THE DEVELOPMENT OF BASAL BODIES AND FLAGELLA IN *ALLOMYCES ARBUSCULUS*

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ABSTRACT

The development of basal bodies and flagella in the water mold *Allomyces arbusculus* has been studied with the electron microscope. A small pre-existing centriole, about 160 mμ in length, was found in an inpocketing of the nuclear membrane in the vegetative hypha. Thus, formation of a basal body does not occur *de novo*. When the hyphal tip started to differentiate into gametangia, the centrioles were found to exist in pairs. One of the members of the pair then grew distally to more than three times its original length, whereas the other remained the same size. The larger centriole would correspond to the basal body of a future gamete. Gametogenesis was usually induced by transferring a "ripe" culture to distilled water. Shortly after this was done, a few vesicles were pinched off from the cell membrane of the gametangium and came in contact with the basal body. Apparently, they fused and formed a large primary vesicle. The flagellum then started to grow by invaginating into it. Flagellar fibers were evident from the very beginning. As the flagellum grew so did the vesicle by fusion with secondary vesicles, thus coming to form the flagellar sheath. The different stages of flagellar morphogenesis are described and the possible interrelationships with other processes are discussed.

INTRODUCTION

A major question in cytology concerns the patterns of morphogenesis in basal bodies, cilia, and flagella. The gametophytic generation of the water mold *Allomyces arbusculus* provides an approach to this problem because the formation and growth of basal bodies and flagella occurs at certain predictable stages of its life cycle (Hatch, 1935). The flagellated gametes possess a well developed basal body which is not apparent in the somatic tissues by ordinary light microscope techniques (Robinow, 1962), thus suggesting either *de novo* synthesis or development from a submicroscopic precursor. The theory of *de novo* synthesis has been used to explain the sudden appearance of basal bodies during meiosis in higher plants with motile gametes (Lepper, 1956).

De novo synthesis has also been suggested in animal systems. Wilson (1925) and more recently Mazia (1961) discussed the possibility of *de novo* formation of centrioles (known to be cytological equivalents of basal bodies) by means of the induction of cell centers in unfertilized sea urchin eggs. Schuster (1963), in an electron microscope study of the ameboflagellate *Naegleria gruberi*, found no evidence for a basal body in the ameboid stage, but found it in the flagellated stage.

Other investigators have postulated that basal bodies or centrioles are self-replicating organelles. This theory received wide support from the early work of Boveri, Van Beneden, and Heidenhain (Wilson, 1925). More recently, Lwoff (1950), after an intensive light microscope study of the

infraciliature of ciliates, came to the conclusion that new basal bodies originated from the old by a process of splitting. Bernhard and de Harven (1958), among the first investigators to study centrioles with the electron microscope, postulated the possible conversion of the centriolar "satellite" bodies into "centrides-filles," which then would develop into "mature" centrioles. Randall and Hopkins (1963), after an electron microscope study of the infraciliature of *Stentor*, also came to the conclusion that production of a new basal body was dependent on synthesis by the old one, although they present a completely different scheme of morphogenesis. The precise mode of synthesis of a basal body still remains a complete mystery, and it was our hope that a study of its formation in *Allomyces* would shed some light on this problem.

The development of cilia and flagella has been investigated in a variety of tissues. In spite of many studies on spermatogenesis in both vertebrates (Burgos and Fawcett, 1956; Fawcett, 1958, 1961; Nagano, 1962; Sotelo and Trujillo-Cenóz, 1958 *a*) and invertebrates (Bertaud and Gatenby, 1960; Gall, 1961; Gatenby, 1961; Sotelo and Trujillo-Cenóz, 1958 *a*), the process of early flagellar development in sperm is still unclear. A lot more is known about the early stages of formation of cilia, particularly from the work of Sotelo and Trujillo-Cenóz (1958 *b*) on neural epithelium of the embryonic chick, and Sorokin (1962) on the formation of rudimentary cilia in differentiating fibroblasts and smooth muscle cells. Both of these systems have the disadvantages of a lack of synchrony, which presents a great problem in the reconstruction of a developmental series from micrographs, and in the case of embryonic fibroblasts and myoblasts the final product is an atypical cilium. On the other hand, in *Allomyces arbusculus*, flagellar formation can be induced more or less synchronously (the whole process taking less than an hour) by the mere imersion of a "mature" agar culture into a fresh aqueous medium, thus allowing one to follow the development of a normal flagellum step by step in time. It is because of the obvious advantages of this system that the present study was undertaken.

MATERIALS AND METHODS

Allomyces arbusculus Butler was obtained from the American Type Culture Collection as a dried hemp seed culture of the sporophytic stage. Purification was achieved by allowing the spores to be released in sterile water and plating the zoospore suspension on Nutrient Agar (Difco Laboratories, Inc., Detroit). After 24 hours of incubation at 25°C, colonies that were free of bacterial contamination were picked and transplanted to fresh medium. Nutrient agar was employed from then on for subculturing.

For the study of hyphal tips and gametangial differentiation, the mold was grown in liquid medium (GY_2), as modified by Turian (1963), on a rotatory shaker, at approximately 70 RPM. After about 2 days, many of the hyphal tips showed different stages of gametangial differentiation. The culture was then fixed for an hour at 5°C with 1 per cent OsO_4 buffered to pH 6.1 with 0.2 M phosphate buffer and containing 10^{-3} M $CaCl_2$ (Blondel and Turian, 1960). Except for the study of hyphal tips, it was found necessary to treat the organism, prior to fixation, with a crude chitinase preparation (Bawden and Pirie, 1946; Neuberger and Pitt Rivers, 1939), to improve the penetration of the fixative through to the thick gametangial wall. This crude chitinase was prepared as follows: the hepatopancreas was removed from five or six helicid snails (*Otala lactea*) and homogenized in 5 ml of cold 0.2 M KH_2PO_4 adjusted to pH 3.6 with 1 N HCl. The homogenate was centrifuged in a refrigerated centrifuge at 2000 RPM for 20 minutes and the supernatant collected and stored in the refrigerator. Cultures were treated for 15 minutes at room temperature with freshly prepared enzyme. This preparation improved fixation without causing noticeable changes in the organism, except that in gametangia returned to water the gamete discharge was delayed for about 10 minutes as compared with untreated controls. The enzyme was toxic to free-swimming gametes, and if stored in the refrigerator for a few days before use, also caused toxic degenerative changes in the gametangia. After fixation, the material was dehydrated in a graded series of cold ethanol, transferred to dried acetone, and embedded in Vestopal W (Ryter and Kellenberger, 1958). Epon 812 was also used but abandoned because of unsatisfactorily low contrast.

Nine-day old nutrient agar cultures were employed to study the fine structure of flagella formation. These cultures showed most gametangia to be ripe for gametogenesis, particularly in the periphery: the nuclei were outlined by cytoplasmic lipid droplets forming the so called "lipid crown" stage (Blondel and Turian, 1960). 1 mm cubes of the culture were cut with a razor blade and transferred to vials containing distilled water. Samples were taken at different time intervals and fixed for the electron microscope using the above-mentioned procedure. Because of the lag period caused by the enzyme treatment, the results may be assumed to represent a stage in time between the initiation of the enzyme treatment and the putting of the sample in the

fixative: for example, a sample 5 minutes in water and 15 minutes in enzyme probably represents the situation as it was between 5 and 20 minutes of development, but not so advanced as if it had been 20 minutes in water. Nevertheless, samples are here referred to according to their time in water.

The blocks were sectioned with a Porter-Blum microtome, and silver and gold sections were placed on carbon-coated copper grids and stained with either 3 per cent uranyl acetate for 12 to 24 hours or 10 per cent phosphotungstic acid for 1 hour. The prolonged staining with uranyl acetate was found necessary to improve the contrast. The sections were examined with an RCA EMU-3C microscope.

