BASAL BODIES, BUT NOT CENTRIOLES, IN *NAEGLERIA*

CHANDLER FULTON and ALLAN D. DINGLE

From the Department of Biology, Brandeis University, Waltham, Massachusetts 02154, and the Department of Biology, McMaster University, Hamilton, Ontario, Canada

ABSTRACT

Amebae of *Naegleria gruberi* transform into flagellates whose basal bodies have the typical centriole-like structure. The amebae appear to lack any homologous structure, even during mitosis. Basal bodies are constructed during transformation and, in cells transforming synchronously at 25°C, they are first seen about 10 min before flagella are seen. No structural precursor for these basal bodies has been found. These observations are discussed in the light of hypotheses about the continuity of centrioles.

INTRODUCTION

Classical, light-microscope observations of centriole behavior led to two basic conclusions (reviewed by Fulton, 1971). The first conclusion, that basal bodies and centrioles are homologous, often interconvertible structures, has been confirmed by electron microscopy. Both organelles have the morphology of a cylinder about 0.2 μ in diameter whose wall is composed of nine parallel and equally spaced triplet microtubules, arranged in transverse section as the vanes of a pinwheel. This structure is herein termed a centriole-like structure (CLS), without regard to its position and function in a cell.

The second classical conclusion is that there is an invariant morphological and genetic continuity of centrioles from generation to generation in cells that contain them. The centrioles of metazoan cells persist as permanent organelles which duplicate and separate as part of each mitotic cycle. As a consequence, as Wilson (1925, p. 1127) noted, the centriole "is often regarded as an autonomous cell-organ arising only by the growth and division of a preëxisting centriole." The same conclusion was extended to the basal bodies of protists. Lwoff (1950) argued "one kinetosome [basal body] is always generated by division of another. We see kinetosomes dividing and have no evidence whatsoever for their formation *de novo*. They are endowed with genetic continuity." Implicit in the hypothesis of continuity by division is the idea that centrioles, or basal bodies, can form only in cells that contain preexisting, parental centrioles. Numerous cases of apparent morphological discontinuity have been described in the past 70 years, but these often have been dismissed on the grounds that the observations were equivocal—how can one prove that something is not present?—as well as challenges to the accepted generalization.

Electron microscopists have found no evidence for division of centrioles, or for reproduction by any template mechanism. Instead, they found that (*a*) even where new centrioles develop next to preexisting ones, the new centrioles form at a right angle to and separated from the old by 50–100 mμ (Gall, 1961; André, 1964; Murray et al., 1965; Robbins et al., 1968); (*b*) centrioles can develop through structurally dissimilar inter-

mediates (Dirksen and Crocker, 1966; Mizukami and Gall, 1966; Sorokin, 1968; Steinman, 1968; Kalnins and Porter, 1969); and (*c*) centrioles are built by the stepwise addition of microtubules (Dippell, 1968; Allen, 1969; Kalnins and Porter, 1969; Steinman, 1968). Although none of these ultrastructural observations are indicative of morphological, or even of genetic, continuity, the old ideas have persisted, and have led to frequent use of terms like "centriole replication" and "parent and daughter centriole."

Morphological persistence, and morphogenesis of new organelles in association with old, are regularly observed in, for example, the basal bodies of ciliates (e.g., Dippell, 1968; Allen, 1969) or the centrioles of vertebrate cells (e.g., Murray et al., 1965; Robbins et al., 1968). There is, however, no evidence that a morphologically similar predecessor is universally essential for the production of CLS, or even that these organelles are "autonomous" or have "genetic continuity." In fact, though centrioles have been referred to as "self-replicating organelles" in many recent discussions, there is little substantive support for that conclusion (Fulton, 1971). This leaves no a priori reason to argue that a preexisting CLS is required for the development of a new one.

A major challenge to the idea of morphological permanence of CLS has come from studies of the amebo-flagellate *Naegleria gruberi*. Amebae of *Naegleria* are able to transform into transient flagellates. Ultrastructural studies of these flagellates (Schuster, 1963; Dingle and Fulton, 1966) have shown that their basal bodies do have the typical CLS (Fig. 1). The question, then, is whether the amebae have preexisting CLS—centrioles—which give rise to basal bodies. Light microscopists have been unable to agree whether or not amebae have centrioles, but light microscopy cannot settle the question because centrioles are too small (see Discussion).

