# THE STRUCTURE, ORIGIN, ISOLATION, AND COMPOSITION OF THE TUBULAR MASTIGONEMES OF THE *OCHROMONAS* FLAGELLUM

G. BENJAMIN BOUCK

From the Biology Department, Yale University, New Haven, Connecticut 06520. Dr. Bouck's present address is the Department of Biological Sciences, University of Illinois, Chicago, Illinois 60680.

ABSTRACT

The structure, assembly, and composition of the extracellular hairs (mastigonemes) of *Ochromonas* are detailed in this report. These mastigonemes form two lateral unbalanced rows, each row on opposite sides of the long anterior flagellum. Each mastigoneme consists of lateral filaments of two distinct sizes attached to a tubular shaft. The shaft is further differentiated into a basal region at one end and a group of from one to three terminal filaments at the free end. Mastigoneme ontogeny as revealed especially in deflagellated and regenerating cells appears to begin by assembly of the basal region and shaft within the perinuclear continuum. However, addition of lateral filaments to the shaft and extrusion of the mastigonemes to the cell surface is mediated by the Golgi complex. The ultimate distribution of mastigonemes on the flagellar surface seems to be the result of extrusion of mastigonemes near the base of the flagellum, and it is suggested that mastigonemes are then pulled up the flagellum as the axoneme elongates. Efforts to characterize mastigonemes biochemically after isolation and purification on cesium chloride (CsCl) followed by electrophoresis on acrylamide gels have demonstrated what appear to be a single major polypeptide and several differentially migrating carbohydrates. The polypeptide is not homologous with microtuble protein. The functionally anomalous role of mastigonemes in reversing flagellar thrust is discussed in relation to their distribution relative to flagellar anatomy and to the plane of flagellar undulations.

The mastigonemes, flimmer, or extraflagellar hairs which are diagnostic of the tinsel flagellum are of increasing interest as their occurrence is documented in an ever broadening range of organisms including members of the Foraminifera (Hedley, Parry, and Wakefield, 1968), sponges (Afzelius, 1961), fungi (e.g. Heath et al., 1970), and algae (Pitelka and Schooley, 1955, for a comprehensive account). Mastigonemes of these organisms seem to fall into two general categories. One type may function by enlarging the effective surface area of the flagellum, as, for example, probably occurs during the breaststroke flagellar movements characteristic of *Chlamydomonas* (Ringo, 1967). A second group of mastigonemes, usually of a more complex architecture, can reverse the thrust of the flagellum. In theory, this occurs because mastigonemes operate as struts which sweep downward passively as the flagellar undulatory waves travel upwards (Jahn, Landman, and Fonseca, 1964). By this interpretation, forward movement occurs because the displacement of medium *towards* the organism by mastigonemes is relatively greater than the displacement of medium *away* from the organism by the action of the flagellum alone. Despite its apparent inefficiency, this concept has gained wide

acceptance and some experimental support (Holwill and Sleigh, 1967). However, it has not been demonstrated directly whether in fact mastigonemes are arranged on the flagellum in the exact planar orientation critical to this hypothesis.

An equally interesting problem is the nature of mastigoneme development. Although other components of the flagellum have been intensively investigated, the origin, composition, and structure of mastigonemes are largely unknown. Recent studies (Bouck, 1969; Leedale, Leadbeater, and Massalski, 1970; Heath, Greenwood, and Griffiths, 1970) suggest that at least some mastigonemes are not assembled *in situ* on the flagellar surface but appear to originate within the nuclear envelope and its contiguous membrane-limited regions (collectively defined here as the perinuclear continuum). How these "presumptive" mastigonemes (Bouck, 1969) within the perinuclear continuum are subsequently released to the flagellar surface has not yet been determined. Similarily, only fragmentary information is available on the composition of mastigonemes. Here knowledge is limited to the observations that mastigonemes are resistant to dissolution by several enzymes and nonspecific reagents (Pitelka and Schooley, 1955), and that colchicine has little apparent effect in their assembly or integrity (Heath, Greenwood, and Griffiths, 1970).

The activities and water currents produced by mastigoneme-bearing flagella have been thoroughly analyzed in the Chrysophyte, *Ochromonas* both by high speed cinematography (Jahn et al., 1964) and by stroboscopy (Holwill and Sleigh, 1967). It seems therefore that a detailed examination of the ontogeny, structure, attachment, and composition of *Ochromonas* mastigonemes might be especially useful for a more complete understanding of mastigoneme function and development. Developmental studies in particular can be facilitated in *Ochromonas* because cells are readily deflagellated (thereby losing extraflagellar mastigonemes) and the flagellum can subsequently regenerate (Dubnau, 1961). Such treatments should reveal the complete assembly and discharge of mastigonemes in suitable thin sections taken during the regenerating period when new mastigonemes must be added to the elongating flagellum.

These and other studies which are detailed in the present report suggest that assembly of mastigonemes in *Ochromonas* involves two cell compartments, the perinuclear continuum and the Golgi saccules, each of which contributes specific morphological components to the mastigoneme. After assembly is completed in the cytoplasm, mastigonemes are then apparently added to the external base of the regenerating flagellum. The final distribution of mastigonemes on the flagellar surface is unbalanced and essentially planar, but the plane of mastigoneme attachment cannot be equated with the plane of flagellar undulations from the available evidence.

In addition to detailing the structure, ontogeny and distribution of *Ochromonas* mastigonemes, a general method for isolation and purification of mastigonemes is described. Preliminary biochemical analysis of these isolates suggests that the structurally complex mastigoneme of *Ochromonas* may consist of only a single polypeptide with several associated carbohydrates.

## MATERIALS AND METHODS

Cultures of *Ochromonas danica* were grown at 25°C in 3-liter flasks containing 400 ml of Aaronson and Baker's (1959) medium as modified by Dubnau (1961). Continuous illumination of about 300 footcandles was used initially, but in later experiments a 16 hr light schedule at the same light intensity was generally employed. Cultures were harvested 4 days after inoculation when cell density was estimated to be about $10^7$ cells/ml.

### *Light Microscopy*

A 1.30 NA planapochromat Zeiss bright-field objective was used with Nomarski optics for viewing and recording flagella. Individual flash photographs of actively swimming cells were obtained with Zeiss microflash illumination of about 0.3–0.5 msec duration, and the images were recorded on Adox KB-14, 35mm film as recommended by Allen et al. (1969).

### *Electron Microscopy*

Cultured cells were pelleted by gentle centrifugation, and the medium was removed and replaced with glutaraldehyde (Polysciences, Inc., Rydal, Pa.) diluted to 3% (v/v) in 0.1 M phosphate buffer at a pH of 7.0. After 2 hr of fixation at room temperature the cells were recentrifuged and the glutaraldehyde solution was replaced with cold phosphate buffer. After four changes of buffer, each of 15 min duration, cells were postfixed with cold 1% osmium tetroxide in phosphate buffer overnight. Sequential dehydration in acetone was usually followed by infiltration in Epon, but in all embeddings of deflagellated and regenerating cells Spurr's (1969) hardest epoxy mixture was substituted for Epon. Following polymerization

at 60°C, the blocks were sectioned with a Dupont diamond knife on either a Sorvall, Porter-Blum MTII, or Reichert ultramicrotome. After staining in uranyl acetate and lead citrate (Reynolds, 1963), the sections were examined and photographed in either a Siemens Elmiskop IA, a JEM6C, or a Philips 300 electron microscope.

Negative contrast was achieved with aqueous solutions of 1% uranyl acetate by adding a drop of "stain" to a drop of suspension on a parlodion-coated, carbon-stabilized copper grid. After removing most of the combined droplets, the grids were allowed to dry at room temperature and then examined immediately.

### *Isolation of Mastigonemes*

Mastigonemes were purified by equilibrium density centrifugation in cesium chloride after their removal from the flagellar surface with the anionic detergent, Sarkosyl. The following procedure was found to give adequate yields of highly purified mastigonemes:

(*a*) 4 days after inoculation the organisms from the medium contained in six to eight culture flasks (2.4–3.2 liters) were concentrated by centrifugation. The pelleted cells were resuspended in 8 ml of medium and deflagellated by agitation of 2 ml samples in a fluted glass tube (Rosenbaum and Child, 1967) for 2 min at top speed in a "Super-mixer" homogenizer (Lab-Line Instruments, Inc., Melrose Park, Ill.). This procedure not only detached flagella from the cell body, but also removed some mastigonemes from the flagellar surface. Intact and deflagellated bodies were then removed from the medium by centrifugation for 5 min at the number five setting of a clinical international centrifuge (about 1000 *g*). The remaining turbid supernatant which contained broken cells, flagella, and mastigonemes was centrifuged at 40,000 rpm (about 149,000 *g* at the tube center) for 30 min in the SW 50 rotor of a Spinco model L ultracentrifuge.

(*b*) Pellets obtained from the high speed centrifugation were resuspended in 20 ml of a solution containing 1% Sarkosyl NL-97 (sodium lauroyl sarcosinate; Geigy Chemical Corp., Ardsley, N.Y.), 0.01 M Tris (Tris [hydroxymethyl] aminomethane), and 0.001 M ethylenediaminetetraacetic acid (EDTA) adjusted to a pH of 7.0. After 30 min this mixture was centrifuged at 40,000 rpm as above, and a brownish pellet was obtained.

(*c*) The brownish pellets were thoroughly resuspended in 60% (w/v) cesium chloride, and centrifuged to equilibrium (usually 22 hr at 40,000 rpm). The resulting whitish band which sometimes appeared to be doubled was adequately separated from an orange-colored surface layer so that the band could be easily collected with a Pasteur pipette. Cesium chloride was removed either by dialysis against distilled water overnight or by dilution of the bands with distilled water followed by pelleting of the mastigonemes at 40,000 rpm for 1 hr. About 50 μg of Lowry-positive material (Lowry et al., 1951), presumably protein, could be obtained from the mastigonemes in 6 harvested flasks (2.4 liters).

### *Preparation of Purified Mastigonemes for Electrophoresis*

Mastigonemes were readily solubilized in a solution of 0.01 M Tris and 0.001 M EDTA containing 8 M urea (pH 7.0). To assure a more complete disaggregation of subunits some samples were reduced in 8 M urea and 0.12 M mercaptoethanol, and then alkylated in iodoacetate as described by Renaud et al., 1968.

### *Acrylamide Gel Electrophoresis*

7.5% acrylamide gels containing 8 M urea were prepared (Davis, 1964) with a spacer gel but without a sample gel. Electrophoresis was performed in 12-cm tubes at a constant current of 2 ma/gel. Samples containing about 20 μg of protein, 3 μl bromphenol blue, and 10% sucrose were applied to the surface of the spacer gel and overlaid with Tris-glycine buffer. After electrophoresis, the gels were removed from the tubes with a jet of running water, fixed in 20% sulfosalicylic acid overnight, and stained with either Coomassie blue or the periodic acid-Schiff (PAS) procedure.

