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Ultrastructural Studies of Microconidium Formation in *Neurospora crassa*

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Microconidiating cultures of "peach-fluffy" (*pe, fl;* Y8743m, L; FGSC #569) were fixed at various times after the initiation of growth and examined with an electron microscope. Hyphae from which microconidia form are markedly vacuolated and show a much more extensive system of rough endoplasmic reticulum than young vegetative hyphae. A bulge in the hypha presages the start of microconidium formation, followed by the rupture of the outermost wall layers. A thick collar forms around the protruding microconidium due to extensive thickening of the inner wall layer of the parent hypha. At this stage, the cytoplasm of the developing microconidium is still continuous with that of the microsporophore cell from which it arises and is contained by a wall which is derived from the thickened collar. The microconidium is finally isolated from the cytoplasm of the microsporophore by a centripetal extension of the collar. Microconidia differ from macroconidia in having a more extensive endoplasmic reticulum and fewer mitochondria, in addition to being smaller and having a single nucleus.

Although its most familiar aspect is conferred upon *Neurospora* by its pinkish-orange monilioid conidia (macroconidia), many strains, including some ordinarily considered to be "aconidial," form a second type of spore, the microconidium. Their discoverer, B. O. Dodge, described their formation and appearance accurately as far back as 1930, and recorded their presence in *N. sitophila, N. crassa,* and *N. tetrasperma,* as well as in several races of each of these species (3). Dodge (2) noted that microconidia are borne laterally and terminally on microsporophores, which are short and blunt branches formed at intervals along hyphae. The cells of the microsporophore were observed to form a "collar-like projection . . . within which it seemed . . . as though there were an opening . . . through which partly formed spores are being extruded."

Microconidia, for which Dodge (2, 3) obtained an average size of 2.5 by 3.5 μ, are smaller and less variable in size and shape than macroconidia. They also have been shown to be uninucleate (1; Baylis and De Busk, *personal communication*), whereas macroconidia usually have several nuclei.

Despite the frequent use of microconidia in genetic experiments and the long time that they have been known, little more is known of the details of their formation and structure than was reported by Dodge. Due to the inadequacy of the light microscope for studying such small objects, these observations were incomplete and often tentative, as the quotation above reveals. We began these studies to describe more fully the ultrastructure and development of microconidia.

MATERIALS AND METHODS

The inositol-less (*inos;* 89601) and peach-fluffy (*pe, fl;* Y8743m, L; FGSC #569) strains of *N. crassa* were grown on petri dishes of inositol-supplemented "minimal" medium (6) at room temperature. Macroconidiation in *inos* began within 24 hr, whereas microconidiation in *pe, fl* required 6 to 7 days for initiation.

Conidia of both types were fixed in 3% Formalin and 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (*p*H 7.4) for 6 hr. After several rinses of cacodylate buffer, the conidia were postfixed in 2% cacodylate-buffered OsO_4 for 1 hr. Several rinses in distilled water were followed by staining in 0.5% uranyl acetate for 6 to 12 hr. The conidia were dehydrated in an ethyl alcohol series and transferred to propylene oxide prior to embedding in an Epon 812 mixture (7). Sections were stained with lead citrate and observed with an RCA model EMU 3G electron microscope at 50,000 ev.

Studies with a light microscope. The appearance of hyphae of *N. crassa* upon which macroconidia and microconidia are formed was compared under a light microscope. Macroconidia of *inos* arise directly from vegetative hyphae and are acropetally formed (Fig. 1) by repeated constriction of the hyphae. Occasionally, as in Fig. 4, these spores are formed laterally, as well as terminally, a process described as "budding" by Turian and Matikian (9). By contrast, microconidia