In an electron microscope study of *Naegleria*, Schuster (1963) found no centrioles in sections of amebae. He also reported that "neither a spindle nor centrioles are apparent in the mitotic stages" but based this on examination of two sections about 0.1 μ thick of dividing amebae about 15 μ in diameter. Dingle and Fulton (1966) also did not find centrioles in amebae. Many have accepted the conclusion that the CLS of basal bodies arises *de novo* in *Naegleria*. However, the evidence to support this

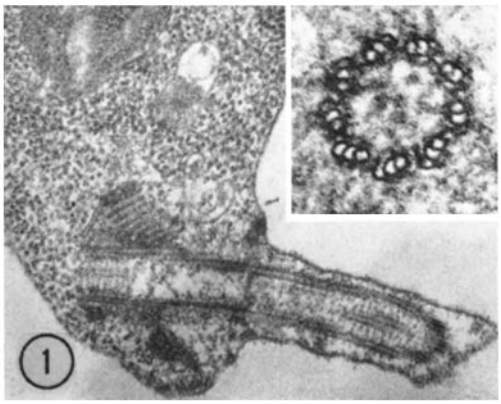

FIGURE 1 *Naegleria* basal bodies. Transverse section, × 100,000; longitudinal section, × 40,000.

conclusion is inadequate, and it has not gone unchallenged (e.g., Renaud and Swift, 1964; de Harven, 1968). The difficulty is that the negative statement "we were unable to find centrioles in amebae" has little meaning. Thus, we were motivated to a further, quantitative search. The search has been unsuccessful; it now seems worthwhile to document and evaluate the evidence that *Naegleria* amebae do not contain any centriole-like precursors for the basal bodies of the flagellates.

METHODS

Naegleria gruberi strain NB-1, methods for cultivation of the amebae, synchronous transformation into flagellates, and measurement of per cent flagellates and of flagella per flagellate are described in Fulton and Dingle, 1967. Basic methods used for electron microscopy were as described previously (Dingle and Fulton, 1966); all OsO_4 fixation was done with the buffer used there. More recently, samples have been fixed in glutaraldehyde (data with tables and figures), postfixed in OsO_4, dehydrated in ethanol, and embedded in Araldite 502 (Luft, 1961). Sections were stained with uranyl acetate in methanol (Stempak and Ward, 1964), often followed by lead citrate (Venable and Coggeshall, 1965), and examined with RCA EMU-3G and Philips 300 microscopes.

RESULTS

Centriole-Like Structures Appear during Transformation

Basal bodies are easily found in thin sections of *Naegleria* flagellates, even when they are scanned at low magnifications such as × 10,000. Identi-

fication of basal bodies is straightforward, regardless of whether they are sectioned transversely, obliquely, or longitudinally, or whether a flagellum is included in the section (micrographs may be found in Schuster, 1963; Dingle and Fulton, 1966; Dingle, 1970).

When we began to look for centrioles in amebae, none could be found. The contrast between our inability to find CLS in amebae and the ease with which they could be found in flagellates was striking. We wished to give the difference a quantitive expression, and also to determine the time and place of the first appearance of basal bodies during transformation of amebae into flagellates. Samples were taken at successive times during a synchronous transformation, and fixed in either Lugol's iodine or buffered OsO_4. The iodine-fixed samples were counted by using phase-contrast optics to determine the proportion of cells with flagella. The OsO_4-fixed samples were embedded and sectioned, and cell profiles were searched to determine the proportion which had CLS. A cell profile was searched only if it was as large as the diameter of a cell (ca. 10–15 μ) by rough visual estimate on the fluorescent screen, and if it contained a major proportion of the nucleus including some of the nucleolus. A total of 250 such cell profiles were scanned for each time of fixation. (Though a flagellum in a section indicates the presence of a basal body, a CLS was counted only if it was itself clear in the section.) The results of these counts are given in Table I and Fig. 2.

The first two counts give 0 CLS per 500 cell profiles, and the last two give 63. If we assume that the cell sections counted represent a random sample, of random orientation—both of which seem likely—the probability that structures of *equivalent visibility* were present in the two samples is negligible ($<10^{-30}$). A major change in visibility of CLS occurs during transformation.

In cells transforming at 25°C, CLS appear about 10 min before flagella are seen (Fig. 2). The percentage of cell profiles with CLS is half-maximal at 62 min; half the cells have visible flagella at 72 min. The two population heterogeneity curves (see Fulton and Dingle, 1967) are parallel throughout. Since flagella are not seen with the light microscope until they reach a length of nearly 2 μ, and since this may be estimated from measurements of flagellum outgrowth to require up to 4 min (Fulton and Dingle, 1967), 10 min is a maximal estimate of the precise time between the appearance of definitive CLS and the outgrowth of flagella. The temporal correlation of all the results obtained by quantitative electron microscopy with those from light microscopy strengthens the evidence that basal bodies, or CLS of equivalent visibility, are not present in *Naegleria* amebae.