## RESULTS

### *Light Microscopy*

Forward movement in *Ochromonas* is accompanied by rotation about the longitudinal axis. Rotation appears to be the result of body shape and not flagellar wave form, as spherical organisms (made spherical with colchicine or pressure; Brown and Bouck, in preparation) swim without rotation. The main propulsive force is generated by a relatively long anterior flagellum (Figs. 1 a–f) whereas the short flagellum is flexed over the anterior eyespot and appears to play little apparent role in normal movements. Flash photographs of the undulating long flagellum suggest that wave propagation is not helical but nearly planar (Fig. 1*b*), as has also been suggested from stroboscopic analysis of flagella from a related species of *Ochromonas* (Holwill and Sleigh, 1967). If the cell is viewed longitudinally from the eyespot side of the cell (arbitrarily defined as the frontal view), the plane of the undulations would appear to bisect the cell

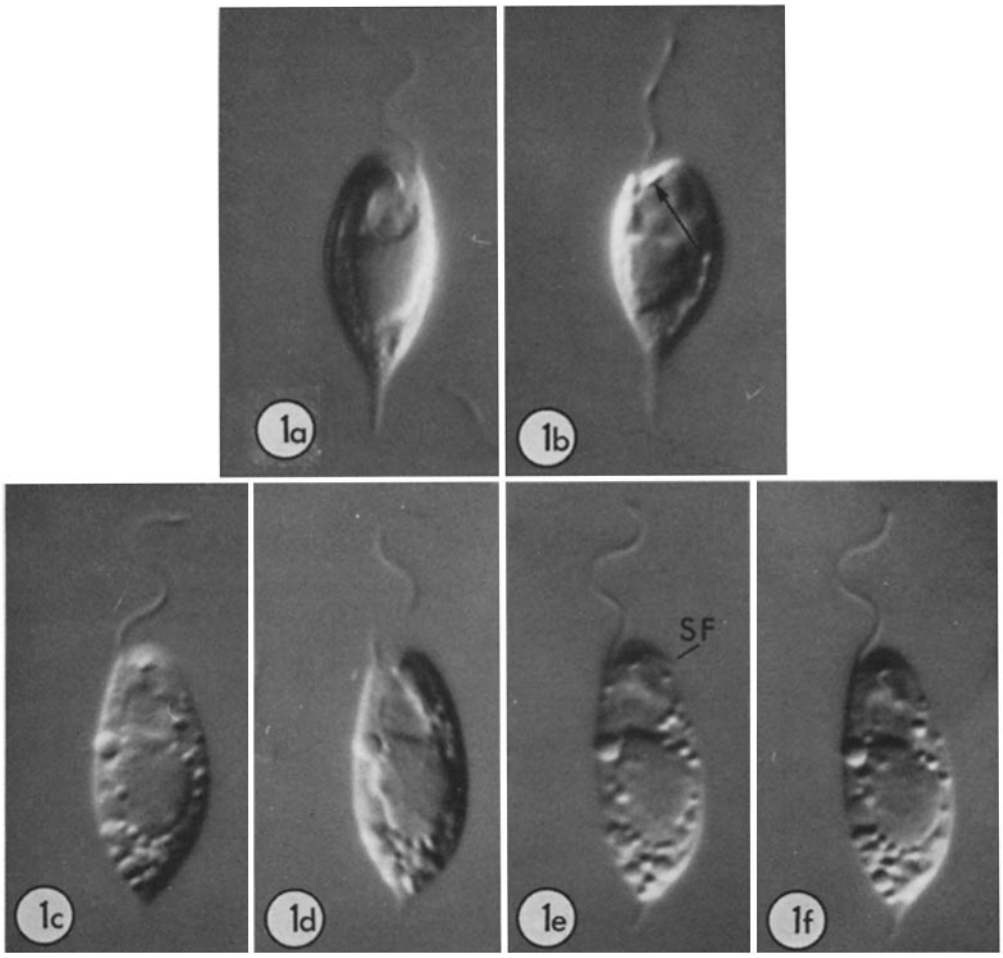



FIGURE 1 *a–b* Microflash photomicrographs of swimming *Ochromonas* illustrating the nearly planar undulations in face view (1 a) and in optical section perpendicular to the planar waves (1 b). An indication of an arch perpendicular to the plane of the undulations may be seen in 1 b. Movement is forward towards the top of the micrograph, flagellar undulations are propagated distally (also towards the top of the micrograph), and the cell rotates around its long axis as it moves forward. Note eyespote in 1 b (*arrow*) × 2200.

FIGURE 1 *c–f* Random flash photographs of a single "crawling" *Ochromonas* in which the posterior tail adheres to the substratum. Medium is propelled towards the body, and movement is in the direction of the top of the micrograph. Flagellar wave form and propagation appears to be similar to that of the free swimming cells. The short flagellum can be seen in 1 e (*SF*). × 2200.

and the eyespot (cf. Fig. 20). During swimming or when stationary, the long flagellum also appears to be arched, but it is not entirely clear whether the arch is in the plane of the undulations, at right angles to the plane of the undulations, or in both planes. Figs. 1 a and 1 b illustrate examples in which a curve of the flagella can be detected in both planes, but the curve is somewhat more pronounced and more consistently found perpendicular to wave propagation so that the flagellum can best be interpreted as undulating in a curved plane. Frame-by-frame analysis of high speed cinematographic films of the flagellar undulations confirms the planar nature of the undulations and also confirms earlier observations that these undulations are propagated distally (equipment for

filming was kindly provided by Dr. T. Jahn; cf. also Jahn et al., 1964), i.e. travel in the same direction as cell movement. Medium, as measured by the motions of debris or introduced graphite particles, is propelled proximally, or towards the cell body. Contrary to earlier reports, no cells were observed with a trailing flagellum despite repeated efforts to find what might be interpreted as a mastigoneme-free individual.

## *Electron Microscopy*

### Mastigoneme Structure

Mastigonemes of *Ochromonas* are of two structural types, fibrous and tubular. The *fibrous* mastigonemes are of small diameter (50–100 A), 1–3 $\mu$ long, and are found on both the long and the short flagella (Fig. 3, 5; also, Pitelka and Schooley, 1955). The *tubular* mastigonemes are about 200 A in diameter, 1 $\mu$ long, possess a tapered basal region attached to a microtubular-like shaft (Figs. 2, 4, 5, 6 a), and are found only on the long flagellum. The lumen of the tubular mastigoneme shaft is hydrophilic, often accumulating uranyl acetate, whereas the basal region appears to be solid. Terminal filaments are found in pairs or triplets at the extreme distal end of the tubular mastigoneme, and are similar to other reports of distal modification on Chrysophyte mastigonemes (Pitelka and Schooley, 1955; Manton 1952; Bradley, 1966; Schnepf and Deichgraber, 1969). Except in dimensions, all these portions of the tubular mastigonemes are remarkably similar to the tubular mastigonemes previously reported in the sperm of flagellum of brown algae (Bouck, 1969). However, additional components appear on the *Ochromonas* mastigoneme in the form of two sets of lateral filaments. The shorter laterals which are 300–400 A long appear, in negatively stained dried preparations, to be attached in two rows on opposite sides of the shaft (Fig. 6 a). However, in sections of mastigonemes these lateral filaments are seen not in two rows but rather positioned around the full diameter of the shaft in at least five or six rows (Fig. 6 b, c). These short lateral filaments clothe the entire mastigoneme shaft except for the basal region, and are of a nearly uniform length. The other set of lateral filaments are regularly inserted among the short lateral filaments. These comprise the long lateral filaments and are of a uniform length of about 2000 A (Fig. 6 a). The precision of their spacings and of their individual lengths suggest that the two kinds of lateral filaments are indeed dissimilar, and that each is a well defined component of the *Ochromonas* tubular mastigoneme.

The complete tubular mastigoneme of *Ochromonas*, then, consists of (*a*) a 2500 A basal attachment region, (*b*) a 1 $\mu$ microtubular-like shaft, (*c*) a few relatively long (0.5 $\mu$) terminal filaments, (*d*) numerous short (400 A) lateral filaments, and (*e*) longer (2000 A) lateral filaments. This complex architecture is remarkably constant from one individual mastigoneme to another.

### Arrangement of Mastigonemes Relative to the Flagellar Surface

The tubular mastigonemes of *Ochromonas* are attached in two unbalanced files on nearly opposite sides of the flagellum (Figs. 5, 7 a–c). One file consists of clusters of two or more mastigonemes separated from adjacent clusters by a distance of 3000 A or less. Clusters of mastigonemes originate from a modified region of the flagellar membrane which appears, in negatively contrasted whole mounts, as a platelike structure (Fig. 7 a), designated the "attachment plate." Sections of the flagellar membrane have not clarified the structure of the attachment plate, but indications of a similar differentiated area can be seen in Mignot's report of mastigoneme attachment in *Peranema* (e.g. Joyon and Mignot, 1969). The number of mastigonemes comprising a cluster in *Ochromonas*, i.e. on one attachment plate, generally decreases distally so that near the tip of the flagellum only one mastigoneme may occupy each plate (Fig. 5). Mastigonemes in the other file are attached singly along the entire length of the flagellum. The spacings between these individually attached mastigonemes in one row do not necessarily coincide with the spacings between the clusters of mastigonemes on the opposite side of the flagellum.

The placement of the finer, fibrous mastigonemes appears to be less precise. Some are associated with the short flagellum, while others are found scattered over the whole length of the long flagellum. A group of fibrous mastigonemes are usually attached to the tip of the long flagellum (Fig. 5).

### Attachment of Mastigonemes Relative to Flagellar Anatomy

Sections of fixed and embedded flagella revealed that mastigonemes may be attached in