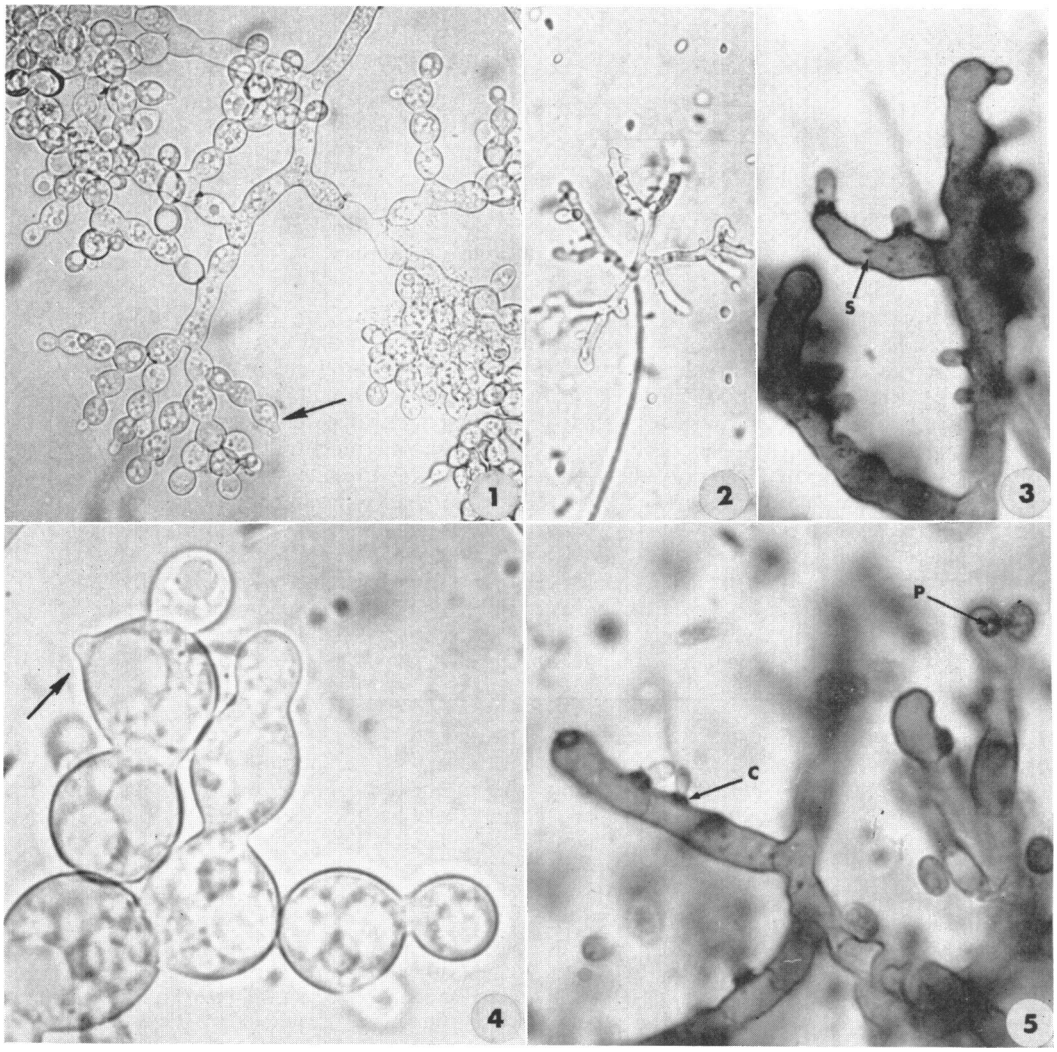

FIG. 1. *Hyphae of Neurospora crassa (inos) upon which macroconidia (arrow) are formed.* × *500.*

FIG. 2. *Hyphae of Neurospora crassa (pe, fl) upon which microconidia are formed.* × *400.*

FIG. 3. *Microconidiophore of Neurospora crassa (pe, fl). Note septum (s), the pore of which is blocked by an inclusion.* × *1,600.*

FIG. 4. *Macroconidia of Neurospora crassa (inos). Arrow indicates point where a conidium is being formed by a "bud" from another conidium. (See also Fig. 1.)* × *1,200.*

FIG. 5. *Microconidiophore of Neurospora crassa (pe, fl). Note collar (c) and pore (p) of cells from which microconidia are formed.* × *1,600.*

FIG. 6. *Tip of actively growing vegetative hypha of N. crassa (pe, fl). Note mitochondrion (m), endoplasmic reticulum (er), nucleus (n) and lipid inclusion (l).* × *18,000.*

FIG. 7. *Early stage in the development of a cell of N. crassa (pe, fl) which will form a microconidium. Note large amount of endoplasmic reticulum and ribosomes.* × *33,000.*

