DEVELOPMENT OF THE FLAGELLAR APPARATUS OF *NAEGLERIA*

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ABSTRACT

Flagellates of *Naegleria gruberi* have an interconnected flagellar apparatus consisting of nucleus, rhizoplast and accessory filaments, basal bodies, and flagella. The structures of these components have been found to be similar to those in other flagellates. The development of methods for obtaining the relatively synchronous transformation of populations of *Naegleria* amebae into flagellates has permitted a study of the development of the flagellar apparatus. No indications of rhizoplast, basal body, or flagellum structures could be detected in amebae. A basal body appears and assumes a position at the cell surface with its filaments perpendicular to the cell membrane. Axoneme filaments extend from the basal body filaments into a progressive evagination of the cell membrane which becomes the flagellum sheath. Continued elongation of the axoneme filaments leads to differentiation of a fully formed flagellum with a typical "9 + 2" organization, within 10 min after the appearance of basal bodies.

INTRODUCTION

The remarkable uniformity of the basic "9 + 2" pattern in the axonemes of cilia and flagella from diverse plant and animal cells has stimulated interest in their structure (Afzelius, 1959; Gibbons and Grimstone, 1960; Fawcett, 1961; Sleigh, 1962) and function (Nelson, 1962; Sleigh, 1962; Gibbons, 1963). Although the development of these organelles has also attracted attention, previous attempts to determine the morphological sequence by which a flagellum is formed have been hampered by the lengthy and asynchronous development in the systems studied. Such systems include the cilia of differentiating neural epithelia (Sotelo and Trujillo-Cenóz, 1958 *b*), the modified cilia of retinal rods (Tokuyasu and Yamada, 1959), and the flagella of sperm tails (Burgos and Fawcett, 1956; Sotelo and Trujillo-Cenóz, 1958 *a*). Of necessity, in these systems the sequence of development has been ordered intuitively, on the basis of increasing size and complexity, in material which is fixed at random stages of differentiation.

A generalized picture of flagellum development has emerged from these observations. One or both of the preexisting centrioles is believed to move to the cell surface, and there, within the ballooning cell membrane, vesicles or filaments condense into an axoneme. In developing fibroblasts and smooth muscle cells, however, Sorokin (1962) found that the cilium and its sheath develop internally. Another developmental sequence has been inferred from a comparison of normal and "developmentally arrested" cilia in the rumen protozoa *Diplodinium* and *Ophryoscolex* (Roth and Shigenaka, 1964); in these organisms, random filaments appear to extend from the numerous basal bodies, become doubled, and are organized into an axoneme cylinder.

Systems in which flagella or cilia can be induced to develop relatively synchronously on all cells provide excellent material with which to describe the development of these organelles. Renaud and Swift (1964) have described the development of flagella in one such system, gametogenesis in the water mold *Allomyces*. Mature flagellated gametes are liberated within an hour after transferring gametangia to aqueous medium. Flagella develop internally by elongation of centrioles to form basal bodies, formation of flagellar sheaths, and condensation of axonemes.

An especially favorable system is the ameba-to-flagellate transformation undergone by cells of *Naegleria gruberi*. The fine structure of *Naegleria* cysts, amebae, and flagellates has been described by Schuster (1963 *a*, *b*) but that study did not include a developmental sequence of transformation. Conditions which permit the rapid, reproducible, and synchronous transformation of *Naegleria* amebae to flagellates have since been devised (Fulton and Dingle, 1966). The *Naegleria* transformation provides an advantageous basis for a quantitative study of the development of flagella and associated organelles.

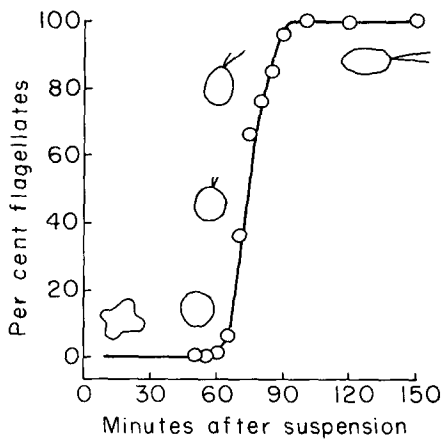


FIGURE 1 Appearance of flagellates in a transforming population. This curve was obtained while collecting samples for electron microscopy. Each point is the number of cells with flagella per 100 Lugol's iodine-fixed cells.

MATERIALS AND METHODS

The cultivation and transformation of *Naegleria*, as well as quantitative methods for evaluating transformation, are described in detail by Fulton and Dingle (1966). To obtain amebae, cysts of *Naegleria gruberi* strain NB-1 were mixed with *Aerobacter aerogenes*, plated on a nutrient agar medium, and incubated overnight at 33–34°C. Under these conditions, the amebae grew to 2 to 4 $\times$ 10^7 cells per plate at stationary phase. For transformation, early stationary phase amebae were suspended at room temperature in 0.002 M Tris (hydroxymethyl) aminomethane at pH 7.4, washed relatively free of *Aerobacter* by differential centrifugation, and resuspended in Tris at a final concentration of approximately 2 $\times$ 10^6 cells per ml. A 125-ml Erlenmeyer flask containing 10 to 30 ml of this suspension was shaken at 100 1-in. strokes per min in a 25.0 $\pm$ 0.2°C water bath. The progress of transformation was evaluated by counting the percentage of flagellates (cells with flagella) in samples fixed at intervals in Lugol's iodine.