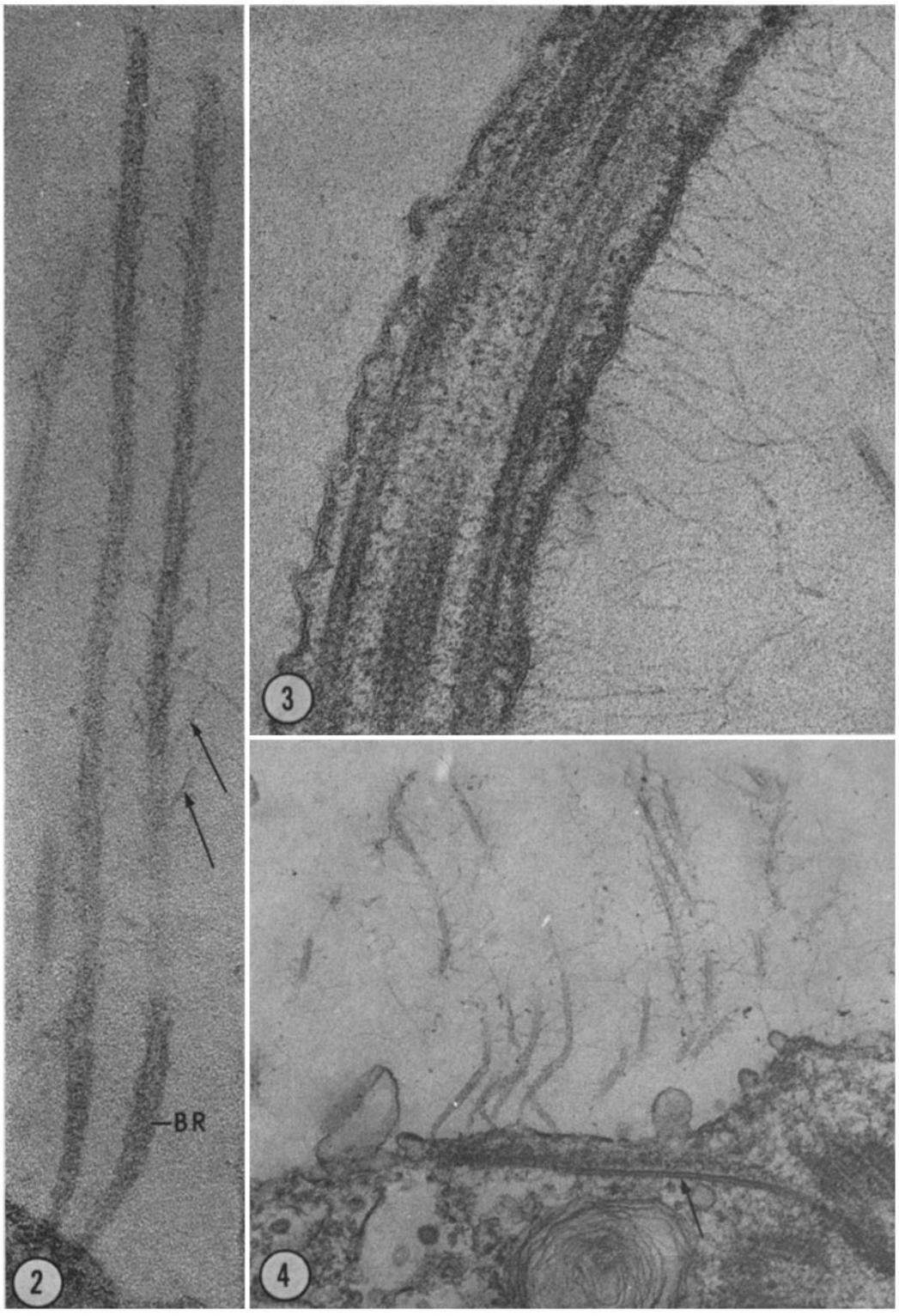
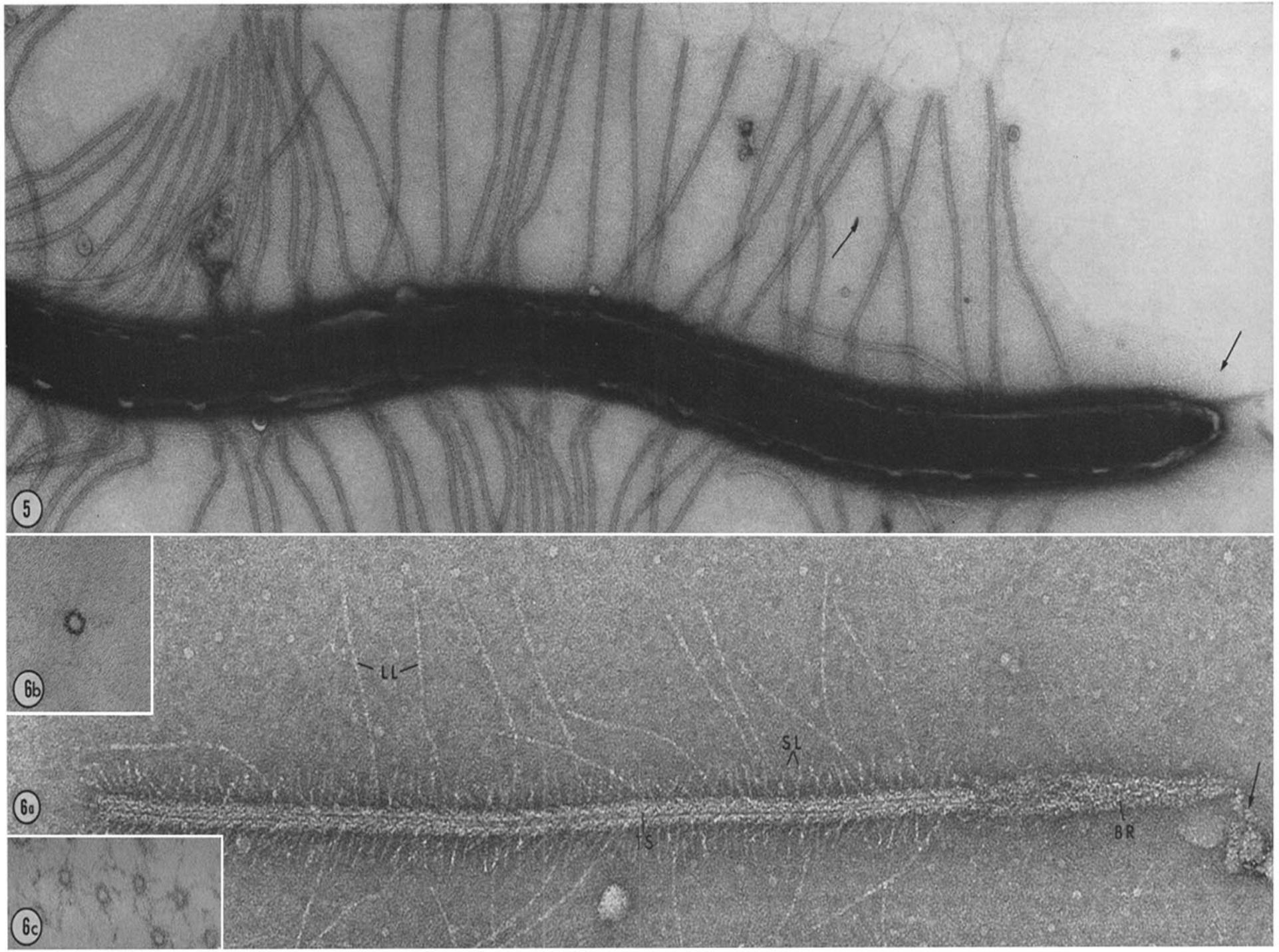



FIGURE 2 Electron micrograph of a section of the long flagellum with attached tubular mastigonemes. Lateral filaments can be identified along the mastigone shaft (*arrows*) but not on the basal attachment region (*BR*). × 154,000.

FIGURE 3 Longitudinal section of flagellum illustrating mat of fibrous mastigonemes attached to the flagellar membrane. × 100,000.

FIGURE 4 Section of *Ochromonas* near the insertions of the flagella. Apparently completely formed tubular mastigonemes are shown attached to the cell surface near the base of the flagellum. The membrane-like structure (*arrow*), which is an extension from the basal body of the short flagellum, often seems to mark the site at which mastigonemes are released to the surface. × 55,000.


5
6b
LL
SL
6a
TS
BR
6c

fairly consistent orientations relative to the plane of the central pair of axonemal microtubules. Suitable micrographs for these determinations were infrequent due to the only occasional coincidence of attachment of mastigonemes on both sides of the flagellum with the level of a random section. Consequently, the sampling of large numbers of flagella with appropriate orientations has proven unfeasible thus far. However, four micrographs in which it was possible to recognize members from both files of mastigonemes are presented in Figs. 8 a–d. Three of these examples indicate attachment in or near the same plane as the central pair (Figs. 8 a–c) of microtubules, while in the fourth illustration (Fig. 8 d) mastigonemes seem to be attached perpendicular to the central pair. This last example, however, may have been damaged during preparation.

Axial rods or other stiffening structures are absent from both the long and the short flagella. Apart from the parabasal body localized on the short flagellum, the only unusual structural modification of the flagellum is the appearance of a short, coiled fiber at the base of both flagella (Fig. 9). This fiber which takes three or four turns around the central pair of axonemal microtubules occupies approximately the same longitudinal region of the flagellum as the stellate pattern of finer overlapping fibers which may occur in many other flagellated plant cells (Lang, 1963; Manton, 1964). Since it is difficult to conceive how this coiled fiber could mediate the profound effects on motility which characterize many mastigoneme-bearing organisms, it is concluded that there are no apparent structural elaborations of flagellar anatomy which might account for the reversal of flagellar thrust in *Ochromonas*. It should also be noted that no evidence for mastigonemes in any stage of construction has been found within the flagellar membrane. Furthermore, an electron-opaque plug of material is present within the axoneme at the level of the cell surface which would seem to preclude passage of formed structures into the axoneme from the remainder of the cytoplasm (Fig. 9).

### Presumptive Mastigonemes

NONREGENERATING CELLS: The internal membranous components of *Ochromonas* have been previously elaborated (Gibbs, 1962; Schuster et al., 1968), and will not be considered further in detail here. However, the membranes which limit the perinuclear continuum are of interest to the present study because of their apparent involvement with the production or storage of presumptive mastigonemes. Dilated portions of the perinuclear continuum in particular are found to contain tubular structures (Figs. 10, 11, 12) which were first reported although not identified by Gibbs (1962). These structures are about 180 A in diameter, with a tapering basal region apparently attached to a portion of the enclosing membrane (Fig. 13 b, for example). Despite some similarities to flagellar mastigonemes of *Ochromonas*, the absence of lateral filaments (Figs. 11, 12) and the somewhat smaller diameter of these structures (180 A) were not consistent with the structure of the extraflagellar mastigoneme. Nor could evidence be found elsewhere in the cell for any other structure possibly related to mastigonemes. The

FIGURE 5 Portion of a negatively stained flagellum illustrating distribution of mastigonemes. Tubular mastigonemes along upper row are generally attached singly at each site, whereas those along bottom row are clumped in groups of 2–5 except near the tip. (See also Fig. 7). Fibrous mastigonemes are found at the tip and elsewhere (*arrows*) × 54,000.

FIGURE 6 *a* Single, isolated, tubular mastigoneme consisting of the basal region (*BR*), tubular shaft (*TS*), and two sets of lateral filaments. Short lateral filaments (*SL*) extend along entire shaft but not the basal region, whereas long lateral filaments (*LL*) are less numerous and are interspersed among the short lateral filaments. The terminal filaments are absent in this preparation. A small membrane fragment from the flagellar membrane can be seen at the arrow. × 260,000.

FIGURE 6 *b* Thin section of a pellet of isolated tubular mastigonemes illustrating tubular nature of the shaft and absence of discernible subunits. × 280,000.

FIGURE 6 *c* Section similar to 6 *b*, but with lateral filaments seen around full diameter of mastigoneme shaft. × 180,000.