RESULTS

The hyphal tip (Fig. 1) is capable of either vegetative growth or gametangial differentiation, depending on the external conditions. In GY_2 liquid cultures, growth predominated initially over gametangial differentiation, the latter occurring mainly in the later phases of the culture, but if a supply of fresh medium was maintained, growth continued to predominate over differentiation. The factors that induce a hyphal tip to differentiate are unknown. The first sign of gametangial differentiation is a bulge occurring at the distal end of the hyphal tip. A septum is then formed between the bulge and the rest of the hypha, followed by a second septum at some distance behind the first, giving rise to two compartments. An approximately equal number of hyphal tip nuclei are trapped into each. The more distal compartment becomes the female gametangium, and the proximal one the male gametangium. Both of these structures grow and increase in volume, becoming subspherical in shape, while the number of nuclei in the male gametangium is duplicated by mitosis. On maturation (Fig. 2) the nuclei are seen surrounded by lipid droplets (the lipid crown stage) and the male gametangium acquires an orange-yellow pigment. The gametangia will now rest in this stage until they receive the proper stimulus that will induce the formation of gametes. This trigger consists of transfer to a fresh aqueous environment (Fig. 3).

Centrioles were found to exist in association with the nuclei of the hyphal tip, in an inpocketing of the nuclear membrane, usually perpendicular to the plane of the latter (Figs. 4 and 5). Not more than one centriole was seen per nucleus. They were relatively small, measuring on the average 160 mμ in length and 170 mμ in width. Since in a number of cases they could be traced in consecutive serial sections, the possibility that they represent tangential sections of larger organelles can be excluded. No structures were seen within the centriole at this stage, except for the nine peripheral fibers. However, a filamentous material was seen filling most of the centriolar interior in longitudinal sections (Fig. 5). Whether the peripheral fibers are singlets, doublets, or triplets is not clear from the micrographs. Tufts of dense material seen in association with the fibers make resolution of fibers difficult (Fig. 4). A relatively ribosome-free area surrounds the centriole like a halo, and is pervaded by a fine filamentous material that seems to radiate from the centriole to the periphery (Fig. 4). Small vesicles, about 30 mμ in diameter, and of a homogeneous content are dispersed in the periphery of this clear area at intervals (V_p, Fig. 4). The neighboring cytoplasm is rich in mitochondria, and also in ribosomes, which tend to occur in clusters. Clusters of smooth vesicles of varying diameter appear sometimes near the centriolar area (Fig. 4).

During the earlier stages of gametangial differentiation, the electron microscope reveals that the centriolar pattern deviates only slightly from that found in the hyphal tip. The centrioles exist in pairs, one pair per nucleus, at an angle of close to 90° to one another (Fig. 6). This represents an apparent doubling in centriole number, although the possibility that centriole pairs may also exist in the hyphae has not been excluded. A tubule or single filament, about 18 mμ in diameter, occurs in the center, from which tenuous filaments radiate and make contact with the peripheral filaments, giving the centriole the aspect of a cartwheel. The peripheral filaments appear to be at least doublets at this level.

By the stage prior to pigment formation in the male gametangium, one of the members of the centriole pair has grown to more than three times its original length, measuring approximately 550 mμ (Figs. 7 and 8). The other centriole remains the original size. The end of the large centriole proximal to the nucleus contains some filamentous material and is associated with tufts of dense material at the sides (Fig. 8). This proximal area measures about 160 mμ in length and resembles morphologically a centriole from a hyphal tip (Fig. 5). The distal end is also associated with tufts of dense material which together with a thin membrane forms a terminal expansion (Figs. 7

and 8). The smaller centriole is seen to occupy a small pocket of its own in the nuclear membrane, and is at an angle of about 60° with respect to the larger one.

In the mature gametangium no further changes occur concerning the larger centriole (Figs. 2 and 9) which is now fully prepared to serve as a basal body for the flagellum of a gamete. When a ripe *Allomyces* culture is put into water, one notices the appearance of some vesicles in the neighborhood of the centriolar area after about 10 minutes (Fig. 10). These vesicles vary in size, but measure around 200 mμ in diameter, and contain some finely filamentous material resembling in texture that found between the plasma membrane and the gametangial wall (Fig. 12). The vesicles come in contact with the terminal expansion of the basal body, and apparently fuse to form a large, single body, here called the primary flagellar vesicle, following Sorokin's (1962) nomenclature (Fig. 11). No vesicles were ever seen in association with the small centriole. In some cases a primary vesicle was observed in direct continuity with the plasma membrane of the gametangium (Fig. 12). All the nuclei within a gametangium are quite precisely synchronized but small variations occur when events in different gametangia are compared. After the culture has been in water for 15 minutes, one sees some material extending from the basal body and invaginating into the primary flagellar vesicle (Fig. 13). Some tenuous filaments in direct continuity with the peripheral fibers of the basal body are seen, in some sections, as part of the invaginating material (Fig. 14). The dense material at the distal end of the basal body has all but disappeared, but the beginnings of a basal plate are already seen (Fig. 14). As the invagination keeps growing, so do the filaments (Fig. 16), and the primary vesicle, apparently by fusion with secondary vesicles (Fig. 15). Ribosome-like particles are seen sometimes within the latter, possibly because some cytoplasm might be trapped inside them owing to their way of formation. The primary vesicle has now come to form a double membranous sheath bounding the growing flagellum. A basal plate is now quite evident. The next step in the development is apparently a club-shaped structure containing some heterogeneous material that looks somewhat vesicular in nature (Fig. 17). By 20 minutes, a short flagellum is already formed (Figs. 3, 18), with the axial filament complex reaching all the way to the tip. The sheath is still in the process of being completed apparently by the fusion of secondary vesicles (Fig. 19). Cross-sections of growing flagella show the sheath as double concentric membranes around the flagellum, but sometimes the outer membrane is incomplete (Fig. 20). This gap will probably be repaired later on by the fusion of more secondary vesicles. Fully grown flagella in mature gametes appear bounded by a single membrane, the outer one presumably having fused to the plasma membrane of the gamete (Fig. 21). A fine, plush-like filamentous material, stainable with phosphotungstic acid, was found in association with both the plasma membrane of the gametangium and the sheath of the flagellum (Figs. 22 and 23). The vesicles that serve as precursors of the cell membrane of the gamete also stain, whereas other membranous components of the cell, like those of the nuclei and mitochondria, do not. The origin and further fate of these vesicles will be discussed in a later publication. A diagrammatic summary of the main events described here is given in Fig. 24.