For electron microscopy, 1.0 ml of the cell suspension was pipetted into a conical centrifuge tube containing 4.0 ml of cold 1% osmium tetroxide in a pH 7.4 buffer of 0.014 M veronal acetate containing 0.012 M NaCl and 0.001 M $CaCl_2$. After 30 to 60 min fixation on ice, the cells were hand centrifuged into a pellet which could thereafter be handled as if it were a piece of tissue. The pellets were rinsed twice with half-strength buffer, and dehydrated through 10-min changes of 25, 50, 75, 95, and 100% (3 changes) ethanols. An equal volume of propylene oxide was added to the third 100% ethanol rinse, and then the cells were given two 15-min changes of propylene oxide and brought to room temperature. The pellets were bathed for 30 min in a 50:50 mixture of propylene oxide and Maraglas (Freeman and Spurlock, 1962), and then embedded in the Maraglas F mixture essentially according to the protocol of Spurlock, Kattine, and Freeman (1963). Cells were impregnated with resin for 12 hr at 10°C in stoppered centrifuge tubes, brought to room temperature, and transferred to fresh Maraglas with accelerator for 2 hr, then embedded in capsules at 60° for 48 to 72 hr.

Silver and gold sections, cut on a Porter-Blum microtome with glass knives and collected on 10% acetone, were spread with chloroform vapor and picked up on 200-mesh grids. The Maraglas sections were strong enough under the electron beam that Formvar or carbon films were unnecessary. Grids were simply coated, without making a film, by dipping them in 0.15% Formvar in ethylene dichloride. The sections were stained by floating grids face down on a saturated solution of uranyl acetate in 50% ethanol for 3 to 6 hr.

Electron micrographs were taken with an RCA EMU-3C at both 50 and 100 kv, with a 50-μ objective aperture and a 250-μ condenser aperture. All measurements were made on prints of the micrographs on high contrast paper, and were calibrated with a carbon grating replica obtained from Baird Atomics Corporation, Cambridge, Massachusetts.

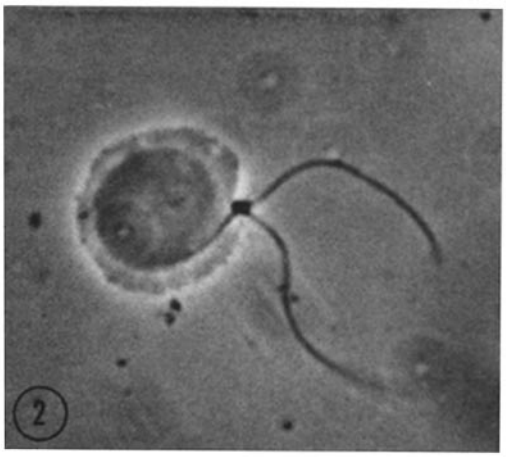


FIGURE 2 Phase contrast micrograph of a *Naegleria* flagellar apparatus obtained by lysis of a flagellate in osmium tetroxide buffered at pH 8.3 with veronal-acetate. The 2 flagella join at the basal bodies, from which the rhizoplast extends as a black line across the nuclear membrane. A large endosome dominates the nucleus. × 2,000.

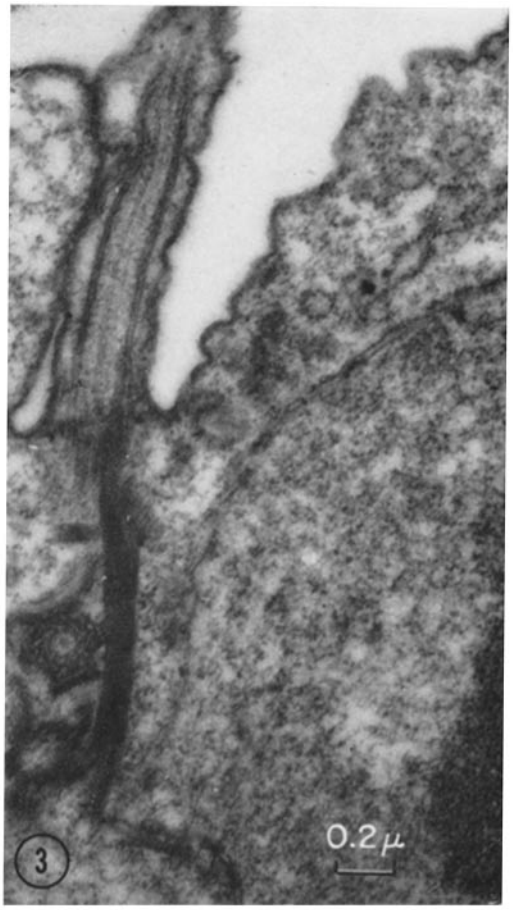


FIGURE 3 Electron micrograph of the anterior end of a flagellate. The nucleus lies close to the rhizoplast, basal bodies, and flagellum. × 40,000.

RESULTS

Transformation from Amebae to Flagellates

Under the transformation conditions described by Fulton and Dingle (1966), amebae begin to round up about 50 min after they are suspended in Tris. The first flagella recognizable with the light microscope appear at about 60 min, and 30 min later virtually all cells have flagella (Fig. 1). Meanwhile, the spherical cells elongate into a flagellate shape.

Structure of the Flagellate

THE FLAGELLAR APPARATUS: In mature flagellates, the flagella, basal bodies, rhizoplast, and nucleus are intimately associated in a unit known as the flagellar apparatus. This association can be clearly seen when flagellates are lysed in osmium tetroxide buffered at high pH (Fig. 2). Nuclei liberated from the cells remain connected to their basal bodies and flagella by a thin thread-like rhizoplast. Though in most flagellates lysed this way the nucleus and basal bodies are adjacent, as in the one shown in Fig. 2, in occasional cells the rhizoplast thread extends for several microns between nucleus and basal bodies.

The fine structure of these components is basically similar to that described for flagellated cells in general (see Sleigh, 1962), and previously for *Naegleria* (Schuster, 1963 *a*). More inclusive observations on the structure and interrelation of these components in *Naegleria* strain NB-1 are presented here as a basis for the description of the development of the flagellar apparatus.