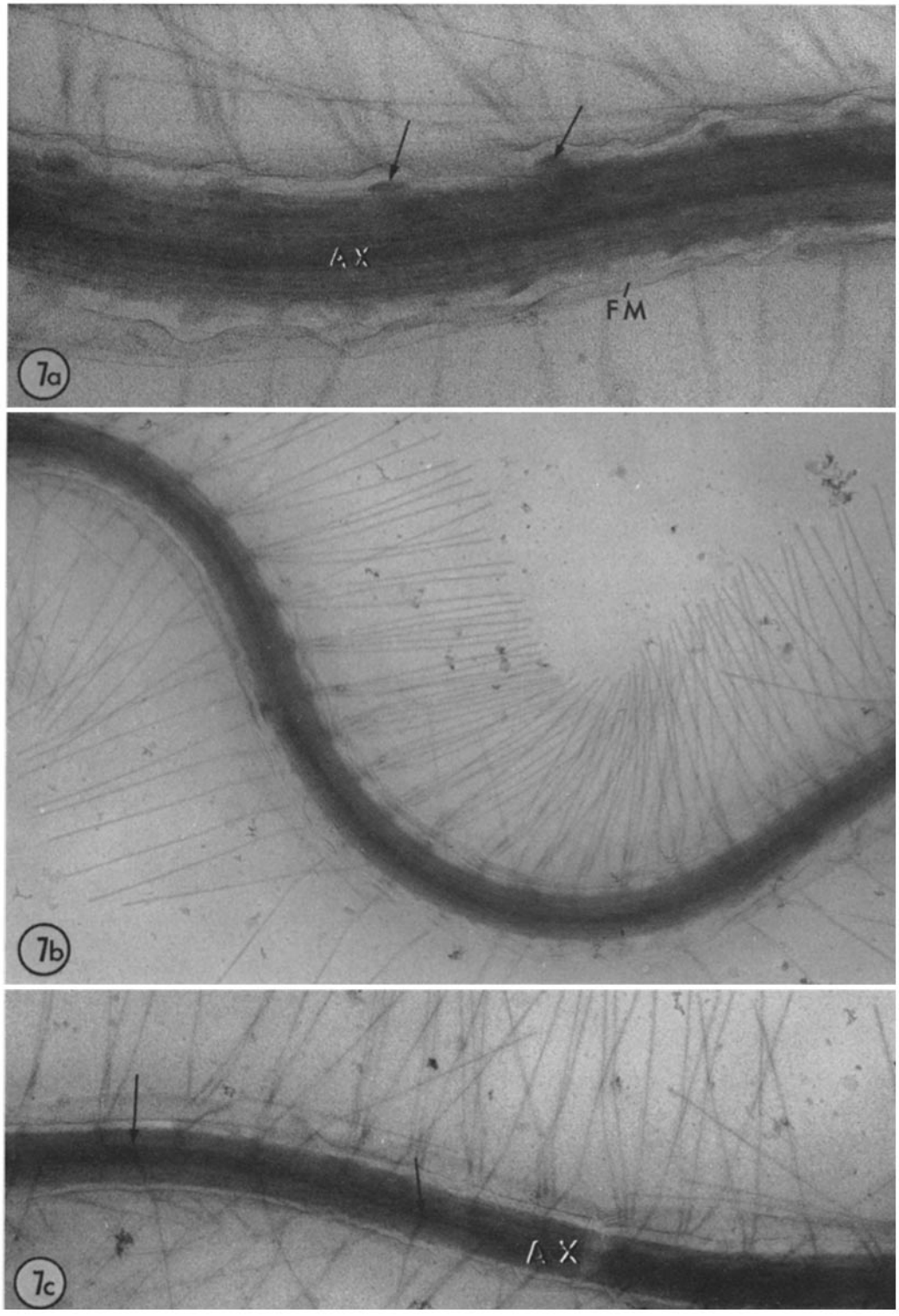



FIGURE 7 *a–c* Lightly stained preparation of *Ochromonas* flagella illustrating site of mastigoneme attachment. Axoneme (*AX*) can be distinguished from the flagellar membrane (*FM*) in these micrographs. 7 *a*: uneven distribution of mastigonemes on opposite sides of flagellum is apparent, and mastigonemes appear to be attached to a disc-like modification of the membrane (*arrows*). × 81,000. 7 *e*: Lower magnification photograph in which a portion of a flagellar wave can be seen. Mastigonemes appear not to be attached along margins of flagella sheath. × 22,000. 7 *c*: The preparation is similar to 7 *b*, with mastigoneme attachment (*arrows*) above and below the axoneme (*AX*). × 33,000. Plane of drying is believed to indicate plane of flagellar flexure and therefore plane of flagellar undulations. In these examples, mastigoneme attachment would appear not to be in the same plane as flagellar flexure.

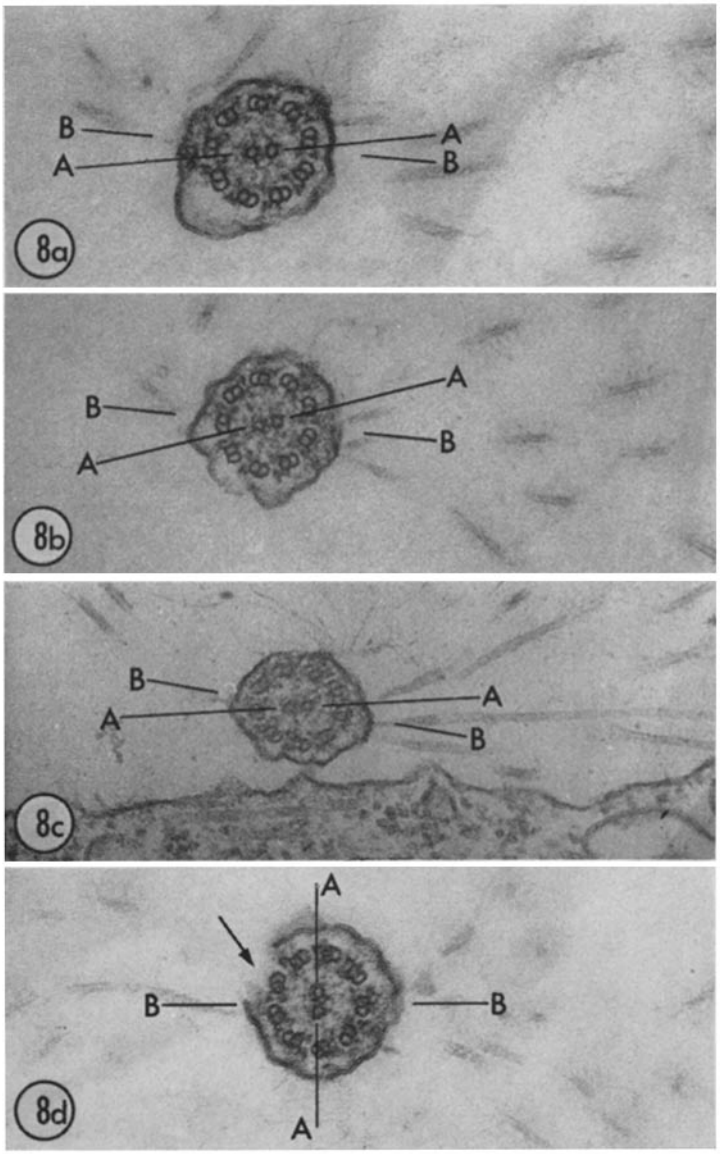



FIGURE 8 *a–d.* Sections of *Ochromonas* flagellum illustrating attachment of mastigonemes relative to central pair of axonemal microtubules. Markers (*A*) indicate plane of central pair, and markers (*B*) define the approximate plane of mastigoneme attachment. In 8 *d*, attachment appears to be a departure from the other examples shown, but the membrane here may be ruptured (*arrow*) and the image distorted during preparation. About × 62,000.

function of these anomalous, presumptive, mastigoneme-like structures became clear only after regenerating cells were examined.

DEFLAGELLATED AND REGENERATING CELLS: Regeneration of flagella after mechanical amputation followed the usual deceleratory kinetics which have been well characterized by Dubnau (1961) and Rosenbaum and Child (1967). The present experiments revealed no significant departure from these earlier reports. Since mastigonemes were found attached to the flagellum from the inception of regeneration, it was of

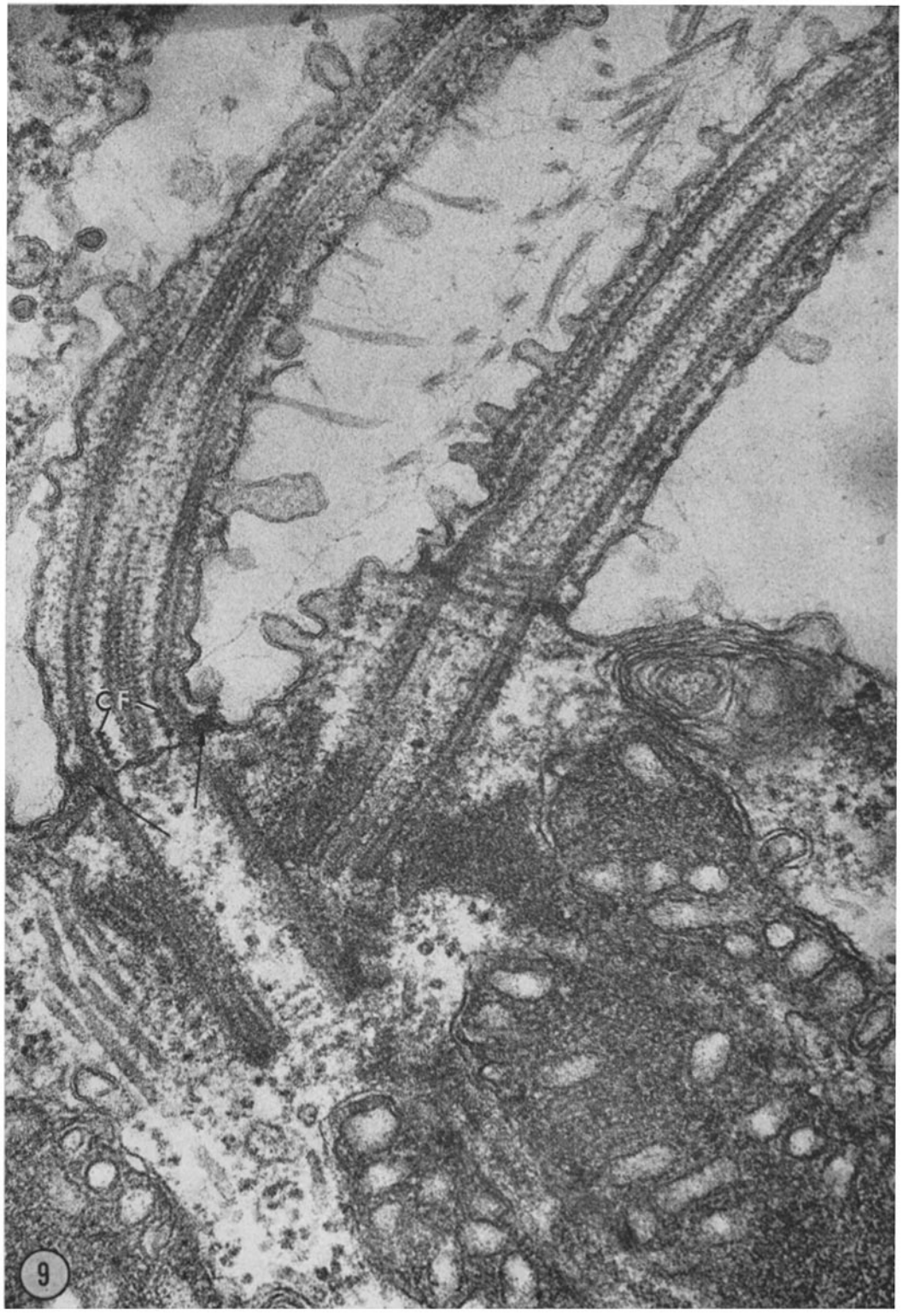



FIGURE 9 Section through short flagellum (right) and long flagellum (left). No special internal modifications of either flagellum is apparent except for coiled fiber at base (*CF*). Mastigonemes cannot be identified within the flagellum, and the partitions and dense materials (*arrows*) at flagellar junction with the cell body would seem to preclude mastigonemes entering the flagellum beyond that point. × 80,000.

