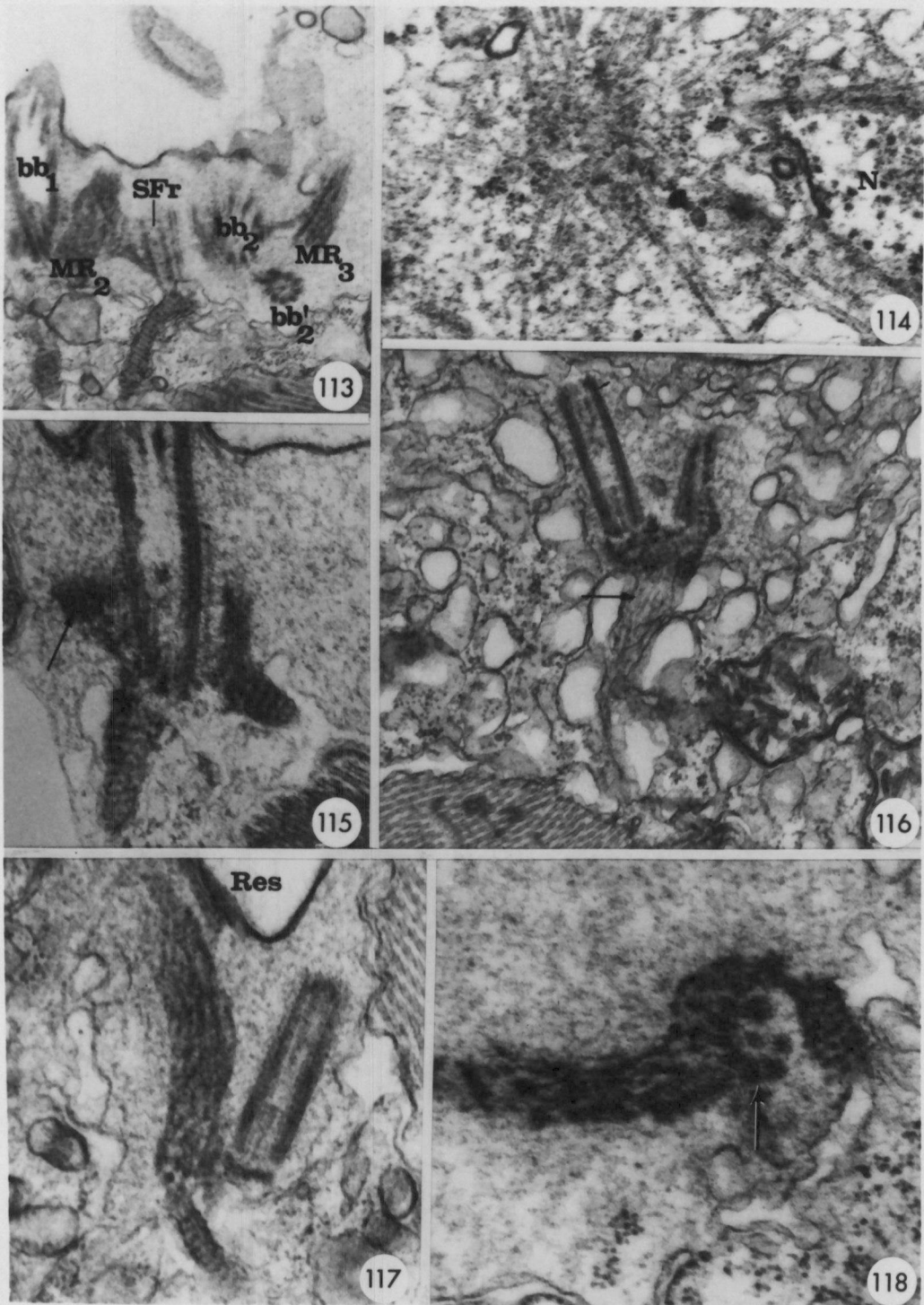


Plate XXV.

the striated roots and the microtubular and flagellar roots are evident in Figures 117 and 118 (Plate XXV). The striated roots extend from these structures to the rodorgan (Plate XXVI, Figure 119).

The striated root may vary from 80-140 nm in diameter throughout its length (Plate XXVI, Figure 120) and is delimited by a membrane which is continuous with elements of endoplasmic reticulum that surround the rodorgan (Plate XXVI, Figures 121-123). Internally the striated root has transverse alternating light and dark bands with a periodicity of 30-38 nm; fine fibrils run perpendicular to the bands (Plate XXVI, Figure 124). More than one fibril or branches of the same fibril appear in some sections (Plate XXVI, Figures 125 and 126); thus the exact number of striated roots in the cell is unknown.

Model of Flagellar Bases and Associated Roots

A diagrammatic representation of the relationships among the components of the flagellar root apparatus is shown in Figure 127 (Plate XXVII). The salient features of the model are summarized as follows: (1) two basal bodies, lying at an angle to each other, are associated with each flagellum; (2) three microtubular roots, with an undetermined number of microtubules in each root, extend anteriorly from the basal body region along the reservoir wall; (3) a striated fiber connects MR_2 to bb_2 ; (4) at least three striated roots extend from the rodorgan and connect with two of the microtubular roots and the striated fiber.

Plate XXVI. Striated roots.

- Figure 119. Outer rod of rodorgan is connected to flagellar base by a striated root similar to the kinetodesmal fibril of ciliates. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 34,900.
- Figure 120. Striated root diameter is not uniform throughout its length. In this section the root broadens at its connection to basal body. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 40,300.
- Figure 121. Striated root is delimited by a single convoluted membrane and arises from dense material adjacent to basal bodies. Portions of a second striated root (arrow) are evident. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 36,600.
- Figure 122. In this section the striated root is frayed into minute fibrils (arrow). The membrane surrounding the root is continuous with common membrane that joins the two rods of rodorgan. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 56,800.
- Figure 123. Oblique section of rodorgan showing continuities of the striated root membrane with membrane surrounding the rodorgan. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 56,800.
- Figure 124. Longitudinal section of two striated roots (or root branches). Each root is 150-200 nm in diameter and has alternating light and dark bands with a periodicity of 30-38 nm. Portions of the lower root diverge from main axis. Note continuities among membranes of two roots and dilated cisterna of endoplasmic reticulum (arrows). Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 105,000.
- Figure 125. Three striated roots (arrows) (or root branches) emanate from the rodorgan shown here in oblique section. Fixed with OsO_4 buffered with sodium cacodylate. TEM thin section. X 37,600.
- Figure 126. Section of branching striated root with five radiating arms (arrows). Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 47,800.

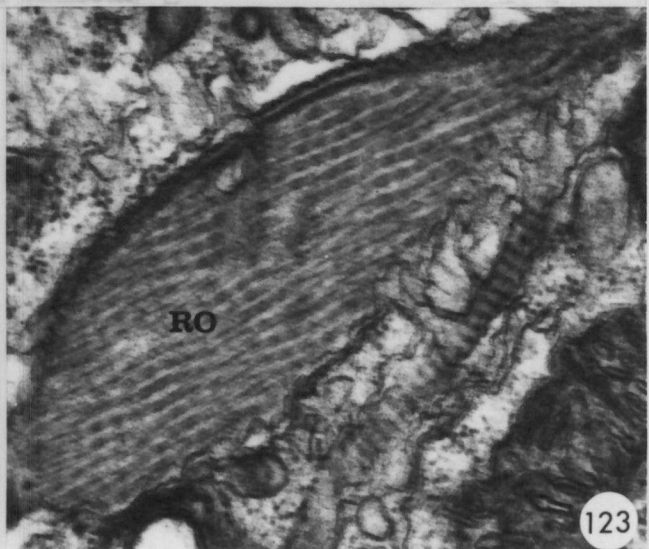


Plate XXVI.

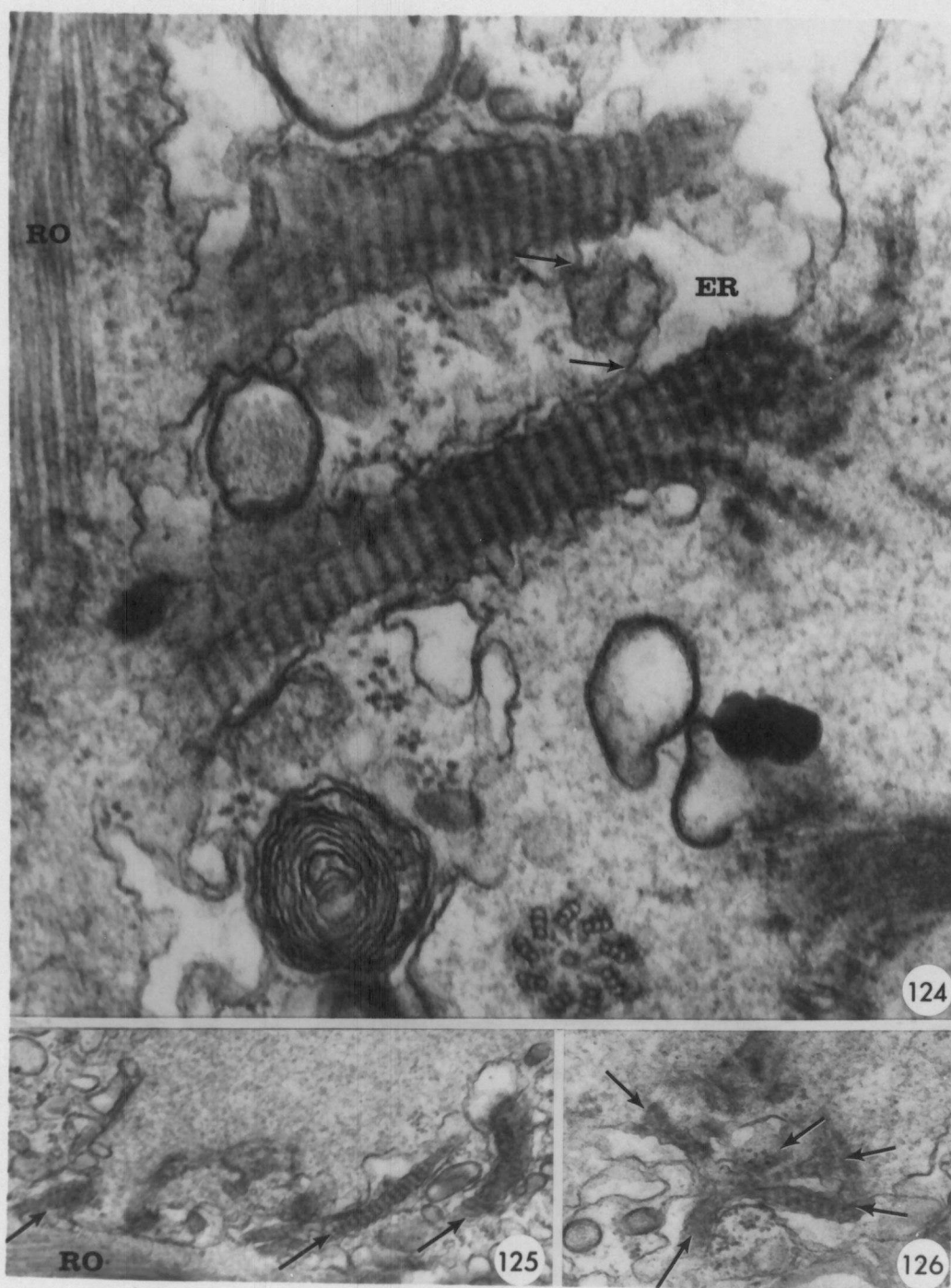
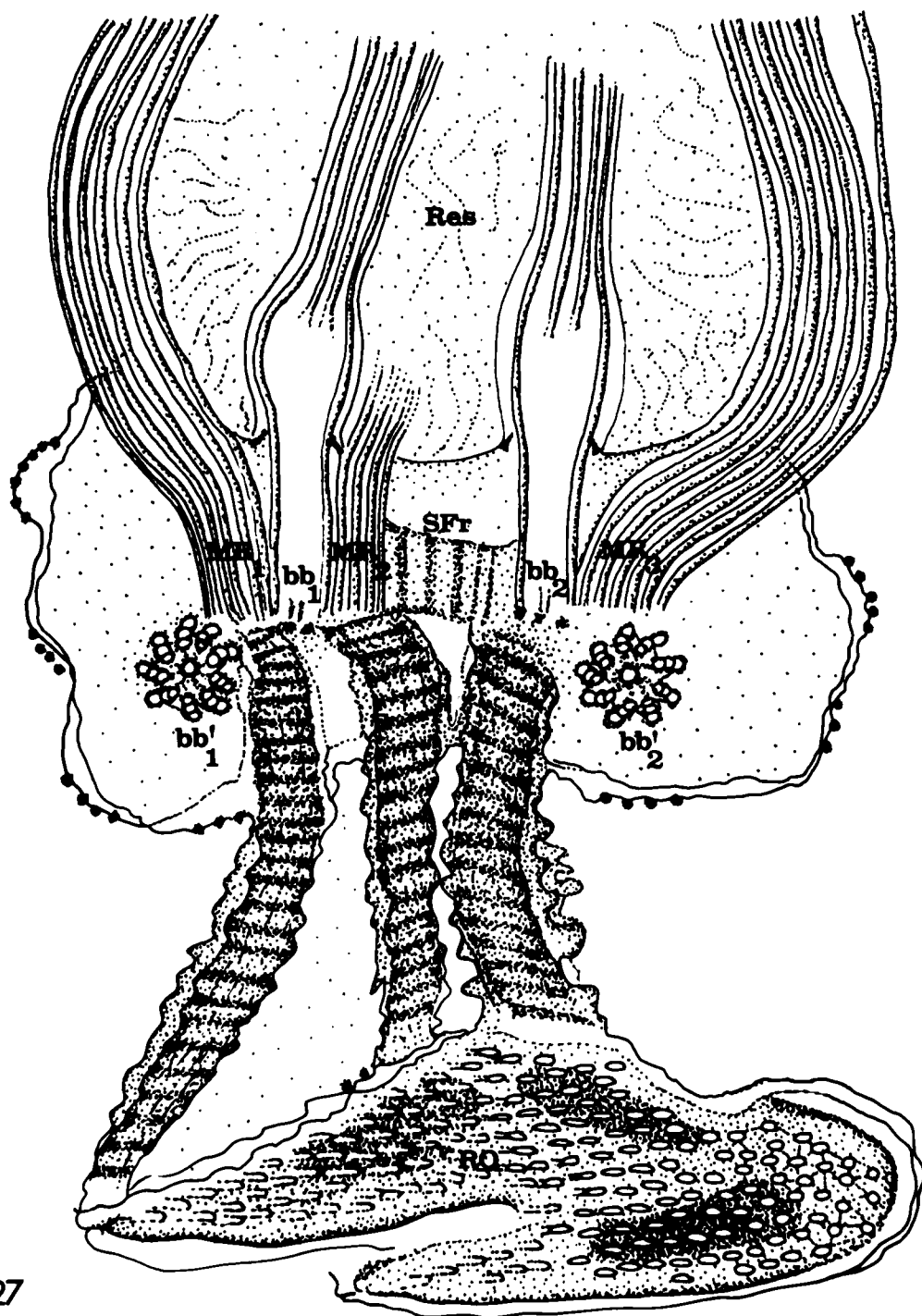


Plate XXVI (Continued).

Plate XXVII. Model of flagellar bases and associated roots.

Figure 127. Diagram illustrating possible arrangement of basal bodies and their microtubular, fibrous and striated roots. Two pairs of basal bodies lie in an area of homogeneous cytoplasm at the base of the reservoir. Each of the two flagella terminates in one member of each basal body pair (bb_1 and bb_2 shown in longitudinal view). Associated with the basal bodies are three microtubular roots each of which extends anteriorly beneath the reservoir membrane. A fibrous root, consisting of electron-dense striations, connects MR2 to bb_2 . Extending from the rodorgan to the basal body region are striated roots. Elements of endoplasmic reticulum are continuous with the membranes surrounding the striated roots and rodorgan.



Contractility of Striated Roots

The striated roots of Peranema are similar in structure (appearance) to rhizoplasts that have been shown to be contractile in other algal flagellates. Salisbury and Floyd (1978) reported that increased extracellular calcium ion concentration induced contraction in the rhizoplast of Platymonas. Since Peranema is routinely grown in a medium with high extracellular calcium ion concentration, it is possible that the striated roots observed in this study are in the contracted state; thus, lowering the calcium ion concentration in the medium could result in alterations in band periodicity indicative of a relaxed state.

The results of studies involving calcium effects on striated roots were inconclusive. Reduced extracellular calcium ion concentrations had no effect on the periodicity of the bands in the root. Cells washed and maintained in distilled water for four days and cells washed with EDTA and left in distilled water overnight had striated roots identical in appearance and dimensions to control cells grown on milk medium with high extracellular calcium.

III. MUCOCYST STRUCTURE AND DEVELOPMENT

Rows of ejectile organelles or mucocysts are located beneath the pellicle. During the transitions from immature or "resting" form to ejected state, the organelles undergo characteristic morphological changes described below.

Immature Mucocysts

In longitudinal view, immature mucocysts are tubular with amorphous contents of low electron density (Plate XXVIII, Figure 128). Small projections with dense tips occur at both ends of the tube. Midway between the tips, the contents infurrow at two electron-dense sites. In transverse section, the organelles are large, single-membrane-bounded vesicles that show continuities with the subpellicular endoplasmic reticulum (Plate XXVIII, Figure 129). Although mucocysts are usually found in longitudinal rows (Plate XXVIII, Figure 130) beneath the pellicle, occasionally immature mucocysts appear near the maturing face of dictyosomes, suggesting a dictyosome-derived origin (Plate XXVIII, Figure 131). Mucocysts are also found in tandem rows parallel to pellicular grooves (Plate XXVIII, Figure 132).

Mature Mucocysts

With incipient infurrowing of the contents, the mature mucocyst assumes an hour-glass configuration and lies within a vesicle (Plate XXIX, Figure 133). In transverse section the mature mucocyst appears as a hollow tube (Plate XXIX, Figure 134). An electron-lucent zone separates the homogeneous contents of varying electron density from the electron-dense granular material at the tips (Plate XXIX, Figures 135-137). Occasionally, the dense tips have knob-like projections that are connected by thin strands to the membrane of the surrounding vesicle (Plate XXIX, Figure 136).

Mucocysts may protrude through the pellicle (Plate XXX, Figure 138) either at the groove (Plate XXX, Figure 139) or along the ridge (Plate XXX, Figure 140) and discharge a ball of mucilaginous material.

Plate XXVIII. Early stages of mucocyst development.

- Figure 128. Longitudinal section of immature mucocyst with homogeneous contents of low electron density. Tube narrows markedly at each end into a small projection with electron-dense granular material at the tips (only one projection appears in this plane of section). Infurrowing of the contents is initiated at two electron-dense sites at the "waist" or middle of the tube. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 63,700.
- Figure 129. Transverse section of immature mucocysts shows membrane-bound structures surrounded by vesicle continuous with endoplasmic reticulum (arrow). Fixed with Paramecium fixative. TEM thin section. X 36,000.
- Figure 130. Row of subpellicular immature mucocysts with incipient infurrowing in longitudinal view. Fixed with Paramecium fixative. TEM thin section. X 28,500.
- Figure 131. Dictyosomes in close association with immature mucocysts. Fixed with Paramecium fixative. TEM thin section. X 36,600.
- Figure 132. Glancing section showing two immature mucocysts in tandem beneath the pellicle. Fixed with Paramecium fixative. TEM thin section. X 36,600.

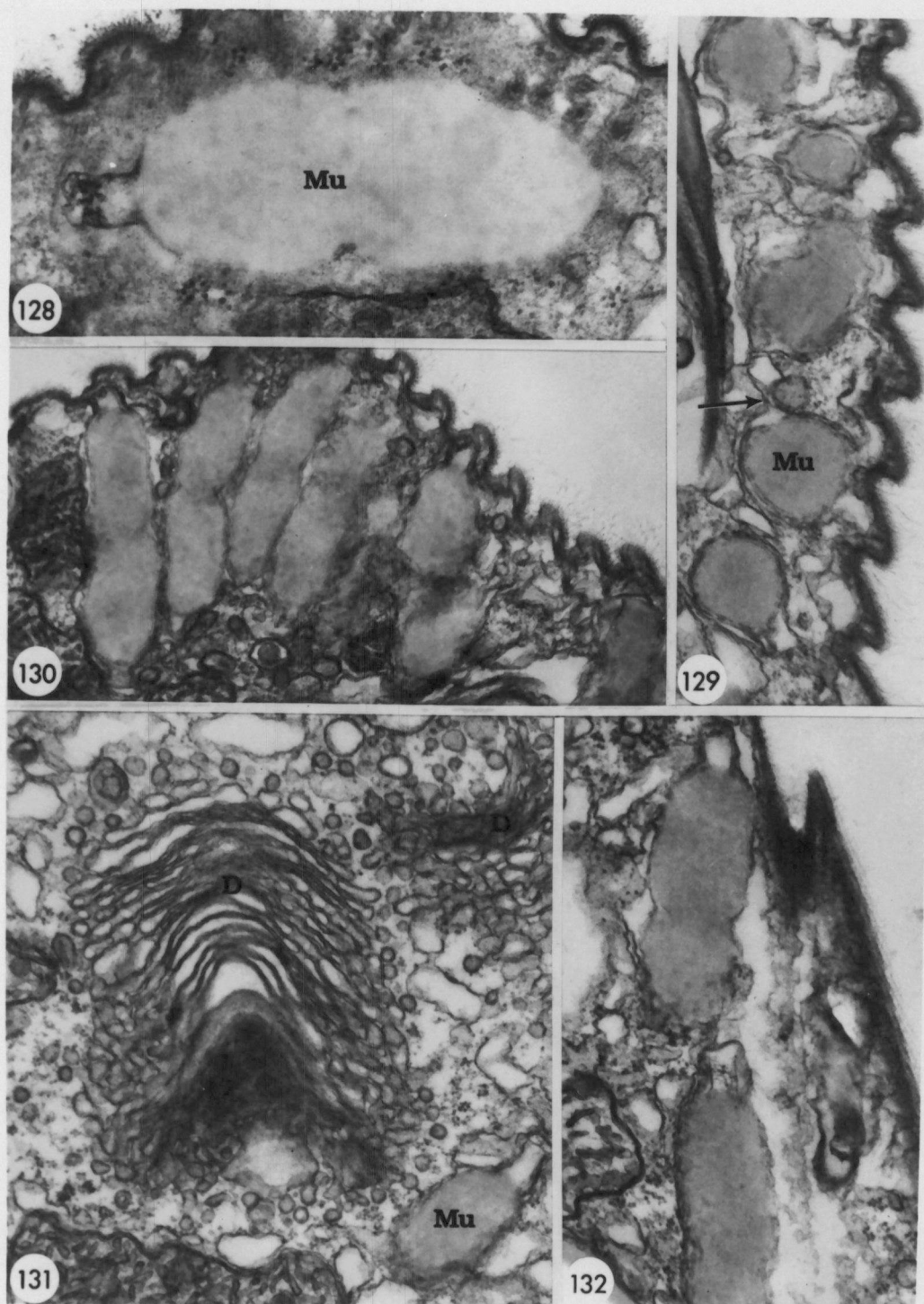


Plate XXVIII.