FIG. 8. *Microconidiophore of Neurospora crassa (pe, fl) showing several cells from which microconidia have been formed (arrows). Note large vacuoles surrounded by remnants of protoplasm in most of these cells.* × *6,000.*

FIG. 9. *Origin of apparatus of Neurospora crassa (pe, fl) from which microconidia will emerge (arrow). Note degeneration of hyphal wall in this region.* × *23,000.*

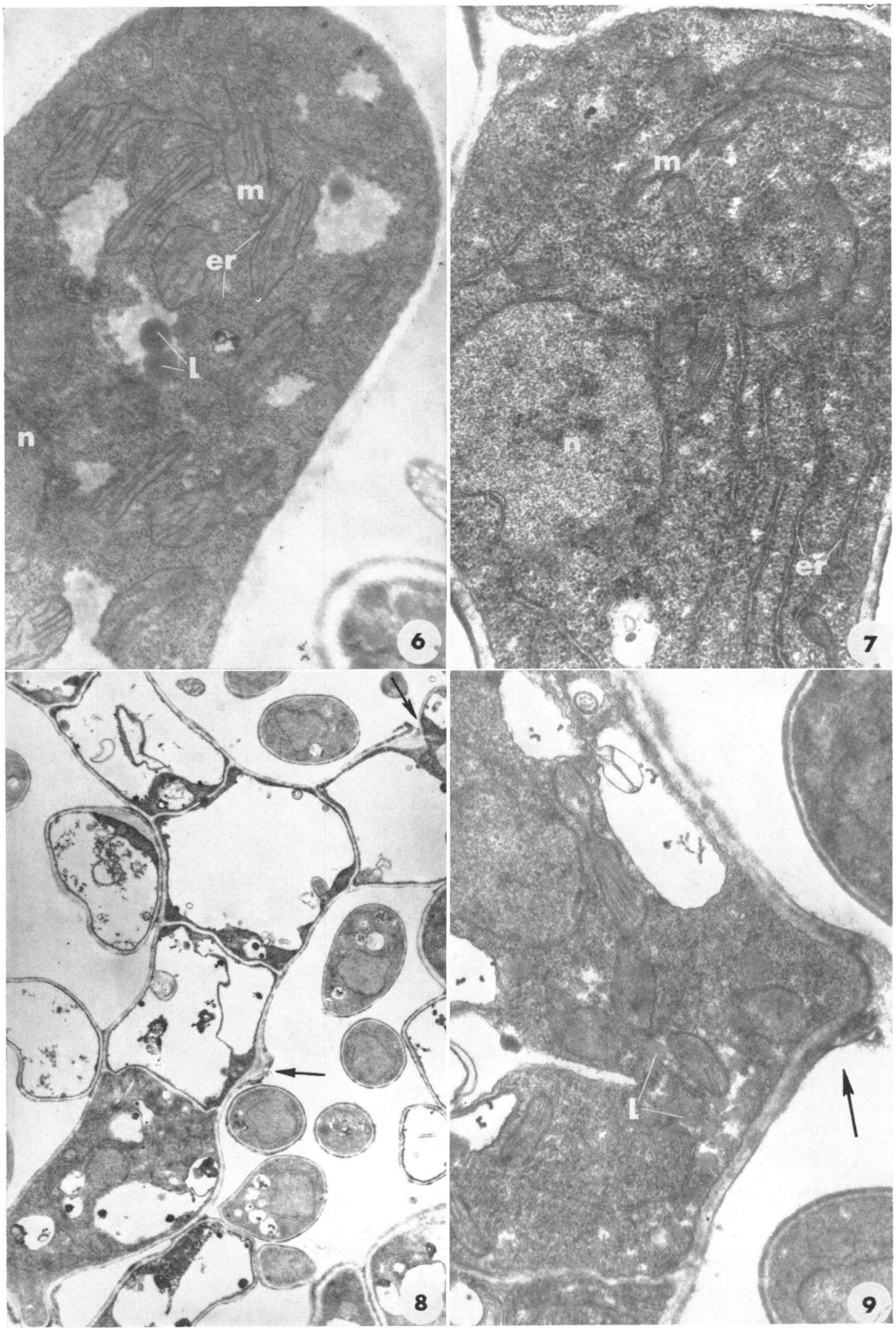

FIG. 6–9