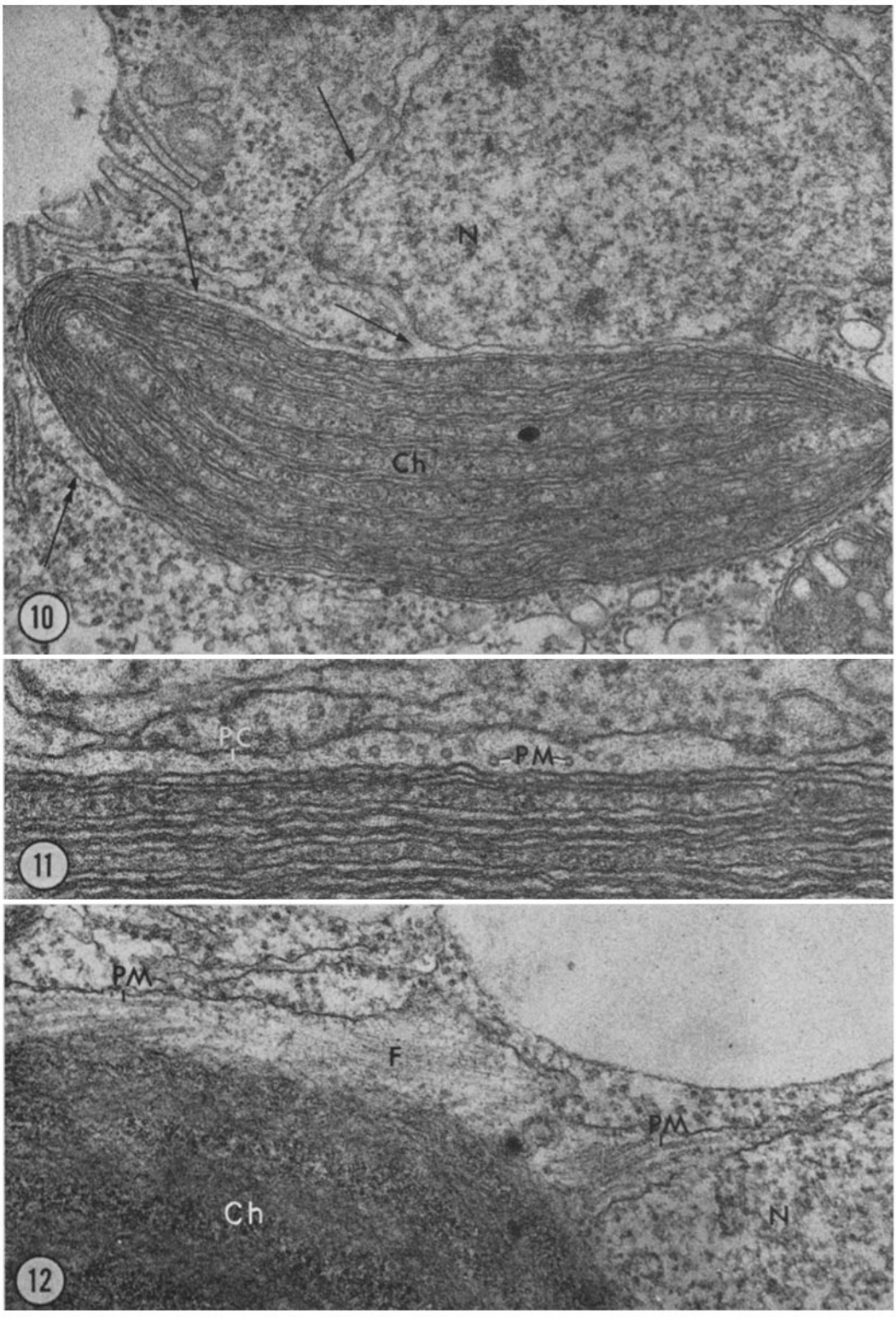



FIGURE 10 Nonregenerating cell with perinuclear continuum (*arrows*) dilated at one region (*double arrow*) adjacent to the chloroplast (*Ch*). Dilated area contains sections through small tubules. *N*, nucleus. × 38,000.

FIGURE 11 Enlarged portion of perinuclear continuum (*PC*) with section of tubules which are believed to be stage I presumptive mastigonemes (*PM*). These structures appear to lack lateral filaments. × 100,000.

FIGURE 12 Glancing section through perinuclear continuum containing incomplete longitudinal sections of stage I presumptive mastigonemes (*PM*) apparently lacking lateral filaments. Fibrous material (*F*) is unidentified but may represent precursors for fibrous mastigonemes. *N*, nucleus; *Ch*, chloroplast. × 53,000.

interest to examine the source and ontogeny of these newly attached mastigonemes. As in nonregenerating cells, presumptive mastigoneme-like structures, lacking lateral filaments, were found within the perinuclear continuum (Figs. 13 a and 13 b). However, in the regenerating cells these structures could also be localized within the cisternae of the Golgi complex and within vesicles free in the cytoplasm (Figs. 13 a, 13 b, and 14). There they possessed all the morphological characteristics of the mature mastigoneme, including lateral filaments. Those presumptive mastigonemes within the Golgi complex were confined to the outer swollen extremities of the Golgi saccules, and often several were present within a single saccule (Fig. 14). In regenerating cells mature mastigonemes could also be found at the cell surface near the base of the flagellum (Fig. 4). The flagellum itself possesses two rows of mastigonemes from the first appearance of the flagellar stub, which indicates that mastigonemes are attached concomitantly with flagellar growth in *Ochromonas*.

### Isolation and Characterization of Tubular Mastigonemes

The marked stability of mastigonemes to mechanical disruption and to relatively high concentrations of detergents facilitated their removal and isolation. Isopycnic density centrifugation in cesium chloride of the pellet of a crude, detergent-treated flagellar preparation yielded a mastigoneme fraction nearly free of other cell debris (Figs. 15, 16). A rerun of this fraction in cesium chloride did not noticeably reduce the minor contamination. Electrophoresis of urea-solubilized mastigonemes gave two or more protein bands (Fig. 17 a). However, after reduction and alkylation only one major and one minor band could be identified with Coomassie blue or fast green (Fig. 18). PAS-stained gels of urea-solubilized mastigonemes gave pronounced staining in the major and minor protein bands, and, in addition, revealed several slow migrating bands which were not stained with Coomassie blue or fast green (Fig. 17 b). Comparison of mastigoneme proteins with purified outer doublet microtubules from flagella of *Chlamydomonas* run on acrylamide gels under identical conditions, after reduction and alkylation of both samples, showed clear differences in major and minor banding (Fig. 18). Under these conditions, mastigoneme protein would not appear to be similar to microtubule protein.

## DISCUSSION

The early development and structure of the mastigonemes of *Ochromonas* is fundamentally similar to that observed in several other related and unrelated organisms. That is, (*a*) tubular mastigonemes can be localized within the cisterna of the perinuclear continuum as well as on the flagellar surface, and (*b*) these mastigonemes are differentiated into a basal region, a microtubular-like shaft, and a group of terminal filaments. The additional elaborations (lateral filaments) of the *Ochromonas* mastigoneme may be significant in increasing the effective surface area of these structures.

In addition to confirming and extending observations on the general anatomy of mastigonemes and the intracellular origin of their apparent precursors, the present study has also sought the answers to three previously unresolved questions: (*a*) How are the presumptive mastigonemes which are found within the perinuclear continuum transported to and attached in precisely spaced rows on the flagellar surface? This question is clearly also related to the localization of growth zones on the flagellar sheath, as well as to the general problem of flagellar morphogenesis. (*b*) Does the biochemical composition of mastigonemes offer new clues to mastigoneme function? In particular, it would be desirable to know if mastigonemes are compositionally similar to either cytoplasmic microtubules or to bacterial flagella which they may resemble in some details. (*c*) Finally, are the distribution of mastigonemes on the flagellar surface and the analysis of wave motions of the whole flagellum consonant with current concepts of a passive oarlike function for mastigonemes?

### *Presumptive Mastigonemes: Their Ontogeny and Attachment to the Flagellum*

From the evidence in this report and elsewhere, the following tentative scheme for the ontogeny of mastigonemes is suggested (Fig. 19): (*a*) Partially assembled structures consisting of at least the tubular shaft and the basal region (stage I presumptive mastigonemes) are constructed within the perinuclear continuum (i.e. the continuous space bounded by the membranes of the nuclear

envelope, the chloroplsast ER, and the cytoplasmic ER). Similar presumptive mastigonemes have been identified within the perinuclear continuum in the mastigoneme-bearing sperm of brown algae (Bouck, 1969), and it now appears probable that this cisternal compartment may be a general site for assembling the basic mastigoneme components (Leedale et al., 1970; Heath et al., 1970). Fibrous mastigonemes may be assembled here as well, since material suggestive of these mastigonemes can also be observed within the perinuclear continuum (Fig. 12).