A longitudinally sectioned flagellum and basal body, and parts of the rhizoplast and nucleus are visible in Fig. 3. Other features of the fine structure of *Naegleria* common to both amebae and flagellates are apparent in this low power micrograph of a flagellate. Ribosomes abound in the cytoplasm; most are single or aggregated in small clusters, but some are distributed along membranes, forming strands and vesicles of a sparse endoplasmic reticulum. A central, granular,

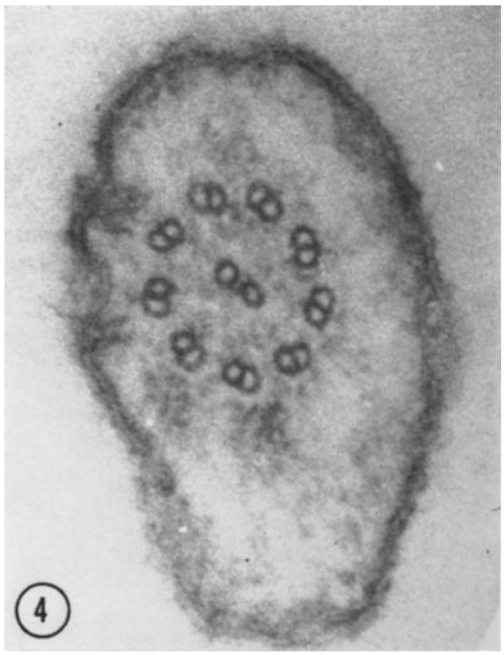


FIGURE 4 Transverse section of a *Naegleria* flagellum. Note the presence of matrix both within and surrounding the axoneme. There are neither secondary filaments nor arms on the peripheral doublets of this flagellum. × 135,500.

electron-opaque endosome dominates the nucleus of both amebae and flagellates (Figs. 3, 7, and 9). No membrane separates this endosome from the less dense, finely granular nucleoplasm. The nucleus is bounded by an envelope whose outer layer is studded with ribosomes. No membranous organelle suggestive of a Golgi complex has been seen in sections of either amebae or flagellates.

FLAGELLA: Flagellates usually have from 1 to 4 (predominantly 2) flagella, which measure from 7 to 17 μ in length and 0.3 to 0.5 μ in diameter. The flagella narrow at the tips where component filaments are lost.

The transversely sectioned axoneme appears as a cylinder, approximately 0.18 μ in diameter, composed of 9 peripheral doublet filaments surrounding 2 single central filaments (Fig. 4). Each of the filaments is composed of a 50- to 60-A thick electron-opaque rim surrounding a less dense core. The central filaments are usually circular, measuring 250 A in diameter, whereas peripheral filaments are often elliptical, the doublet measuring 340 × 240 A. Electron-opaque condensations, similar to the arms described by Afzelius (1959) and found subsequently on a variety of flagella (Gibbons and Grimstone, 1960; Fawcett, 1961), often can be seen extending from the peripheral doublets (Fig. 5 *b*). There is an irregularly aggregated matrix throughout the interior of the axoneme of *Naegleria* flagella (Figs. 4 and 5 *b*), but no structures could be resolved in this matrix. The axoneme is bounded by a double membrane approximately 100 A wide. This membrane is continuous with the general cell membrane (Figs. 3, 5 *a*, and 10).

BASAL BODIES: The 9 peripheral doublets of a flagellum are continuous with a cylinder of 9 triplet filaments situated just beneath the cell surface (Fig. 5 *a*, *e*). The basal body varies from 0.7 to 2.0 μ in length; transverse sections measure approximately 0.2 μ in diameter, but the basal body tapers gently from 0.24 μ proximally to 0.21 μ distally, merging ultimately with the 0.18 μ diameter axoneme.

The pattern of 9 triplets generally associated with centrioles and basal bodies is seen throughout most of the *Naegleria* basal body; each 500- × 300-A triplet is composed of three 170- × 300-A filaments (Fig. 5 *e*). Along most of its length the lumen of the cylinder is devoid of filaments or other recognizable structures, but contains granular material of intermediate density, similar to the matrix in which the basal body is embedded.

There are striking structural modifications along the length of the basal body. Distally, at the transitional zone between flagellum and basal body, a thin membrane lying just within the cylinder of peripheral doublets circumscribes the 2 central filaments (Fig. 5 *c*). At this level, electron-opaque fibers extend from the juncture of the doublet filaments toward the flagellum membrane. Slightly beneath this level, these or similar fibers become elongate and diffuse, creating a pinwheel effect in the cytoplasm surrounding the basal bodies (Fig. 5 *d*). The central filaments terminate at or before this point; in the center of the cylinder is a diffuse area of intermediate electron-opacity, which is presumably the basal granule or basal plate. The axial filament complex is intermediate in structure between that of the flagellum and that of the median basal body at this level; some of the peripheral filaments are doubled and others are tripled.

Running directly up the center of the proximal third of the basal body is a 250- to 300-A wide