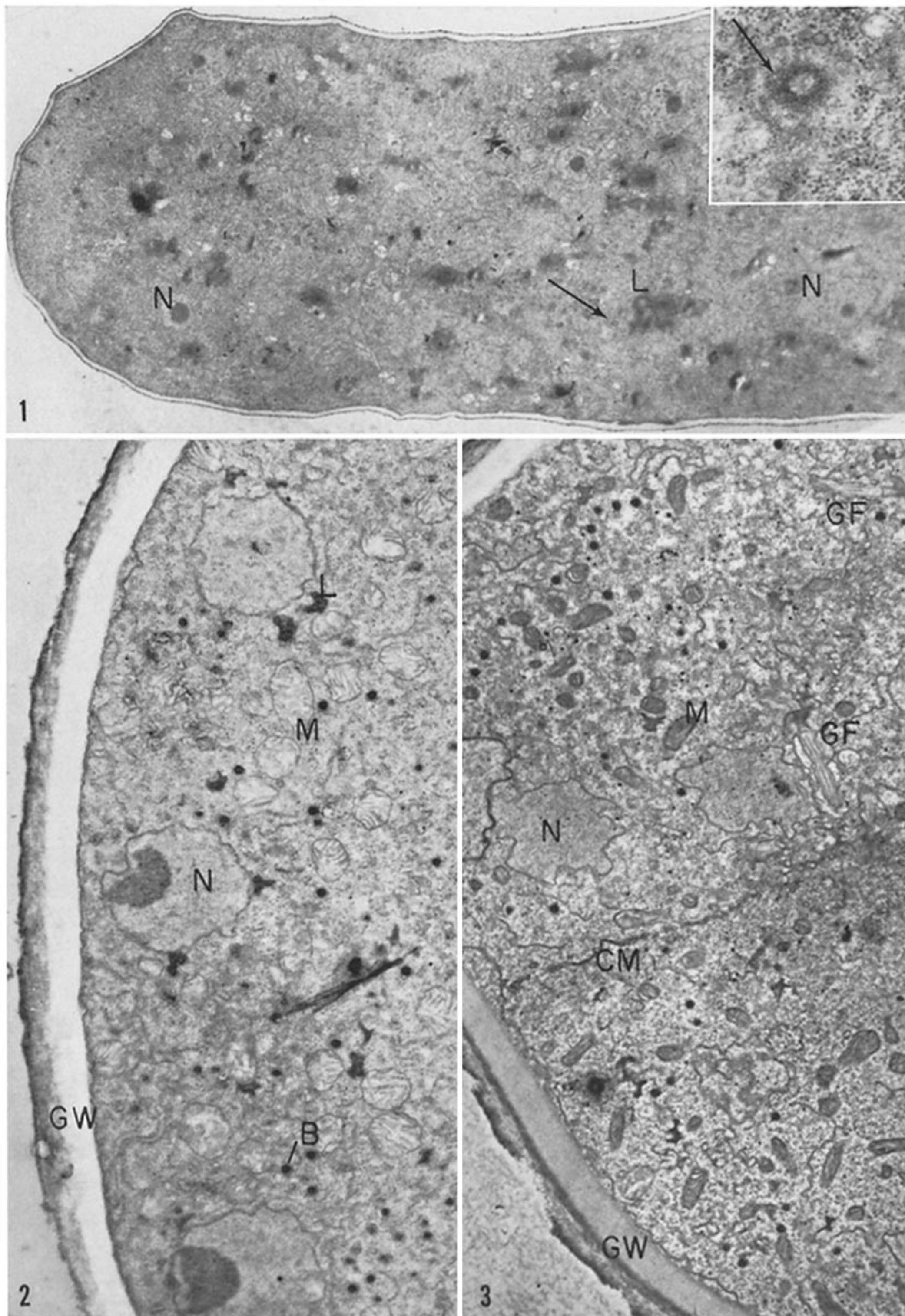
DISCUSSION

The finding of centrioles in the hyphae of *Allomyces* leads to some interesting conclusions. First of all, formation of a basal body in the gamete is not by

FIGURE 1 Hyphal tip. Note the nuclei (*N*) distributed throughout, the numerous mitochondria, and lipid droplets (*L*). A centriole is seen at arrow and at higher magnification in the inset. × 5700; inset, × 49,000.

FIGURE 2 Mature gametangium. The nuclei (*N*) are distributed in the periphery with lipid droplets (*L*) around them. The nuclei show the usual orientation, with the nucleolus and centriolar inpocketing near the cell membrane. *B*, membrane-bounded dense bodies of unknown function; *GW*, gametangial wall; *M*, mitochondrion. × 10,000.

FIGURE 3 Mature gametangium after 20 minutes in water. Cell membrane formation and flagellation are well under way. The mitochondria (*M*) have decreased in volume and become denser. *CM*, forming cell membrane; *GF*, growing flagellum; *GW*, gametangial wall; *N*, nucleus. × 8,400.


N
L
N
1
L
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B
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3

de novo synthesis, but results from the growth of an already existing small centriole. Only when it grows during the process of maturation of the gametangium does the centriole become visible by ordinary techniques of light microscopy. The dramatic nature of the change could very easily lead to belief in a *de novo* synthesis. These results suggest that the sudden formation of a basal body or "blepharoplast" during the meiotic divisions of higher plants producing motile gametes might also occur by the same process. This possibility had already been suggested by Gall (1961), when he stated that, in the above mentioned systems, a pro-centriole might always be present, which would mature only during gametogenesis. We feel, however, that the small centriole in the hypha of *Allomyces* deserves the full-fledged title of centriole, for it does not represent a stage in the development of a larger organelle in the hypha, but is a fully formed structure. Robinow and Marak (unpublished), in an electron microscope study of mitosis in the somatic nuclei of *Allomyces macrogynus*, found a small centriole at the spindle poles similar to that found in the hypha of *Allomyces arbusculus*. Spindle fibers were found in association with it. However, that the pattern of basal body development in *Allomyces* might not be applicable to other plants is suggested by the work of Gall and Mizukami (1963) on the fern *Marsilea*, in which they were able to trace the origin of the basal bodies to compact spheres of radially oriented tubules found at the poles of the mitotic spindle, but of unknown origin.

Concerning the possibility of *de novo* synthesis of basal bodies in animal cells, it could be that, in organisms such as the amebo-flagellate *Naegleria*, a closer examination might reveal a phenomenon like that in *Allomyces*. On the other hand, the theory of *de novo* synthesis of centrioles in artificially activated sea urchin eggs requires a different explanation. It rests on the assumption that the egg lacks "active" centrioles which become functional in the mitotic spindle of the zygote. Mazia (1961) points out how odd it would seem for centrioles to "die" after having operated normally in the meiotic divisions of the oocytes. On the other hand, the fact that cytasters can be induced in enucleated pieces and even in eggs where the whole spindle has been removed, and the fact that the number of cytasters induced can be greater than the number of centrioles usually found in a cell, do suggest a *de novo* synthesis. Brachet (1957) argued that cytasters could be formed around any cytoplasmic granule, not necessarily centrioles, but Dirksen (1961), in an electron microscope study, found centrioles associated with the cytasters of artificially activated sea urchin eggs. She suggested that they might originate from precursor material occurring in the cytoplasm in a disperse form, perhaps similar in nature to pericentriolar satellites, but pointed out that the question is still far from answered.

FIGURE 4 Tangential section of a hyphal tip nucleus (*N*) showing a centriole (*C*) in cross-section in an inpocketing of the nuclear membrane. V_p, pericentriolar vesicles; V_s, smooth vesicles. × 50,000.