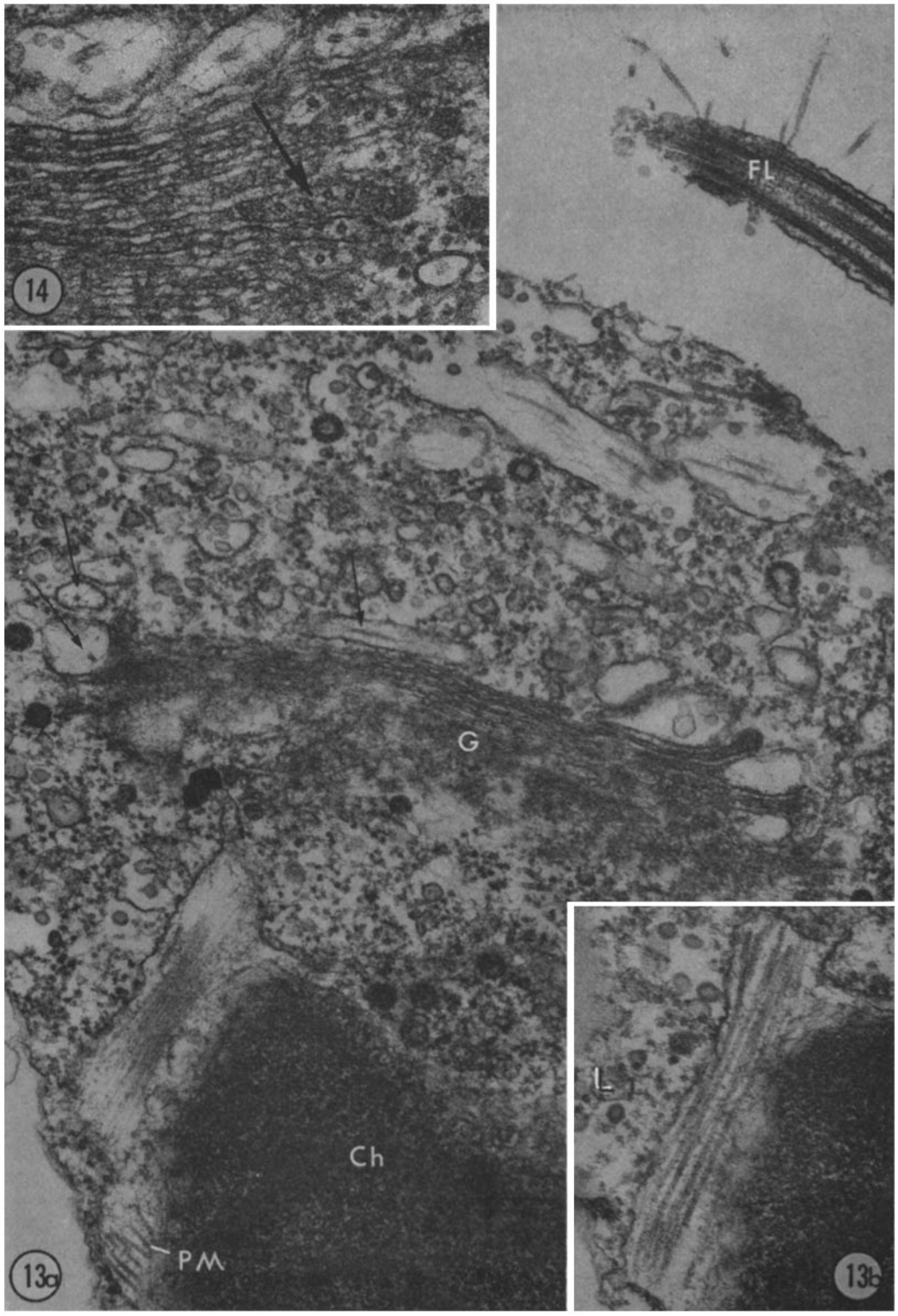
Although the perinuclear space has not previously been generally recognized as an assembly site for extracellular materials, indirect evidence has suggested that pools of materials destined for secretion may be derived from vesicles blebbed from the nuclear envelope in other organisms (e.g. Bouck, 1965). The crystals (Marquet and Sobel, 1969) and tubular invaginations (Meek and Moses, 1961) which have been reported within the perinuclear space of certain cells of goldfish and crayfish, respectively, are present apparently only under pathological conditions. In some plants, however, crystals may be found within the perinuclear space in normal cells (Wergin et al., 1970), but their function here is unknown. (*b*) In *Ochromonas* the stage I presumptive mastigonemes are next transferred intact from the perinuclear continuum to the margins of the Golgi cisternae. Within these marginal and dilated portions of the Golgi apparatus, lateral filaments are added, creating the stage II presumptive mastigoneme. Since stage II presumptive mastigonemes have been found thus far only in deflagellated cells undergoing regeneration, it might be argued that this may not represent the normal course of development. However, in untreated cells of *Synura*, a Chrysophyte related to *Ochromonas*, mastigoneme-like structures (although not identified as such) have been reported in both the perinuclear continuum and the Golgi vacuoles (Schnepf and Deichgraber, 1969). Furthermore, Manton et al. have specifically pointed out that mastigonemes may be present within the Golgi saccules of the phytoflagellate, *Heteromastix* (Manton et al., 1965). These latter two reports would seem to confirm the conclusions of the present study that the Golgi apparatus participates in mastigoneme production in at least some organisms.

The absence of stage II presumptive mastigonemes in the nonregenerating *Ochromonas* can be useful in estimating the initial rate of stage II assembly in regenerating cells, and also suggests a close relationship between Golgi apparatus activity and the length of the *Ochromonas* flagellum. The 15–20 min lag period characteristic of flagellar regeneration kinetics in these cells (Dubnau, 1961; Rosenbaum and Child, 1967) might represent the period necessary to convert stage I presumptive mastigonemes present in the perinuclear continuum to the stage II presumptive mastigonemes within the Golgi apparatus plus the time needed for extrusion at the cell surface. The consistently fewer mastigonemes found at the flagellar tip might then be explained by assuming that initially only those stage I presumptive mastigonemes that are present in the perinuclear continuum at the time of deflagellation are avail-

FIGURE 13 *a* Section through a regenerating cell fixed 1 hr after deflagellation. Presumptive mastigonemes (*PM*) lacking lateral filaments can be identified in the perinuclear continuum. In the Golgi saccules similar structures but with lateral filaments attached (stage II presumptive mastigonemes) are seen at arrows, and in the cytoplasm near the margins of the cell free sacs with stage II presumptive mastigonemes are also present. Flagellum (*FL*) with mature mastigonemes is also sectioned in this micrograph. *G*, Golgi apparatus; *Ch*, chloroplast. × 50,000.

FIGURE 13 *b* Section, adjacent to that shown in 13 *a*, in which the presumptive mastigonemes are found in complete longitudinal section. The tapering basal region of the stage I presumptive mastigoneme appears to be attached to a flattened portion of the membrane. Stage I presumptive mastigoneme are oriented in two directions but parallel to each other so that attachment sites are present on both ends of the enclosing membranous container. × 61,000.

FIGURE 14 Golgi vesicles containing up to four (*arrow*) presumptive mastigonemes per cisternum. × 70,000.


14
FL
G
Ch
PM
13a
L
13b

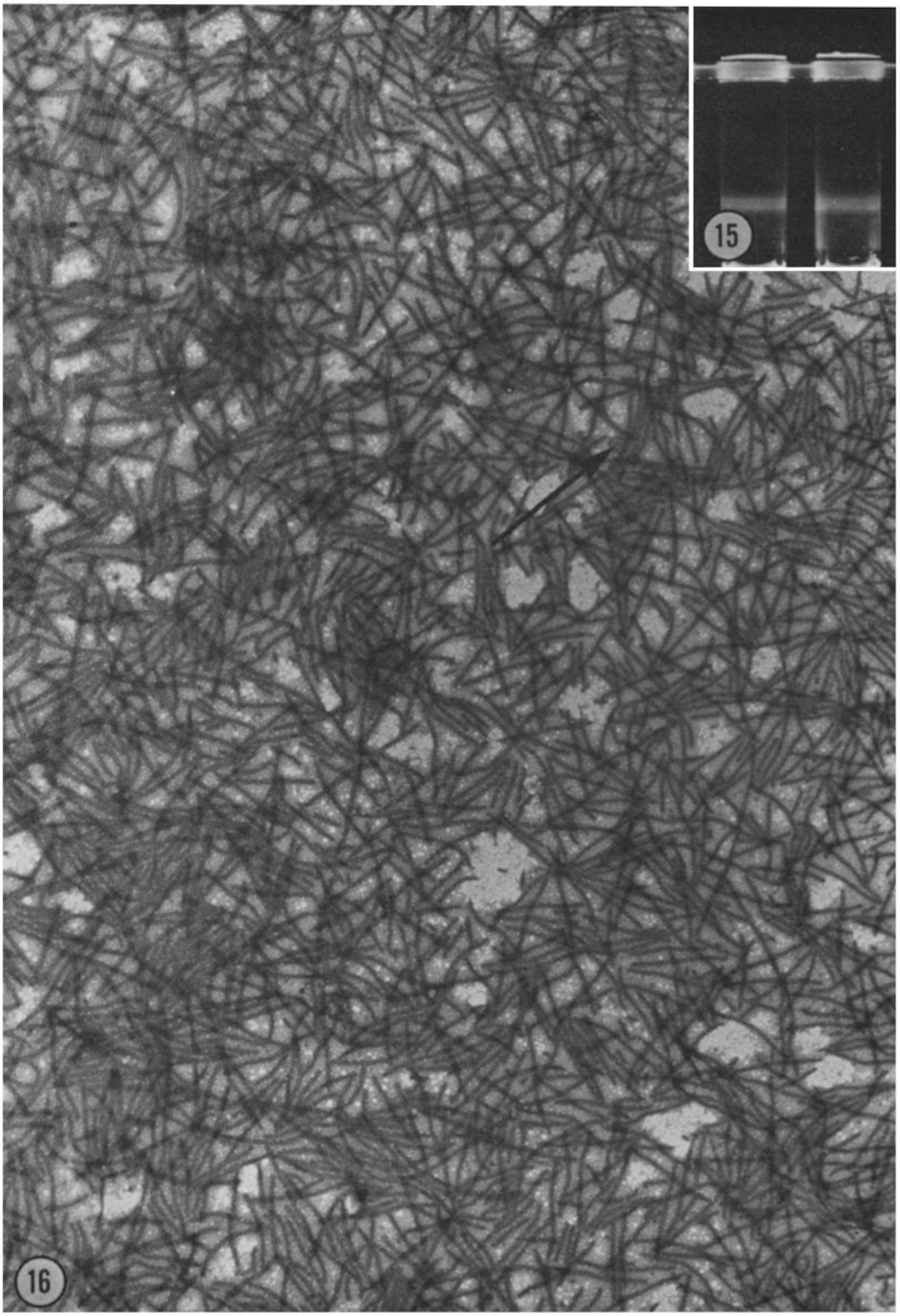



FIGURE 15 Duplicate samples of isolated mastigonemes purified by equilibrium centrifugation in cesium chloride.

FIGURE 16 Negatively stained preparation from bands of Fig. 15. Contamination (*arrow*) is minor. × 12,000.

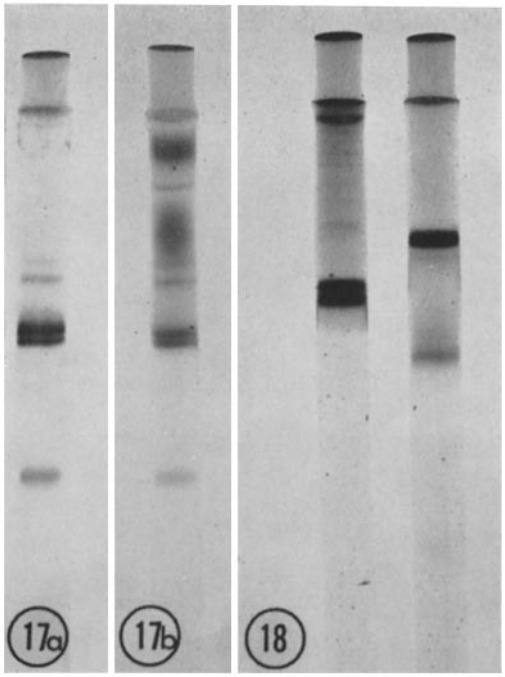



FIGURE 17 *a–b* Purified mastigonemes solubilized in urea, Tris EDTA, and electrophoresed on urea-containing 7.5% acrylamide gels. 17 *a*: Stained with Coomassie blue. 17 *b*: Stained with periodic acid-Schiff procedure. Note additional bands revealed with PAS.