Plate XXIX. Later stages of mucocyst development.

- Figure 133. Longitudinal section of immature mucocyst on left and one in later stages of development on right. The latter is hour-glass shaped and lies within a vesicle. An electron-lucent zone separates the homogeneous contents from the electron-dense tips. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 52,100.
- Figure 134. Transverse section of mature mucocyst shows hollow tube enclosed within a membrane-bounded vesicle. Fixed with glutaraldehyde/ OsO_4 . TEM thin section. X 38,200.
- Figure 135. Contents of mature mucocysts may appear highly electron dense, contrasting sharply with the more electron-lucent ends. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 53,400.
- Figure 136. Longitudinal section of two adjacent mucocysts, an immature one on right and a mature one on left. Tip of mature mucocyst has knob-like projections connected by thin strands to membrane of surrounding vesicle (arrow). Fixed with Paramecium fixative. TEM thin section. X 48,200.
- Figure 137. Oblique section shows orderly array of subpellicular mature mucocysts. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 38,700.

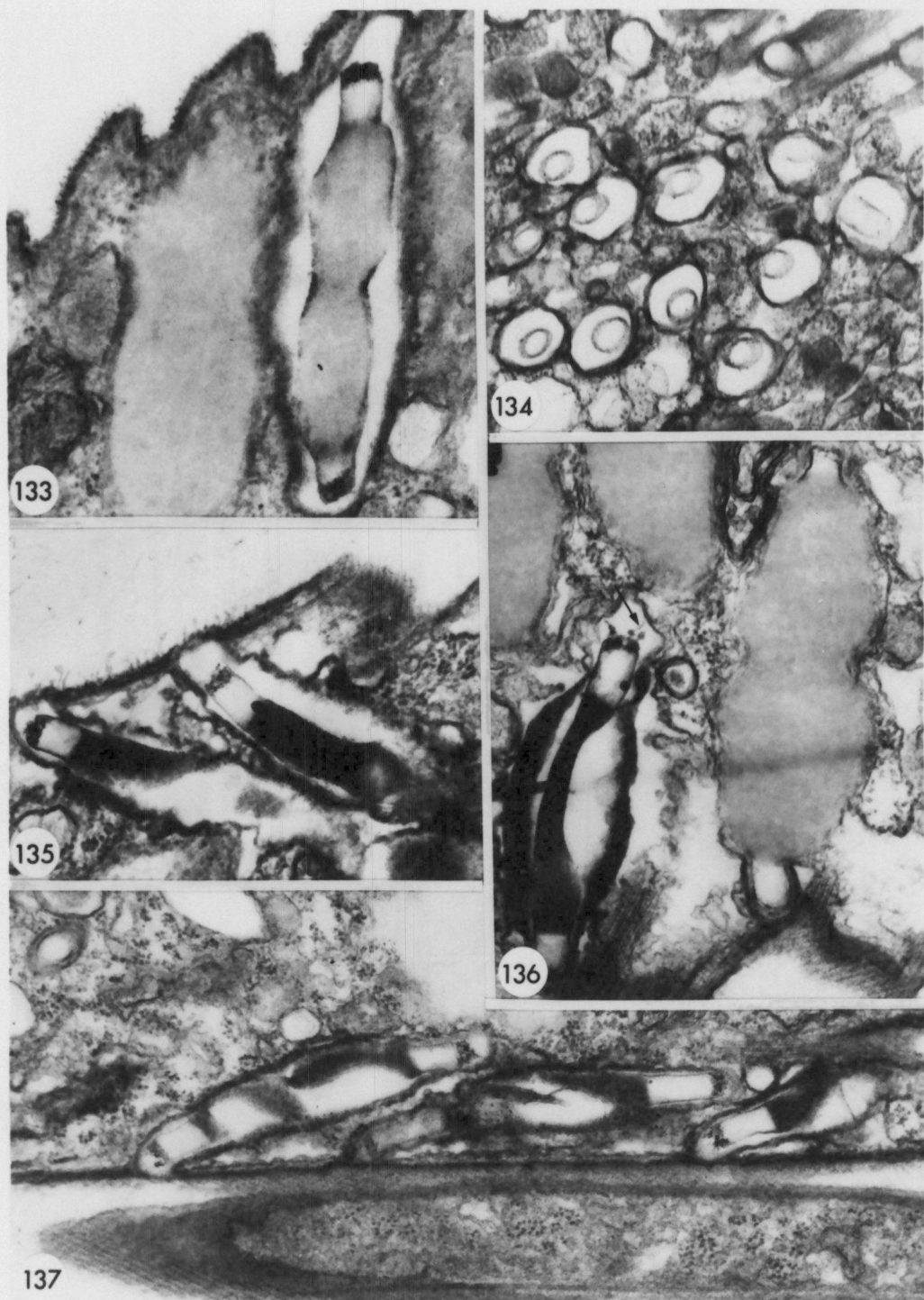


Plate XXIX.

Plate XXX. Discharge of mucilaginous material from mucocysts.

- Figure 138. Oblique section of pellicle showing one end of mucocyst protruding through pellicle and plasma-lemma, with discharged bubble of mucilaginous material. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 124,000.
- Figure 139. Mucocyst protrudes through groove between two pellicular striae; other end of mucocyst within cell is seen in oblique view. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 48,200.
- Figure 140. Oblique section of pellicle showing mucocyst protruding through top of pellicular ridge, with discharged material. Electron-dense material surrounds apical rim of mucocyst. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 48,200.
- Figure 141. Longitudinal section of discharged mucocyst with bubble of mucilaginous material at one end. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 63,700.
- Figure 142. Negative-stain preparation of discharged mucocyst with bubble of mucilaginous material at one end. Unfixed. TEM. Negative-stain with 1% aqueous uranyl acetate. X 84,400.

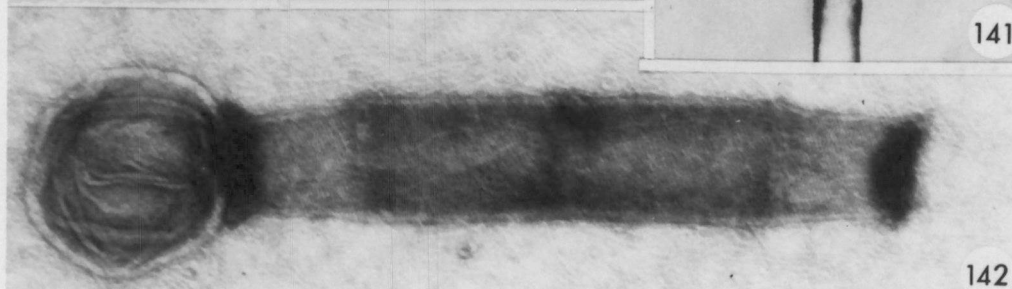
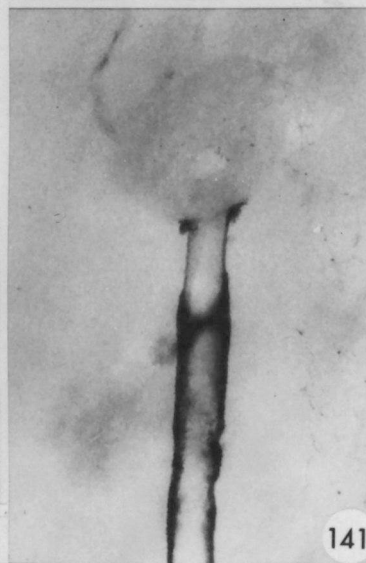
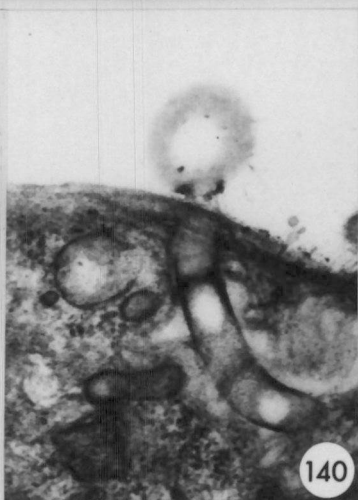
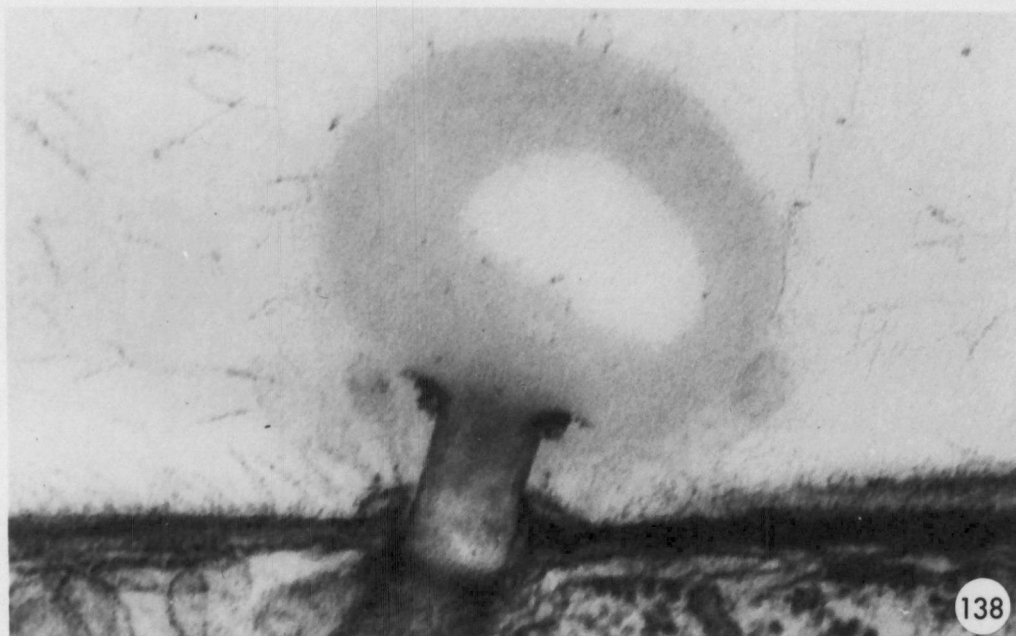


Plate XXX.

This phenomenon is particularly evident when cells are fixed in the cold, a result suggesting that cold shock may be a stimulus for discharge. Tubular mucocysts outside the cell, in both thin sections (Plate XXX, Figure 141) and negative-stain preparations (Plate XXX, Figure 142), also show discharged mucilage at only one end, never at both ends.

Ejected Mucocysts

Mucocysts that subtend the pellicular articulations (Plate XXXI, Figure 143) may be ejected from the cell through the grooves (Plate XXXI, Figure 144). In contrast to the heterogeneous appearance and size of their intracellular counterparts, discharged mucocysts are uniform in structure in thin sections (Plate XXXI, Figures 145 and 146), shadow-cast (Plate XXXI, Figure 147) and negative-stain preparations (Plate XXX, Figure 142); in all these preparations they can be recognized by their regular tubular form, helical striations and characteristic dense middle band.

Details of mucocyst fine structure are most evident in negative-stain preparations (Plate XXXII, Figures 148-150). The mucocyst consists of three distinct regions: (1) an inner tube 1.2 μm long and 0.18 μm wide with helical striations; (2) an outer tube 0.77 μm long and 0.21 μm wide with diamond-shaped pattern; and (3) a dense middle band 0.07 μm wide. A crown of dense material may occur at the tips of the mucocyst (Plate XXXII, Figure 149). Fine fibrils 8-9 nm in diameter are associated with mucocysts and appear to emanate from their tips (Plate XXXII, Figure 148). They are similar in size and appearance to the strands that run parallel

Plate XXXI. Structure of discharged mucocysts.

- Figure 143. Oblique section of cell periphery shows array of mucocysts lying in pellicular grooves. Fixed with Paramecium fixative. TEM thin section. X 38,200.
- Figure 144. Negative-stain preparation shows array of overlapping mucocysts that have been discharged from cell. Unfixed. TEM. Negative-stain with 2% PTA. X 38,200.
- Figure 145. Thin section of ejected mucocyst with one end still within pellicular groove; note the tubular shape and characteristic electron-dense middle band (arrow). Fixed with glutaraldehyde/OsO₄. TEM thin section. X 80,900.
- Figure 146. Longitudinal and oblique sections of discharged mucocysts showing electron-dense tips, dark middle band and outer jacket with helical striations. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 80,900.
- Figure 147. Shadow-cast preparation of discharged mucocyst shows helical striations on outer jacket. Unfixed. TEM. Shadow-cast with Au/Pd at 5°. X 48,400.

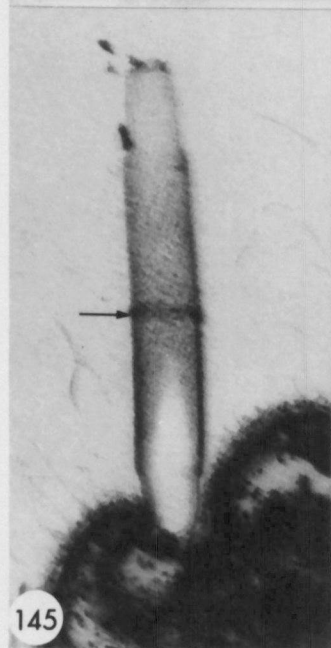
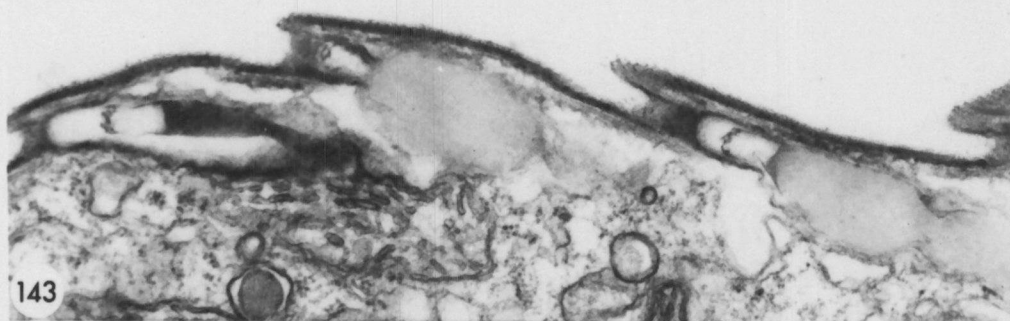


Plate XXXI.

Plate XXXII. Details of mucocyst fine structure.

- Figure 148. Inner tube of mucocyst measures $1.2\ \mu\text{m}$ long and $0.18\ \mu\text{m}$ wide; outer tube measures $0.77\ \mu\text{m}$ long and $0.21\ \mu\text{m}$ wide; electron-dense middle band is $0.07\ \mu\text{m}$ in width. Helical striae of inner tube are traversed by minute strands parallel to long axis of tube (arrow). Numerous fine fibrils $8\text{--}9\ \text{nm}$ in diameter emanate from mucocyst tip. Unfixed. TEM. Negative-stain with 1% uranyl acetate. X 110,200.
- Figure 149. Face view of mucocyst tip shows crown of electron-dense material. Outer jacket has diamond-shaped pattern. Unfixed. TEM. Negative-stain with 1% uranyl acetate. X 110,200.
- Figure 150. Minute longitudinal strands of inner tube are absent in lower portion of tube, thus making helical striations more readily apparent. Unfixed. TEM. Negative-stain with 1% uranyl acetate. X 99,900.

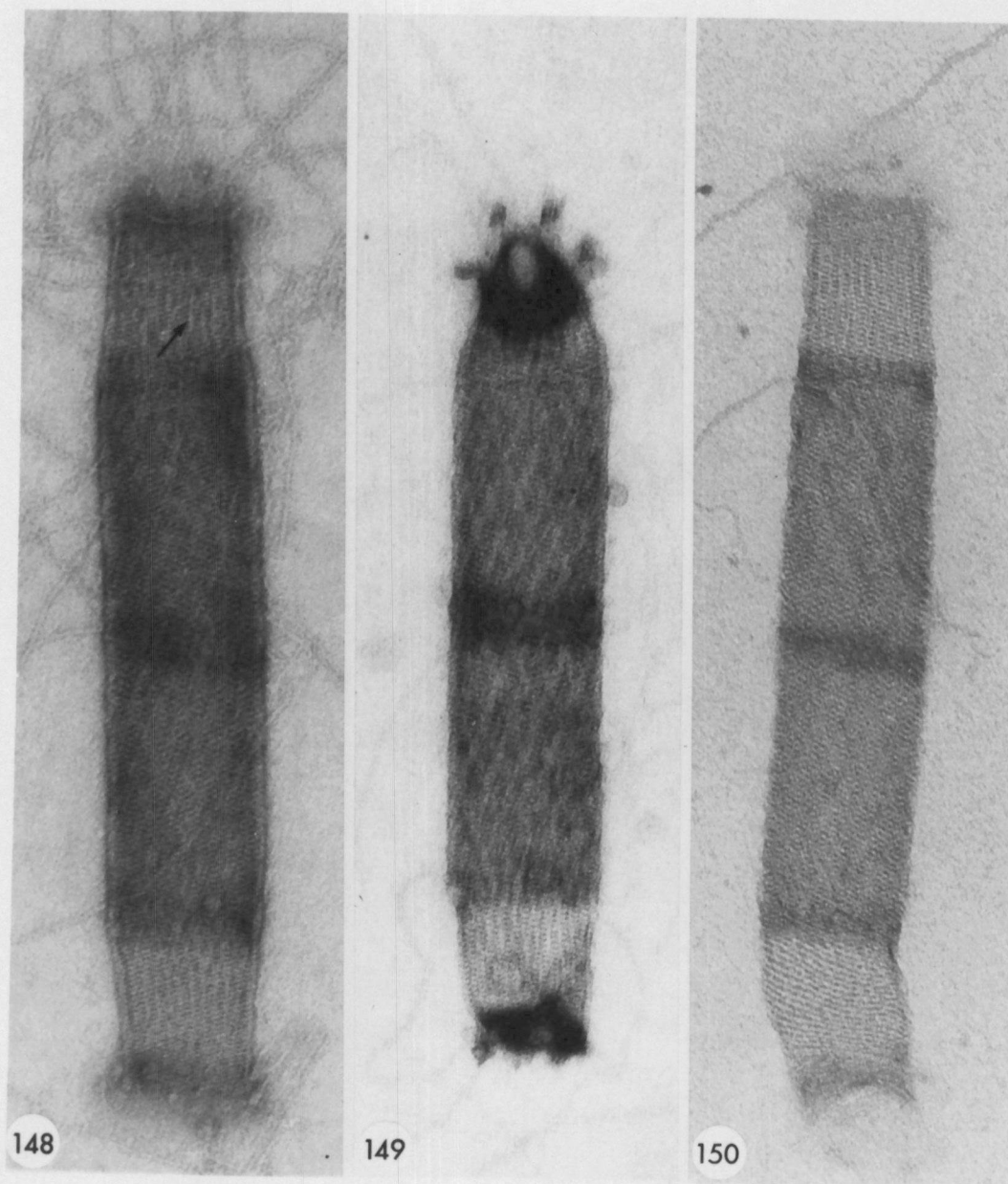


Plate XXXII.

to the long axis of the inner tube and may thus be structural components of the organelle.

IV. PHAGOCYTOSIS

Fine Structure of Food Vacuoles and Autophagosomes

Although Peranema is a voracious phagotroph that ingests a wide variety of food particles, the feeding process is difficult to observe with the light microscope due to the organism's contortive euglenoid movement during engulfment of prey. During feeding the rodorgan is inflexible and moves as a unit backward and forward in the canal opening. Carmine particles, heat-killed yeast cells (Plate XXXIII, Figures 151 and 152), latex beads (Plate XXXIII, Figure 153), and fungal spores are engulfed whole and appear intact inside food vacuoles. Prey such as nonliving Peranema and Euglena acus cells are sometimes first broken open by a rasping motion of the rodorgan over the prey surface; the cellular contents are then engulfed in less than 10 minutes. Since Peranema is cannibalistic, autophagosomes (Plate XXXIII, Figures 154 and 155) and food vacuoles containing ingesta from cannibalism (Plate XXXIII, Figure 156) are often indistinguishable.

Surface View of Ingestion

Occasionally, Peranema will cannibalize another cell and engulf the organism whole (Plate XXXIV, Figures 157 and 158). The canal region greatly expands to accommodate the incoming ingesta. Whole yeast cells (Plate XXXIV, Figure 159) and unidentified particles (Plate XXXIV, Figure 160) may also be engulfed. Many individual

Plate XXXIII. Food vacuoles and autophagosomes.

- Figure 151. Cell that was fed heat-killed yeasts for 20 minutes shows numerous food vacuoles containing the ingested yeasts in different stages of digestion. One vacuole contains both an intact yeast cell and one that has been partially digested (arrow). Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 4800.
- Figure 152. Two vacuoles containing yeast; fibrillar components may represent elements of the decomposing yeast wall. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 13,800.
- Figure 153. Vacuole containing latex bead ca. 2 μm in diameter. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 29,600.
- Figure 154. Presumptive autophagosome with extension of cytoplasm into interior of organelle. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 17,200.
- Figure 155. Autophagosome containing multivesiculate electron-dense material and smaller vesicle with cytoplasmic elements. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 18,300.
- Figure 156. Large food vacuole containing fragments from another *Peranema* cell that has been ingested. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 4700.