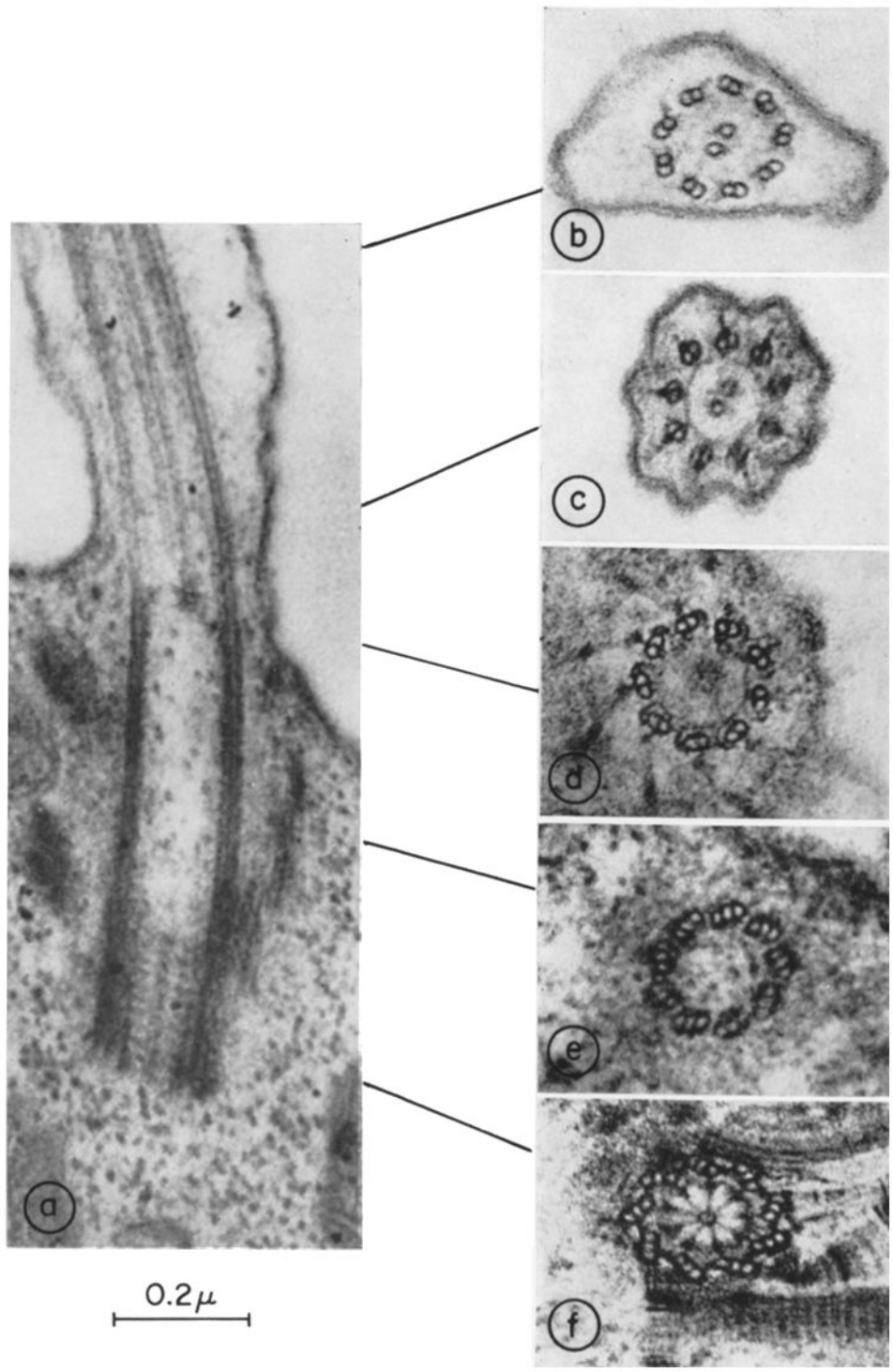


FIGURE 5 Longitudinal and transverse sections of the flagellum and basal body of *Naegleria*, illustrating the proximodistal structural differentiation of the basal body. Fig. 5 *a*, longitudinal section; Fig. 5 *b*, typical flagellum axoneme; Fig. 5 *c*, flagellum at the level of the basal plate; Fig. 5 *d*, transitional region between flagellum and basal body; Fig. 5 *e*, typical basal body structure; Fig. 5 *f*, proximal basal body, showing the single central filament, spokes, and triangular condensations within the cylinder of triplets. All micrographs, approximately × 97,000.