FIGURE 18 Reduced and alkylated *Ochromonas* mastigoneme protein (right). Similarly treated, outer doublet microtubular protein from *Chlamydomonus* (left). Both preparations were electrophoresed simultaneously and stained in Coomassie blue.

able for passage through the Golgi complex, addition of lateral filaments, and extrusion. As regeneration progresses, however, stage II presumptive mastigonemes can be readily detected in vesicles in the cytoplasm, indicating that the supply of presumptive mastigonemes exceeds the demands of the regenerating flagellum.

An approximation of the number of mastigonemes required from the Golgi system per unit time can be calculated from available data. For example, during the most active phase of flagellar regeneration the flagellum elongates approximately 1 $\mu$ in 20 min. Except at the tip, this 1 $\mu$ of flagellum has been found to average about 17 attached mastigonemes (similar to values for *Ochromonas malhamensis* reported by Holwill and Sleigh, 1967). Therefore, 17 presumptive mastigonemes (stage II) would be required every 20 min of flagellar growth, or less than one mastigoneme per min. It is therefore of interest that sections of *Ochromonas* cells taken during these actively regenerating periods reveal as many as 30 Golgi-related, stage II presumptive mastigonemes. Such excesses of presumptive mastigonemes suggest that the act of deflagellation stimulates intensive Golgi

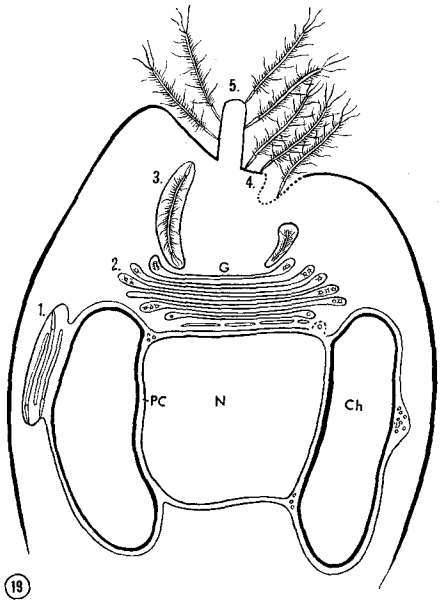
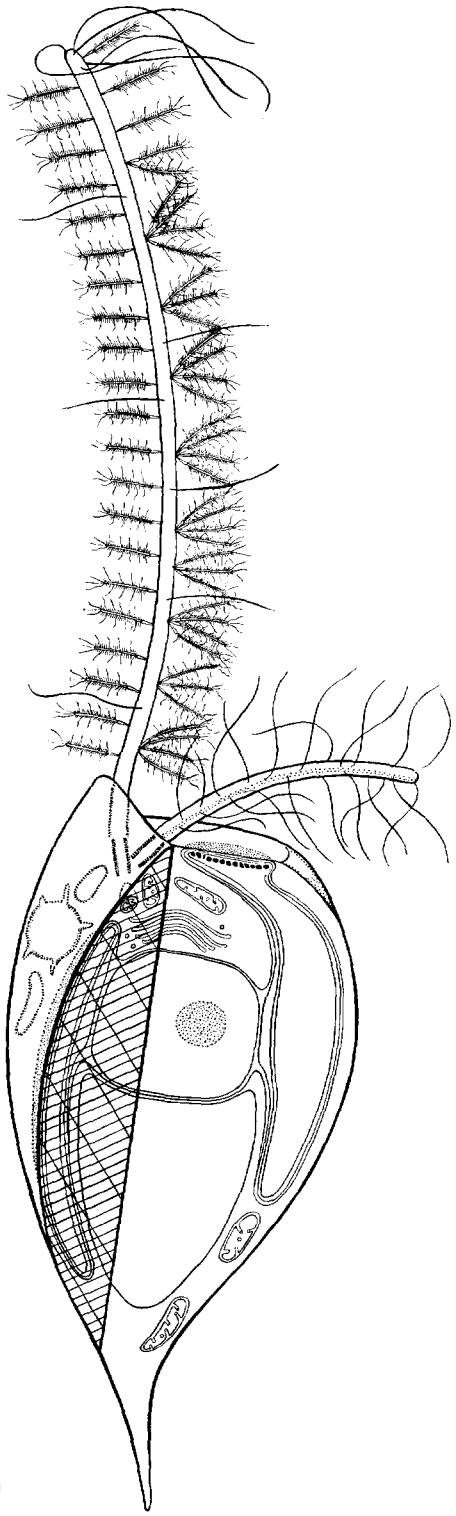



FIGURE 19 Schematic diagram depicting the probable sequence of mastigoneme assembly and discharge in regenerating *Ochromonas* cells. Stage I presumptive mastigonemes are initially assembled within the perinuclear continuum (*PC*), and are attached (or polymerized) by a site on the enclosing membrane (*1*). Presumptive mastigonemes are then transferred to the Golgi cisternae where they acquire lateral filaments of two types which are attached to the tubular shaft of the presumptive mastigoneme (*2*). After assembly in the Golgi complex these stage II presumptive mastigonemes are transported by means of an inflated sac to the cell surface in the vacinity of the regenerating flagellum. (*3*). As the flagellum elongates, new mastigomes and probably the membrane to which they are attached are added to the base of the flagellum (*4*). The oldest mastigonemes would therefore be those attached nearest the flagellar tip (*5*). The mastigoneme appears to have no free-floating stage, i.e. the basal region may be attached to a membranous site from its inception in the perinuclear continuum. G, Golgi apparatus; *N*, nucleus; *Ch*, chloroplast.



apparatus activity. On the other hand, since few stage II presumptive mastigonemes appear to remain in the cytoplasm after the flagellum is fully formed, a restraint on Golgi apparatus activity must be exerted as the flagellum approaches its regeneration limit. Thus a rather precise feedback mechanism seems to operate among Golgi apparatus activity, mastigoneme production, and the stage of growth of the flagellum. (*c*) The final steps in extrusion of the mastigoneme from the Golgi saccule to the flagellar surface presumably result from fusion of the Golgi membranes with the plasma membranes at the cell surface, although such transitions have not yet been observed in either regenerating or nonregenerating organisms. However, discharge occurs near the flagellar base as judged from the presence of mastigonemes on the cell surface. Several additional observations also bear on the question of whether newly synthesized mastigonemes are attached at the flagellar base or at the flagellar tip: a) Mastigonemes are present on the flagellum from the first appearance of the regenerating stub; b) in no section was a presumptive mastigoneme ever found within the flagellum, and c) mastigonemes are arranged in two rows and are more or less evenly spaced within those rows either as clumps or as singlets. These observations, together with those discussed in the previous section, suggest that mastigonemes are added as fully formed structures to the outside of the flagellum by addition at the base of the flagellum. It does not seem likely that they then migrate to the tip of the flagellum because the mastigonemes attached along the flagellum show an orderly arrangement, i.e. there is no indication of files of moving mastigonemes among the permanently attached members. It is concluded therefore that, although the *Ochromonas* flagellum as a whole has been characterized as tip growing (Rosenbaum and Child, 1967), mastigonemes, or more probably, the flagellar sheath as marked by the presence of attached mastigonemes, is added

FIGURE 20 Diagram of *Ochromonas danica* showing distribution of tubular mastigonemes in two unequal rows on the long flagellum. The longitudinal section through the eyespot and including at least one basal body is believed to define the plane of flagellar undulations. The short flagellum lies in this plane as well. Mastigonemes are illustrated somewhat oversized in order to include fine details.

by basal growth. By this interpretation the transfer of mastigonemes from the basal region of the flagellum would be the result of passive displacement of the sheath as the axoneme elongates.

It is also noteworthy that the point of attachment of the mastigoneme to the envelope of the perinuclear continuum may remain intact throughout the entire assembly process, and therefore ultimately comprise a portion of the flagellar membrane. This interpretation is supported by the apparent similarity between the mastigoneme attachment plate on the flagellar surface and the attachment site of the presumptive mastigoneme within the perinuclear continuum, and also by the observation that the presumptive mastigoneme appears to have no unattached developmental stage. However, this equating of attachment sites to initiator sites for mastigonemes remains yet to be verified by reassembly of isolated, depolymerized mastigonemes in the presence of such sites.

### *The Properties of Isolated Mastigonemes*

In the absence of previous studies on the biochemical composition of mastigonemes, it was first necessary to perfect techniques for mastigoneme isolation and purification. The anionic detergent Sarkosyl proved to be especially useful for solubilizing unwanted portions of the cell while having little apparent effect on the integrity of mastigonemes even in high concentrations (up to 5% by weight). The resistance of mastigonemes to dissolution by a variety of other reagents has been noted in earlier studies (Pitelka and Schooley, 1955). Urea, however, rapidly denatured mastigonemes of *Ochromonas*. Urea-solubilized mastigonemes required reduction in mercaptoethanol and alkylation in iodoacetate to prevent aggregation by disulfide cross-bridging (Sela et al., 1959) during electrophoresis on acrylamide gels. Double bands visible on nonreduced and nonalkylated samples may indicate dimerization or further aggregation of the basic polypeptide, but the denatured, reduced, and carboxymethylated mastigoneme fraction yielded only a single, Coomassie blue-staining band, which suggested that this fraction consisted of single polypeptide. The homogeneity of the mastigoneme polypeptide was unexpected in view of the morphological complexity of the *Ochromonas* mastigoneme, although the presence of a heterogeneous group of PAS-positive carbohydrates may explain some of this complexity.

Neither the exact nature nor the relationship of the carbohydrates to the mastigoneme polypeptide is yet known. Even so, some observations of mastigoneme development suggest how and where they may be added. In view of the substantial evidence for the utilization of the Golgi apparatus for attachment of carbohydrate moieties to proteins subsequently extruded (e.g. Rambourg et al,. 1969; Berlin, 1967; Wise and Flickinger, 1970), it seems possible that a similar function for the Golgi apparatus might operate here as well. Since the lateral filaments are added to the mastigoneme shaft in the Golgi complex, it is reasonable that these lateral filaments are wholly or in part carbohydrate. Extending this argument further, it might be expected therefore that organisms that lack lateral filaments on their mastigonemes (brown algae, for example; Bouck, 1969) would not utilize the Golgi apparatus in discharging mastigonemes to the cell surface. Thus, the relative infrequency of association of the Golgi apparatus with mastigoneme production reported thus far may be due to a real absence of such participation rather than being, as was the case with *Ochromonas*, simply a question of examining cells during the appropriate period of flagellar growth.

It cannot be maintained, from the details revealed thus far, that close biochemical similarities exist between mastigonemes and cytoplasmic microtubules. The insensitivity of mastigonemes to colchicine (Heath et al. 1970), which has been confirmed in the course of the present work, would also indicate an absence of any such relationship.