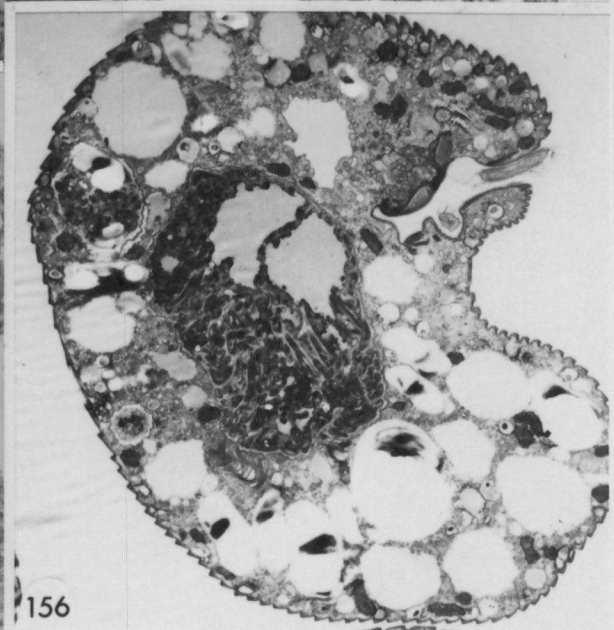
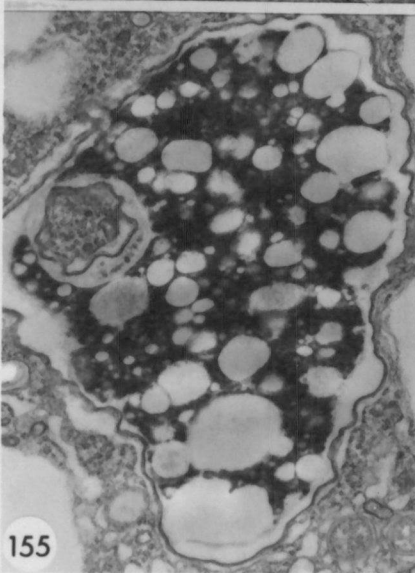
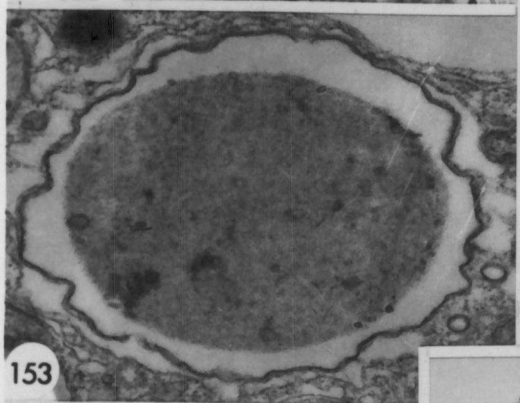
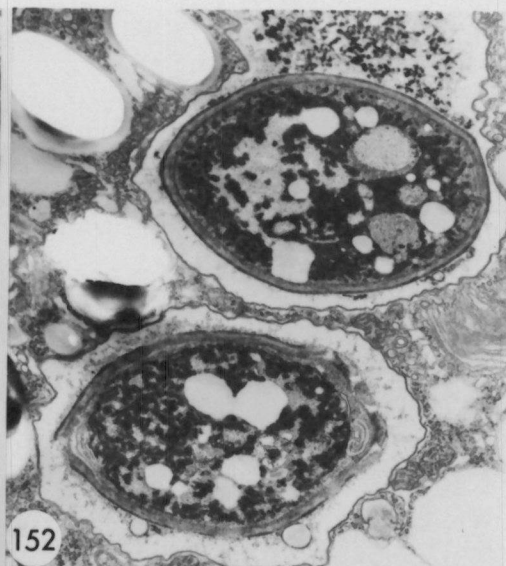
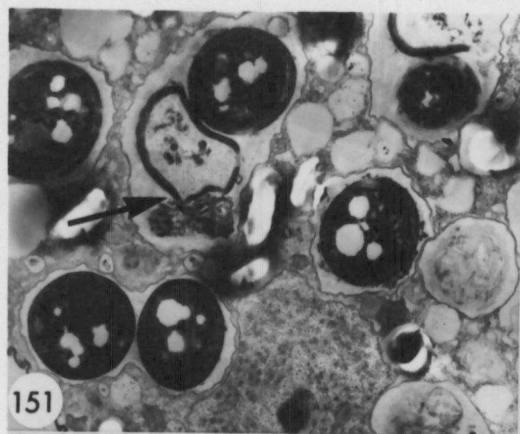


Plate XXXIII.

Plate XXXIV. Scanning electron microscopy of phagocytosis.

- Figure 157. Cannibalism in Peranema, showing engulfment of entire shriveled, dead organism by a predatory cell. Flagella of other Peranema cells are in contact with the predator. Fixed with OsO₄/glutaraldehyde. CPD. SEM. X 2100.
- Figure 158. Anterior view of predator shown in Figure 157 showing canal opening greatly expanded to accommodate the entrance of the ingested cell. Fixed with OsO₄/glutaraldehyde. CPD. SEM. X 4000.
- Figure 159. Ingestion of yeast cell by Peranema showing expanded canal region and anterior flagellum. Fixed with OsO₄. CPD. SEM. X 9800.
- Figure 160. Engulfment of unknown particle by Peranema. Fixed with OsO₄/glutaraldehyde. CPD. SEM. X 4600.

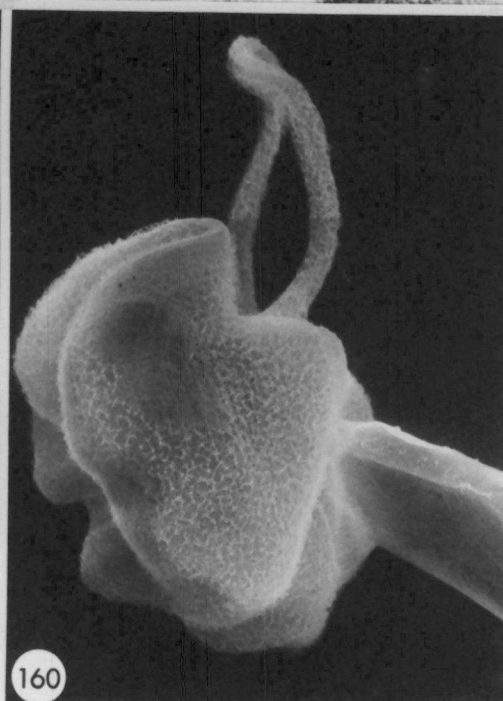
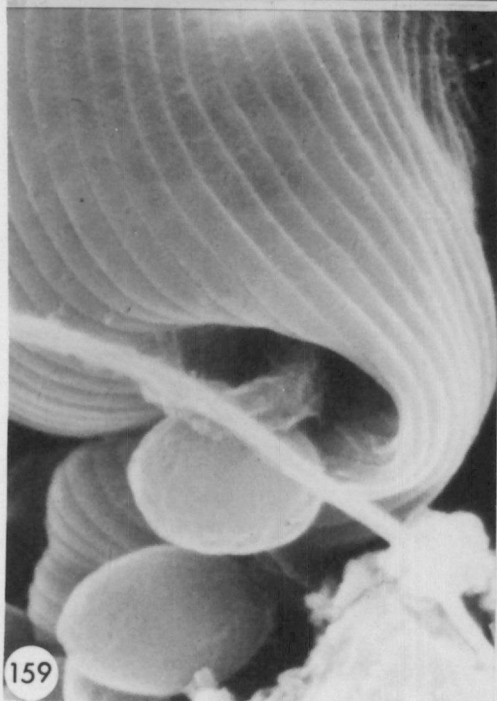
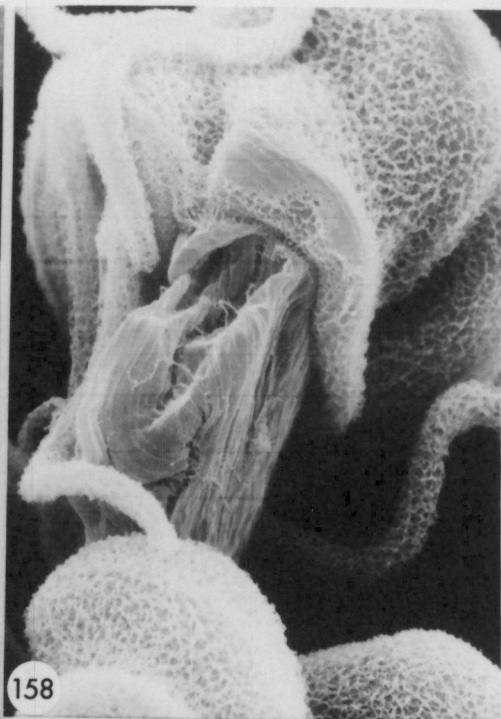
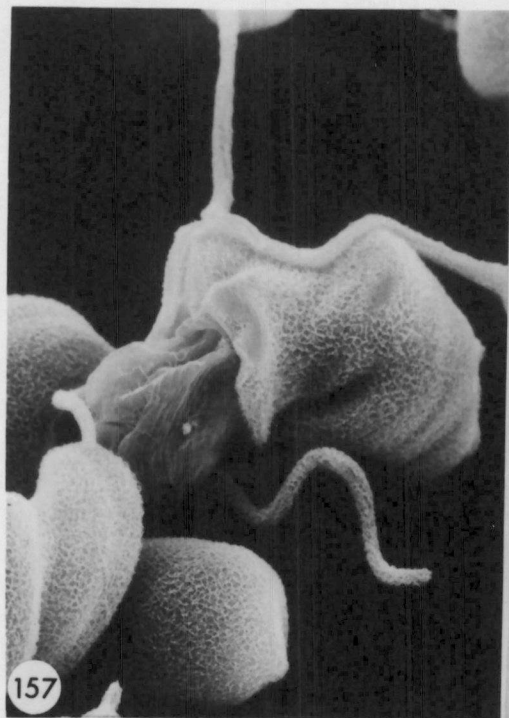


Plate XXXIV.

cells are usually assembled around feeding sites, suggesting the existence of chemotactic irritability in Peranema. Although Chen (1950) reported egestion of indigestible material through an "area of defaecation," this phenomenon was not observed in the present study.

Colchicine Effects

Colchicine, a plant alkaloid that disrupts microtubules through its ability to bind with and prevent polymerization of tubulin (Wilson et al., 1974), had no effect on phagocytosis or motility in Peranema. Cells that were exposed to concentrations ranging from 1.5×10^{-6} to 1.0×10^{-2} M for 102 hours remained viable, motile and able to ingest yeast cells.

V. FEEDING APPARATUS

The capability of Peranema to ingest particulate food is made possible by an elaborate movable feeding apparatus that consists of the rodorgan and associated lamellar structures. The ultrastructure of these ingestion organelles will be described below using the terminology of Nisbet (1974).

Rodorgan

At its basal portion, the rodorgan consists of two separate outer and inner rods, the outer rod being nearer the cell surface and the inner rod being in a more central position (Plate XXXV, Figure 161). At their distal ends, the two rods are connected, forming a single blunt-ended structure which separates the cavities of the reservoir and cytostome (Plate XXXV, Figure 162).

Plate XXXV. Structure of the rodorgan.

- Figure 161. Basal portion of rodorgan showing outer and inner rods and their relative position to reservoir base. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 17,900.
- Figure 162. At their distal ends, the two rods are united by a bridge connection into a single structure surrounded by a thick sheath. Microtubules, seen here in oblique section, occur both in the rods and in the bridge connection at this level. Fixed with Paramecium fixative. TEM thin section. X 31,200.
- Figure 163. Basal portion of rodorgan shows that each rod contains 160-200 microtubules interconnected by fine fibrils and embedded in a matrix of electron-dense and electron-lucent areas. Longitudinal lamellae border on one side of each rod, one band curving out of a deep groove in the rod matrix. Surrounding each rod are enlarged cisternae of endoplasmic reticulum. Fixed with unbuffered OsO_4 . TEM thin section. X 75,800.
- Figure 164. Longitudinal section of the outer rod showing its anterior connection with outer anchoring lamella, from which several other lamellae diverge. Microtubules terminate in the distal margin of the rod. One element of the longitudinal lamellae curves out of the groove and away from the rod (arrow). If transverse sections of the rod were taken at levels a and b, a change in the relative proportions of the matrix and a redistribution of the microtubules would result, as shown in Figure 165 and 163 respectively. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 10,300.
- Figure 165. Transverse section of rodorgan corresponding to level a of Figure 164 shows that most of the microtubules are confined to the electron-lucent core and to the periphery of the electron-dense area. The longitudinal lamellae show no direct connection to the rods at this level. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 60,300.

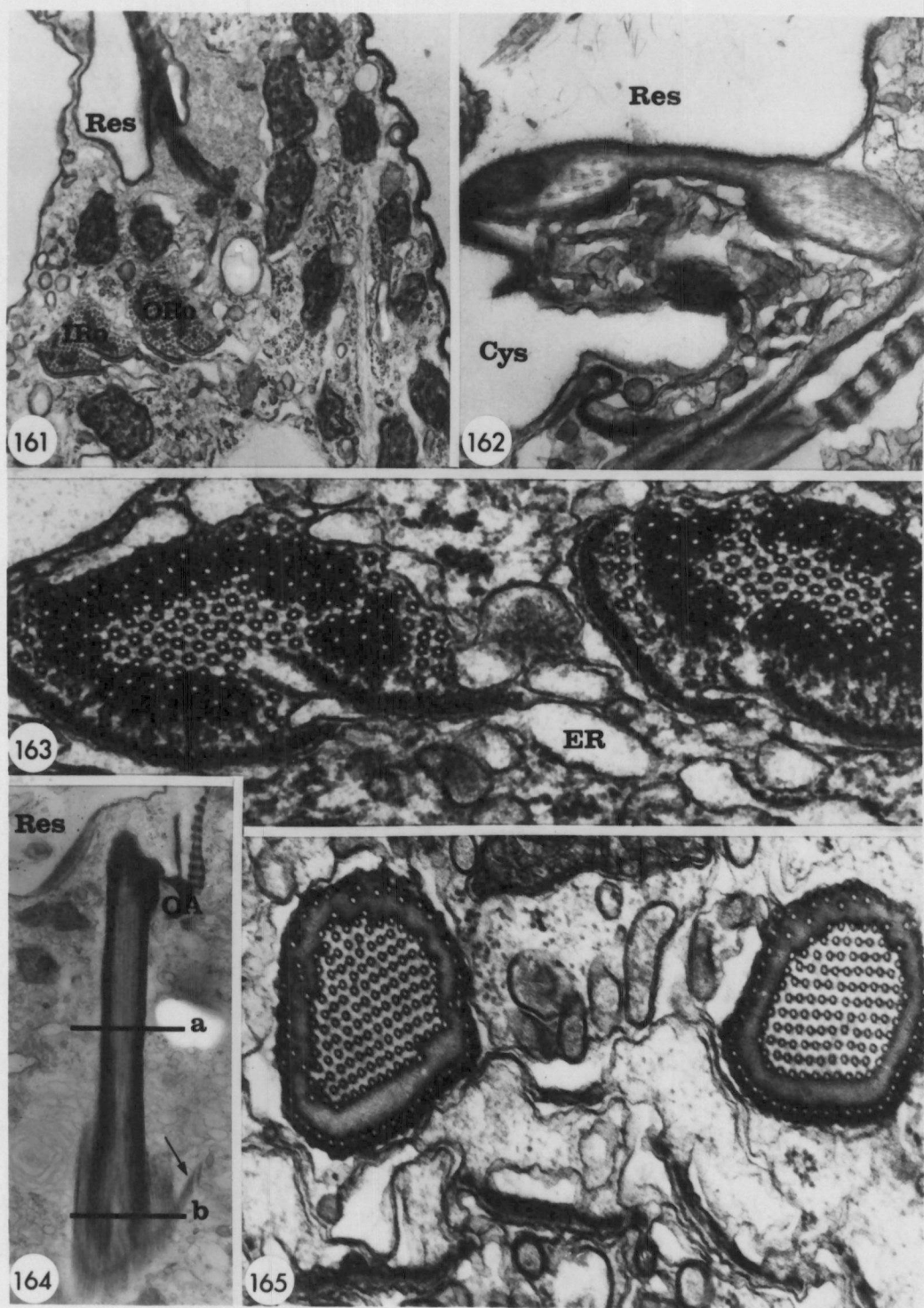


Plate XXXV.

Each rod lies in an enlarged cisterna of endoplasmic reticulum and contains 160-200 microtubules interconnected by fine fibrils and embedded in a matrix of varying electron density (Plate XXXV, Figure 163). Transverse sections of the rodorgan at different levels show a change in the matrix and a redistribution of the microtubules (Plate XXXV, Figures 163-165). A transverse section near the base of the rods shows a thick sheath bordering the grooved side of each rod; microtubules occur in irregular rows in both matrices (Plate XXXV, Figure 163). A more distal section shows the thick sheath encircling each rod; the grooved region is not present (Plate XXXV, Figure 165). Microtubules occur in rows in the electron-lucent matrix but are confined to the periphery of the denser matrix.

Longitudinal Lamellae

In the grooved basal end of each rod, the rod sheath splits into two sheet-like bands and forms the longitudinal lamellae (Plate XXXVI, Figure 166). The lamellae diverge from the rods and occur as separate structures in more distal sections (Plate XXXVI, Figure 167). Oblique sections of the lamellae show a honeycomb appearance (Plate XXXVI, Figure 168), with each fenestration in the honeycomb apparently connected to a small cytoplasmic vesicle (Plate XXXVI, Figure 169). At their distal ends, the longitudinal lamellae curve toward the rim of the cytostome (Plate XXXVI, Figure 170).

Canal and Reservoir

A short anterior canal lined with pellicular striae serves as a common opening for both the reservoir and cytostome. Encircling the canal are two concentric rings of different densities with the denser

Plate XXXVI. Longitudinal lamellae.

- Figure 166. In the grooved region near the basal end of each rod, two segments diverge from the rod sheath and form sheet-like longitudinal lamellae. The two sheets connected with the outer rod form the outer longitudinal lamellae; the two with the inner rod form the inner longitudinal lamellae. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 33,300.
- Figure 167. The longitudinal lamellae curve away from the rod bases and become separate structures. Note the "honeycomb" areas in the oblique sections of the lamellae. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 26,900.
- Figure 168. Higher magnification of "honeycomb" areas in obliquely sectioned longitudinal lamella. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 86,000.
- Figure 169. Fenestrations of the "honeycomb" are connected to small cytoplasmic vesicles (arrows). Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 51,100.
- Figure 170. At the distal end, the inner longitudinal lamellae (arrows) curve in toward the rim of the cytostome. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 53,400.

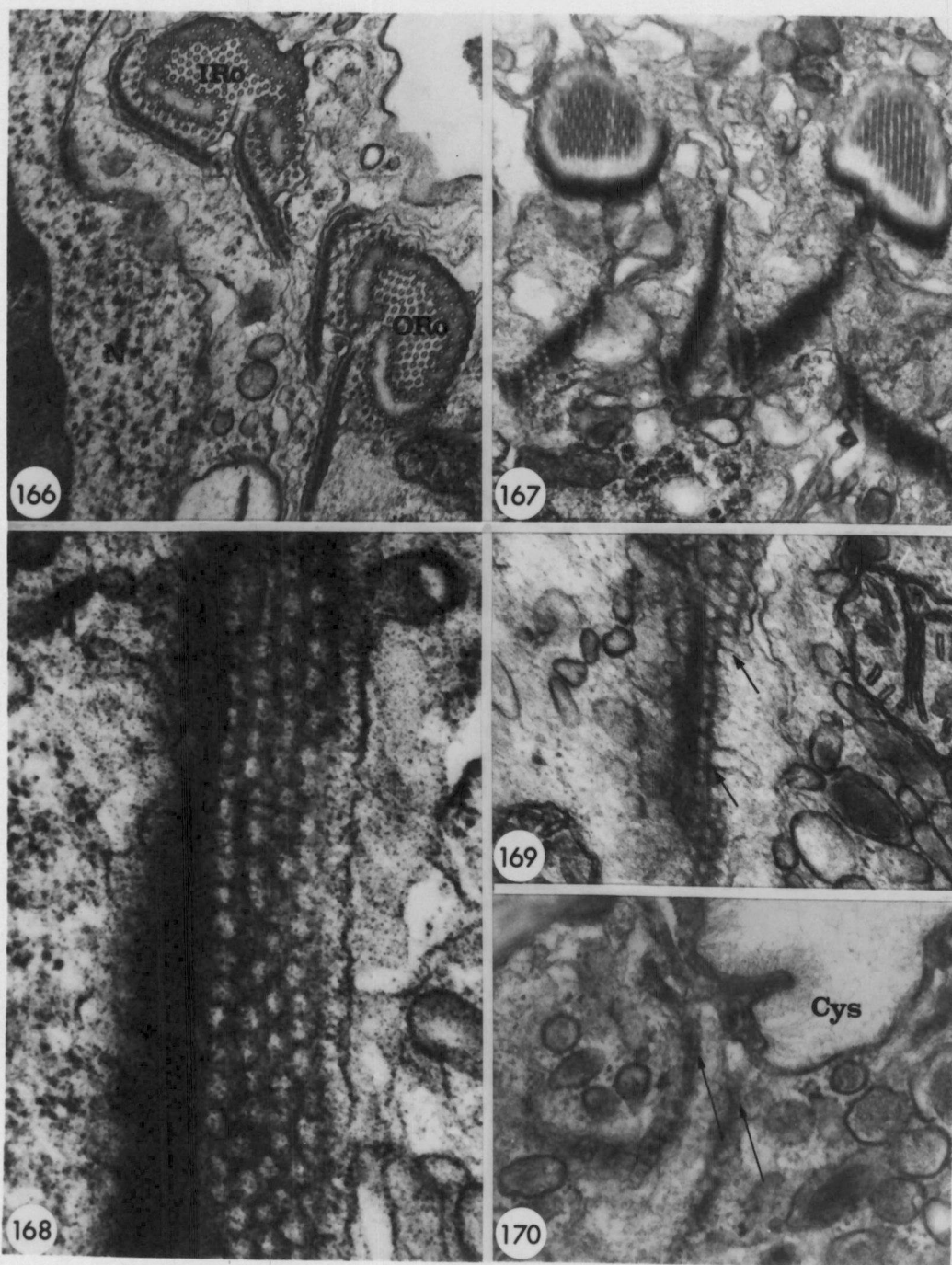


Plate XXXVI.

layer toward the pellicle (Plate XXXVII, Figure 171). At the proximal end of the canal, a cytoplasmic ridge containing the rodorgan separates the reservoir from the cytostome (Plate XXXVII, Figures 172-176). The thick rod sheath extends laterally, forming two protuberances into the cytostome (Plate XXXVII, Figures 172-174). During feeding the cytostome is capable of enlarging to accommodate the incoming solid ingesta (Plate XXXVII, Figure 175).