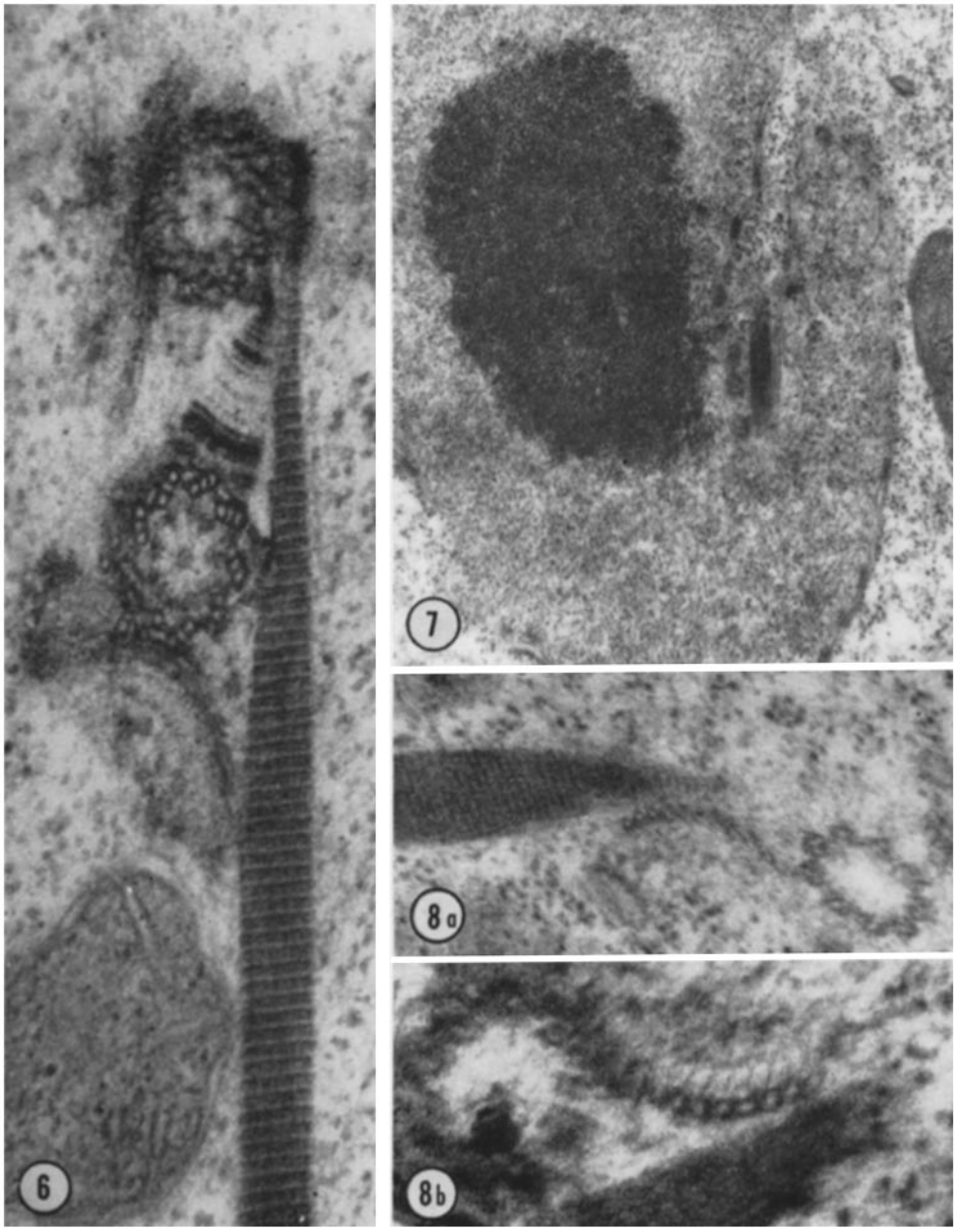


FIGURE 6 Part of a rhizoplast and 2 basal bodies in the cytoplasm of a flagellate. × 113,000.

FIGURE 7. A portion of the nucleus of a flagellate, showing part of a rhizoplast which extends into a deeply invaginated pocket of the nucleus, but which does not penetrate the nuclear membrane. × 33,000.

FIGURE 8 Connecting filaments between the rhizoplast and basal bodies. Fig. 8*a* shows a chain between the 2 organelles (× 88,000), and Fig. 8 *b* illustrates more clearly the structure of the filaments in cross tangential section (× 107,000).

electron-opaque filament (Figs. 5 *a*, *f*). From this central filament, 9 fine spokes radiate to triangular condensations immediately within the cylinder of triplets. Ten to 15 layers of these spokes, each approximately 150 A in depth, and separated by 100 A interspaces, run across the basal body.

RHIZOPLAST AND CONNECTING FILAMENTS: Connecting the nucleus of the flagellate to the basal bodies and flagella is a narrow thread-like rhizoplast. This rhizoplast may vary tremendously in width—from 50 to 240 mμ in the same organelle—and may stretch over 5 μ in the cell. Alternating light and dark (electron-opaque) bands along its length confer on the rhizoplast a highly ordered, periodic pattern (Fig. 6). The light band maintains a relatively constant 40-A width, both within a given rhizoplast and among 11 rhizoplasts measured. Although the width of the dark band was also relatively constant in a given rhizoplast, it varied from 60 to 195 A with a mean of 120 A in the rhizoplasts measured. The periodicity is not constant and "collagen-like" as suggested by Schuster (1963 *a*). No membranes define the limits of the rhizoplast.

The physical continuity of nucleus, rhizoplast, and basal bodies, demonstrated by partial lysis (Fig. 2), is not paralleled by any distinctive morphological continuity at the points of union. The rhizoplast runs along the nuclear membrane, but does not enter the nucleus. Usually the rhizoplast appears to terminate within an infolded pocket of the nucleus in which the periodic structure becomes indistinct and then disappears (Fig. 7), but some rhizoplasts seem to continue beyond the nucleus and end free in the cytoplasm. At the other end, the rhizoplast sometimes splays into branches which reach out toward each of the basal bodies. An interconnected series of filaments occurs regularly at the rhizoplast-basal body junction (Fig. 8). The 200- to 300-A diameter filaments are similar to flagellar filaments in transverse section, but form shallow palisades of 10 or 12 filaments joined side by side. We cannot relate the chain of filaments ("spur" of Schuster, 1963 *a*) in any obvious way to the connection of basal bodies to the rhizoplast, since there is a gap at both ends of the chain.

Development of the Flagellar Apparatus

STRUCTURE OF AMEBAE: Except for the total absence of the components of the flagellar apparatus, the fine structure of *Naegleria* amebae resembles that of the flagellates. One distinctive feature of amebae is the presence of numerous digestion vacuoles containing ingested bacteria and intricately lamellated figures thought to be residual membranes of these bacteria (Fig. 9). These vacuoles, which are very prominent in amebae fixed on growth plates, disappear from cells during transformation.

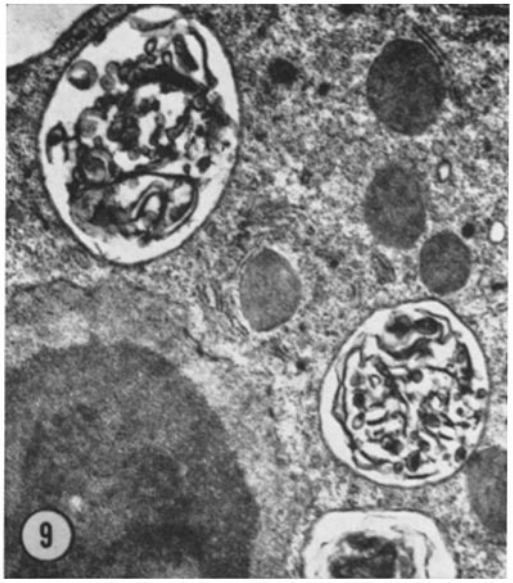


FIGURE 9 Portion of an ameba fixed on a growth plate. Note the vacuoles containing numerous lamellae and vesicles thought to be remnants of ingested bacteria. × 10,500.