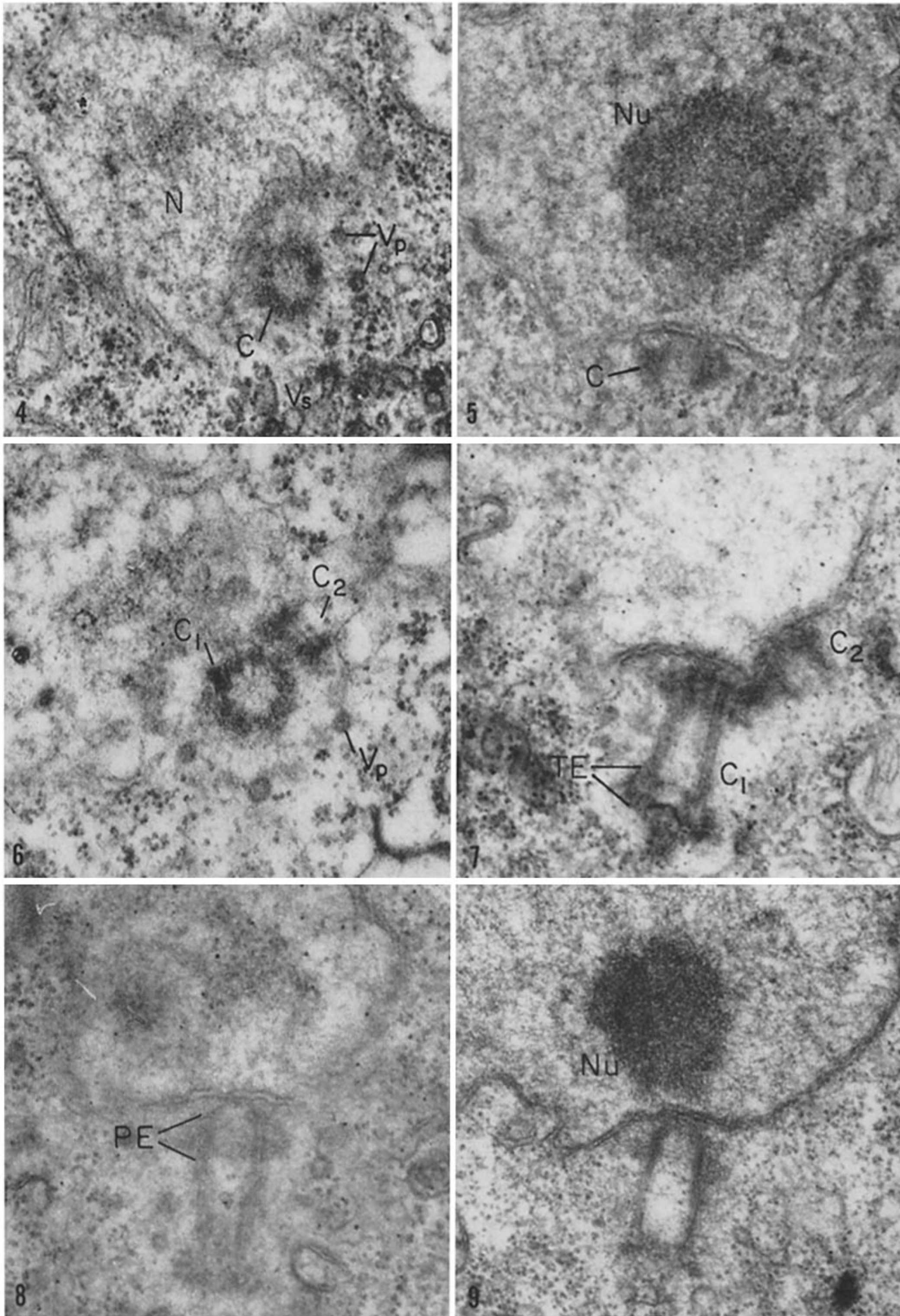
FIGURE 5 Same as Fig. 4, but with the centriole (*C*) perpendicular to the plane of the nuclear membrane. Note the filamentous material filling the centriole interior. *Nu*, nucleolus. × 50,000.

FIGURE 6 Centrioles as they occur in an immature gametangium, at an angle of close to 90° to one another. C_1, transverse section of a centriole (note the cartwheel pattern); C_2, centriole in longitudinal section; V_p, pericentriolar vesicles. × 56,000.

FIGURE 7 Centriole complement of an immature gametangium, later than Fig. 6, at a stage just prior to pigment formation in the male. One of the members of the pair has grown to more than three times its original length (C_1). The second centriole (C_2) remains unchanged. *TE*, terminal expansion. × 49,000.

FIGURE 8 Same as Fig. 7, but showing the large centriole only. Note the similarity between the proximal end (*PE*) and the hyphal tip centriole seen in Fig. 5. × 49,000.

FIGURE 9 Nucleus and large centriole from a mature gametangium. *Nu*, nucleolus. × 44,000.


N
Vp
C
Vs
4
Nu
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C2
C1
Vp
6
C2
TE
C1
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PE
8
Nu
9

It is evident that the two centrioles in mature gametangia are not equivalent, for when stimulated to flagellar growth the large centriole becomes associated with a primary vesicle and is active in flagellar growth, while the small centriole shows no activity. The question as to why the centriole in *Allomyces* should first grow before functioning as a basal body could be approached by Gall's theory of centriolar polarization, derived from his study of *Viviparus* spermatogenesis. He found that a pro-centriole only 70 mμ in length is formed, sometime before pachytene, at right angles to the proximal part of a full-sized centriole (330 mμ long). This pro-centriole grows later to a full size and its "new" distal end gives rise to the sperm flagellum. He postulated that the distal end is responsible for organizing flagella and other related organelles, while the old proximal end represents the old pro-centriole, which remains as a "germinal" center in some way organizing another centriole at right angles to it. Since the centriole in *Allomyces* is apparently not capable of functioning as a basal body unless it grows a "new" distal end, the centriole in the hypha could be considered to be the equivalent of a proximal end of a basal body, presumably playing some germinal role in its own duplication. This is further substantiated by the occurrence in it of the cartwheel pattern found to be characteristic of the proximal end of basal bodies by Gibbons and Grimstone (1960) and also by Gall (1961). The fibers forming the cartwheel pattern probably correspond to the filamentous material which is seen filling the interior of the hyphal tip centriole in longitudinal sections, and which in the proximal end of the basal body marks the area occupied by the former small centriole. It is interesting to note that, since Robinow and Marak (unpublished) found only a small centriole at the poles of the spindle of somatic mitoses of *Allomyces macrogynus*, spindle fiber formation is not dependent on centriolar growth.

Other interesting instances of unequal centriolar growth, analogous to that of *Allomyces*, are found in bird (Nagano, 1962) and mammalian (Fawcett, 1958) spermatogenesis. In the latter case, the distal centriole becomes highly modified and grows into a funnel-shaped structure resting against an inpocketing of the nuclear membrane, whereas the proximal centriole remains the original size.

Our developmental sequence for flagellar growth closely parallels that found by Sorokin (1962) for rudimentary cilia in chick fibroblasts and developing myoblasts, with a few interesting differences. In our work, the primary flagellar vesicle apparently originates from the fusion of a few smaller vesicles. This suggests that a minimal size must be attained by the vesicle to make it suitable for the flagellar invagination. In addition, filaments are evident in *Allomyces* flagella from the very early stages. In Sorokin's material, no filaments are evident until the cilium reaches the cell

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FIGURE 10 Mature gametangium after 10 minutes in water. Secondary vesicles (V_2) are formed near the centriolar area, and become associated with the terminal expansion of the large centriole (C_1) to form the primary vesicle (V_1). *GW*, gametangial wall; C_2, small centriole. × 34,000.