A striated fibril 140 nm wide with transverse striations of 20 nm periodicity lies beneath the "left" wall of the reservoir (Plate XXXVIII, Figures 177-180). In transverse sections the fibril is partially surrounded by an enlarged cisterna of endoplasmic reticulum (Plate XXXVIII, Figure 178). On either side of the fibril lies a row of microtubules, one row interlinked to a narrow electron-dense structure which separates the fibril from the reservoir membrane (Plate XXXVIII, Figures 178 and 179).

Lamellar Components of Cytostome

A complex array of lamellae arches over the rim of the cytostome and connects structures from the outer and inner rods (Plate XXXIX, Figures 181 and 182). The outer rod is joined by a fibrous connection to an outer anchoring lamella which diverges into two structures: (1) an upper structure of variable appearance, either differentiated into nodes (Plate XXXIX, Figures 181 and 182) or a double serrated marginal lamella (Plate XL, Figure 183); and (2) a lower articulating lamella (Plate XL, Figures 183-186). The latter structure consists of two dense plates which connect to an inner dense body beneath the left wall of the reservoir (Plate XXXIX, Figure 182) and to an outer

Plate XXXVII. Canal and cytostome.

- Figure 171. Longitudinal section of anterior canal showing pellicular striae lining canal wall and concentric thickenings encircling canal opening (arrows). Canal thickenings consist of two layers of varying electron densities with the denser layer toward the pellicle. In transverse section (insert, X 17,200), the thickenings are shown to form an incomplete ring around the canal opening. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 22,000.
- Figure 172. The canal serves as a common opening for the reservoir and cytostome. Inner dense body and inner anchoring lamella are shown near left wall of reservoir. Portions of rodorgan and outer anchoring lamellae are associated with cytostomal area. Lateral extensions of the thick sheath surrounding each rod form two protuberances (arrows) into the cytostome. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 17,200.
- Figure 173. At its distal end, the rodorgan lies within a cytoplasmic ridge which separates the reservoir from the cytostome. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 24,700.
- Figure 174. Transverse section showing the cytoplasmic ridge containing the rodorgan. Note the expanded cytostome (cf. Figures 172 and 173). Fixed with Paramecium fixative. TEM thin section. X 20,400.
- Figure 175. Section showing organism in act of cannibalism. Reservoir is displaced to left and cytostome is expanded to accommodate incoming ingesta. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 6400.
- Figure 176. Oblique section of anterior end showing portions of distal end of rodorgan within the reservoir/cytostome region. No microtubules are evident within the dense margins of the rodorgan (cf. Figure 164). Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 14,100.

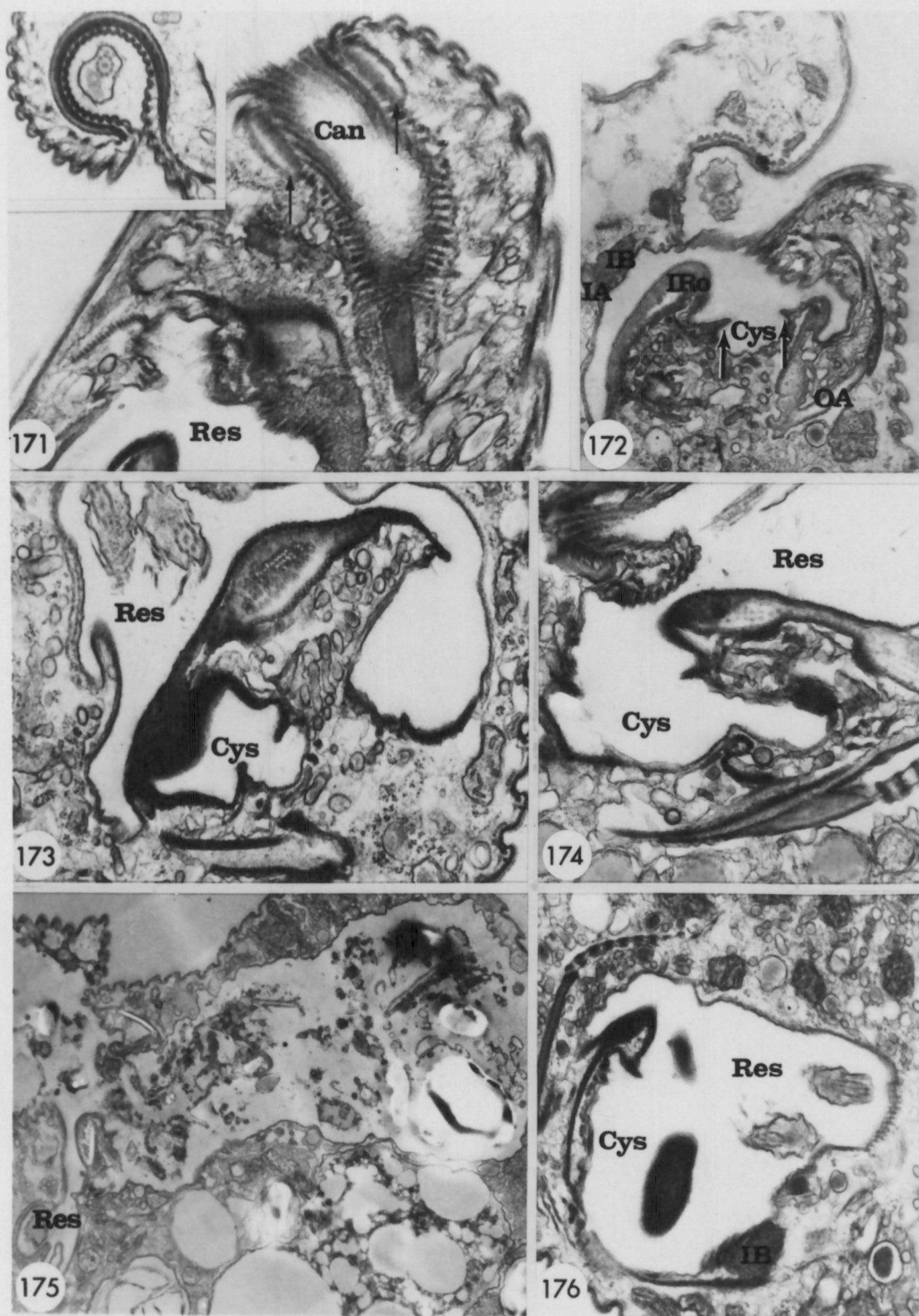


Plate XXXVII.

Plate XXXVIII. Striated fibril in reservoir wall.

- Figure 177. Striated fibril runs parallel with left wall of reservoir, extending from canal toward base of reservoir. Fixed with glutaraldehyde/OsO₄. TEM thin section. X 16,200.
- Figure 178. Transverse section shows fibril as dense ovoid structure partially surrounded by enlarged cisterna of endoplasmic reticulum. A row of microtubules flanks each side of fibril, four tubules on right and eight on left. Narrow electron-dense structure (arrow) separates reservoir membrane and fibril and interconnects row of four microtubules. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 93,000.
- Figure 179. In longitudinal aspect, the fibril is ca. 140 nm wide and has transverse striations with a periodicity of ca. 20 nm. Electron-dense structure (arrow) lies between reservoir membrane and fibril and corresponds to the structure that lies between membrane and fibril shown in Figure 178. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 101,600.
- Figure 180. Longitudinal section of fibril and oblique section of adjacent row of microtubules. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 99,900.

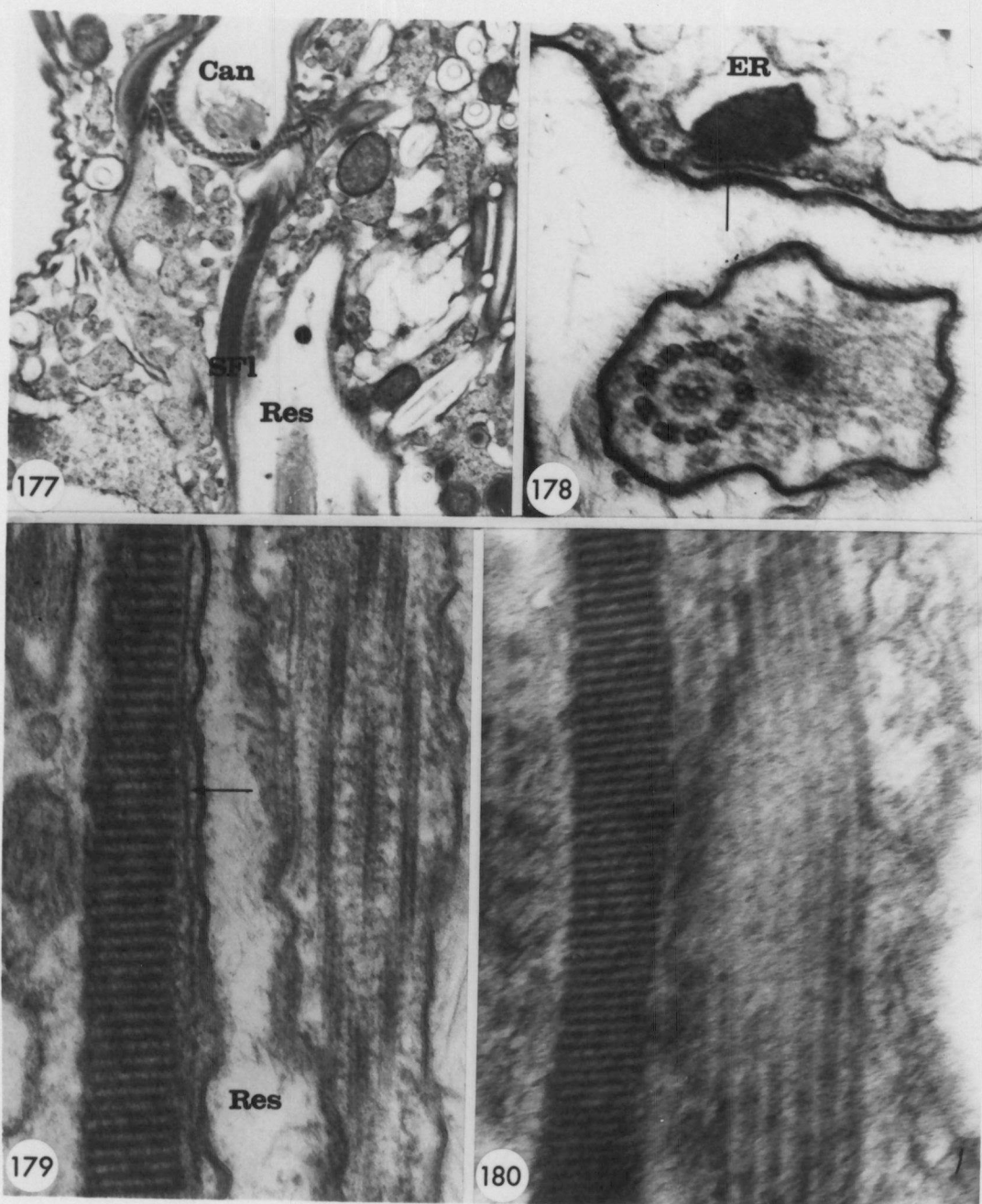


Plate XXXVIII.

Plate XXXIX. Cytostome-associated lamellae.

- Figure 181. The outer rod, shown in oblique section, joins with the outer anchoring lamella which diverges into two structures: the top structure, differentiated into nodes or joints at one level, is layered next to the double serrated marginal lamella; the bottom structure is the articulating lamellae. A thick sheath is continuous between the two rods. Fixed with glutaraldehyde/OsO₄. TEM thin section. X 24,100.
- Figure 182. At a different level in the same cell shown in Figure 181, the fibrous connection (arrow) between the outer rod and the outer anchoring lamella is evident. The jointed structure from the outer anchoring lamella which arches over the cytostome rim is continuous with the inner anchoring lamella. The articulating lamella connects with the inner dense body. Fixed with glutaraldehyde/OsO₄. TEM thin section. X 24,100.

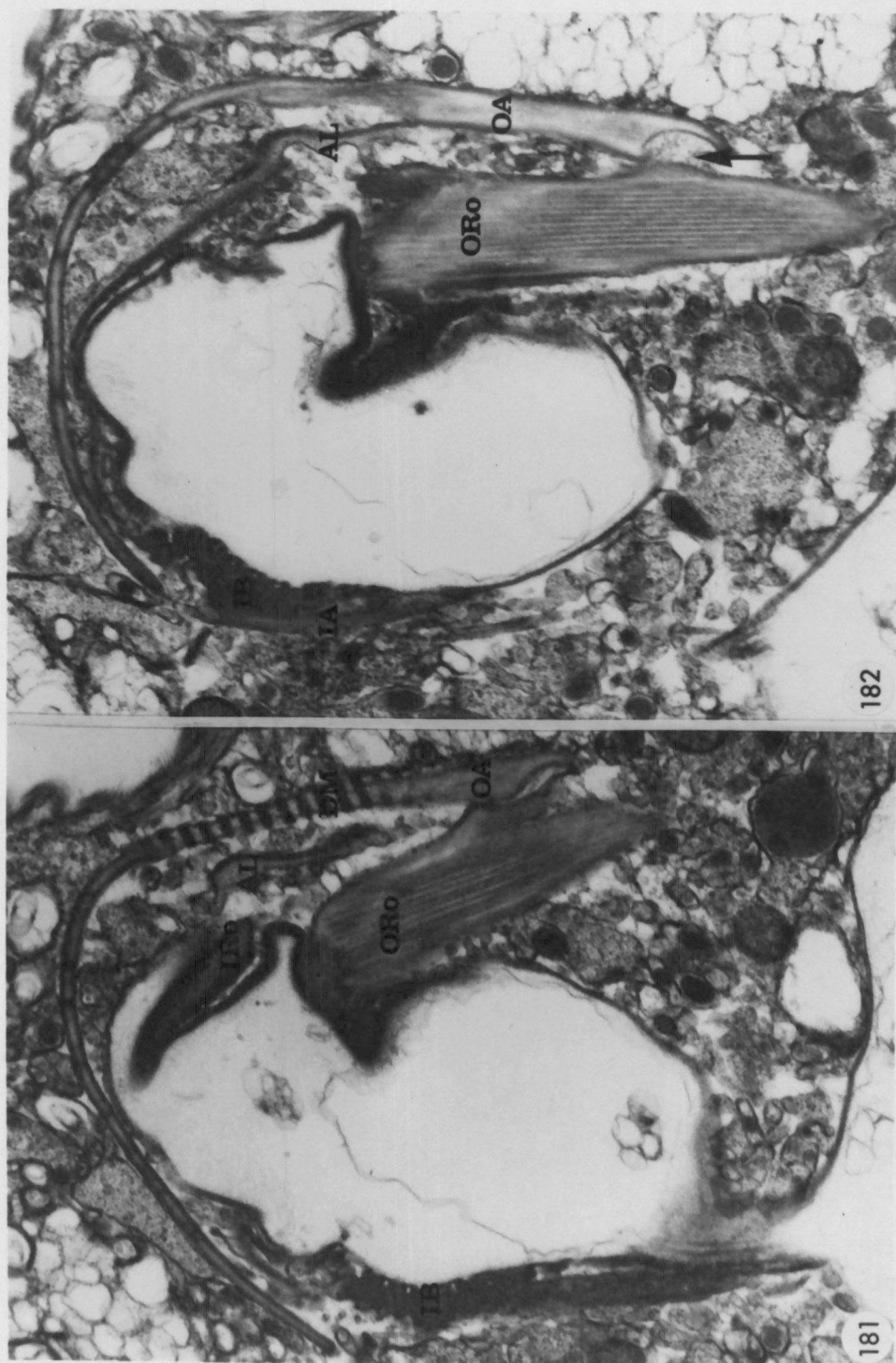


Plate XXXIX.

Plate XL. Articulating lamellae and outer rod connections.

- Figure 183. Oblique section showing the two plates of the articulating lamellae which extend from the outer anchoring lamella to the inner dense body (not shown in this plane of section). A row of microtubules occurs along the lower lamella (arrow). Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 19,900.
- Figure 184. Oblique section of cytostome rim showing row of microtubules. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 40,900.
- Figure 185. Transverse section of microtubules associated with lower plate of inner articulating lamella. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 96,400.
- Figure 186. Oblique section of two plates of outer articulating lamellae (arrows) showing connection to outer anchoring lamella. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 53,400.
- Figure 187. Transverse section showing fibrous connection extending from knob-like projection on outer rod to outer anchoring lamella. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 26,900.
- Figure 188. Longitudinal section of rod showing fibrous connection from rod to outer anchoring lamella. Fixed with OsO₄/glutaraldehyde, then OsO₄. TEM thin section. X 52,100.

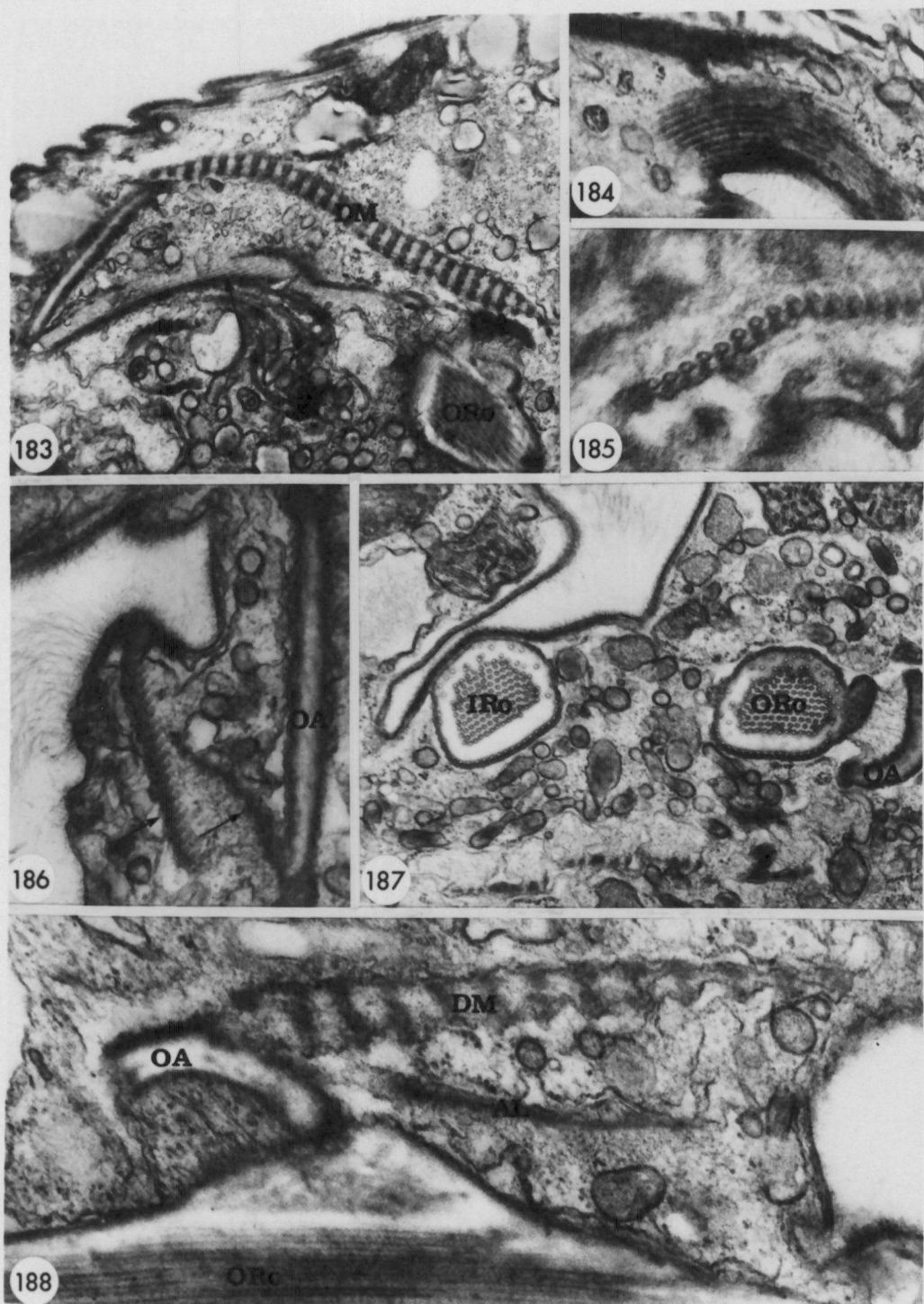


Plate XL.

anchoring lamella associated with the outer rod (Plate XL, Figure 186). Associated with the lower plate of the articulating lamella is a row of 16-20 microtubules (Plate XL, Figures 184-186).

A knob-like extension from the sheath of the outer rod (Plate XL, Figures 187 and 188) is united to the outer anchoring lamella by the fibrous connection. There is no connection between the inner rod and inner anchoring lamella corresponding to this fibrous connection that unites the outer rod and outer anchoring lamella; a deep groove separates the inner rod from the inner anchoring lamella (Plate XXXVII, Figure 172).

The prominent double serrated marginal lamella, consisting of alternating electron-dense and electron-lucent bands with a periodicity of 120-130 nm, is a structure of variable appearance (Plate XLI, Figures 189-196). In some sections, the lamella is membrane-bounded and lies within an expanded cisterna of endoplasmic reticulum (Plate XLI, Figure 190), while in other sections parts of the lamella appear as small electron-dense areas (Plate XLI, Figures 189, 191 and 192). Its connection with the outer anchoring lamella (Plate XLI, Figure 193) appears as a hinge joint in some sections (Plate XLI, Figure 194). The diameter of the lamella is not uniform throughout its length and is variable from cell to cell, ranging from 130 nm (Plate XLI, Figure 190) to 950 nm (Plate XLI, Figure 195). Although the diameter is greater in some lamellae (Plate XLI, Figures 195 and 196), the periodicity of the bands is the same.

Plate XLI. Double serrated marginal lamella.

- Figure 189. Oblique section of reservoir/cytostome complex showing rodorgan separating the two cavities. Arching over the rim of the cytostome is a complex array of lamellar structures, one of which is the conspicuous double serrated marginal lamella (arrow). Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. $\times 16,100$.
- Figure 190. Higher magnification reveals the double serrated marginal lamella as a membrane-bounded structure of ca. 130 nm diameter consisting of alternating dark and light bands with a periodicity of 120-130 nm. The lamella lies within a profile of endoplasmic reticulum. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. $\times 53,400$.
- Figure 191. In the cytoplasm near one end of the lamella, electron-dense areas and portions of the cytoplasmic matrix accumulate in profiles of endoplasmic reticulum (arrow). Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. $\times 33,300$.
- Figure 192. Section showing presumptive electron-dense areas of lamella adjacent to cisterna of endoplasmic reticulum. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. $\times 51,700$.
- Figure 193. Double serrated marginal lamella diverges from outer anchoring lamella. Adjacent outer dense body connects with rodorgan through dense knob-like projection on outer rod (arrow). Inner dense body and inner anchoring lamella are separated from inner rod by a deep groove. Dense material forms bridge between two rods of rodorgan. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. $\times 32,300$.
- Figure 194. Junction between outer anchoring lamella and double serrated marginal lamella appears as a "hinge joint" (arrow). Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. $\times 53,400$.
- Figure 195. Oblique section showing double serrated marginal lamella with diameter up to 950 nm (to right of micrograph) which is ca. seven times greater than those shown in Figures 189-191. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. $\times 26,300$.