The question of equivalent visibility is important, since it is one of several factors which

TABLE I

Appearance of CLS in Transforming Cells

For each time point, counts were made of the number of CLS per 250 cell profiles, selected from random sections using the criteria described in the text. Transforming cells (Fig. 2) were fixed, embedded, and stained as described in Dingle and Fulton, 1966, and searched for CLS using an RCA EMU-3G microscope.

Minutes after suspension	Number of CLS	
	In deep cytoplasm	Near cell surface
25	0	0
45	0	0
55	1	3
60	2	13
70	6	22
80	0	34
120	0	30
150	0	33

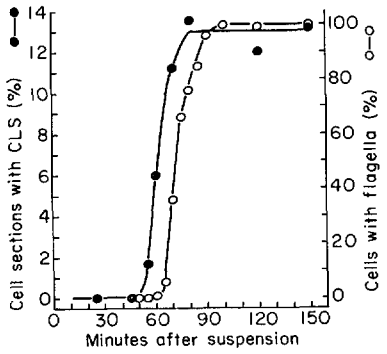

FIGURE 2 Time of appearance of CLS and of flagella during transformation. Cells transforming in Tris buffer at 25°C were sampled at intervals into one of two fixatives. Lugol's-iodine–fixed samples were counted by light microscopy for cells per 100 with flagella (○). OsO_4-fixed samples were counted by electron microscopy for cell sections per 250 with CLS; the results (Table I) are converted to per cent for this figure (●).

could influence the probability of seeing CLS in amebae versus flagellates:

(1) INCREASE IN NUMBER:

Since the average number of flagella per flagellate is about two (Fulton and Dingle, 1967), the number of CLS would have to increase either from zero or from one to two per cell.

(*a*) A twofold change from one to two is insufficient in itself to account for the observed increase.

(*b*) Increase from zero is the possibility that CLS are not present in amebae.

(2) CONSTANT NUMBER, INCREASE IN RELATIVE VISIBILITY:

(*a*) One obvious possibility is that in flagellates the flagella and rhizoplasts (rootlets) provide arrow-like indicators pointing to areas in cell sections which are likely to have basal bodies. This could bias an observer to find more CLS in flagellates, and thus could affect the counts. However, since basal bodies are readily found in sections without flagella or rhizoplasts, the presence of these structures does not determine whether CLS are seen. We consider it unlikely that these structures even affect the counts significantly.

(*b*) CLS could be "hiding" somewhere in amebae and change their position in cells during transformation. Our data are suggestive of a change in position in the cytoplasm (Table I). Since most of the cytoplasm (excluding mitochondria and food vacuoles) and most of the nucleoplasm (excluding nucleoli) are of similar density, it does not seem probable that a change in position outside of mitochondria, food vacuoles, and nucleoli would greatly affect relative visibility, and it seems unlikely that CLS reside inside these organelles.

(*c*) CLS could change in stability to fixation. Though it is conceivable that CLS in amebae are labile to OsO_4 fixation, this seems improbable in view of the generally observed stability of centriolar microtubules (de Harven, 1968). However, this possibility was considered further in an additional search of amebae, described below.

(*d*) CLS could increase in length or in staining intensity. The basal bodies of *Naegleria* flagellates are about 0.2 μ in diameter and about 0.8 μ in length. Their component microtubules stain intensely, and thus stand out in sections of flagellates. A shorter, less intensely staining CLS would be harder to see, and would occupy fewer thin sections. CLS, if present in amebae, might be shorter and stain less than the basal bodies of flagellates. This has been observed in the water mold *Allomyces*, where very short, relatively indistinct centrioles increase in length and staining intensity during their conversion into basal bodies (Renaud and Swift, 1964).

Only two of these possibilities seem likely to influence relative visibility sufficiently to explain the appearance of CLS during transformation: (1 *b*) CLS are not present in amebae or (2 *d*) CLS are present but very short or of low staining intensity. These possibilities are both equivalent to saying that no *definitive* CLS is present in amebae. The question of equivalent visibility is important, however, since if the visibility of CLS in amebae were reduced sufficiently relative to the visibility in flagellates, our results could be explained not as a *de novo* origin of CLS but as the maturation of a preexisting CLS.