FORMATION OF BASAL BODIES: Although basal bodies were seen frequently in sections of *Naegleria* flagellates, no basal-bodylike structures were ever seen in amebae, confirming the report of Schuster (1963 *a*). In transforming cells, basal bodies were first seen in samples fixed 55 min after suspension. We conclude tentatively that basal bodies are structured during transformation. However, despite intensive scanning of sections of cells fixed 55 to 70 min after suspension, no structures which could reasonably be interpreted as developing basal bodies were seen. All recognizable basal bodies in these samples already had the definitive structure of 9 triplets, and most were found at or near the cell surface.

FLAGELLUM DEVELOPMENT: A sequence of flagellum development has been inferred by fixing cells at successive times during transformation, and arranging longitudinally sectioned basal

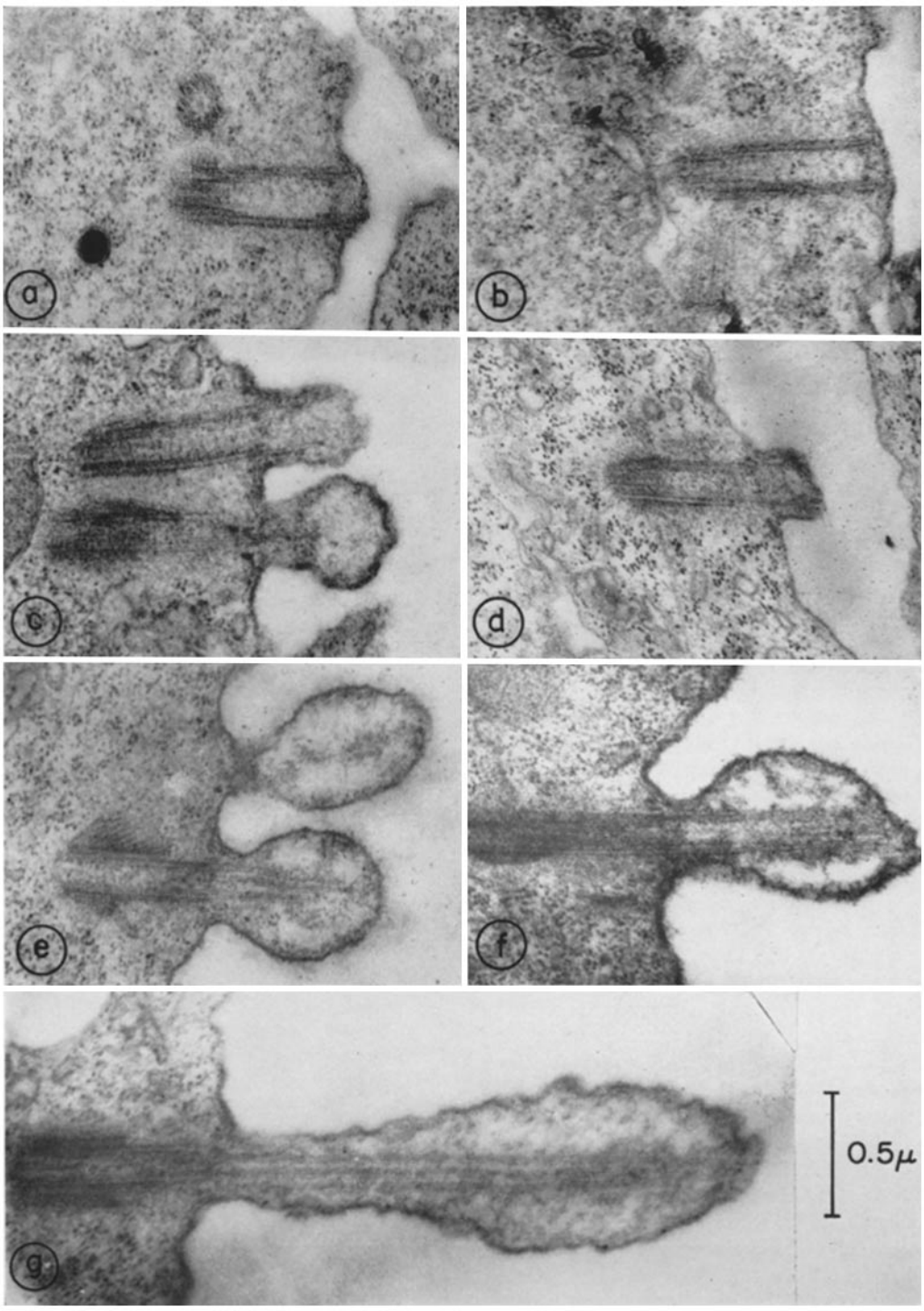


FIGURE 10 Longitudinally sectioned basal bodies and flagella showing sequence of flagellum development from the appearance of the basal body at the cell surface to the relatively well differentiated, elongating flagellum. Figs. 10 *a* and *b* are developmental stage 1; Figs. 10 *c* and *d*, stage 2; Figs. 10 *e* and *f*, stage 3; Fig. 10 *g*, stage 4. All micrographs, approximately × 36,500.

bodies and flagella on the basis of increasing size and complexity (Fig. 10). The continuum of shape changes can be arbitrarily divided into 4 stages:

Stage 1. In the earliest stage of flagellum development, following basal body formation, the basal bodies lie immediately beneath the cell surface with their filaments perpendicular to the cell membrane (Figs. 10 *a*, *b*). No flagellar filaments extend from the basal body.

Stage 2. Flagellar filaments extend from basal bodies at the surface of the cell into a slightly elevated cell membrane (Fig. 10 *c*, *d*).

Stage 3. Axial filaments extend from the basal bodies into ballooning membrane buds. These outpocketing membranes typically contain a conglomerate of loosely aggregated cytoplasmic material surrounding the developing axonemes (Figs. 10 *e*, *f*).

Stage 4. Maturing flagella have typical axonemes through most of their length. The membrane draws closer to the axial filaments at the base of the flagellum, but remains distended at the tip (Fig. 10 *g*). These flagella are visible in the light microscope.