Plate XLI. (Continued)

- Figure 196. Small portion of oblique section of double serrated marginal lamella from different cell than shown in Figure 195. Although the diameter is greater than that of the lamella shown in Figure 190, the periodicity of the bands in the two lamellae is the same. Fixed with OsO_4 /glutaraldehyde, then OsO_4 . TEM thin section. X 36,000.

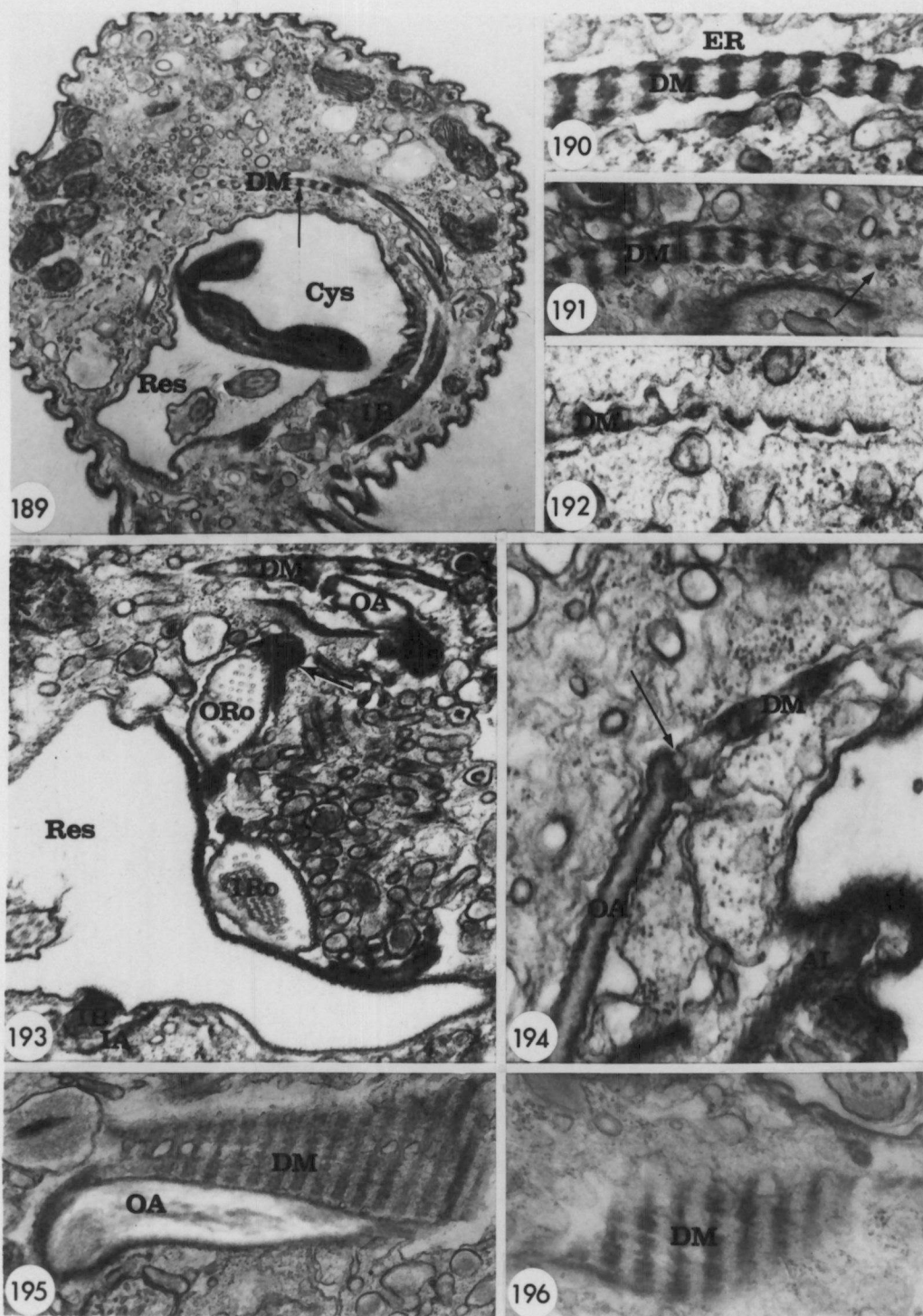


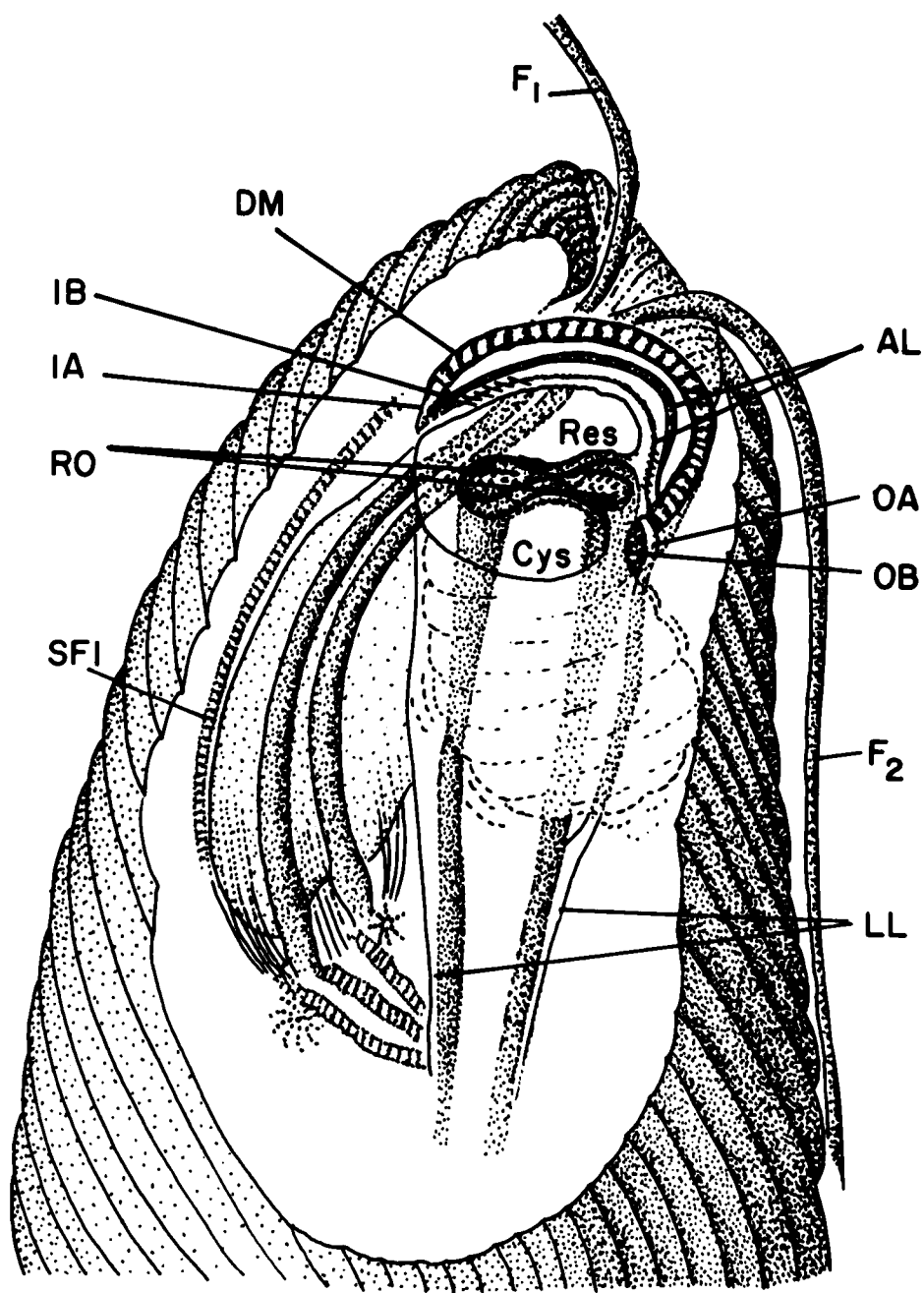
Plate XLI.

Model of the Feeding Apparatus

A comprehensive diagram of the components of the feeding apparatus of Peranema is shown in Figure 197 (Plate XLII). The major features of the model are summarized as follows: (1) an anterior subapical canal, from which the two flagella emerge, serves as a common opening for both the reservoir and cytostome; (2) the pyriform reservoir is a rigid structure with fixed dimensions, whereas the cytostome is capable of expanding to accommodate incoming food; (3) the rodorgan, lying in a cytoplasmic ridge which separates the reservoir from the cytostome, is connected by the outer dense body to the outer anchoring lamella; (4) the outer anchoring lamella diverges into (a) the double serrated marginal lamella, which connects to the inner anchoring lamella, and (b) two plates of the articulating lamella, which connect with the inner dense body; (5) a row of 16-20 microtubules extends along the lower plate of the articulating lamella; (6) longitudinal lamellae, arising from the base of the rodorgan, diverge from the rods and terminate in the rim of the cytostome; (7) a striated fibril lies beneath the left wall of the reservoir; (8) the base of the rodorgan is connected to the flagellar bases by striated roots.

Plate XLII. Model of feeding apparatus.

Figure 197. Diagrammatic representation of feeding apparatus in dorsal view, adapted from Mignot (1966) and Nisbet (1974). The rodorgan lies in a cytoplasmic ridge which separates the reservoir from the expandable cytostomal sac. The outer rod is joined to the outer dense body which in turn connects with the outer anchoring lamella. Diverging from the outer anchoring lamella are (a) the double serrated marginal lamella, which arches dorsally and joins with the inner anchoring lamella at the left side of the cytostome, and (b) the articulating lamella, consisting of two dense plates which connect with the inner dense body. Associated with the lower plate of the articulating lamella is a single row of microtubules. A prominent striated fibril extends along the left wall of the reservoir. Striated roots connect the base of the rodorgan to the flagellar roots.



197

Plate XLII.

CHAPTER IV

DISCUSSION

I. FLAGELLAR STRUCTURE

Mastigoneme Organization

Although mastigonemes from different taxa vary in structure, they can be broadly categorized as tubular, nontubular or striated. Tubular mastigonemes, ca. 20 nm in diameter, are differentiated into three regions: a tapering basal area attached to the flagellar membrane; a tubular shaft which may be smooth or ornamented with lateral filaments; and one to three terminal filaments. Nontubular mastigonemes are smaller in diameter than tubular mastigonemes and are a heterogeneous group. Striated mastigonemes, identified in Chlamydomonas and some prasinophycean algae, show a striking periodicity in negative-stain and shadow-cast preparations.

Euglenoid flagellates have nontubular mastigonemes of two different lengths: long, fine mastigonemes 2-3 μm long and a "felt" of shorter, fibrous material that coats the flagella (Leedale, 1967). Previous reports on the mastigonemes of Peranema have attempted to define the felt material, which was recognized by Chen (1950) as a "sheath" consisting of numerous fibrils that radiated from the axis, giving the flagellum a "test-tube brush" appearance. Pitelka and Schooley (1955) further characterized the sheath as two cross-striated ribbons that are easily separated from the axoneme. The ribbons, ca. 400 nm in width, are sharply delimited laterally, and striations occur with a periodicity of 100 nm. The present study clearly

demonstrates that this periodicity is due to overlapping tiers of short mastigonemes such as those shown in Plate XX, Figure 96, page 72.

Roth (1959) interpreted the ribbons seen in transverse thin section as striated, layered material in two crescent-shaped halves (similar to the image shown in Plate XV, Figure 79, page 60 and Plate XVI, Figure 82, page 63). He identified four to six layers, each layer ca. 10 nm thick and separated from the adjacent layer by 10 nm or more. Although the sheath appears multilayered in some thin sections, the present study shows that the mastigonemes occur in only two overlapping tiers. Roth also recognized that the short mastigoneme ribbons are absent on the trailing flagellum and on the anterior flagellum inside the reservoir.

Mastigoneme units. In a study employing a combination of techniques such as thin-section TEM, negative staining, and selective solubilization of the flagellar surface membrane and isolated mastigonemes of Euglena gracilis, Bouck et al. (1978) found that the sheath consists of overlapping mastigoneme units arranged parallel to the flagellar surface in a presumptive helical spiral. The present study shows that in Peranema, the sheath also consists of overlapping mastigoneme tiers that are parallel to the flagellar surface. Instead of the helical spiral described in Euglena, however, the mastigonemes in Peranema form two lateral ribbons. Although structures corresponding to the elaborate mastigoneme "units" in Euglena described by Bouck et al. (1978) were not found in Peranema, certain comparisons can be drawn between the mastigonemes of both organisms. The mastigoneme units 280 nm long in Euglena are comparable to the 250-nm-long

tiers in Peranema (Plate XX, Figure 96, page 72). Both in the units in Euglena and the tiers of Peranema, mastigonemes overlap longitudinally by ca. 140 nm (i.e., one half of a unit or tier length). The laterally aligned, regularly spaced components seen in surface view and in more centripetal sections of ribbons in Peranema (Plate XVIII, Figures 90 and 91, page 67) correspond to the unit shafts in Euglena. The fine fibrillar elements (Plate XVIII, Figure 91, page 67) correspond to the fine lateral elements that extend from the unit shafts of Euglena.

Major components of the mastigoneme units of Euglena are two loops, one of which is easily detached. Although no comparable structures were found in Peranema, there are areas in dissociated ribbons that appear to contain "loops" (Plate XXI, Figure 98, page 74). Bouck et al. (1978) postulated that the larger loop of the mastigoneme units in Euglena function to bind overlapping units together. Since the two ribbons in Peranema remain intact during isolation, structures similar to the loops in Euglena may be responsible for binding overlapping tiers.

Long mastigonemes. Long mastigonemes ranging from 0.8 μm - 2.8 μm in length are independent of the short mastigoneme ribbons in Peranema. Leedale (1967) stated that most euglenoids bear a unilateral row of long, fine mastigonemes. Further support for a unilateral distribution is cited by Bouck et al. (1978), who suggested that longer mastigonemes of Euglena arise singly or in small clusters of three to four attached to a paraflagellar rod. In tangential sections of the flagellum in Peranema (Plate XVII, Figure 84, page 65), small tufts of

long mastigonemes are evident, but their lateral distribution is not clear. In shadow-cast and negative-stain preparations of intact flagella, long mastigonemes appear on both sides of the flagellum. In transverse sections, however, the long mastigonemes have been found only on the side of the flagellum opposite the paraflagellar rod. Thus, the long mastigonemes actually may be arranged in a unilateral array, or they may represent part of a spiral tier of long mastigonemes (e.g., Plate XVII, Figure 85, page 65), which is consistent with Mignot's (1966) findings of a spiral band that encircles the flagellum throughout most of its length.

The nature of the attachment of long mastigonemes to the flagellum is not clear. After mild detergent treatment on flagella self-stuck to carbon-coated EM grids, long mastigonemes of Euglena appear to be attached to the paraflagellar rod (Bouck et al., 1978). In dismembered flagella of Peranema, the mastigonemes are often associated with strips or lumps of accessory material (Plate XXI, Figure 100, page 74) as shown also in Euglena (Leedale, 1967). Other evidence that the mastigonemes pass through the flagellar membrane and attach to intraflagellar structures can be seen in transverse thin sections in which long mastigonemes appear to be connected to axonemal microtubules (Plate XVII, Figure 86, page 65).

Chemistry of mastigonemes. Mastigonemes of Euglena gracilis are reported to consist of at least one major glycoprotein in which the major carbohydrate moiety is xylose, with possibly a small amount of glucose and an unknown minor component (Bouck et al., 1978). The large glycoprotein consists of several subspecies of glycoproteins

apparently aggregated by disulfide bonds. These properties are similar to those of complex mucus glycoproteins obtained from animal sources. In the present study, the mastigonemes stain with ruthenium red which binds selectively to acid mucopolysaccharides. That the mastigonemes are resistant to trypsin digestion (Pitelka and Schooley, 1955) and to solubilization by the anionic detergent Sarkosyl (Bouck et al., 1978, and present study) provides evidence for non-protein components.

Origin of mastigonemes. In all major algal and fungal groups that have tubular mastigonemes, except the Bacillariophyceae, presumptive tubular mastigonemes have been identified in the perinuclear continuum that consists of the nuclear envelope, chloroplast endoplasmic reticulum cisternae, and cytoplasmic endoplasmic reticulum cisternae. These compartments are the sites for assembly of mastigonemes (Bouck, 1972). The Golgi apparatus is also involved in mastigoneme production in some of the Chrysophyceae. For example, in Ochromonas, additional lateral filaments are found only on those presumptive mastigonemes that are within the Golgi cisternae or within vesicles of Golgi origin (Bouck, 1972).

In contrast to the well-documented perinuclear continuum/Golgi apparatus routes for the development of tubular mastigonemes in the Chrysophyceae, the intracellular origin of nontubular mastigonemes is less certain in the Euglenophyceae. Structures containing presumptive nontubular mastigonemes are usually found near the reservoir in Pernanema (Plate IX, Figure 44, page 45), but it is unclear whether these are separate vacuoles or sections of channels of the reservoir.

The fine filaments that line the reservoir may represent the final assembly of nontubular mastigonemes which may occur within the reservoir or directly on the flagellar surface (Bouck et al., 1978). The intrareservoir filaments, the flagellar mastigonemes and the mucilage strands on the pellicle all stain with ruthenium red in Peranema, but there is no evidence that all three structures are similar in composition.

The question of how mastigonemes are distributed along the length of the flagellum, i.e., whether they are added at the base or the tip, has been addressed by Bouck (1972). Bouck speculates that if new mastigonemes are added at the growing tip, which is the site of axonemal growth, then they must migrate along the flagellum independently of the membrane. If, however, mastigonemes are extruded at the base, they could remain attached to a portion of the original vesicular membrane after extrusion and be "dragged up" from their point of exocytosis by the "moving" membrane of the flagellum as the flagellum elongates distally. The absence of short mastigoneme ribbons on flagella inside the reservoir of Peranema would tend to argue against the latter hypothesis, although assembly and polymerization of mastigonemal elements might take place once they are outside the reservoir. Studies on regenerating flagella could provide helpful insights into the origin of mastigonemes.

Functions of mastigonemes. The function of the nontubular mastigonemes in euglenoids is unknown. Unlike the stiff tubular mastigonemes in Ochromonas which reverse the thrust of the flagellar movements (Jahn and Bovee, 1968; Bouck, 1972), nontubular mastigonemes

are more flexible and elastic. The flexibility of the nontubular mastigonemes allows them to unwind between successive turns of the flagellum which beats in the form of an interrupted helix (a series of single turns interrupted by straight sections between the turns). The unwinding serves to increase the total viscous drag of the flagellum and thus the efficiency of propulsion.

Flagellar Bases

In contrast to many other chlorophyll a- and b-containing eucaryotic plants that have a characteristic stellate pattern in the flagellar transition zone (Moestrup, 1978), the nine outer doublets of the axoneme are the only structural elements in the transition zone of euglenoids (Leedale, 1967; Leedale and Hibberd, 1974; Dynesius and Walne, 1975). In both longitudinal and transverse sections of the flagellum of Peranema, (Plate XIV, Figures 71 and 72, page 58), the central doublet is absent.

The striated material in the flagellum shown in Plate XIV, Figure 72, page 58, is reminiscent of a similar structure in the locomotory flagellum of Menoidium (Leedale and Hibberd, 1974). The position of this material coincides with the position of the flagellar swelling (presumptive photoreceptor) found in green euglenoids and in colorless forms with an eyespot such as Khawkinea. The material in Peranema is thus interpreted as a possible degenerate photoreceptor, supporting a hypothesis that colorless euglenoids are derived from phototrophic forms (Leedale, 1967).

At the base of the reservoir in Peranema, the two flagella terminate in a specialized area of homogeneous cytoplasm delimited by

elements of endoplasmic reticulum. This region appears to contain a presumptive microtubular organizing center (Pickett-Heaps, 1969; Osborn and Weber, 1976; Raff, 1979), a diffuse, amorphous mass from which microtubular arrays radiate in all directions (Plate XXV, Figure 114, page 86). There are two pairs of basal bodies in this region, the members of each pair lying at an angle to each other. In a biflagellated cell, pairs of basal bodies are found only in dividing cells; thus the significance of the four basal bodies in Peranema is unknown, although Nisbet (1974) described one member of the pair as a "barren basal body" but gave no explanation. Four basal bodies were also found in another colorless biflagellated euglenoid, Entosiphon (Mignot, 1963).

Flagellar Roots

Microtubular roots. Three microtubular roots evident in Peranema are typical of euglenoids, having also been found in Entosiphon (Mignot, 1966), Menoidium and Rhabdomonas (Leedale and Hibberd, 1974) and Eutreptiella (Moestrup, 1978). These roots in Peranema pass from the flagellar bases and continue anteriorly along the reservoir wall; they are readily observed in transverse sections of the reservoir (Plate VI, Figure 31, page 38). The number of microtubules in each root is undetermined; transverse sections at the flagellar bases show a 4:6:8-stranded configuration while the reservoir shows a 10:4:6 arrangement. The fact that three microtubular arrays are seen in dividing Peranema cells at the base of the canal, a site for duplication of pellicular microtubules in euglenoids

(Sommer and Blum, 1965), suggests that the roots also give rise to subpellicular microtubules.

In transverse sections of the roots in the reservoir wall, the four-stranded root is separated from the six-stranded root by a thin plate-like structure that lies between a striated fibril and the reservoir membrane. A similar plate-like structure was reported to separate a four-stranded root from a layer of evenly spaced microtubules covering the eyespot of Eutreptiella (Moestrup, 1978). This association could be either a structural connection between the eyespot and flagella or a physiological connection transferring impulses between the flagella and eyespot.