Centrioles Are Absent in Mitotic Amebae

The apparent absence of CLS in amebae, and their appearance during transformation, raised the question of whether centrioles might also appear temporarily in amebae during mitosis. Mitosis in *Naegleria* amebae is characterized by conventional chromosome behavior, but the nuclear envelope does not break down at any time during division and the nucleolus divides without complete dissolution (Fulton, 1970).

For a search of mitotic amebae, we required populations enriched for dividing cells. It was found possible to obtain a single synchronized division by treating growing amebae at a temperature sufficiently high to prevent division but permitting cell growth (Fulton and Guerrini, 1969). After this treatment about 80% of the amebae divided in less than one-fourth of the normal doubling time. This provided samples in which 60–70% of the amebae were in mitosis (late prophase to telophase) at the time of fixation. Samples of these amebae were fixed in a variety of fixatives in the hope that if CLS were present the variations might preserve them or enhance their visibility. Fixation series 1 (Table II) was the same as used in the preceding study. Series 2–5 were variations of glutaraldehyde fixatives; these, as well as the glutaraldehyde-acrolein fixative (series 6), were selected because in other organisms these fixatives give good preservation of microtubules (e.g., Ledbetter and Porter,

TABLE II

Mitotic Amebae Searched for Centrioles

Amebae were sampled from a culture in synchronized mitosis, when about 60% of the amebae had division figures, and fixed as indicated. The OsO_4 fixative and veronal-acetate buffer are given in Dingle and Fulton, 1966; glutaraldehyde (GA) fixatives were made up in veronal-acetate or in 0.05 M phosphate, pH 7. Amebae were fixed in iced fixative, except series 4 was fixed at room temperature. Series 2–6 were postfixed in buffered OsO_4.

Series	Fixative	Central sections searched
1	OsO_4 in veronal-acetate	114
2	1% GA in phosphate	105
3	3% GA in phosphate	68
4	3% GA in phosphate, room temp.	100
5	3% GA in veronal-acetate	100
6	3% GA-3% acrolein in phosphate	50
Total		537

1963; Sandborn et al., 1964; Tilney and Porter, 1965).

The procedure used for scanning these mitotic amebae varied with the fixation series, observer, and microscope, but in every case was designed to optimize the likelihood of seeing any definite CLS present in a cell. Under the conditions used, basal bodies in flagellates were obvious, and spindle microtubules were evident in glutaraldehyde-fixed mitotic amebae. Cell profiles with prominent nuclei, judged by their large size to be central sections (cells > 11 μ, nuclei > 5 μ), were selected for examination (see Fig. 3). Each selected cell profile was then systematically scanned at magnifications of × 70–80,000 (including binocular magnification of × 7 or 10). Special attention was paid to the nucleus and adjacent cytoplasm, which were scanned at higher magnifications. All condensations which could conceivably be CLS were photographed at initial magnifications of × 20–30,000 for further study. (With the RCA microscope it proved necessary to photograph many possible candidates for CLS, which could be ruled out—as aggregates of ribosomes, nuclear pores, etc.—on negatives and prints. This was less necessary with the Philips microscope, where decisions could usually be made while looking at the image on the fluorescent screen.)

The different fixatives gave three distinct images. OsO_4-fixed amebae (series 1, Table II) could be recognized as mitotic only in sections of suitable orientation (see Fulton and Guerrini, 1969). In general, the appearance of these cells was comparable to that of the amebae and flagellates studied previously (Dingle and Fulton, 1966). No mitotic microtubules were seen, confirming Schuster (1963). The glutaraldehyde-fixed cells (series 2–5), while similar to one another, differed markedly from the OsO_4-fixed cells. Amebae could be recognized as mitotic by the presence of bundles of spindle microtubules (Fig. 4), each about 25 mμ in diameter, as well as by the appearance of the nucleus and its nucleolus (Fulton, 1970). In series 4, for example, 72% of the cell profiles examined were scored as mitotic; spindle microtubules were seen in all of these. Spindle microtubules were also preserved in glutaraldehyde-acrolein (series 6), but