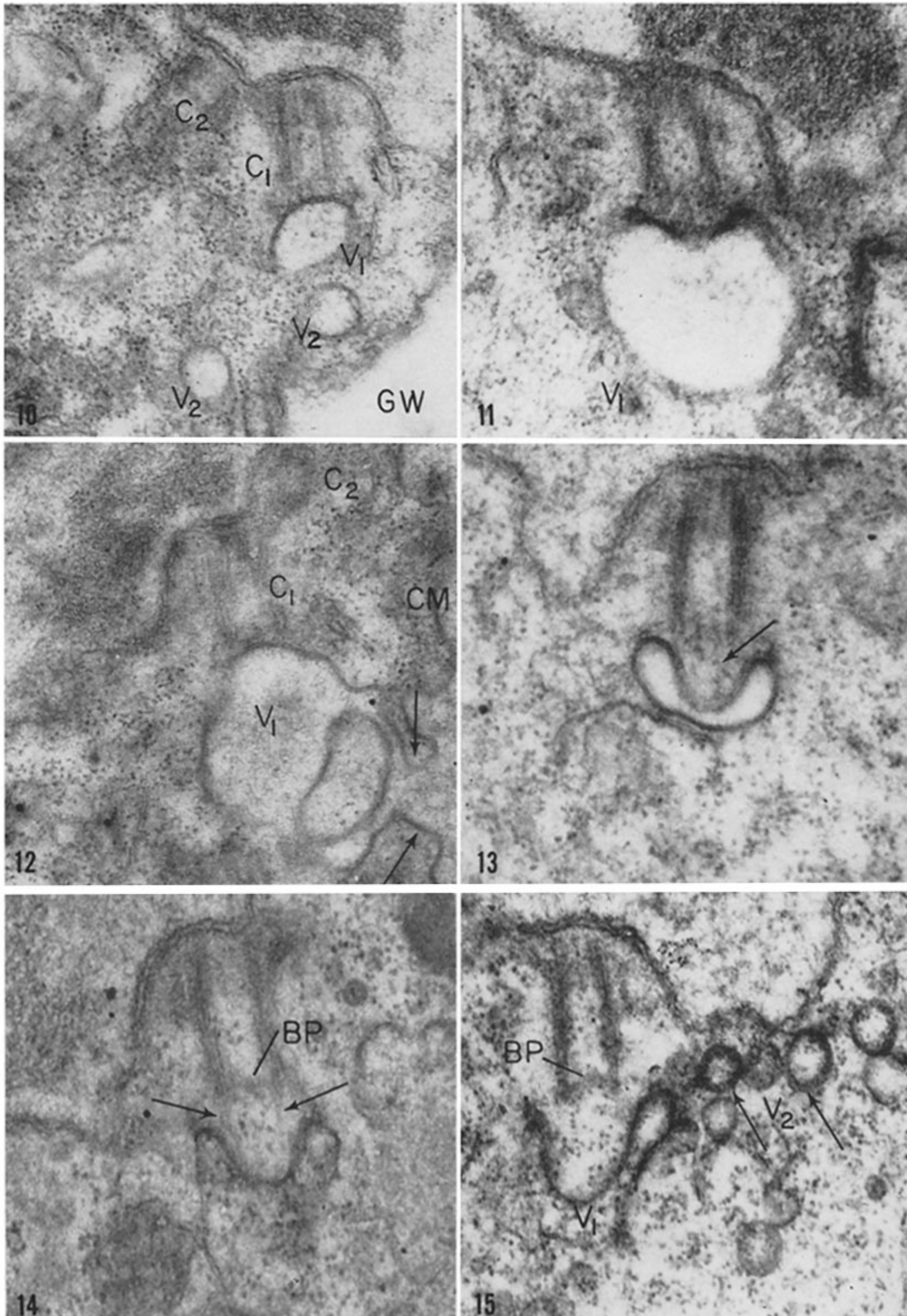
FIGURE 11 Mature gametangium after 10 minutes in water. A primary vesicle (V_1) is seen in association with the terminal expansion of the large centriole. × 54,000.

FIGURE 12 Same stage as Fig. 11. Large centriole (C_1) in association with a primary vesicle (V_1) which is continuous with the cell membrane of the gametangium (*CM*) at points marked by arrows. C_2, small centriole. × 42,000.

FIGURE 13 Mature gametangium after 15 minutes in water. Some material (arrow) is seen projecting from the large centriole and invaginating into the primary vesicle. This centriole is now functioning as a basal body. × 51,000.

FIGURE 14 Mature gametangium 15 minutes in water. Note tenuous fibers in the invagination (arrows). A forming basal plate (*BP*) is apparent at this stage. × 51,000.

FIGURE 15 Same material as Fig. 14, but showing many secondary vesicles (V_2), probably in the process of fusion with the enlarging primary vesicle (V_1). *BP*, basal plate. × 51,000.


C2
C1
V1
V2
V2
GW
10
V1
11
C2
C1
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BP
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BP
V2
V1
15

surface and then only in the basal part of the cilium. A club-shaped rudimentary cilium, full of vesicles and heterogeneous material, is the end product in Sorokin's system, whereas in *Allomyces* a club-shaped structure containing somewhat similar material is found only in the very early stages. As the occurrence of this structure in *Allomyces* was very rare, it could represent a deviation from the normal pattern of development. It is not clear why a flagellum would develop from the stage represented in Fig. 16 *via* a club-shaped structure. However, club-shaped structures have been found as developmental stages of retinal rods and cones by Tokuyasu and Yamada (1959), the photoreceptors of a tree frog by Eakin and Westfall (1961), and cilia by Sotelo and Trujillo-Cenóz (1958 *b*). The latter authors suggested that the heterogeneous material took part in the formation of the ciliary fibers. This club-shaped stage may represent the stage in organelle ontogeny in which the morphogenetic patterns of cilia and flagella, photoreceptors, and retinal rods and cones start to diverge.

The formation of a basal plate seems to be in some way connected to the activity of the basal body, for in both our work and that of Sotelo and Trujillo-Cenóz (1958 *b*) a basal plate is seen only in basal bodies engaged in cilio- or flagellogenesis.

In *Allomyces*, primary and secondary vesicles seem to originate from the cell membrane of the gametangium. This is evidenced by the continuity shown occasionally (Fig. 12) between a primary vesicle and the plasma membrane of the organ and by the similarity between the content of the vesicles and the material found between the cell membrane and the gametangial wall. This suggests that the vesicles are pinched off from the cell membrane by a process analogous to vesicle formation in pinocytosis. In addition both the vesicles and the cell membrane show an association with a finely filamentous material stainable with phosphotungstic acid, which suggests some biochemical similarity between them. Sorokin (1962) suggested that the primary and secondary vesicles originated in the Golgi apparatus, and that therefore contact between the basal body and the cell membrane was not necessary for the induction of ciliogenesis, as had been suggested by Sotelo and Trujillo-Cenóz (1958 *b*). If, as suggested by our findings, the primary vesicle originates from the cell membrane, the theory of Sotelo and Trujillo-Cenóz might apply even to cases where the basal body does not make contact with the cell surface proper. What induces the cell membrane to pinch off the vesicles is unknown. These are not produced at random, for they always originate in the vicinity of a centriole. This suggests that the centriole exerts some inductive influence on the cell membrane. However, centrioles are always present near the cell membrane in the mature gametangia, but it is not until the culture is put in a fresh aqueous environment that the cell membrane becomes capable of pinching off flagellar vesicles. Thus the aqueous environment seems to make the cell membrane susceptible to the centriolar influence, perhaps by increasing the oxygen available to the gametangium or by hydrating the filamentous coat of the cell membrane. This external surface coat seems to play an important role in the formation of pinocytotic vesicles in amoebae (Brandt and Pappas, 1960).