The plate-like structure in Peranema is closely associated with the striated fibril in the left wall of the reservoir. Whether the striated fibril is associated structurally with the flagellar bases is unknown, but it might have a role in photoreception. That Peranema responds to light is evident in its shock reaction to high-intensity illumination. When the organism is subjected to rapidly increased light intensity, the rate of flagellar beat is increased, and there is a sharp deflection of the anterior end, followed by a reorientation on a new course 90° to the original; although the entire organism is sensitive to light, the flagellum is most sensitive (Shettles, 1937). Since there are no pigments or eyespot in Peranema, the striated fibril might function as a photoreceptor, with the plate-like structure serving to transfer impulses between the fibril and the flagellar bases. Future studies with serial sectioning could define the extreme limits of the striated fibril and elucidate connections, if any, with other cellular components.

Fibrous roots. The striated fibrous material (Plate XXV, Figure 113, page 86) forms a bridge between the two flagellar bases analogous to the proximal connecting fibers between the flagellar bases in Chlamydomonas (Ringo, 1966), the kineto-kinetosome fiber of Ochromonas (Bouck and Brown, 1973), the unstriated fibrous bridge of Dinobryon (Kristiansen and Walne, 1977) and the basal aggregate of Poterioochromonas (Schnepf et al., 1977). A similar structure was called a rhizoplast in Entosiphon (Mignot, 1963). Possible functions of this bridge are the anchoring and maintenance of position of the basal bodies (Kristiansen and Walne, 1977) and flagellar coordination (Bouck and Brown, 1973).

Striated roots. The striated roots connecting the flagellar bases and the rodorgan in Peranema are similar to banded kinetodesmal fibrils in ciliates and rhizoplasts of other flagellates (Pitelka, 1963). All these banded fibrils originate at basal bodies or centrioles and are commonly thought to be anchoring and supportive structures that counterbalance the force of the beating cilium or flagellum (Pitelka, 1969). The root fibrils might also serve an ancillary role as a tether to restrain and maintain correct orientation of organelles such as the rodorgan, which maintains a fixed location adjacent to the reservoir despite its rasping movements.

The flagellum-striated root-rodorgan interrelationship in Peranema also suggests that the root might be involved in impulse transfer. Chen (1950) reported that the tip of the anterior flagellum is mechanically irritable; the whole organism contracts spasmodically in response to an obstacle touched only by the flagellum. Thus,

response to the presence of food by the flagellum in Peranema might be transferred via the root to the rodorgan, promoting its forward movement and opening the canal to the cytostome, letting solid ingesta pass into the organism.

In addition to the anchorage, maintenance of position and impulse transfer roles postulated for striated roots, a fourth role ascribes contractile properties to the organelle because of its structural similarity to rhizoplasts. Salisbury and Floyd (1978) showed that the rhizoplast of the green alga Platymonas undergoes cyclic contraction and extension in solutions containing calcium and adenosine triphosphate. These authors suggested that the rhizoplast acts as a mechanical aid in initiation, coordination and direction of the beat of the four flagella. Contraction of the rhizoplast was induced and sustained by addition of 0.1 to 5.0 mM calcium chloride to the medium. Rhizoplast extension occurred in cells that received no calcium or that had been washed in solutions containing EGTA without additional calcium.

Since Peranema is routinely grown in a milk medium containing 7.0 mM additional calcium sulfate, the striated root shown in Plate XXVI, Figure 124, page 89, was postulated to be in the contracted state. Thus, if calcium were removed from the medium, the striated root should then appear in the extended state similar to that shown for Platymonas. Dilution of calcium from the medium by daily washing and maintenance of cells in distilled water and by washing in EGTA had no effect on the appearance or the periodicity of the striated root. Since the intracellular calcium levels could not be determined,

it is possible that these levels were unchanged by manipulation of the medium. Future studies on the contractile nature of striated roots might utilize ionophores such as A23187, compounds that facilitate transport of divalent cations across membranes (Plattner and Fuchs, 1975; Ferreira and Lew, 1976; Pfeiffer et al., 1978). Since these ionophores abolish calcium ion gradients, they could provide a means for manipulating intracellular calcium ion concentrations and for studying the effects on striated roots.

II. MUCOCYSTS

In his review of protistan extrusive organelles (extrusomes), Hausmann (1978) defined them as membrane-bounded structures usually located in the cortical cytoplasm, which are readily discharged upon mechanical, electrical or chemical stimulation. During discharge and transition from the resting state to ejected form, the organelles undergo characteristic morphological changes. Of the ten types of extrusomes described by Hausmann, the extrusive organelles found in Peranema, previously designated "trichocyste muqueux" (Mignot, 1966), "muciferous bodies" (Leedale, 1967), or "mucigenic bodies" (Nisbet, 1974), most closely resemble mucocysts.

Although mucocysts occur in both ciliates and flagellates, their origin and structure are different in the two groups. Mucocysts of ciliates arise from the endoplasmic reticulum as small vesicles with granular contents which become crystalloid as the organelle increases in size. Mucocysts of flagellates have granular to amorphous contents and arise either from the Golgi apparatus, as in the chloromonad

Chattonella (Hausmann, 1978), or from the mitochondria, as in the dinoflagellate Noctiluca (Soyer, 1969).

The mucocysts in Peranema most closely resemble the unique mucocyst found in another colorless euglenoid, Entosiphon sulcatum (Mignot and Hovasse, 1973). In Entosiphon, the mucocysts increase in length and width upon discharge from the cell. During this expansion, two sets of ordered filaments become visible in the ejected mucocysts. Unlike the mucocysts of Entosiphon, the mucocysts of Peranema do not increase in size upon discharge from the cell. In fact, immature mucocysts have a wider diameter and exhibit less uniformity in size and shape than do ejected mucocysts. Like those of Entosiphon, however, the mucocyst filaments of Peranema become visible only after discharge. A similar situation is also found in the trichocysts of Paramecium aurelia (Jurand and Selman, 1969), in which the mature intracellular trichocysts have no visible internal structures. On discharge, however, the shaft appears regularly cross-banded at intervals of 55 nm. Because of the change in structure upon extrusion, Jurand and Selman postulated that the mature intracellular trichocysts must in fact possess a regular ultrastructure which has not been observed.

The filaments which are visible only after mucocyst discharge in Peranema may function in the mechanism of discharge. For example, discharge of spindle trichocysts in ciliates is due to a rapid stretching and unfolding of a network of filaments with a concomitant increase in length of the organelle (Hausmann, 1978). Since the mucocyst filaments of Peranema are only visible upon discharge, it is not

known whether they are internal components of the mucocyst or are structural components of the tubular shaft. In any case, the filaments may burst out of the shaft upon stimulation, bringing with them the entire mucocyst.

In addition to the discharge of filaments, the mucocyst in Peranema also expels a ball of mucilage that stains with ruthenium red. Although the mode of discharge is unknown, it may be similar to that described for the mucocysts in Tetrahymena (Tokuyasu and Scherbaum, 1965). In the latter, the mature mucocysts in the resting state show a crystalline pattern. Stimulation causes hydration of the crystalline contents to an amorphous mucoid substance with a concomitant increase in volume. Since the mucocyst itself does not change in size, the expanding contents are propelled to the outside.

Although one function proposed for mucocysts is to provide raw material for the formation of the cyst wall in some organisms (Hausmann, 1978), no cysts have been identified in Peranema. Mucilage strands on the pellicle surface, however, could conceivably have their origin from the mucocysts.

Mucus derived from extruded mucocysts may be involved in the feeding process in Tetrahymena, in which components of the growth medium are adsorbed by extruded mucocysts and are ingested into food vacuoles (Nilsson, 1979). Soyer (1970) found that mucocysts bordering the tentacle in the dinoflagellate Noctiluca miliaris make the tentacle sticky, thus allowing adherence of food particles. A similar phenomenon might take place in Peranema since aggregates of mucocysts are sometimes found in the surrounding medium of contaminated cultures.

The mucocysts might function to adsorb bacteria or other small food particles for phagocytosis.

III. FEEDING APPARATUS

Canal

Although the cytostome and ingestion apparatus form an independent system lateral to the canal and reservoir, both the cytostome and reservoir share a common opening (Roth, 1959; Nisbet, 1974). In most green euglenoids, a cylindrical sheath of endoplasmic reticulum delimits several layers of microtubules which encircle the canal, presumably adding rigidity to the structure (Leedale, 1967). The absence of encircling microtubules in Peranema (Mignot, 1966; Nisbet, 1974) would allow the canal to be more elastic and extensible, enlarging to accommodate solid ingesta which passes into the cytostome.

Origin of the Feeding Apparatus

Leedale and Hibberd (1974) postulated that the ingestion apparatus of colorless, phagotrophic euglenoids could have evolved from the fusion and lateral displacement of the microtubules encircling the canal in phototrophic forms. They described the structure of the canal region in the colorless osmotrophs, Menoidium and Rhabdomonas, as an evolutionary link between phototrophs and phagotrophs. In the colorless osmotrophs, a single layer of canal microtubules is fused laterally, lined with amorphous material and delimited by a cylinder of endoplasmic reticulum. Such split cylinders could perhaps be laterally displaced from the canal and lie as separate structures encircled by endoplasmic reticulum. The cylinders could then serve

as the structural basis for evolution of the ingestion rods of Entosiphon and Peranema. The three rods in Entosiphon form a siphon to one side of the reservoir (Mignot, 1966). Each rod contains regularly arranged rows of microtubules. In the center of the siphon are structures similar to the longitudinal lamellae of Peranema. Unlike Peranema, the rods are not fused at their anterior ends and are not delimited by a membrane.

Lateral displacement of the microtubules encircling the canal could also have given rise to the microtubules associated with the lower plate of the articulating lamella in Peranema (Plate XL, Figure 184, page 125). These microtubules arch over the rim of the cytostome, perhaps functioning to give support.

Model of the Feeding Apparatus

Nisbet (1974) described some details of the ingestion apparatus of Peranema and her work is confirmed and extended by the present study. She provided the following working hypothesis for the action of the ingestion organelles. Contraction of the longitudinal lamellae attached near the rod bases is transmitted through the dense bodies to the anchoring lamellae. The pull of the inner anchoring lamella, located to the left side of the canal, moves the rodorgan forward to occupy a ventral position in the canal, closing the passage to the reservoir and making the canal opening continuous with the cytostome. The flagella are displaced and the canal is extended laterally such as shown in Plate XXXVII, Figure 175, page 119. The dense bodies act as "hinge joints" as the rodorgan moves forward and backward during feeding. Food is drawn in by suction and forced into the cytostome

by contraction of the double serrated marginal lamella at its upper rim (Nisbet, 1974).

Implicit in the Nisbet model described above is the contractile nature of several of the lamellar systems. Although such contractility has not yet been demonstrated, the striated pattern of the double serrated marginal lamella suggests such a function because of its structural similarity to rhizoplasts, organelles which have recently been shown to contract (Salisbury and Floyd, 1978). Future studies on the chemical composition and presumptive contractility of the lamellae may provide further insight into the action of the ingestion organelles.

IV. DIAGNOSTIC EUGLENOPHYCEAN FEATURES

Pellicle Structure and Cytochemistry

One characteristic that Peranema trichophorum shares with other euglenophycean flagellates is its helical symmetry. The pellicular striae, whorled in a left-handed (S) helix, are reduced in number at the posterior end of the cell by a tucking of one stria under another in the same manner as described for Euglena by Guttman and Ziegler (1974). The arrangement of striae can be modified, and such positional modifications account for the myriad cell shapes resulting from euglenoid movement. Microtubules in the ridges act as a cytoskeleton, allowing the cell to resume its characteristic form after the shape changes. Leedale reported a correlation between thickness of pellicular striae and pellicle flexibility. Those species, such as Peranema, Astasia and Eutreptia, with thin striae undergo violent euglenoid

movement, whereas organisms such as Phacus and Euglena spirogyra with thicker pellicles exhibit a more rigid shape (Leedale, 1967).

The mechanisms responsible for pellicle flexibility are unknown. Sliding of pellicular striae past one another may account for cell bending but not for the more pronounced distortions of cell shape characteristic of euglenoid movement (Leedale, 1967). That the striae contain contractile elements responsible for cell shape plasticity is suggested by the following observations: (1) the pellicle reportedly is 80% proteinaceous (Barras and Stone, 1965) and thus may contain elastic proteins and (2) the striated layer shown in freeze-etch preparations of the pellicle of Euglena gracilis (Schwelitz et al., 1970) and in thin sections of the pellicle of Peranema (Plate V, Figure 24, page 35) suggests the presence of highly organized contractile elements. Additional studies on the chemical composition of the pellicle are needed to elucidate its role in euglenoid movement.

The architecture of the cell surface in Peranema is generally obscured by mucilage strands on the pellicle. Although glusulase removes the mucilage from some cells, the enzyme is neither always effective nor always necessary for mucilage removal. Other authors have reported an absence of surface mucilage under various conditions. Guttman (1971) found that incubation of living Euglena cells in distilled water removed external mucilage. Gomez et al. (1974) observed a decrease in surface mucilage in Euglena cells from older cultures, presumably the result of mucilage strands being sloughed into the medium as the cells aged. Rosowski and Glider (1977) reported that excessive heat in preparative methods used to coat euglenoid specimens

for SEM altered the appearance of mucilage strands. Thus the absence of mucilage on some Peranema cells may be due either to preparative techniques, such as washing or metal evaporation onto specimens, or to physiological variation in cells in culture.

Although the chemical nature of the mucilage strands is unknown, their heavy labeling with ruthenium red suggests acid mucopolysaccharide constituents. Similar morphology and staining properties were shown in mucilage strands and in knob-like protrusions from the grooves of Euglena pellicles (Gomez et al., 1974) and in thick fibrils on the walls of higher plants (Colvin and Leppard, 1973). The latter fibrils contained an α 1-4 polymer of D-galacturonic acid.

The origin of the mucilage is presumed to be the subpellicular muciferous bodies associated with each pellicular stria. Studies on other euglenoids have shown the muciferous bodies to be peripheral elements of the endoplasmic reticulum which open to the outside through pores in the pellicle (Arnott and Walne, 1967; Leedale, 1967). Labeling of these internal bodies with ruthenium red, a putative extracellular stain which can penetrate crevices and small openings at the cell surface (Chambers, 1973), supports the view that they are channels opening to the outside. Similarly, the presence of ruthenium red label in both the reservoir and peri-reservoir vesicles indicates that material from the reservoir is sequestered in the vesicles, a result suggesting that they function in pinocytosis by recovering large molecules from the medium through the reservoir membrane (Kivic and Vesik, 1974).

The extracellular mucilage may function as a lubricant between pellicular articulations, facilitating the sliding of the ridges along

grooves during euglenoid movement (Leedale, 1967) and/or as a surface that provides traction between the organism and the substratum along which it glides (Jahn and Bovee, 1968). Other algal organisms such as motile diatoms glide over self-secreted mucus trails (Drum and Hopkins, 1966), but it is not known how the tractile force between the cell surface and the extruded mucus is generated.

Dictyosomes

Dictyosome profiles in euglenoid flagellates are distinctive in having an exceptionally large number of 20-30 curved, flattened or discoid cisternae. Elements of endoplasmic reticulum and polyribosomes are associated with the forming face (Leedale, 1967). Colorless euglenoids often have two to three very large dictyosomes anterior to the nucleus. Still others are found in Peranema regularly positioned near the contractile vacuole and the reservoir base. Leedale (1967) postulated that the contractile vacuole/dictyosome positioning suggests a role for dictyosome-associated vesicles in the water relations of the cell, perhaps through formation of alveolate vesicles.

Acid phosphatase activity has been localized in dictyosome cisternae and in subpellicular elements of endoplasmic reticulum of Peranema (Plate XII, Figure 59, page 52, and Plate V, Figure 21, page 35). In Euglena cells that had formed an induced acid phosphatase by exposure to a phosphate-deficient medium, the induced activity was localized at the notch of each pellicle complex and near a vesicle of the endoplasmic reticulum, a result suggesting a continuity between subpellicular endoplasmic reticulum and dictyosomes (Sommer and Blum, 1965). An active role of the dictyosome in the origin and/or

processing of the hydrolytic enzymes in lysosomes is suggested by acid phosphatase reaction product in dictyosomes and associated vesicles of Euglena (Brandes et al., 1964; Brandes and Bertini, 1964; Sommer and Blum, 1965; Palisano and Walne, 1972; Gomez et al., 1974).

Mitochondria

In contrast to the randomly aligned cristae in mitochondria of other euglenoids, many mitochondria in Peranema contain stacked cristae. This unusual arrangement of stacked cristae was also observed in the mitochondria of other colorless euglenoids including Distigma proteus (Mignot, 1966) and the colorless mutant W_3 BUL derived from Euglena gracilis (Palisano and Walne, 1976). Palisano and Walne speculated that the major metabolic switch from phototrophism to heterotrophism in these cells could explain the ultrastructural changes associated with the mitochondria. Stacked cristae have not been observed in mixotrophically or phototrophically cultured cells, but whether the phenomenon is a general occurrence in colorless heterotrophic euglenoids is unknown since relatively few have been investigated.

Nucleus

The interphase nucleus of Peranema contains large nucleoli (endosomes) characteristic of euglenoids. However, in contrast to most euglenoids in which chromosomes are heterochromatic, i.e., permanently condensed structures throughout the cell cycle (Leedale, 1968), there are no such heterochromatic structures in the interphase nucleus of Peranema. West (1977) also observed nuclei without stainable heterochromatin in Trachelomonas and interpreted this

non-condensed condition as indicative of interphase nuclei. Since no dividing nuclei were observed in the present study, it was not possible to compare the interphase nucleus with nuclei in mitotic stages. If the 177 chromosomes reported for Peranema (Leedale, 1958a) were permanently condensed, at least some of them should be visible in the interphase nucleus. The fact that they are not suggests that the Peranema nucleus may deviate from this diagnostic euglenophycean feature.

Endosymbiotic Bacteria

The presence of endocyttoplasmic and/or endonuclear bacteria in euglenoid flagellates is a fairly widespread phenomenon, occurring both in colorless species including Peranema (Roth, 1959; Mignot, 1966) and in such green species as Euglena, Strombomonas, Trachelomonas and Lepocinclis (Leedale, 1969). Both the endocyttoplasmic and the endonuclear bacteria in these euglenoids are similar in size, shape and ultrastructural appearance to the symbionts reported here in Peranema: all are rod-shaped, ca. 0.3 μm wide and 1 μm long, double-membrane-bounded and encapsulated in an electron-lucent area which Leedale (1969) interpreted as a mucilaginous sheath.

The DNase-sensitive and filamentous central component in the cytoplasm of endocyttoplasmic bacteria in Trachelomonas (Leedale, 1969) is probably analogous to the fine central fibrils in the endosymbiont of Peranema shown in Plate XIII, Figure 65, page 55. Both structures represent the bacterial genome. Although Leedale found that the endonuclear bacteria in green euglenoids multiply to keep pace with host division, putative division of the Peranema endosymbiont was not accompanied by division of the host.

Roth (1959) found several hundred endonuclear bacteria in Peranema but reported no cytoplasmic symbionts. In the present study, however, only one endonuclear bacterium was observed; all other symbionts occurred in the cytoplasm. Differences in strains and culture conditions in the two studies of Peranema may account for their different locations.

The ultrastructural appearance of the euglenoid endosymbionts bears a striking resemblance to the Gram-negative mu particles, type 540, found in species of killer paramecia (Beale and Jurand, 1966). These endosymbionts, which have been isolated and cultured free of their hosts, contain α -diaminopimelic acid and muramic acid (Stevenson, 1967), constituents that occur only in bacterial cell walls. The bacterial cell wall is described as the outer dense "membrane" of the double membrane of the endosymbionts of Paramecium (Preer et al., 1974).

Attempts to isolate euglenoid endosymbionts and to characterize them by various bacteriological stains have been unsuccessful (Leedale, 1969). More detailed information on the endosymbiont-euglenoid association requires the isolation of the bacteria into pure culture, but at present there is no evidence that the flagellates either benefit or are harmed by the association.

CHAPTER V

SUMMARY AND CONCLUSIONS

This investigation of Peranema trichophorum, a colorless, phagotrophic, euglenoid flagellate, involved the ultrastructural characterization of cellular organization, in general, and of organelles specialized for motility, secretion and ingestion, in particular. Primary research emphasis was placed on (1) the organization of the flagella, flagellar mastigonemes and flagellar roots; (2) the structure and development of mucocysts, which are extrusive organelles; and (3) the interrelationships among the organelles and lamellar systems of the feeding apparatus.

I. FLAGELLA, FLAGELLAR MASTIGONEMES AND FLAGELLAR ROOTS

Flagella

Both the anterior and the trailing flagella extend from the base of the reservoir and emerge from the cell through the anterior canal opening. Proximal portions of the two flagella are identical in appearance; however, outside the cell the anterior flagellum has a greater overall diameter due to the presence of tufts of long non-tubular mastigonemes, two lateral ribbons of short mastigonemes and a paraflagellar rod. In contrast, the distal portion of the trailing flagellum outside the cell lacks the rod, has no lateral ribbons and fewer mastigonemes.

Flagellar Mastigonemes

Evidence from thin section and from shadow-cast and negative-stain preparations of intact and dissociated flagella suggests the following model of mastigonemal organization around the anterior flagellum: (1) tufts of mastigonemes 0.8-2.8 μm in length emanate from spiral rows and curve anteriorly toward the distal end of the flagellum; (2) two short mastigoneme ribbons, consisting of overlapping tiers 250 nm in length, form a bilateral array parallel to the long axis of the flagellum; short mastigonemes in the tiers are spaced 25 nm apart and are linked by fine fibrils. Reaction product precipitate from ruthenium red labeling, indicative of acid mucopolysaccharide constituents, is localized in the mastigonemes.

The model of mastigoneme tiers in Peranema presented in this dissertation is similar to the model of mastigoneme units in Euglena described by Bouck et al. (1978) in the following ways: (1) the tiers and units are both arranged parallel to the long axis of the flagellum and overlap longitudinally by ca. 140 nm (one-half of a unit or tier length); (2) the laterally aligned mastigonemes and fine fibrillar elements in Peranema correspond to the unit shafts and fine lateral elements in Euglena; (3) tiers and units both contain areas with loops, although the loops in Peranema are not part of an elaborate structure as in Euglena. The major difference in the two models is in the arrangement of the mastigonemal arrays: in contrast to the spirally arranged units in Euglena, the tiers in Peranema are arranged in a bilateral array.