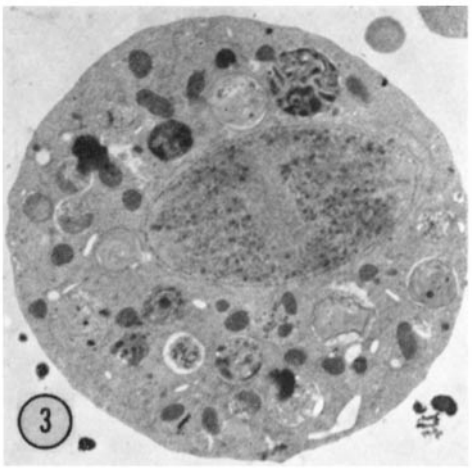

FIGURE 3 A mitotic ameba (from fixation series 2, Table II). The appearance of the nucleus is characteristic of glutaraldehyde-fixed cells in late metaphase or early anaphase. The nuclear envelope is intact. Bundles of microtubules run through the nucleus, though only their vague position can be seen at this magnification (see Fig. 4). On the basis of light microscope studies the chromosomes are in the relatively clear area in the center of the nucleus. The nucleolus is dispersed throughout the two denser areas toward the poles. The cytoplasm is full of food vacuoles containing bacteria in various stages of digestion. × 4000.

the cytoplasm was so dense that we are less confident of our search of these sections.

The search revealed no entities with CLS, or even any reasonable candidates. None were found in the two places suggested by light microscope observers: at the poles or in the nucleolus. Nucleoli were carefully searched, but neither in the dense material nor in nucleolar vacuoles were CLS found. The search was facilitated during karyokinesis because then the dense material