### *The Role of Mastigonemes in Locomotion*

Since forward movement in *Ochromonas* appears to be primarily the result of the activities of the long anterior flagellum, the problems of interpreting the mechanisms of motility are reduced to understanding the kinetics of this single flagellum. Unlike the situation in most motile sperm, the distally propagated waves of the *Ochromonas* flagellum pull rather than push the cell through its medium. This apparent contradiction has been attributed, in the case of *Ochromonas*, to the presence of mastigonemes whose total action has been described as an "inefficient hydrodynamic reversing gear" (Jahn, 1965). By current interpretations mastigonemes can perform this function passively, provided they are relatively stiff (or bend in some preferred direction) and are properly oriented relative to the planar undulations of the flagellum (Jahn et al., 1964). The principal ob-

servational support for this hypothesis, however, comes not from mastigonemes, but by analogy with the recorded activities of polychete worms which send undulatory waves forward and move in the same direction. The presence of lateral parapodia arranged in the plane of the undulations is the theoretical and demonstrated rationale for this apparently anomalous behavior (Taylor, 1952). However, it may be misleading to compare mastigonemes and parapodia, since parapodia are attached to internal body muscles and, in fact, the more heavily muscled species of polychetes can locomote more efficiently (Clark and Clark, 1960). The mastigoneme is neither muscled nor does it appear to be attached to axonemal components. Taylor's calculations do not require musculature (appropriate "roughness" is sufficient), but the fact remains that the validity of these calculations is yet to be directly demonstrated in any organism lacking the ability to power its lateral appendages. Also, to support theory, mastigonemes must be arranged in two opposing rows in the *same* plane as the undulatory waves of the flagellum (Holwill and Sleigh, 1967). While mastigonemes are clearly attached in the requisite opposing rows, it is not yet certain whether these rows are in the necessary flagellar plane.

For example, in dried preparations the flagellum lies so that its plane of flexure (presumably therefore its plane of undulations) is flat on the supporting surface. Mastigonemes viewed in such flattened preparations are often attached to the undersides and top rather than to the margins of the flagellum, suggesting orientation in a plane closer to perpendicular to the plane of undulations (Figs. 7 a–c). While these determinations are relatively crude in that gross distortions undoubtedly occur during the drying process, they may gain added credence by a second series of observations. That is, in sections of flagella, mastigonemes are usually found attached parallel to the central pair of microtubules (Figs. 8 a–c), and arrangements similar to those of *Ochromonas* have been reported for the mastigonemes of sponges (Afzelius, 1961), as well as in brown algae (Manton, 1959). Since a line drawn between the central pair of microtubules often (but not invariably, e.g. Ringo, 1967) defines the plane of flagellar undulations (Gibbons, 1961), mastigoneme attachment might, by the consistency of all the available reports, again appear to be perpendicular to the plane of the undulations. Also, a pronounced arch is clearly seen in swimming *Ochromonas*, and this arch appears in flash photomicrographs to be primarily in a plane perpendicular to the plane of flagellar undulations. The simplest explanation for this arch would be an asymmetric distribution of mastigonemes (see Results) perpendicular to the flagellar wave. None of these observations provide unequivocable evidence against the theory (as pointed out by Holwill and Sleigh, 1961, the plane of mastigoneme attachment may more properly give information on the direction of flagellar undulations rather than the reverse), but they do indicate that there may be some basis for considering alternatives.

Apparently, the mechanisms of mastigoneme action will remain unsettled until the crucial question of attachment relative to the plane of undulations has been fully resolved. In particular, some reliable morphological marker of the direction of flagellar beat would be especially useful. It is believed, however, that sufficient evidence is presented in this report to warrant consideration of alternatives to the hypothesis presented by Jahn et al., 1964. Not the least of the interesting possibilities to explain mastigoneme functioning might be in an analogy to the bacterial flagellum which some mastigonemes resemble, at least superficially (cf. discussion in Bouck, 1969). Further biochemical analyses and additional comparative studies should also be helpful in resolving the profound effects of mastigonemes on cell locomotion.

A portion of this investigation was carried out while the author held a visiting appointment at the University of California (Berkeley). The author is grateful for the use of the electron microscope facility and the generous help of the facility staff. He is also indebted to Dr. J. Rosenbaum for samples of purified outer doublet microtubules from *Chlamydomonas*, for collaboration with the initial deflagellation experiments, and for many useful discussions. Dr. L. Simone's skillful drawing created the two illustrations, whereas Dr. D. L. Brown performed deflagellation experiments which were used to confirm some aspects of this work. Dr. F. L. Schuster and Mr. R. Berman kindly provided cultures of *Ochromonas*, and Mr. I. Price gave excellent technical assistance in the early phases of this work. A preliminary account of this report was given at the 1969 ASCB meetings in Detroit. (*J. Cell Biol.* **43**:14 a)

This research was supported by grant no. GM 11100 from the United States Public Health Service.

*Received for publication 6 October 1970, and in revised form 10 February 1971.*

## REFERENCES


AARONSON, S., and H. BAKER. 1959 A comparative biochemical study of two species of *Ochromonas*. *J. Protozool.* **6**:282.

AFZELIUS, B. A. 1961. Flimmer flagellum of the sponge. *Nature (London).* **191**:1318.

ALLEN, R. D., G. B. DAVID, and G. NOMARSKI. 1969. The Zeiss-Nomarski differential interference equipment for transmitted-light microscopy. *Z. Wiss. Mikrosk.* **69**:193.

BERLIN, J. D. 1967. The localization of acid mucopolysaccharides in the Golgi complex of intestinal goblet cells. *J. Cell Biol.* **32**:760.

BOUCK, G. B. 1965. Fine structure and organelle associations in brown algae. *J. Cell Biol.* **26**:523.

BOUCK, G. B. 1969. Extracellular microtubules: The origin, structure, and attachment of flagellar hairs in *Fucus* and *Ascophyllum* antherozoids. *J. Cell Biol.* **40**:446.

BRADLEY, D. E. 1966. The ultrastructure of the flagella of three Chrysomonads with particular reference to the mastigonemes. *Exp. Cell Res.* **41**:162.

CLARK, R. B., and CLARK, M. E. 1960. The ligomentary system and segmented musculature of *Nephtys*. *Quart. J. Microsc. Sci.* **101**:149.

DAVIS, B. J. 1964. Disc electrophoresis—II. Method and application to human serum proteins. *Ann. N.Y. Acad. Sci.* **121**:404.

DUBNAU, D. A. 1961. The regeneration of flagella by *Ochromonas danica*. Ph.D. Thesis. Columbia University, New York.

GIBBONS, I. R. 1961. The relationship between the fine structure and direction of beat in gill cilia of a lamellibranch mollusc. *J. Biophys. Biochem. Cytol.* **11**:179.

GIBBS, S. P. 1962. Chloroplast development in *Ochromonas danica*. *J. Cell Biol.* **15**:343.

HEATH, I. B., A. D. GREENWOOD, and H. B. GRIFFITHS. 1970. The origin of Flimmer in *Saprolegnia, Dictyuchus, Synura,* and *Cryptomonas*. *J. Cell Sci.* **7**:445.

HEDLEY, R. H., D. M. PARRY, and J. ST. J. WAKEFIELD. 1968. Reproduction in *Boderia turneri* (Foraminifera). *J. Natur. Hist.* **2**:147.

HOLWILL, M. E. J., and M. A. SLEIGH. 1967. Propulsion in hispid flagella. *J. Exp. Biol.* **47**:267.

JAHN, T. L., M. D. LANDMAN, and J. R. FONSECA. 1964. The mechanism of locomotion of flagellates. II. Function of the mastigonemes of *Ochromonas*. *J. Protozool.* **11**:291.

JAHN, T. L. 1965. Hydrodynamic principles in the locomotion of microorganisms. *Excerpta Med. Int. Congr. Ser.* **91**:18.

JOYON, L., and J. P. MIGNOT. 1969. Données récentes sur la structure de la cinétide chez les protozoaires flagelles. *Année Biol.* **8**:1.

LANG, N. T. 1963. An additional ultrastructural component of flagella. *J. Cell Biol.* **19**:631.

LEEDALE, G. F., B. S. C. LEADBEATER, and A. MASSALSKI. 1970. The intracellular origin of flagellar hairs in the Chrysophyceae and Xanthophyceae. *J. Cell. Sci.* **6**:701.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265.

MANTON, I. 1952. The fine structure of plant cilia. *Symp. Soc. Exp. Biol.* **6**:306.

MANTON, I. 1959. Observations on the internal structure of the spermatozoid of *Dictyota*. *J. Exp. Bot.* **10**:448.

MANTON, I. 1964. The possible significance of some details of flagellar bases in plants. *J. Roy. Microsc. Soc.* **82**:279.

MANTON, I., D. G. RAYNS, and H. ETTL. 1965. Further observations on green flagellates with scaly flagella: the genus *Heteromastix* Korshikov. *J. Mar. Biol. Ass. U. K.* **45**:241.

MARQUET, E., and H. J. SOBEL. 1969. Crystalline inclusions in the nuclear envelope and granular endoplasmic reticulum of the fish spinal cord. *J. Cell Biol.* **41**:774.

MEEK, G. A., and M. J. MOSES. 1961. Microtubulation of the inner membrane of the nuclear envelope. *J. Biophys. Biochem. Cytol.* **10**:121.

PITELKA, D. R., and C. N. SCHOOLEY. 1955. Comparative morphology of some protistan flagella. *Univ. Calif. Publ. Zool.* **61**:79.

RAMBOURG, A., W. HERNANDEZ, and C. P. LEBLOND. 1969. Detection of complex carbohydrates in the Golgi apparatus of rat cells. *J. Cell Biol.* **40**:395.

RENAUD, F. L., A. J. ROWE, and I. R. GIBBONS. 1968. Some properties of the protein forming the outer fibers of cilia. *J. Cell Biol.* **36**:79.

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.

RINGO, D. L. 1967. Flagellar motion and fine structure of the flagellar apparatus in *Chlamydomonas*. *J. Cell Biol.* **33**:543.

ROSENBAUM, J. L., and F. M. CHILD. 1967. Flagellar regeneration in protozoan flagellates. *J. Cell Biol.* **34**:345.

SCHNEPF, E., and G. DEICHGRÄBER. 1969. Über die Feinstruktur von *Synura pertersenii* unter besonderer Berücksichtigung der Morphogenese ihrer Kieselschuppen. *Protoplasma.* **68**:85.

SCHUSTER, F. L., B. HERSHENOV, and S. AARONSON. 1968. Ultrastructural observations on aging of stationary cultures and feeding in *Ochromonas*. *J. Protozool.* **15**:335.

SELA, M., F. H. WHITE, JR., and C. B. ANFINSEN. 1959. The reductive cleavage of disulfide bonds

and its application to problems of protein structure. *Biochim. Biophys. Acta.* **31**:417.

SPURR, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26**:31.

TAYLOR, G. 1952. Analysis of the swimming of long and narrow animals. *Proc. Roy. Soc. Ser. A.* **214**:158.

WERGIN, W. P., P. J. GRUBER, and E. H. NEWCOMB. 1970. Fine structural investigation of nuclear inclusions in plants. *J. Ultrastruct. Res.* **30**:533.

WISE, G. E., and C. J. FLICKINGER. 1970. Cytochemical staining of the Golgi apparatus in *Amoeba proteus*. *J. Cell Biol.* **46**:620.