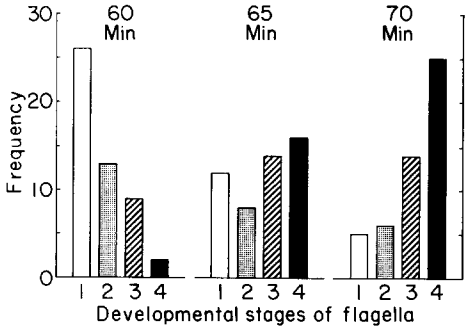
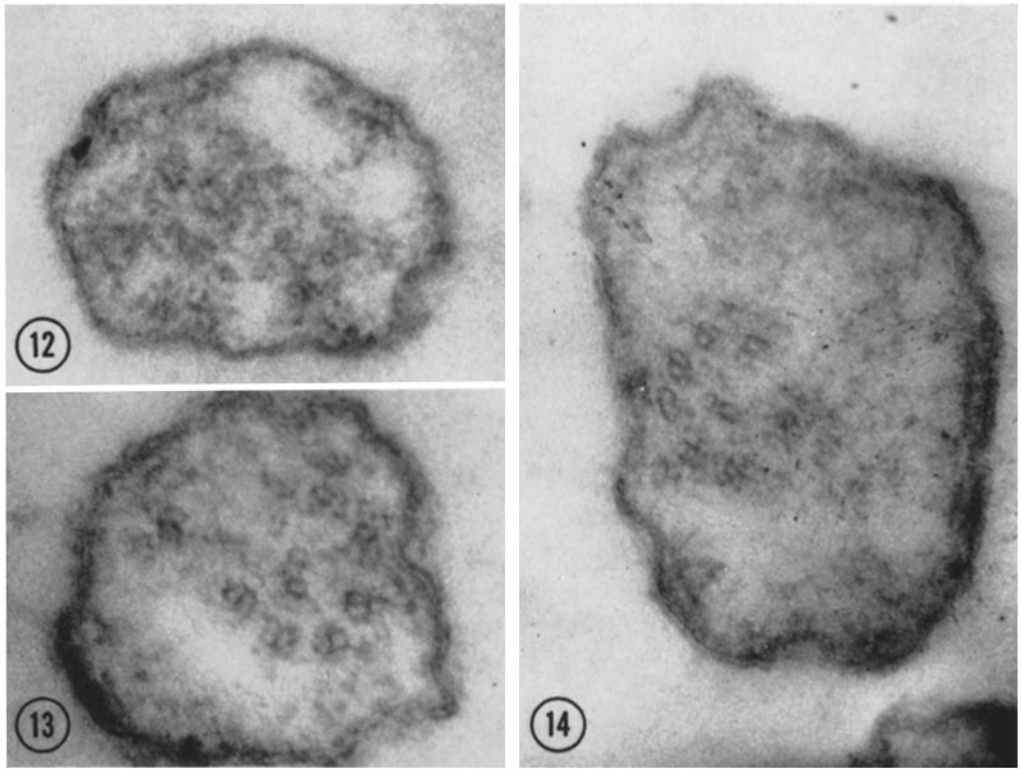


FIGURE 11 Change in the distribution of flagellum stages within the population during transformation. Samples of 50 longitudinally sectioned basal bodies and flagella were classified as to developmental stage at 60, 65, and 70 min after the initiation of transformation.



FIGURES 12 TO 14 Transverse sections, believed to be developing flagella. In Fig. 12, numerous single filaments are distributed randomly in the flagellum matrix. Axoneme cylinders are clearly discernible but still incomplete in Figs. 13 and 14. Note the greatly expanded flagellum membranes, especially in Fig. 14. All micrographs, × 113,000.

This description of flagellum development, though intuitively appealing, could be incorrect for various reasons. (1) The illustrations have been highly selected, and might not represent a typical sequence of development. (2) Some heterogeneity in time of transformation of individual cells exists even though transformation of the population is relatively synchronous (Fig. 1; for discussion, see Fulton and Dingle, 1966). A sample fixed at a given time during transformation, therefore, contains some of each stage. (3) Sectioning artifacts could result in misinterpretation of stages. For example, a fully formed flagellum, if sectioned obliquely, could be mistaken for a stage 2 flagellum.

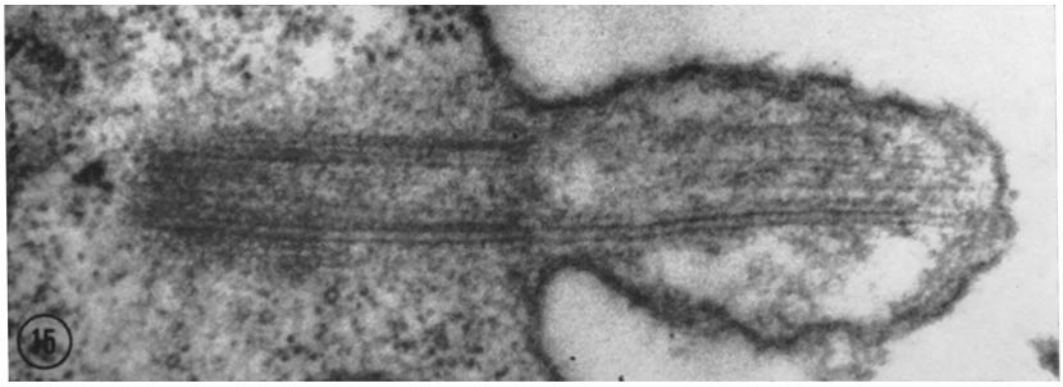


FIGURE 15 Median longitudinal section through the basal body and axoneme of a stage 3 developing flagellum. Both central and peripheral filaments extend to the limit of the flagellum membrane; no vesicles are present within the developing flagellum. × 74,000.