Flagellar Roots

Associated with the flagellar bases are two pairs of basal bodies, the members of each pair lying at an angle to each other. The flagellar root apparatus, heretofore undescribed, consists of microtubular, fibrous and striated roots. Three microtubular roots, with an undetermined number of microtubules in each, extend from the flagellar bases and continue anteriorly beneath the reservoir membrane. Three microtubular roots have also been reported in other euglenoids, including Entosiphon (Mignot, 1966), Menoidium and Rhabdomonas (Leedale and Hibberd, 1974) and Eutreptiella (Moestrup, 1978). Since the number of microtubular roots has been found to be consistent not only in euglenoids but also in other groups of chlorophyll a- and b-containing organisms, the flagellar apparatus can be an important phylogenetic indicator in flagellated algae and protozoans.

Striated roots, similar to banded kinetodesmal fibrils of ciliates and the rhizoplasts of flagellates, extend from the flagellar bases to the rodorgan. Although contractile properties for the striated roots are postulated, contractility was not demonstrated in the present study. Utilization of ionophores might be helpful in future studies on the contractile nature of striated roots.

II. MUCOCYSTS

Ejectile organelles or mucocysts are usually found in longitudinal rows beneath the pellicle. Occasionally, immature mucocysts are observed near the maturing face of dictyosomes and are similar in structure and electron density to other dictyosome-associated

vesicles, suggesting a dictyosome-derived origin for mucocysts. Immature mucocysts are tubular structures with amorphous contents of low electron density. As the mucocyst develops, the electron-dense homogeneous contents are separated from the dense tips by electron-lucent zones. Two phenomena are described in the present study for the first time: (1) Mucocysts may protrude through the pellicle and discharge a ball of mucilaginous material which stains with ruthenium red, indicating the presence of acid mucopolysaccharide constituents. (2) Sometimes the mucocyst itself is ejected from the cell. Ejected mucocysts show a uniform structure consisting of (a) an inner tube 1.2 μm long and 0.18 μm wide with helical striations; (b) an outer tube 0.77 μm long and 0.21 μm wide with a diamond-shaped pattern; and (c) a dense middle band. Fine fibrils 8-9 nm in diameter emanate from the tips of ejected mucocysts. The subpellicular mucocysts of Peranema most closely resemble the mucocysts of another colorless euglenoid, Entosiphon sulcatum (Mignot and Hovasse, 1973).

Although the function of the mucocysts is unknown, possible roles include providing mucilage strands on the cell surface and/or adsorbing bacteria and other small food particles for phagocytosis by the cell. Future studies are needed to resolve questions concerning the chemical composition, mode of discharge and function of mucocysts.

III. FEEDING APPARATUS

The major features of the feeding apparatus are summarized in the following model adapted from Mignot (1966) and Nisbet (1974) and are extended in this model developed in the present study: (1) an

anterior subapical canal, from which the two flagella emerge, serves as a common opening for both the reservoir and cytostome; (2) the pyriform reservoir is a rigid structure with fixed dimensions, whereas the cytostome is capable of expanding to accommodate incoming food; (3) the rodorgan lies in a cytoplasmic ridge which separates the reservoir from the cytostome and is connected by the outer dense body to the outer anchoring lamella; (4) the outer anchoring lamella diverges into the double serrated marginal lamella, which connects to the inner anchoring lamella and two plates of the articulating lamella, which connect with the inner dense body; (5) a row of 16-20 microtubules extends along the lower plate of the articulating lamella; (6) longitudinal lamellae arise from the base of the rodorgan, diverge from the rods and terminate in the rim of the cytostome; (7) a striated fibril lies beneath the left wall of the reservoir; (8) the base of the rodorgan is connected to the flagellar bases by striated roots.

Several structures associated with the above model of the feeding apparatus are described in Peranema for the first time: (1) A row of microtubules is associated with the lower of the two plates of the articulating lamella, which underlies the double serrated marginal lamella. (2) In longitudinal aspect, the longitudinal lamellae have a honeycomb appearance, with the margins of the fenestrae connecting to small cytoplasmic vesicles. (3) A thin plate-like structure lies between the striated fibril and the reservoir membrane. A similar structure was observed in Eutreptiella (Moestrup, 1978) and was postulated to have connections with the flagella. (4) Striated roots are characterized by transverse alternating light and dark bands with

a periodicity of 30-38 nm; fine fibrils run perpendicular to the bands. Possible functions of the striated roots include their serving (a) as contractile elements of the movable feeding apparatus; (b) as a mechanism for impulse transfer; (c) as anchoring and supporting structures that counterbalance the force of the beating cilium or flagellum (Pitelka, 1969); (d) as a tether to restrain and maintain correct orientation of organelles such as the rodorgan (Pitelka, 1969).

IV. GENERAL CELLULAR ORGANIZATION

The present study shows that typical euglenoid diagnostic features in Peranema include (1) left-handed (S) helical symmetry; (2) pellicular striae with associated subpellicular microtubules and endoplasmic reticulum in which acid phosphatase reaction product is localized; (3) pyriform reservoir with accessory vacuoles and alveolate vesicles; (4) dictyosomes with stacks of 20-25 curved or flattened cisternae.

Structures found in the present study which are atypical of most euglenoids include (1) stacked cristae in some mitochondria, a phenomenon also described for other colorless euglenoids (Mignot, 1966; Palisano and Walne, 1976); (2) nuclei without stainable heterochromatin indicative of permanently condensed chromosomes; (3) rod-shaped endosymbiotic bacteria 1 μm long and 0.3 μm wide; (4) an unidentified cytoplasmic dense body 1 μm long.

LIST OF REFERENCES

LIST OF REFERENCES

- Allen, J. R., J. J. Lee, S. H. Hutner and J. Storm. 1966. Prolonged culture of the voracious flagellate Peranema in antioxidant-containing media. J. Protozool. 13:103-108.
- Archibald, A. R., W. L. Cunningham, D. J. Manners, J. R. Stark and J. F. Ryley. 1963. Studies on the metabolism of the protozoa. 10. The molecular structure of the reserve polysaccharides from Ochromonas malhamensis and Peranema trichophorum. Biochem. J. 88:444-451.
- Arnott, H. J. and P. L. Walne. 1967. Observations on the fine structure of the pellicle pores of Euglena granulata. Protoplasma 64:330-334.
- Barras, D. R. and B. A. Stone. 1965. The chemical composition of the pellicle of Euglena gracilis var. bacillaris. Biochem. J. 97:14P-15P.
- Beale, G. H. and A. Jurand. 1966. Three different types of mate-killer (mu) particle in Paramecium aurelia (Syngen 1). J. Cell Sci. 1:31-34.
- Berjak, P. 1972. Lysosomal compartmentation: ultrastructural aspects of the origin, development, and function of vacuoles in root cells of Lepidium sativum. Ann. Bot. 36:73-81.
- Bouck, G. B. 1972. Architecture and assembly of mastigonemes. Adv. Cell Mol. Biol. 2:237-271.
- Bouck, G. B. and D. L. Brown. 1973. Microtubule biogenesis and cell shape in Ochromonas. I. Distribution of cytoplasmic and mitotic microtubules. J. Cell Biol. 56:340-359.
- Bouck, G. B., A. Rogalski, and A. Valaitis. 1978. Surface organization and composition of Euglena. II. Flagellar mastigonemes. J. Cell Biol. 77:805-826.
- Brandes, D., D. E. Buetow, F. Bertini and D. B. Malkoff. 1964. Role of lysosomes in the cellular lytic process. I. Effects of carbon starvation in Euglena gracilis. Exp. Mol. Pathol. 3: 583-609.
- Brandes, D. and F. Bertini. 1964. Preliminary notes. Role of the Golgi apparatus in the formation of cytolsomes. Exp. Cell Res. 35:194-217.
- Bray, D. F. and E. B. Wagenaar. 1978. A double staining technique for improved contrast of thin sections from Spurr-embedded tissue. Can. J. Bot. 56:129-132.

- Chambers, V. C. 1973. The use of ruthenium red in an electron microscope study of cytophagocytosis. *J. Cell Biol.* 57:874-878.
- Chayen, J., L. Bitensky, R. G. Butcher and L. W. Poulter. 1969. *A Guide to Practical Histochemistry*. Oliver and Boyd, Edinburgh.
- Chen, Y. T. 1950. Investigations of the biology of Peranema trichophorum (Euglenineae). *Q. J. Microsc. Sci.* 91:279-308.
- Christen, H. R. 1963. Zur Taxonomie der farblosen Eugleninen. *Nova Hedwigia* 4:437-464.
- Colvin, J. R. and G. G. Leppard. 1973. Fibrillar, modified polygalacturonic acid in, on and between plant cell walls. In: F. Loewus, ed. *Biogenesis of Plant Cell Wall Polysaccharides*. Academic Press, N.Y., pp. 315-330.
- Curds, C. R. and A. Cockburn. 1970. Protozoa in biological sewage-treatment processes. I. A survey of the protozoan fauna of British percolating filters and activated-sludge plants. *Water Res.* 4:225-236.
- De Luca, H. F. and P. P. Cohen. 1964. Preparation of Krebs-Ringer phosphate and bicarbonate solutions. In: W. W. Umbreit, R. H. Burris, and J. E. Stauffer, eds. *Manometric Techniques*, 4th ed. Burgess, Minneapolis, p. 132.
- Drum, R. W. and J. T. Hopkins. 1966. Diatom locomotion: an explanation. *Protoplasma* 62:1-33.
- Dujardin, F. 1841. *Histoire Naturelle des Zoophytes-Infusoires*. Paris: Roret.
- Dynesius, R. A. and P. L. Walne. 1975. Ultrastructure of the reservoir and flagella in Phacus pleuronectes (Euglenophyceae). *J. Phycol.* 11:125-130.
- Ehrenberg, C. G. 1838. *Die Infusionsthierchen als vollkommene Organismen*. Leipzig: Verlag von Leopold Voss.
- Ferreira, H. G. and V. L. Lew. 1976. Use of ionophore A23187 to measure cytoplasmic Ca buffering and activation of the Ca pump by internal Ca. *Nature* 259:47-49.
- Franke, W. W., S. Krien, and R. M. Brown, Jr. 1969. Simultaneous glutaraldehyde-osmium tetroxide fixation with postosmication. *Histochemie* 19:162-164.
- Gomez, M. P., J. B. Harris, and P. L. Walne. 1974. Studies of Euglena gracilis in aging cultures II. Ultrastructure. *Brit. Phycol. J.* 9:175-193.

- Gomori, G. 1952. Microscopic Histochemistry. University of Chicago Press, Chicago.
- Guttman, H. N. 1971. Internal cellular details of Euglena gracilis visualized by scanning electron microscopy. Science 171:290-292.
- Guttman, H. N. and H. Ziegler. 1974. Clarification of structures related to function in Euglena gracilis. Cytobiologie 9:10-22.
- Hall, R. P. 1929. Reaction of certain cytoplasmic inclusions to vital dyes and their relation to mitochondria and Golgi apparatus in the flagellate Peranema trichophorum. J. Morphol. 48:105-121.
- Hall, R. P. 1934. A note on the flagellar apparatus of Peranema trichophorum and the status of the family Peranemidae Stein. Trans. Am. Microsc. Soc. 53:237-244.
- Hall, R. P. and W. N. Powell. 1928. Morphology and binary fission of Peranema trichophorum (Ehrbg.) Stein. Biol. Bull. 54:36-65.
- Hänel, V. K. 1979. Systematik and Ökologie der farblosen Flagellaten des Abwassers. Arch. Protistenkd. 121:73-137.
- Hartmann, M. and C. Chagas. 1910. Flagellaten-Studien. Mem. Inst. Osw. Cruz 2:64-125.
- Hausmann, K. 1978. Extrusive organelles in protists. Int. Rev. Cytol. 52:197-276.
- Hayat, M. A. 1970. Principles and Techniques of Electron Microscopy, Vol. 1. Van Nostrand Reinhold Co., N.Y.
- Huber-Pestalozzi, G. 1955. Das Phytoplankton des Süßwassers. 4. Euglenophyceen. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.
- Jahn, T. L. and E. C. Bovee. 1968. Locomotive and motile response in Euglena. In: D. E. Buetow, ed. The Biology of Euglena, Vol. 1. Academic Press, N.Y., pp. 45-108.
- Jahn, T. L., J. R. Fonseca and M. Landman. 1963. Mechanisms of locomotion of flagellates. III. Peranema, Petalomonas and Entosiphon. J. Protozool. 10(Suppl):11.
- Jensen, M. S. and D. F. Bainton. 1973. Temporal changes in pH within the phagocytic vacuole of the polymorphonuclear neutrophilic leukocyte. J. Cell Biol. 56:379-388.
- Jeon, K. W. 1965. Simple method for staining and preserving epoxy resin-embedded animal tissue sections for light microscopy. Life Sci. 4:1839.

- Johansen, D. A. 1940. Plant Microtechnique. McGraw-Hill Book Co., Inc., N.Y.
- Jurand, A. and G. G. Selman. 1969. The Anatomy of Paramecium aurelia. Macmillan and Co., Ltd., London.
- Kivic, P. A. and M. Vesik. 1974. Pinocytotic uptake of protein from the reservoir in Euglena. Arch. Microbiol. 96:155-159.
- Kristiansen, J. and P. L. Walne. 1977. Fine structure of photo-kinetic systems in Dinobryon cylindricum var. alpinum (Chrysophyceae). Br. Phycol. J. 12:329-341.
- Korschikow, A. A. 1923. Über den Bau und die Aggregation der Geisseln bei den Volvocales und den Flagellaten. Russkii Arkhiv Protistologii 2:198-205.
- Lackey, J. B. 1933. Studies in the life histories of Euglenida. III. The morphology of Peranema trichophorum Ehrenberg, with special reference to its kinetic elements and the classification of the Heteronemidae. Biol. Bull. 65:238-248.
- Lackey, J. B. 1940. Some new flagellates from the Wood's Hole area. Am. Midl. Nat. 23:463-471.
- Leedale, G. F. 1958a. Mitosis and chromosome numbers in the Euglenineae (Flagellata). Nature 181:502-503.
- Leedale, G. F. 1958b. Nuclear structure and mitosis in the Euglenineae. Arch. Mikrobiol. 32:32-64.
- Leedale, G. F. 1959. Periodicity of mitosis and cell division in the Euglenineae. Biol. Bull. 116:162-174.
- Leedale, G. F. 1967. Euglenoid Flagellates. Prentice-Hall, Inc. Englewood Cliffs, N.J.
- Leedale, G. F. 1969. Observations on endonuclear bacteria in euglenoid flagellates. Österr. Bot. Z. 116:279-294.
- Leedale, G. F. 1978. Phylogenetic criteria in euglenoid flagellates. BioSystems 10:183-187.
- Leedale, G. F. and D. J. Hibberd. 1974. Observations on the cytology and fine structure of the euglenoid genera Menoidium Perty and Rhabdomonas Fresenius. Arch. Protistenkd. 116:319-345.
- Lillie, R. D. 1969. H. J. Conn's Biological Stains. Williams and Wilkins Co., Baltimore.

- Luft, J. H. 1971. Ruthenium red and violet. Chemistry, purification, methods of use for electron microscopy and mechanism of action. *Anat. Rec.* 171:347-368.
- Malawista, S. E. and P. T. Bodel. 1967. The dissociation by colchicine of phagocytosis from increased oxygen consumption in human leukocytes. *J. Clin. Invest.* 46:786-796.
- Mast, S. O. and B. Hawk. 1936. Response to light in Peranema trichophorum. I. Relation between dark-adaptation and sensitivity to light. *Biol. Bull.* 70:408-412.
- Mazia, D., G. Schatten and W. Sale. 1975. Adhesion of cells to surfaces coated with polylysine. *J. Cell Biol.* 66:198-200.
- Mignot, J. P. 1963. Quelques particularités de l'ultrastructure d'Entosiphon sulcatum (Duj.) Stein, Flagellé Euglénien. *Comptes rendus des séances de l'Académie des Sciences* 257:2530-2533.
- Mignot, J. 1966. Structure et ultrastructure de quelques Euglenomonadines. *Protistologica* 2:51-117.
- Mignot, J. P. and R. Hovasse. 1973. Nouvelle contribution a la connaissance des trichocystes: les organites grillagés d'Entosiphon sulcatum (Flagellata, Euglenida). *Protistologica* 9: 373-391.
- Moestrup, Ø. 1978. On the phylogenetic validity of the flagellar apparatus in green algae and other chlorophyll a and b containing plants. *BioSystems* 10:117-144.
- Nilsson, J. R. 1979. Phagotrophy in Tetrahymena. In: S. H. Hutner and A. Lwoff, eds. *Biochemistry and Physiology of Protozoa*, Vol. 2, 2nd ed. Academic Press, Inc., N.Y., pp. 339-379.
- Nisbet, B. 1974. An ultrastructural study of the feeding apparatus of Peranema trichophorum. *J. Protozool.* 21:39-48.
- Nisbet, B. 1977. Flagellar regeneration in Peranema trichophorum. *J. Protozool.* 24:7A.
- Osborn, M. and K. Weber. 1976. Cytoplasmic microtubules in tissue culture cells appear to grow from an organizing structure towards the plasma membrane. *Proc. Nat. Acad. Sci. USA* 73: 867-871.
- Palisano, J. R. and P. L. Walne. 1972. Acid phosphatase activity and ultrastructure of aged cells of Euglena granulata. *J. Phycol.* 8:81-88.

- Palisano, J. R. and P. L. Walne. 1976. Light and electron microscopy of two permanently bleached cell lines of Euglena gracilis (Euglenophyceae). Nova Hedwigia XXVII:455-480.
- Parducz, B. 1967. Ciliary movement and coordination in ciliates. Int. Rev. Cytol. 21:91-128.
- Pearse, A. G. E. 1968. Histochemistry. Little, Brown and Co., Boston.
- Pease, D. C. 1964. Histological Techniques for Electron Microscopy. Academic Press, N.Y.
- Pfeiffer, D. R., R. W. Taylor and H. A. Lardy. 1978. Ionophore A23187: cation binding and transport properties. Ann. N.Y. Acad. Sci. 307:402-423.
- Pickett-Heaps, J. D. 1969. The evolution of the mitotic apparatus: an attempt at comparative ultrastructural cytology in dividing plant cells. Cytobios 3:257-280.
- Pitelka, D. R. 1945. Morphology and taxonomy of flagellates of the genus Peranema Dujardin. J. Morphol. 76:179-192.
- Pitelka, D. R. 1963. Electron-microscopic Structure of Protozoa. Macmillan Co., New York.
- Pitelka, D. R. 1969. Fibrillar systems in protozoa. In: T. T. Chen, ed. Research in Protozoology, Vol. 3. Pergamon Press, Oxford, pp. 282-388.
- Pitelka, D. R. and C. N. Schooley. 1955. Comparative morphology of some protistan flagella. Univ. Calif. Publ. Zool. 61:79-128.
- Plattner, H. and S. Fuchs. 1975. X-ray microanalysis of calcium binding sites in Paramecium with special reference to exocytosis. Histochemistry 45:23-47.
- Preer, J. R., Jr., L. B. Preer and A. Jurand. 1974. Kappa and other endosymbionts in Paramecium aurelia. Bacteriol. Rev. 38:113-163.
- Pringsheim, E. G. 1963. Farblosen Algen. Gustav Fischer Verlag, Stuttgart.
- Raff, E. C. 1979. The control of microtubule assembly in vivo. Int. Rev. Cytol. 59:1-96.
- Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17:208-212.

- Ringo, D. L. 1966. Form and function of flagellar apparatus: a study of Chlamydomonas reinhardi. Ph.D. Dissertation. University of Texas, Austin.
- Rosowski, J. R. and W. V. Glider. 1977. Comparative effects of metal coating by sputtering and by vacuum evaporation on delicate features of euglenoid flagellates. SEM/IITRI/1977/1:471-480.
- Roth, L. E. 1959. An electron-microscope study of the cytology of the protozoan Peranema trichophorum. J. Protozool. 6:107-116.
- Salisbury, J. L. and G. L. Floyd. 1978. Calcium-induced contraction of the rhizoplast of a quadriflagellate green alga. Science 202:975-977.
- Schnepf, E., G. Deichgräber, G. Röderer and W. Herth. 1977. The flagellar root apparatus, the microtubular system and associated organelles in the chrysophyceae flagellate, Poterioochromonas malhamensis Peterfi (syn. Poterioochromonas stipitata Scherffel and Ochromonas malhamensis Pringsheim). Protoplasma 92:87-107.
- Schwelitz, F. D., W. R. Evans, H. H. Mollenhauer and R. A. Dilley. 1970. The fine structure of the pellicle of Euglena gracilis as revealed by freeze-etching. Protoplasma 69:341-349.
- Shettles, L. B. 1937. Response to light in Peranema trichophorum with special reference to dark-adaptation and light-adaptation. J. Exp. Zool. 77:215-249.
- Shortess, G. S. 1942. The relation between temperature, light, and rate of locomotion in Peranema trichophorum and response to changes in temperature. Physiol. Zool. 15:184-195.
- Sleigh, M. A. 1973. The Biology of Protozoa. Edward Arnold, London.
- Sommer, J. R. and J. J. Blum. 1965. Cell division in Astasia longa. Exp. Cell Res. 39:504-527.
- Sommer, J. R. and J. J. Blum. 1965. Cytochemical localization of acid phosphatases in Euglena gracilis. J. Cell Biol. 24:235-251.
- Soyer, M. 1969. Étude ultrastructurale des inclusions paracrystallines intra-mitochondriales et intra-vacuolaires chez Noctiluca miliaris S. dinoflagellé, Noctilucidae et observations concernant leur rôle dans la genèse des trichocystes fibreux et muqueux. Protistologica 5:327-334.
- Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31-43.

- Stein, F. R. von. 1878. Der Organismus der Infusionsthier. III. Der Organismus der Flagellaten. I. Leipzig: Verlag von Wilhelm Engelmann.
- Stevenson, I. 1967. Diaminopimelic acid in the mu particles of Paramecium aurelia. Nature Lond. 215:434-435.
- Storm, J. and S. H. Hutner. 1953. Nutrition of Peranema. Ann. N.Y. Acad. Sci. 56:901-909.
- Tamm, S. L. 1964. Flagellar development in the protozoan Peranema trichophorum. J. Exp. Zool. 164:163-186.
- Tokuyasu, K. and O. H. Scherbaum. 1965. Ultrastructure of mucocysts and pellicle of Tetrahymena pyriformis. J. Cell Biol. 27: 67-81.
- West, L. K. 1977. Cellular organization and biomineralization of the envelope of Trachelomonas hispida var. coronata. Ph.D. Dissertation, The University of Tennessee, Knoxville.
- Wilson, L., J. R. Bamburg, S. B. Mizel, L. M. Grisham and K. M. Creswell. 1974. Interaction of drugs with microtubule proteins. Fed. Proc. 33:158-166.