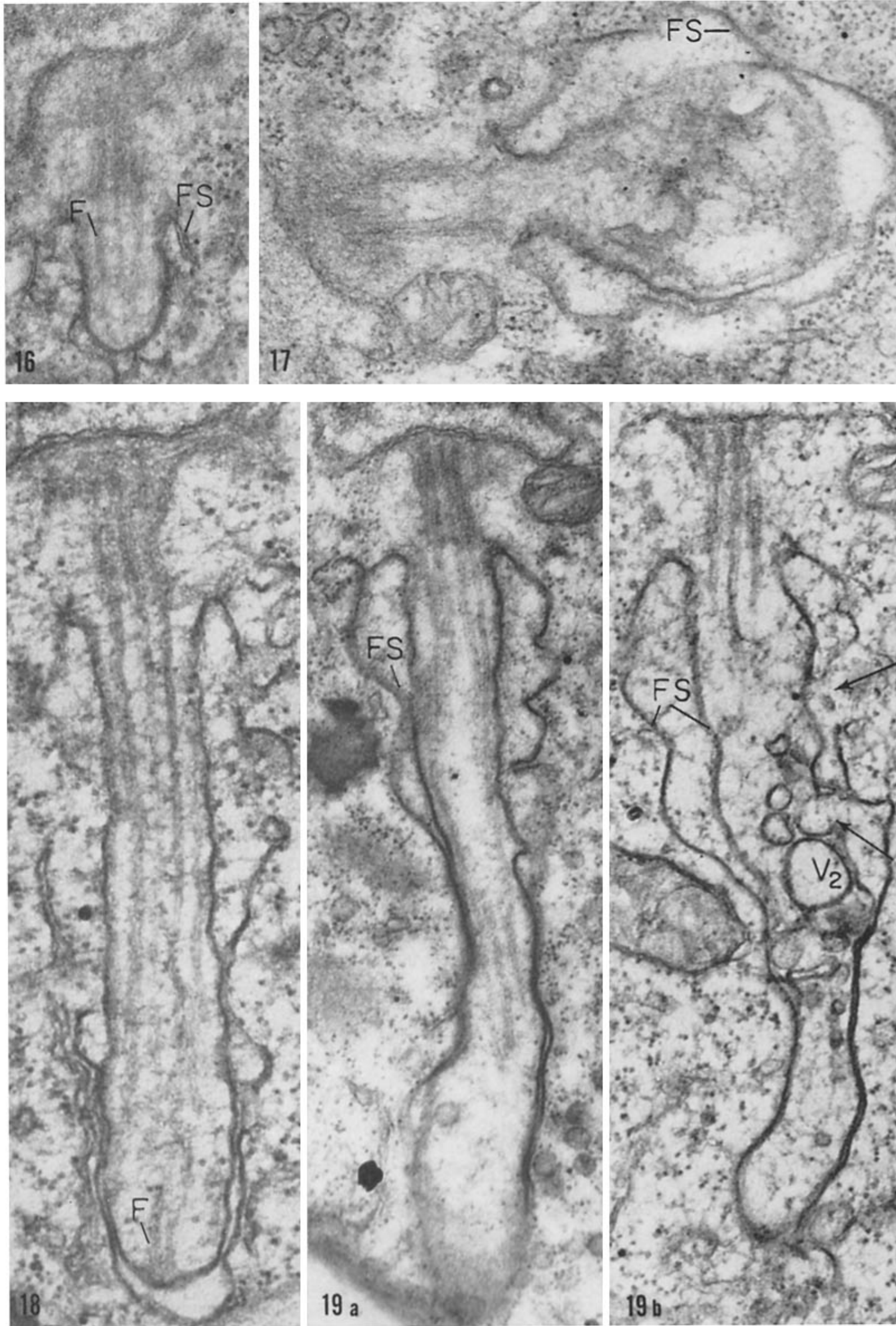
Some other investigators have reported work that suggests that a similar pattern of flagellar formation may be present in other systems as well. Bertaud and Gatenby (1960), studying the spermatogenesis of a cave cricket, show pictures of

FIGURE 16 Gametangium after 15 minutes in water. Fibers (*F*) apparently extend from the basal body to the tip of the invagination. The primary vesicle now forms the flagellar sheath (*FS*). × 48,000.

FIGURE 17 Gametangium after 20 minutes in water, showing a club-shaped structure containing heterogeneous material. *FS*, flagellar sheath. × 54,000.

FIGURE 18 Gametangium after 20 minutes in water. A short flagellum is already formed. Note that the flagellar fibers (*F*) extend to the tip of the flagellum. × 62,000.

FIGURE 19 Same as Fig. 18. Figs. 19 *a* and *b* represent longitudinal sections of the same flagellum. Note that in Fig. 19*b* the sheath (*FS*) is still incomplete at several points (arrows) and is associated with a number of secondary vesicles (V_2). Fig. 19 *a*, × 41,000; Fig. 19 *b*, × 51,000.


F
FS
16
FS—
17
F
18
FS
19 a
FS
V2
19 b

what we would interpret to be early stages of flagellar invagination into a primary vesicle. The authors stated that the origin and function of these vesicles were unknown. Grassé (1961), studying the centrioles and flagella of *Trypanosoma equiperdum*, presented what could be considered intermediate stages of flagellar growth. No primary vesicle was reported; instead, the flagellum seems to start growing when the basal body makes contact with the bottom surface of the reservoir, in which a mature flagellum is already inserted. As the flagellum grows, the cell membrane is evaginated, and becomes the sheath, the reservoir acting as the primary vesicle. Bernhard and de Harven (1958), in chick spleen, presented pictures of a growing cilium bounded by a double membranous sheath, still beneath the cell surface. They suggested that the external layer of the sheath fuses to the cell membrane when the cilium reaches the cell surface, and presented some micrographs that suggest that this is indeed the case. On the other hand, Sotelo and Trujillo-Cenóz (1958 *b*), presented a completely different scheme of ciliogenesis in chick neural epithelium. They came to the conclusion that when the basal body reaches the cell surface it induces the formation of an immature cilium. This organelle is then retracted into the cell, bringing the cell membrane along with it to serve as a sheath. The axial filaments are formed, and then it comes back to the surface, the sheath then fusing with the rest of the cell membrane.

There exist other reports in the literature that indicate that invagination into a growing vesicle might not be the only way in which a flagellum is put together. Manton (1959), studying the spermatozoid of *Dictyota*, found that before the liberation of the spermatozoid the whole flagellum is coiled spirally within the cell, with no signs of possessing a membranous sheath. When uncoiling begins, the flagellum pushes the cell membrane, on its way out, which "stretches" and forms the flagellar sheath. Schuster (1963), in a study of the amebo-flagellate *Naegleria*, also observed what looks like an axial complex lying free in the cytoplasm, with no sheath bounding it.

Although there are differences in the process of vesicle and sheath formation in these various systems, they all involve smooth membrane components, whether derived from Golgi apparatus, pinocytotic vesicles, or direct contact with the cell membrane. It has been pointed out, *e.g.* by Dalton (1961), that these three different components, at least in some cells, may well be phases of a single Golgi-vesicle-cell membrane system in which individual parts are continuously exchanging. If true, the differences in ciliary or flagellar sheath formation as shown by Grassé, Sotelo and Trujillo-Cenóz, and Sorokin may be more apparent than real.