A definite progression through the proposed developmental sequence was demonstrated by classifying flagellum stages in samples fixed at intervals during transformation. Sections were examined for cells with midlongitudinally sectioned basal bodies and flagella. Care was taken to score only those flagella in which the limiting double membrane indicated even sectioning and whose central filaments indicated median sections. By intensive scanning of grids, 50 flagella were "staged" at 60, 65, and 70 min after suspension. Even in these short intervals, a marked shift toward later flagellum stages is evident (Fig. 11). The sequence of flagellum development is correct as presented in Fig. 10.

With the transformation conditions described, less than 10 min elapses from the appearance of a basal body to the elaboration of a stage 4 flagellum. Though the argument for this can be developed more precisely, it suffices that the first basal bodies are not seen until 55 min, and stage 4 flagella are observed by 60 min after suspension. The progression from stages 1 to 4 is even more rapid (Figs. 1 and 11). Light microscope observations of fixed cells indicate that the outgrowth of flagella is also rapid, with an average rate of elongation of at least 0.5 μ per min.

The axonemes of developing flagella are noticeably less electron opaque than those of mature flagella. A conglomerate of irregular and unstructured matrix of intermediate density generally fills early flagellum buds and the tips of maturing flagella (Fig. 10 and Figs. 12 to 15). Single and double filaments appear within this matrix, sometimes randomly (Fig. 12), but usually in the rough outline of an axoneme cylinder (Figs. 13, 14). There is no apparent difference in the rate of outgrowth of central and peripheral filaments; their formation occurs essentially simultaneously (Figs. 10 *g*, 15), although the condensation of either component may precede that of the other in a given flagellum section.

We have seen no suggestion of the vesicles reported by Sotelo and Trujillo-Cenóz (1958 *b*) within the centrioles and preceding filament condensation in the developing ciliary buds of chick neural epithelium, and seen by Tokuyasu and Yamada (1959) extending beyond the tips of the formed filaments of developing mammalian retinal rods. Rather, the filaments of developing *Naegleria* flagella extend uninterrupted from the

basal bodies to their distal terminus at the limiting flagellum membrane (Fig. 15). Because of the lack of vesicles, filaments, or other formed structures which could be traced into flagellum buds or anywhere in the vicinity of the basal bodies, no inferences can be drawn about the source of the axial filament material.

DEVELOPMENT OF THE RHIZOPLAST: The narrow, elongate form of the rhizoplast precludes quantitative electron microscope studies of its development. In early transformation stages when rhizoplasts are present but quite short, the probability of encountering one in thin sections would be much lower than in later samples in which the rhizoplast may extend over 5 μ in length. Consequently, the time sequence of development is tentative. Rhizoplasts are not often seen in sections of flagellates before 80 min, and are not prevalent until about 120 min after suspension. The earliest rhizoplast was seen in a sample fixed 60 min after suspension, in which only 1% of the cells had visible flagella.

We do not know whether the rhizoplast arises at the nuclear membrane or opposite the basal bodies. Some sections of early flagellates have rhizoplastlike condensations extending from the basal bodies and appearing to terminate free in the cytoplasm, but others suggest that the initial condensation occurs at the nuclear membrane. In every partial rhizoplast thought to be a developing stage, the periodically banded pattern was seen throughout the structure.

DISCUSSION

The structures of the flagellum and basal body of *Naegleria* are similar to those of other flagellates. The basal bodies apparently are constructed during transformation, which is unexpected since in most systems basal bodies arise from preexisting centrioles. These observations confirm those of Schuster (1963 *a*). The origin and development of basal bodies in transforming cells is being studied.

The development of flagella in *Naegleria*, in which synchronous transformation and quantitative measurements have permitted close examination, is remarkably straightforward: a basal body appears at the cell surface, and filaments extend from the basal body into a sheath which is a morphological extension of the cell membrane. Filament growth and membrane evagination are concurrent. There is no apparent involvement of other morphological structures, such as vesicles, in the formation of either filaments or sheath. The simple morphological sequence suggests that (1) the association of basal body and cell membrane is involved in flagellum development, (2) the axoneme pattern is ordered under the influence of the basal body pattern, and (3) the flagellum sheath is derived from the cell membrane.

One hypothesis about flagellum development in *Naegleria* can definitely be discarded. Schuster (1963 *a*) observed flagellum axonemes lying free in the cytoplasm of his transforming cells, and suggested that the axoneme may be formed within the cell and later extruded. We have never observed intracellular axonemes in cells which are developing flagella, but have found some in cells fixed 150 min or more after suspension, by which time some flagellates have begun to revert to amebae (Fulton and Dingle, 1966). During the 4-hr period when Schuster fixed cells, some flagellates undoubtedly reverted to amebae; resorption of their flagella could account for the intracellular axonemes he observed.

Flagellum development in *Allomyces* seems to proceed like that in *Naegleria*, except that axoneme filaments extend from the basal body into a large intracellular vesicle formed by coalescence of small vesicles moving internally from the cell membrane. This vesicle becomes the flagellum sheath (Renaud and Swift, 1964). Such differences have been found in other systems, e.g., a similar sequence is described by Sorokin (1962) in developing fibroblasts and smooth muscle cells in which flagella develop internally and the sheath is formed by the coalescence of vesicles. However, neither the vesicles described by Sotelo and Trujillo-Cenóz (1958 *b*) nor the filaments described by Roth and Shigenaka (1964) appear to be involved in axoneme formation in *Naegleria* or in *Allomyces*. In view of the apparent diversity of developmental patterns, it seems all the more remarkable that there should be such uniformity of structure in the cilia and flagella of various plants and animals. It is possible that the observation of such vesicles and filaments reflects the use of rudimentary, developmentally arrested, or modified nonmotile cilia, and that the pattern of flagellum axoneme formation suggested by the studies on *Naegleria* and *Allomyces* will be found to prevail in normal cilia and flagella.

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