APPENDICES

APPENDIX A

CULTURE MEDIUM

Milk Medium-0.1% w/v CaSO_4 + 1% v/v milk (Chen, 1950)

For flasks:

- | | |
|---------------------------|---------|
| 1. CaSO_4 | 1.5 g |
| 2. Double-distilled water | 1500 ml |

Use 250-ml Erlenmeyer flasks. Dispense 150 ml of above solution into each of 10 flasks. Add 1.5 ml fresh homogenized milk to each flask and swirl to mix. Steam sterilize solutions for 1 hour on 3 consecutive days.

For test tubes:

- | | |
|---------------------------|--------|
| 1. CaSO_4 | 0.2 g |
| 2. Double-distilled water | 200 ml |

Use 15-ml test tubes. Dispense 8 ml of above solution into each of 25 tubes. Add 3-4 drops of fresh homogenized milk to each tube and mix on Vortex mixer. Steam sterilize solutions for 1 hour on 3 consecutive days.

The addition of calcium sulfate (CaSO_4) causes the milk to coagulate during sterilization, leaving a less-turbid solution and thus permitting better observation of the organisms.

APPENDIX B

COMMON CHEMICALS USED IN ELECTRON MICROSCOPY

Buffers

1. Sodium Cacodylate Stock Solution (0.2 M)
Sodium cacodylate (Polysciences, FW:214) 21.4 g
Double-distilled water 500 ml
Adjust pH to 7.0. Store in refrigerator.
2. Sodium Cacodylate Working Solution (0.1 M)
Sodium cacodylate stock solution (0.2 M) 1 part
Double-distilled water 1 part
3. Sodium Cacodylate Working Solution (0.05 M)
Sodium cacodylate working solution (0.1 M) 1 part
Double-distilled water 1 part

Fixatives

1. Osmium Tetroxide Stock Solution (4%)
Osmium tetroxide (OsO_4) 1 g
Double-distilled water 25 ml

Use fume hood and polyvinyl gloves. Measure 25 ml of distilled water into heavy-walled glass reagent bottle. Drop a vial containing 1 g of OsO_4 crystals into reagent bottle. Fit bottle with ground glass stopper and swirl vigorously to break the vial. Wrap the bottle with aluminum foil (or use brown glass bottle) and leave at room temperature for 12 hours to allow the OsO_4 crystals to dissolve. Store in refrigerator.
2. Glutaraldehyde Stock Solution (TAAB Laboratories, 25% in water)
Store in refrigerator. Discard if pH drops below 3.5.
3. Glutaraldehyde Working Solution (4% in 0.2 M buffer)
Glutaraldehyde stock solution 4 ml

Sodium cacodylate stock solution (0.2 M) 21 ml

Store in refrigerator.

Paramecium Fixative (Jurand and Selman, 1969)

- | | |
|----------------------------|---------|
| 1. Sodium barbitone | 0.167 g |
| 2. Sodium acetate | 0.11 g |
| 3. 0.1 N Hydrochloric acid | 11.4 ml |
| 4. OsO_4 | 1.0 g |
| 5. Sucrose | 1.27 g |
| 6. Calcium chloride (dry) | 0.03 g |

Use fume hood and polyvinyl gloves. Add above constituents to ca. 50 ml distilled water and bring volume up to 100 ml. Pour fixative into heavy-walled glass reagent bottle. Wrap bottle in aluminum foil (or use brown glass bottle). Store in refrigerator.

Parducz Fixative (Parducz, 1967)

- | | |
|--|---------|
| 1. 2% aqueous OsO_4 | 6 parts |
| 2. Saturated aqueous mercuric chloride (HgCl_2) | 1 part |

Use fume hood and polyvinyl gloves. Measure above constituents into heavy-walled glass reagent bottle. Wrap bottle with aluminum foil (or use brown glass bottle). Store in refrigerator.

Spurr's Embedding Medium (Spurr, 1969)

- | | |
|--|--------|
| 1. VCD (Polysciences)
Vinylcyclohexene dioxide (epoxy resin) | 5.0 g |
| 2. DER 736 (Polysciences)
Diglycidyl ether of polypropylene glycol (flexibilizer) | 3.0 g |
| 3. NSA (Polysciences)
Nonenyl succinic anhydride
(anhydride hardener) | 13.0 g |
| 4. S-1 (Polysciences)
Dimethylaminoethanol (accelerator) | 0.2 g |

Measure all of the above chemicals gravimetrically into a 120-ml polypropylene beaker. Either acetone or propylene oxide may be used as a solvent during infiltration. For final embedding, use the above stock solution. Stock solutions may be stored for several weeks at 0° C. Dispense stock solution into 12-ml aliquots in snap-cap vials and store in freezer. Preheat BEEM capsules used for final embedding in 60° C drying oven with desiccants to prevent moisture.

Post-Stains for Ultrathin Sections

1. Aqueous Uranyl Acetate (Pease, 1964)

Uranyl acetate (Baker Chemical Co.) 0.5 g

Distilled water 100 ml

Dissolve uranyl acetate in distilled water. Filter using 0.45 μ m pore Millipore filter and store in refrigerator. Warm to room temperature before use. Solution should be clear. Do not use if turbid.

2. Saturated Methanolic Uranyl Acetate (Hayat, 1970)

Saturate methanol (25% or 50% in distilled water) by adding uranyl acetate gradually. Filter using 0.45 μ m pore Millipore filter and store in refrigerator. Warm to room temperature before use. Discard if solution becomes turbid.

3. 1% Potassium Permanganate (Bray and Wagenaar, 1978)

Potassium permanganate 1.0 g

Distilled water 100 ml

This stain is excellent for providing contrast to cells embedded in Spurr's plastic, but stain is susceptible to contamination by precipitation.

4. Reynold's Lead Citrate (Reynolds, 1963)

Lead nitrate 0.666 g

Sodium citrate 0.880 g

Distilled water 15 ml

Combine the above three ingredients by shaking mixture vigorously for 1 minute in a 25-ml volumetric flask. Let stand with intermittent shaking to dissolve and ensure complete conversion of lead nitrate to lead citrate. After 30 minutes, add 4 ml of

1.0 N sodium hydroxide (carbonate-free). Dilute mixture to 25 ml with distilled water. Mix by inversion. Solution should clear. Discard if solution becomes turbid.

APPENDIX C

STAINED YEASTS FOR STUDIES OF PHAGOCYTOSIS

Modified Krebs-Ringer Phosphate (KRP) Solution (Malawista and Bodel, 1967)

Stock solutions (DeLuca and Cohen, 1964)

1. 0.90% NaCl (0.154 M)

NaCl	9 g
Distilled water	1000 ml

2. 1.15% KCl (0.154 M)

KCl	1.15 g
Distilled water	100 ml

3. 0.244% CaCl_2 (0.02 M)

CaCl_2	0.244 g
Distilled water	100 ml

4. 0.76% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.03 M)

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.76 g
Distilled water	100 ml

5. 0.1 M phosphate

$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$	17.8 g
1 N HCl	20 ml

Mix 17.8 g $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ and 20 ml 1 N HCl. Dilute to 1 liter.

To prepare KRP solution, mix together the above solutions in the following amounts:

100 parts of solution 1

4	"	"	"	2
3	"	"	"	3

1 part of solution 4

20 " " " 5

Adjust pH to 7.3.

After mixing, the KRP solution is gassed with either O_2 , H_2 or air. A precipitate of calcium phosphate forms; this is suspended by shaking before use.

Staining of Yeasts (Jensen and Bainton, 1973)

Add 0.5 g dried baker's yeast to 10 ml KRP and heat at $100^\circ C$ for 60 minutes. Add 0.05 g neutral red. Centrifuge, wash with KRP and resuspend. Use within 2 days.

APPENDIX D

REPRESENTATIVE FIXATION PROCEDURE

Simultaneous Glutaraldehyde-OsO₄ Fixation in the Cold (Franke et al., 1969)

Procedure:

1. Pipette cells from the surface of the milk medium, gently centrifuge to concentrate (400 X g for 3 minutes) and wash once in distilled water. Immerse tubes containing cells in ice for ca. 1 minute before adding cold fixative.
2. Combine equal volumes of cold OsO₄ stock solution (4%) and cold glutaraldehyde working solution (4% in 0.2 M sodium cacodylate buffer). To this cold fixative immediately add an equal volume of chilled cells (final concentration of the fixative will be 1% OsO₄:1% glutaraldehyde buffered in 0.05 M sodium cacodylate). Keep the tubes containing the cells in fixative on ice for 30 minutes.
3. Wash cells twice with cold 0.1 M sodium cacodylate.
4. Combine equal volumes cold OsO₄ stock solution (4%) and cold sodium cacodylate stock solution (0.2 M). Post-fix cells in this fixative for 3 hours on ice.
5. Wash cells three times in cold 0.1 M sodium cacodylate.
6. Dehydration in cold acetone series (ca. 10 minutes each concentration in the cold)
 - a. 10% acetone
 - b. 25% acetone
 - c. 50% acetone
 - d. 70% acetone
 - e. 95% acetone
 - f. 100% acetone
 - g. 100% acetone
 - h. 100% acetone
7. Dehydrate in two changes of 100% propylene oxide at room temperature (15 minutes each).
8. Infiltration at room temperature
 - a. 1:1 (v/v) propylene oxide:Spurr's plastic 12 hours
 - b. 100% Spurr's plastic 8 hours

9. Embedding

Decant 100% plastic from fixation vessel and add fresh 100% plastic. Mix plastic with cells. Pellet the cells by centrifugation. Draw the pellet into a Pasteur pipette and add the cells to a small amount of 100% plastic in pre-dried BEEM capsules (00). Add sufficient 100% plastic to fill BEEM capsule. Centrifuge and polymerize at 60° C for 12-48 hours.

Pipette a small drop of the residual pellet containing the cells onto a glass slide, mount a coverslip, and examine the embedded cells with light microscopy. Check quality of fixation by looking for distortions (obvious swelling or shrinkage) that may have occurred during processing.

APPENDIX E

PREPARATIVE PROCEDURES AND STAINS FOR THICK SECTIONS

Slide Preparation for Examination of Thick Sections

1. Dissolve 1 g gelatin in 1000 ml of hot distilled water. Allow solution to cool; then add 0.1 g chromium potassium sulfate.
2. Clean glass slides in 70% ethanol and wipe clean. Dip the slides several times in the above solution. Air dry and store in a dust-free environment.

Stains for Thick Sections

1. Methylene Blue (Lillie, 1969)
(Stains pectin and cellulose purple)

Methylene blue	1.0 g
----------------	-------

Distilled water	100 ml
-----------------	--------

Dissolve stain in distilled water and filter using 0.45 μ m pore Millipore filter.

2. Azure II (Jeon, 1965)
(Stains membranes blue)

Azure II	0.1-0.5 g
----------	-----------

Borax	1.0 g
-------	-------

Distilled water	100 ml
-----------------	--------

Dissolve Azure II in 1% borax solution; filter using 0.45 μ m pore Millipore filter. Store in dark glass or polyethylene bottle.

3. Crystal Violet (Johansen, 1940)

Crystal violet	1.0 g
----------------	-------

Distilled water	100 ml
-----------------	--------

Dissolve stain in distilled water and filter using 0.45 μ m pore Millipore filter.

APPENDIX F

PROCEDURES FOR ULTRASTRUCTURAL CYTOCHEMISTRY

Ruthenium Red (RR) Staining (Chambers, 1973)

Reagents:

1. 0.2% RR Stock Buffer Solution

RR (ICN Pharmaceuticals, Inc.)	0.1 g
Sodium cacodylate working solution (0.1 M) pH 7.2	50 ml
2. 0.2% RR:4% Glutaraldehyde Working Solution

Glutaraldehyde stock solution (TAAB Laboratories, 25% in water)	2.0 ml
0.2% RR stock buffer solution	10.5 ml
3. 0.1% RR:2% OsO₄ Working Solution

OsO ₄ stock solution (4%)	1 part
0.2% RR stock buffer solution	1 part

Procedure:

1. Concentrate cells to a loose pellet by gentle centrifugation (400 x g for 3 minutes) and wash in distilled water. Combine cells and 0.2% RR:4% Glutaraldehyde Working Solution 1:1 (v/v). Final concentration of fixative is 2% glutaraldehyde, 0.1% RR buffered in 0.05 M sodium cacodylate. Fix cells for 1 hour at room temperature.
2. Wash cells twice in 0.05 M sodium cacodylate.
3. Post fix cells in 0.1% RR:2% OsO₄ Working Solution for 3 hours at room temperature.
4. Wash cells in 0.05 M sodium cacodylate.

5. Dehydration in acetone series at room temperature (ca. 10 minutes each concentration)

a. 10% acetone	e. 95% acetone
b. 25% acetone	f. 100% acetone
c. 50% acetone	g. 100% acetone
d. 70% acetone	h. 100% acetone
6. Dehydrate in two changes of 100% propylene oxide (15 minutes each).
7. Infiltration

a. 1:1 100% propylene oxide:Spurr's plastic	12 hours
b. 100% Spurr's plastic	8 hours
8. Embedding

Decant 100% plastic and add fresh 100% plastic. Pellet the cells by centrifugation. Draw the pellet into a Pasteur pipette and add the cells to a small amount of 100% plastic in pre-dried BEEM capsules (00). Centrifuge and polymerize at 60° C for 12-48 hours.

Acid Phosphatase Labeling (Gomori, 1952; Pearse, 1968; Chayen et al., 1969; Berjak, 1972)

Reagents:

1. Sodium Acetate Buffer Stock Solution (0.1 N)

Sodium acetate	13.6 g
Distilled water	1000 ml

Adjust pH to 5.0 using 0.1 N acetic acid or 0.1 N sodium hydroxide.
2. Sodium Acetate Buffer Working Solution (0.05 N)

Sodium acetate buffer #1	1 part
Distilled water	1 part

3. Substrate Solution for Gomori Reaction Medium (3% or 0.1 M)

Sodium- β -glycerophosphate	3 g
Distilled water	100 ml

4. Gomori Reaction Medium (Gomori, 1952)

Sodium acetate buffer working solution (0.05 N) #2	500 ml
---	--------

Lead nitrate	0.6 g
--------------	-------

Adjust pH to 5.0. The Gomori reaction medium may be used as a control without substrate.

5. Gomori Reaction Medium Plus Substrate (Gomori, 1952)

Substrate solution #3	50 ml
-----------------------	-------

Gomori reaction medium #4	500 ml
---------------------------	--------

The reaction medium plus substrate is left for 24 hours at 37° C, during which time a white precipitate should form. Cool the solution and filter using 0.45 μ m pore Millipore filter. Use the medium immediately after filtration.

6. Gomori Reaction Medium with Sodium Fluoride Inhibitor Used as Control (Berjak, 1972)

Sodium fluoride	0.42 g
-----------------	--------

Gomori reaction medium #4	100 ml
---------------------------	--------

7. Ammonium Sulfide Coupler

Ammonium sulfide	0.02-0.1 ml
------------------	-------------

Distilled water	100 ml
-----------------	--------

Use a fume hood during preparation of the solution. Use fresh solution immediately.

Procedure:

1. Concentrate cells to a loose pellet by gentle centrifugation (400 x g for 3 minutes). Wash once in distilled water.
2. Combine equal volume of cells with equal volume of 2% glutaraldehyde buffered in 0.1 M sodium cacodylate, pH 7.0, and fix for 1 hour at room temperature. Final fixative concentration is 1% glutaraldehyde buffered in 0.05 M sodium cacodylate.

3. Wash cells three times in 0.05 M sodium cacodylate, pH 7.0.
4. Distribute cells in tubes labeled the following: (a) Gomori reaction medium; (b) Gomori reaction medium plus substrate; (c) Gomori reaction medium with sodium fluoride as inhibitor.
5. Wash cells four times in 0.05 N sodium acetate buffer.
6. Add the respective Gomori reaction media to the labeled tubes and incubate in a water bath at 37° C for 1-2 hours.
7. Centrifuge and decant Gomori reaction media. Wash cells three times in distilled water.
8. Saturate cells with 0.02% ammonium sulfide #7 for 3 minutes.
9. Centrifuge and decant ammonium sulfide solutions. Wash cells four times in distilled water.
10. Post-fix cells in 1% OsO₄ buffered in 0.05 M sodium cacodylate for 2 hours or overnight at room temperature.
11. Wash cells once in 0.1 M sodium cacodylate buffer.
12. Dehydrate in graded acetone series for 10 minutes in each concentration and in two changes of 100% propylene oxide for 15 minutes each.
13. Infiltrate sequentially in the following:
 - a. 1:1 100% propylene oxide:Spurr's plastic 12 hours
 - b. 100% Spurr's plastic 8 hours
14. Embedding

Decant 100% plastic and add fresh 100% plastic. Pellet the cells by centrifugation. Draw the pellet into a Pasteur pipette and add the cells to a small amount of 100% plastic in pre-dried BEEM capsules (00). Centrifuge and polymerize at 60° C for 12-48 hours.

APPENDIX G

PREPARATIVE PROCEDURE FOR SCANNING ELECTRON MICROSCOPY

Polylysine-coated glass coverslips (Mazia et al., 1975)

Poly-D-lysine (United States Biochemical) 10 mg

Distilled water 10 ml

Use 12-mm round glass coverslips. Dip coverslips into above solution, rinse once with distilled water and let dry.

Procedure:

1. Concentrate cells to a loose pellet by gentle centrifugation (400 X g for 3 minutes), wash once in distilled water, and fix for 15 minutes either in 2% unbuffered OsO₄ or Parducz fixative. Wash three times in distilled water. Place a polylysine-coated coverslip in the bottom of a Petri dish and pipette a drop of cells onto the coverslip. Allow cells to settle and adhere to the glass for 2-3 minutes.

The following manipulations are carried out while the cells are attached to the glass coverslip. Cells must remain submerged at all times to prevent drying.

2. Wash the cells by adding distilled water to the Petri dish containing the glass coverslip. Pipette off most of the water but leave enough to cover the cells. Gradually add 10% acetone and pipette off the solution until solution consists entirely of 10% acetone. Leave cells in 10% acetone for ca. 10 minutes.
3. Follow the above procedure for 25%, 50%, 70%, 95% and three changes of 100% acetone.
4. Transfer the coverslip to a 13-mm coverslip holder immersed in 100% acetone. Load the holder into an acetone-filled specimen chamber of a critical point drying (CPD) apparatus (Bomar SPC-900/EX). Purge the acetone and replace with liquid CO₂ according to the CPD directions.
5. Mount the glass coverslip on an SEM pedestal with Neolube (a dry film conductive lubricant). Leave overnight in a vacuum desiccator.
6. Coat specimens with gold or gold/palladium before examining in the scanning electron microscope.

APPENDIX H

ISOLATION OF FLAGELLA AND MASTIGONEMES

Procedure (after Bouck et al., 1978):

1. Concentrate cells to a loose pellet by gentle centrifugation (400 X g for 3 minutes) and wash once in distilled water.
2. Resuspend cells in ca. 2 ml of cold distilled water and deflagellate by agitation in a Super-Mixer (Lab-Line Instruments, Inc.) for 2 minutes at top speed.
3. Separate whole cells from suspension by centrifugation (1000 X g) in an International Clinical Centrifuge for 30 seconds.
4. Discard pellet of whole cells and centrifuge supernatant containing flagella in a Beckman Model J-21C centrifuge at 17,500 rpm (37,000 X g) for 30 minutes.
5. Discard supernatant and resuspend white pellet in 1% (v/v) Sarkosyl NL-30 in distilled water or in 1.5% (w/v) Sarkosyl NL-100 in distilled water. Leave overnight at room temperature.
6. Centrifuge Sarkosyl solution at 40,000 rpm (192,000 X g) in a SW 50.1 rotor in Beckman L3-50 Ultracentrifuge for 30 minutes.
7. Discard supernatant. Take aliquots of pellet and pipette samples onto carbon/Formvar-coated grids. Stain with 1% aqueous uranyl acetate and examine with transmission electron microscopy.

VITA

Lula Lail Hilenski, daughter of Julius L. and Catherine H. Lail, was born in Gastonia, North Carolina, on December 5, 1947. She attended elementary school in that city and was graduated from Hunter Huss High School in June 1966. The following August, she entered Pfeiffer College, Misenheimer, North Carolina, and in May 1970, graduated cum laude with a Bachelor of Arts degree in Biology.

She received the Master of Arts in College Teaching degree with a major in Botany from The University of Tennessee, Knoxville in June 1974. During the M.A.C.T. program she held a fellowship through the Education Professions Development Act (1970-72) and a Graduate Teaching Assistantship from the Department of Botany (1972-74). Her thesis topic was "Characterization of lipids from isolated eyespot granules of Euglena gracilis var. bacillaris."

She entered the doctoral program at The University of Tennessee, Knoxville in 1974, and subsequently held the position of Graduate Teaching Assistant in the Department of Botany (1974-77), Hilton A. Smith Graduate Fellow (1977-78) and Research Assistant in Agricultural Engineering (1978-79). In August 1980, she completed the Ph.D. degree in Botany with major areas in phycology and cell biology and is a Postdoctoral Research Associate in the Department of Plant and Soil Science, Institute of Agriculture, The University of Tennessee, Knoxville. Her membership in professional and honorary societies include American Association for the Advancement of Science, The American Society for Cell Biology, Electron Microscopy Society of America, The Society of Sigma Xi and Phi Kappa Phi. Publications

and published abstracts of papers presented at regional and national scientific meetings include the following:

- Hilenski, L. L., P. L. Walne and F. Snyder. 1976. Aliphatic chains of esterified lipids in isolated eyepots of Euglena gracilis var. bacillaris. Plant Physiol. 57:645-646.
- Hilenski, L. L. and P. L. Walne. 1979. Some cytological features of the phagotrophic euglenoid flagellate Peranema. J. Phycol. 15 (Suppl.):16.
- Hilenski, L. L., P. L. Walne, F. Snyder and M. Blank. 1974. Characterization of eyespot lipids in Euglena gracilis var. bacillaris. J. Phycol. 10(Suppl.):6.
- Hilenski, L. L., P. L. Walne, F. Snyder and E. A. Cress. 1974. Partial characterization of the lipid constituents from isolated eyespot granules of Euglena gracilis var. bacillaris. ASB Bull. 21:61.
- Hilenski, L. L., P. L. Walne, F. Snyder and E. A. Cress. 1973. Preliminary characterization of lipid components from isolated eyespots of Euglena gracilis var. bacillaris. J. Phycol. 9 (Suppl.):20.

She is married to Ferdinand Alexi Hilenski II of Knoxville, Tennessee.