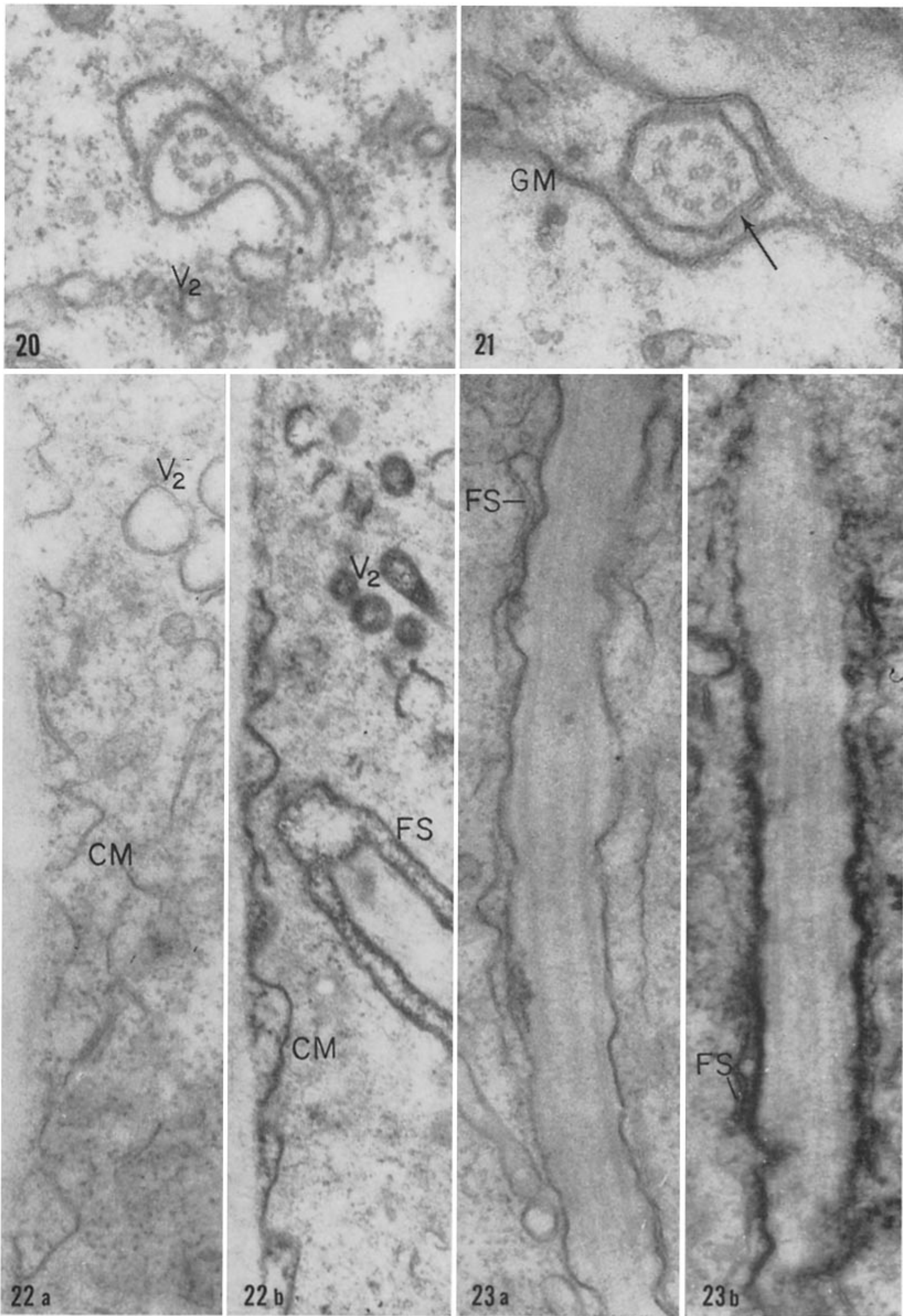
In conclusion, it could be said that in *Allomyces* two important morphogenetic events occur having to do with the growth of a flagellum, one of them preparatory to the process, and the other of an inductive nature. The preparatory event consists of the development of a basal body. This organelle does not originate *de novo* but from a preexisting small centriole that grows a new distal end during the process of gametangial differentiation. Once the gametangium has reached its mature state,

FIGURE 20 Cross-section of a growing flagellum surrounded by a double-membranous sheath, the outer layer apparently incomplete on one side. Secondary vesicles (V_2) are probably in the process of fusion to the forming sheath. × 51,000.

FIGURE 21 Cross-section of the flagellum from a mature gamete, still in the gametangium. The sheath consists of a single membrane, although some indication of doubleness is shown at arrow. *GM*, cell membrane of a mature gamete. × 64,000.

FIGURE 22 Cell membrane of gametangium 20 minutes in water. in Fig. 22 *a*, the material is unstained, while in Fig. 22 *b* it is stained with phosphotungstic acid. Note staining of the cell membrane (*CM*) of the gametangium and of the secondary vesicles (V_2) that serve as precursors for the cell membrane of the gamete. *GW*, gametangial wall; *FS*, probable tangential section of a flagellar sheath. × 51,000.

FIGURE 23 Longitudinal sections of flagella from the same material seen in Fig. 22. Fig. 23 *a*, unstained; Fig. 23 *b*, stained with phosphotungstic acid. Note staining of the flagellar sheath (*FS*). × 51,000.


V2
20
GM
21
V2
CM
22 a
V2
FS
CM
22 b
FS
23 a
FS
23 b

morphogenesis comes to a temporary halt. The basal body remains inactive until the proper stimulus is applied, namely, the transfer of the culture to a fresh aqueous environment. This results in the second or inductive event: a primary vesicle, apparently derived *via* pinocytosis from the cell membrane, comes in contact with the basal body and induces it to form a flagellum. Undoubtedly, growth of a flagellum is a complex process, and many questions remain unanswered, such as what is the origin of the flagellar protein and the nature of the organizing role of the basal body both in the attraction to the primary vesicle and as the center for flagellar growth.

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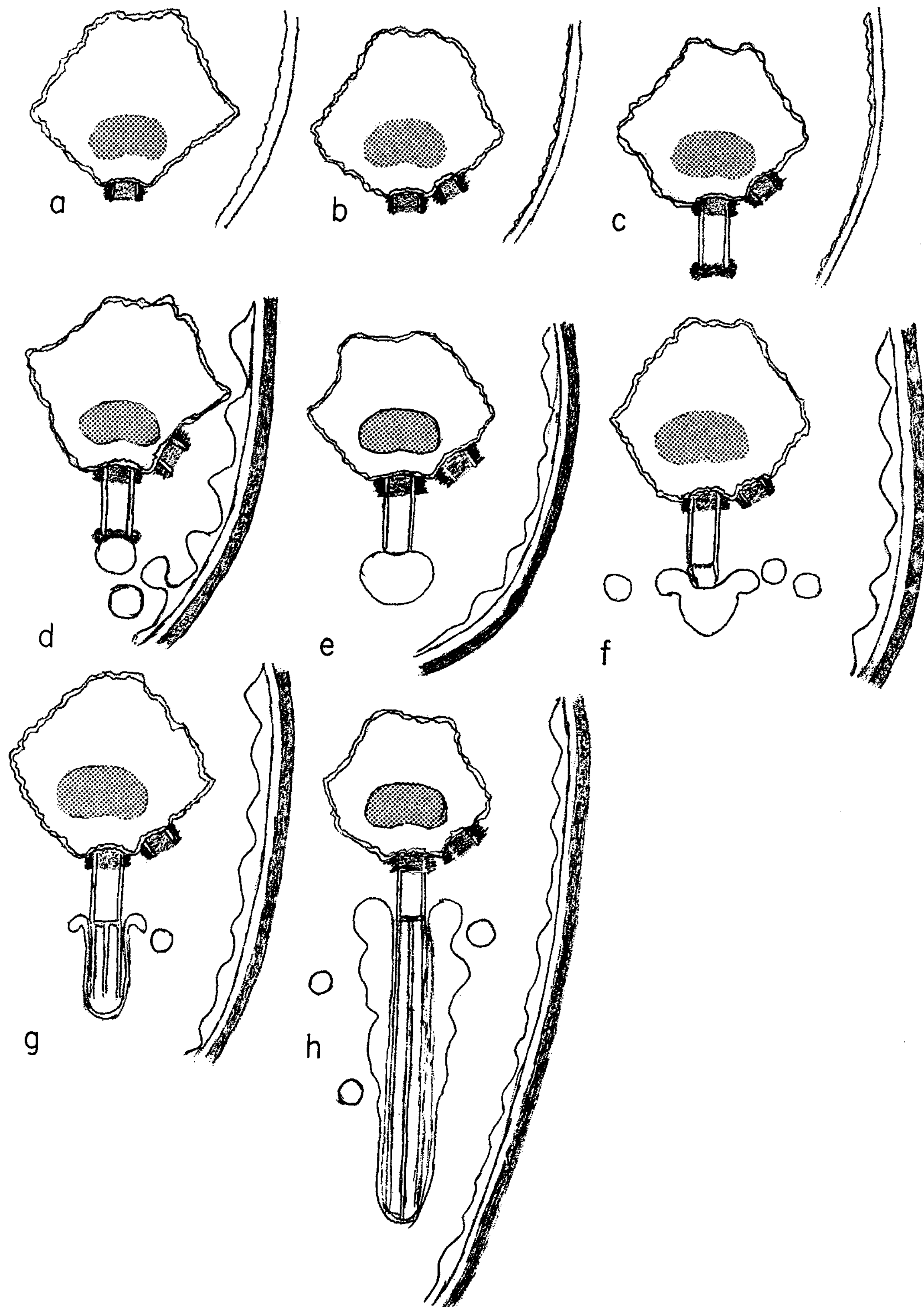
For References, See page 354.