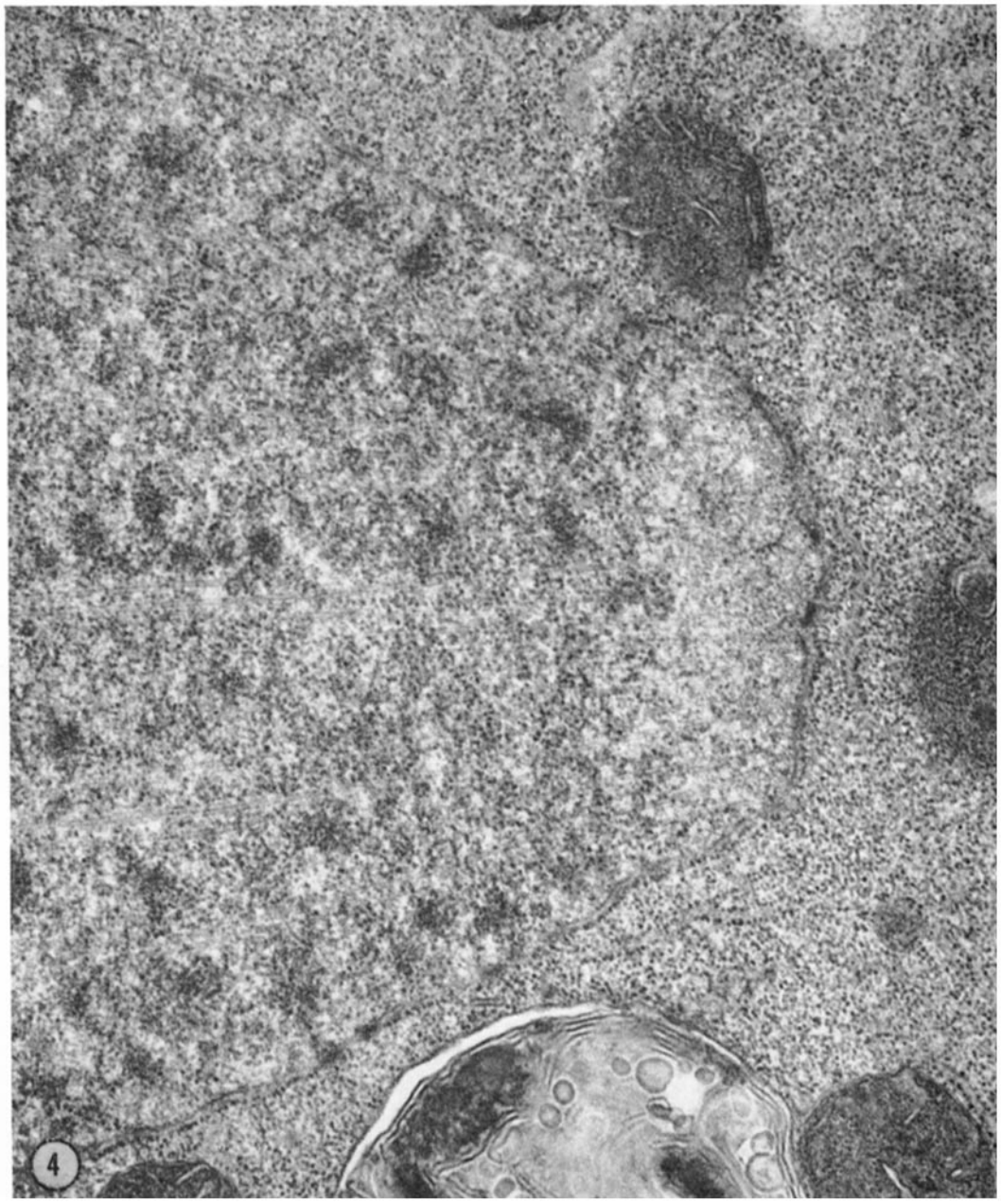

FIGURE 4 A mitotic apex from an ameba in a stage similar to that shown in Fig. 3 (from fixation series 4, Table II). The spindle microtubules do not come to a focal point, but rather run straight toward the nuclear envelope. × 40,000.

of the nucleolus is more diffuse in glutaraldehyde-fixed cells (Fulton, 1970). The apexes of mitotic figures were also examined with special care, and many were photographed for further study, but no CLS were found near these apexes, either apposed to the nuclear envelope or in the adjacent cytoplasm. The apexes were bluntly rounded or flattened (e.g., Fig. 4); none were encountered which came to a focal point as is seen in metazoan mitoses where centrioles are involved. In glutaraldehyde-fixed cells the microtubules did not come to a focus near the poles, but instead seemed to go straight toward the nuclear envelope (Fig. 4). In contrast, in mammalian cells one can see the convergence of microtubules toward the centrioles even when the centrioles are not included in the section (Robbins and Gonatas, 1964). All the observations suggested an anastral mitosis, such as is found in higher plants, where centrioles are absent. Not too much weight should be attached to this observation, however, since mitosis in *Naegleria* occurs within an intact nuclear envelope and might be expected to have a different appearance. In water molds, which also have intranuclear mitosis with parallel microtubules, centrioles have been found in the cytoplasm outside the apexes (Ichida and Fuller, 1968; Lessie and Lovett, 1968). No such structure has been found near the apexes in *Naegleria*. Not even any especially dense areas, comparable to the "centriolar plaques" observed in certain fungi (Robinow and Marak, 1966; Robinow and Caten, 1969) and in an ameba (Bowers and Korn, 1968), were observed where the spindle microtubules touch the nuclear envelope.

Since about 13% of central sections of flagellates were found to have CLS (Fig. 2), and since no CLS were found in a rigorous search of 537 amebae, 60–70% of which were in mitosis, we are convinced that no CLS are present in mitotic amebae.

Failure To Find Developing Basal Bodies

Definitive CLS appear during transformation. If we could trace their origin and morphogenesis, the conclusion that CLS are absent in amebae would be strengthened. The effort to do this has been unsuccessful. Sections of cells fixed during the 15 min interval from 55 to 70 min, when all the basal bodies appear (Fig. 2), were carefully scanned for indications of developing basal bodies. No structures were ever seen which could be reasonably interpreted as basal bodies forming; all basal bodies recorded had the definitive structure to the extent that orientation in the section would permit this structure to be seen. There was some suggestion that basal bodies arise deep in the cytoplasm and move to the cell surface, where flagellum outgrowth occurs (Dingle and Fulton, 1966). Eight CLS were seen in these samples which, as far as could be told from the cell profiles, were deep in the cytoplasm. These all were observed during the time of basal body appearance, 55–70 min; the CLS found in later samples were at the cell surface (Table I). No CLS was ever seen touching the nuclear envelope, but since such small numbers of CLS were seen away from the cell surface any conclusion about origin remains tenuous.

A continued search for developing basal bodies was made using glutaraldehyde-fixed cells, mainly from transformations where flagellates are produced with more than twice the normal number of basal bodies and flagella (Dingle, 1970), a procedure which should increase the probability of finding developing stages. Some *conceivably* developing stages were found, but none that were unequivocal. Cells contained frequent dense condensations, such as have been implicated in other cases of centriole morphogenesis (Dirksen and Crocker, 1966; Sorokin, 1968; Steinman, 1968; Kalnins and Porter, 1969), but these were much too numerous to be accounted for entirely as stages in basal body morphogenesis. Occasional examples were found where the microtubules appeared disordered, or in incomplete array, but all of these can be explained as sections which were slightly oblique or which just grazed one end of a complete CLS. Rare longitudinal sections of short CLS were found. These were probably developing basal bodies, as in other organisms, but since there was no way to know whether they had the definitive CLS in transverse section, they do not help answer the question of origin of CLS.

DISCUSSION

No centriole, or other definitive CLS, is present in *Naegleria* amebae, even during mitosis. We are confident of this negative conclusion, and believe our search has been sufficient to find even short centrioles, such as the ones 160 mμ in length that

Renaud and Swift (1964) found in *Allomyces*. On the other hand, our search does not exclude the possibility that relatively undefined "procentrioles" (Gall, 1961), "protocentrioles" (Perkins, 1970), or similar elements may be present to serve as structural precursors for the basal bodies that appear during transformation. Of particular interest would be structural precursors with ninefold rotational symmetry, as has been observed in procentrioles (Mizukami and Gall, 1966). To the present, however, procentrioles have been seen only in association with centrioles, basal bodies, or intermediate developmental stages. Examination of the figures in the papers on centriole morphogenesis (e.g., Gall, 1961; Mizukami and Gall, 1966; Dirksen and Crocker, 1966) makes it clear that what are unequivocally procentrioles when seen in association with specific organelles often would not be recognized if alone, especially if they were not sectioned transversely. A developing CLS can be as short as 70 mμ in length—an annulus just sufficient to be seen in a single section (Gall, 1961; Dippell, 1968). Such an annulus with granular ninefold symmetry pressed against the nuclear envelope or free in the cytoplasm could easily escape detection in even the most exhaustive search. Obviously, in our study an "undetected structural precursor" and "no precursor" are indistinguishable.

It is worth noting that serial sections would not solve this problem. Between 100 and 200 serial sections would be required to traverse a single cell, only about a third of which would include any substantial part of the nucleus. Even if several cells were serially sectioned, and each section carefully examined, there would be no way of knowing whether an imagined procentriole was in the correct orientation to permit recognition. Our search of random cell profiles has permitted us to examine more potential sites, in various orientations, and thus is more decisive.

The morphogenesis of centrioles and basal bodies has been studied mainly in organisms where the new structures develop next to old ones or where many are developing simultaneously. In one study of the production of new centrioles next to old, Murray et al. (1965) found morphogenesis "only twice in over 150 profiles." In ciliates and ciliated epithelia, hundreds of centrioles are developing simultaneously, which greatly increases the probability of finding intermediates in centriole assembly. Equally important is the development of new centrioles in a predictable position relative to old, which permits recognition of early stages because of position (see Dippell, 1968). Since in *Naegleria* we have no such indicators, and since only about two basal bodies develop, our failure to find developing basal bodies is not surprising. It does suggest, however, that these organelles go from "undetected" to definitive form fairly quickly — even short basal bodies have been observed only rarely.

Most of the many reports of *de novo* formation of centrioles and basal bodies are based on light microscope studies. Unfortunately, the small size of CLS places them at or near the resolution of the light microscope, and whether or not they are seen at all depends largely on what surrounds them (Fulton, 1971). Because of this technical problem any discontinuities observed by light microscopy require reevaluation today. In contrast, it is easy to resolve CLS with the electron microscope but it is possible to search only thin sections rather than entire cells.

The only definite reports of centrioles in *Naegleria* amebae placed them in the nucleolus, often in the nucleolar vacuole of interphase amebae (Wasielewski and Hirschfeld, 1910; Wilson, 1916). These reports closely followed publication of Nägler's (1909) widely considered hypothesis (see Fulton, 1970) that organisms like *Naegleria* have "promitosis" in which the nucleolus is a division center containing centrioles. Many described intranucleolar centrioles in other organisms (Wilson, 1925), but no one has ever found intranuclear centrioles in any organism with the electron microscope. At this point it seems reasonable to consider the early observations of intranuclear centrioles as artifacts of light microscopy.

Other amebo-flagellates besides *Naegleria* probably also have morphological discontinuity of their basal bodies. In *Tetramitus rostratus*, an organism closely related to *Naegleria* (Fulton, 1970), no centrioles have been found in amebae with either the light or electron microscope (Outka and Kluss, 1967). Although Outka and Kluss (1967) emphasized that "developmental stages of kinetosomes *have* been identified," study of their text and figures reveals that the few presumed developing basal bodies are all longi-

tudinal sections, and are all fairly long. None of their micrographs even suggests immature stages in development of the centriolar pinwheel. In the plasmodial stage of the life cycle of the true slime molds, Myxomycetes, mitosis is intranuclear and centrioles have never been found with the electron microscope—though no systematic search has been made—whereas in the myxamoebae and flagellated swarm cells CLS are evident (Schuster, 1965; Aldrich, 1967, 1969; McManus and Roth, 1968; Guttes et al., 1968).