FIGURE 24 Diagram of the development of the basal body and flagellum in *Allomyces*. The nuclei have been drawn disproportionately small for convenience of representation.

a. Hyphal tip nucleus and centriole.
b. Centriole complement in early stages of gametangial differentiation.
c. Elongation of one of the members of the centriole pair in later stages of gametangial differentiation.
d. Initiation of gamete formation, 10 minutes after immersion in water. Vesicles arise from the cell membrane and fuse with the basal body to form the primary vesicle.
e. Primary vesicle stage.
f. Start of flagellar fiber formation; enlargement of the primary vesicle by fusion with secondary vesicles.
g. and *h*. Further stages in elongation of the fibers and flagellar sheath.


a
b
c
d
e
f
g
h

REFERENCES

Bawden, F. C., and Pirie, N. W., 1946, The virus content of plants suffering from tobacco mosaic, *Brit. J. Exp. Pathol.*, **27**, 81.

Bernhard, W., and de Harven, E., 1958, L'ultrastructure du centriole et d'autres éléments de l'appareil achromatique, *Internat. Conf. Electron Microscopy, 4th*, Berlin, **2**, 217.

Bertaud, W. S., and Gatenby, J. B., 1960, The mitochondrial nebenkern and centriole complex in *Pachyrhamma fasifer* (Orthoptera), *Cellule*, **61**, 153.

Blondel, B., and Turian, G., 1960, Relation between basophilia and fine structure of cytoplasm in the fungus *Allomyces macrogynus* Em., *J. Biophysic. and Biochem. Cytol.*, **7**, 127.

Brachet, J., 1957, Biochemical Cytology, New York, Academic Press, Inc.

Brandt, P. W., and Pappas, G. D., 1960, An electron microscopic study of pinocytosis in Ameba: I The surface attachment phase, *J. Biophysic. and Biochem. Cytol.*, **8**, 675.

Burgos, M., and Fawcett, D. W., 1956, An electron microscope study of spermatid differentiation in the toad, *Bufo arenarum* Hensel., *J. Biophysic. and Biochem. Cytol.*, **2**, 223.

Dalton, A. J., 1961, Golgi apparatus and secretion granules, *in* The Cell, (J. Brachet and A. E. Mirsky, editors), New York, Academic Press, Inc., **2**, 603.

Dirksen, E. R., 1961, The presence of centrioles in artificially activated sea urchin eggs, *J. Biophysic. and Biochem. Cytol.*, **11**, 244.

Eakin, R. M., and Westfall, J. D., 1961, The development of photoreceptors in the stirnorgan of the tree frog, *Hyla regilla*, *Embryologia*, **6**, 84.

Fawcett, D. W., 1958, The structure of the mammalian spermatozoon, *Internat. Rev. Cytol.*, **7**, 195.

Fawcett, D. W., 1961, Cilia and flagella, *in* The Cell, (J. Brachet and A. E. Mirsky, editors), New York, Academic Press, Inc., **2**, 217.

Gall, J. G., 1961, Centriole replication. A study of spermatogenesis in the snail *Viviparus*, *J. Biophysic. and Biochem. Cytol.*, **10**, 163.

Gall, J., and Mizukami, J., 1963, Centriole replication in the water fern *Marsilea*, *J. Cell Biol.*, **19**, 26A.

Gatenby, J. B., 1961, The electron microscopy of centriole, flagellum and cilium, *J. Roy. Micr. Soc.*, **79**, 299.

Gibbons, I. R., and Grimstone, A. V., 1960, On flagellar structure in certain flagellates, *J. Biophysic. and Biochem. Cytol.*, **7**, 697.

Grassé, P. P., 1961, La reproduction par induction du blépharoplaste et du flagelle de *Trypanosoma equiperdum*, *Compt. rend. Acad. sc.*, **252**, 3917.

Hatch, W. R., 1935, Gametogenesis in *Allomyces*, *Ann. Bot.*, **49**, 623.

Lepper, R., 1956, The plant centrosome and the centrosome blepharoplast homology, *Bot. Rev.*, **22**, 375.

Lwoff, A., 1950, Problems of Morphogenesis in Ciliates, New York, John Wiley and Sons.

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

Mazia, D., 1961, Mitosis and the physiology of cell division, *in* The Cell, (J. Brachet and A. E. Mirsky, editors), New York, Academic Press, Inc., **3**, 77.

Nagano, T., 1962, Observations on the fine structure of the developing spermatid in the domestic chicken, *J. Cell Biol.*, **14**, 193.

Neuberger, A., and Pitt Rivers, R. V., 1939, The hydrolysis of glucosaminides by an enzyme in *Helix pomatia*, *Biochem. J.*, **33**, 1580.

Randall, J., and Hopkins, J. M., 1963, Studies of cilia, basal bodies and some related organelles, Part IIa: Problems of genesis, *Proc. Linnean Soc. London*, **174**, pt. 1, 37.

Robinow, C. F., 1962, Some observations on the mode of division of somatic nuclei of *Mucor* and *Allomyces*, *Arch. Mikrobiol.*, **42**, 369.

Ryter, A., and Kellenberger, E., 1958, L'inclusion au polyester pour l'ultramicrotomie, *J. Ultrastruct. Research*, **2**, 200.

Schuster, F., 1963, An electron microscope study of the amoebo-flagellate *Naegleria gruberi* (Schardinger). I. The amoeboid and flagellate stages, *J. Protozool.*, **10**, 297.

Sorokin, S., 1962, Centrioles and the formation of rudimentary cilia by fibroblasts and smooth muscle cells, *J. Cell Biol.*, **15**, 363.

Sotelo, J. R., and Trujillo-Cenóz, O., 1958 *a*, Electron microscope study of the kinetic apparatus in animal sperm cells, *Z. Zellforsch.*, **48**, 565.

Sotelo, J. R., and Trujillo-Cenóz, O., 1958 *b*, Electron microscope study on the development of ciliary components of the neural epithelium of the chick embryo, *Z. Zellforsch.*, **49**, 1.

Tokuyasu, K., and Yamada, E., 1959, The fine structure of the retina studied with the electron microscope. IV. Morphogenesis of outer segments of retinal rods, *J. Biophysic. and Biochem. Cytol.*, **6**, 225.

Turian, G., 1963, Synthèse différentielle d'acide ribonucleique et différenciation sexuelle chez l'*Allomyces*, *Dev. Biol.*, **6**, 61.

Wilson, E. B., 1925, The cell in Development and Heredity, New York, The MacMillan Company.