Morphological discontinuity of centrioles may be common among the eucaryotic protists and lower plants, as originally emphasized by Sharp (1921) and Lepper (1956). One of the best-known cases is Mizukami and Gall's (1966) study of *de novo* formation of basal bodies in the fern *Marsilea*. Perkins (1970) has described the *de novo* formation of centrioles during zoosporulation in the marine protozoan *Labyrinthula*. In this organism the vegetative cells seem to have no centrioles. They form protocentrioles, dense granular masses 200–300 mμ in diameter, which develop a hub-and-spokes cartwheel with ninefold symmetry. The protocentrioles apparently give rise to the centrioles, though no intermediate stages have been observed. Electron microscopists have reported other examples of probable discontinuity in algae (Turner, 1968; Randall et al., 1967), fungi (King and Butler, 1968), bryozoans (Moser and Kreitner, 1970), and a cycad (Mizukami and Gall, 1966). In none of these cases has a systematic search for CLS been reported.

There have been extensive debates about the discontinuity of centrioles in eggs, especially those of sea urchins (Wilson, 1925; Briggs and King, 1959; Mazia, 1961; Went, 1966; Fulton, 1971). Boveri and Van Beneden developed the idea that the centriole is lost during the final stages of oogenesis, and is restored by the sperm on fertilization. When it was found that asters, and even parthenogenetic development, could be induced in eggs without sperm, this suggested the *de novo* formation of centrioles. Dirksen (1961; also Van Assel and Brachet, 1966; Sachs and Anderson, 1970) demonstrated by electron microscopy the presence of CLS in artificially activated eggs, which can be interpreted to mean either that CLS were present in the eggs or that they formed *de novo*. The question of *de novo* formation of CLS in eggs remains unsettled, though it appears that the eggs of some species may contain centrioles (Longo and Anderson, 1969).

This survey of the literature suggests that the discontinuity found in *Naegleria* may prove as widespread as it once appeared to classical cytologists. Many of the protists and lower plants seem to use their CLS exclusively as basal bodies and not as division centers (see Pickett-Heaps, 1969; Friedländer and Wahrman, 1970; Fulton, 1971). It may turn out that in many such organisms basal bodies are only constructed during flagellated stages of the life cycle.

The evidence for *Naegleria* is among the best for morphological discontinuity of centrioles. Though no well-formed centrioles are present to serve as precursors for the centriole-like basal bodies which develop, we cannot say with confidence that no precursors are present. The specific question of precursors remains unsettled, but perhaps the question itself has relatively little force. There never was any evidence that CLS are present throughout the entire life cycle of *Naegleria*. Most of the evidence which originally led to the notions of self-replication of centrioles and basal bodies—including the evidence that they contain DNA—has not withstood contemporary scrutiny (Fulton, 1971). There is no overwhelming reason why one should *expect* an organism that forms CLS to always have CLS—though many organisms always do seem to have them (just as some always have flagella), and regularly do seem to form the new ones near the old ones. From this viewpoint it is not necessarily disturbing if some organisms, such as *Naegleria*, can produce CLS without morphological precursor templates. Though the terminology and the image resolution have changed, the problems are not very different than they appeared to E. B. Wilson in 1925. After discussing the conflict between the ideas of self-replication and of *de novo* formation of centrioles, he wrote: "In the very fact of such a double mode of origin (if it can be accepted) lies the peculiar interest of central bodies" (Wilson, 1925, p. 672).

The absence of centrioles in *Naegleria* amebae raises the question of whether any precursor, unrecognized in our search and perhaps unrecognizable by electron microscopy, is found in these amebae. Such a precursor might act as a nucleating center around which a basal body forms, and the number of precursors in a cell would then determine the number of flagella which can develop during transformation. Alternatively, the basal bodies might develop without any such precursor entity, and the number of flagella

per flagellate might be determined by some other mechanism (see Dingle, 1970; Fulton, 1971). Clearly, techniques other than electron microscopy will be required to approach the precursor question. If a precursor can be found by another approach, information about its properties might make it possible to trace the basal body to its origin, and there to ask whether any recognizable structure, and especially any traces of CLS, can be found in the precursor. The absence of centrioles in *Naegleria* amebae is thus not an end, but only a beginning toward a new evaluation of the origin and reproduction of CLS.

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