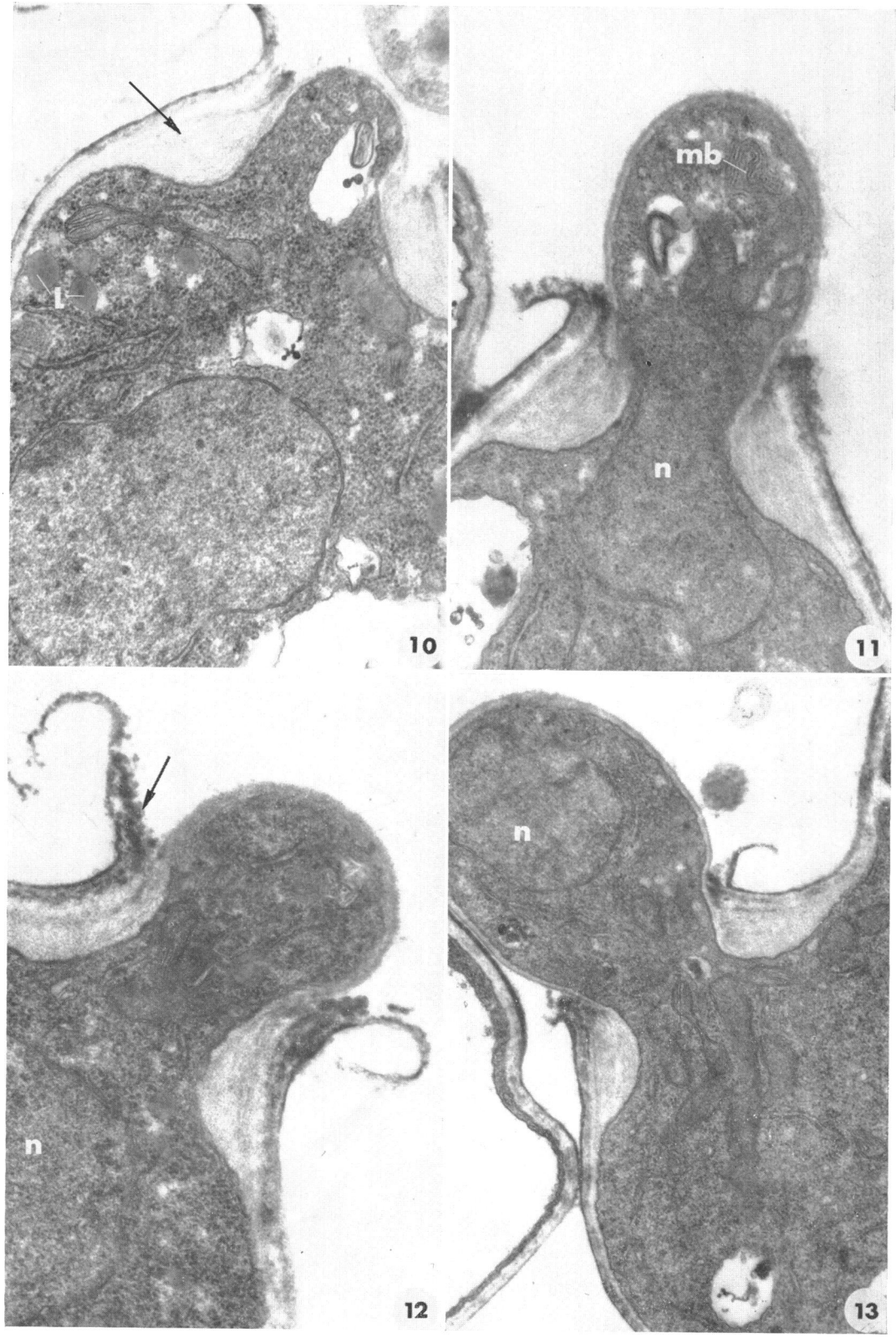

FIG. 10–13

of *pe, fl* arise from specialized hyphae, called microsporophores or microconidiophores (Fig. 2, 3, 5), which are borne on the vegetative mycelium. Microsporophores consist of several cells, some of which may branch (Fig. 2 and 3). These cells are separated by septa whose pores frequently are obstructed (Fig. 3), as described by Tsuda and Tatum (8). The pores surrounded by collars described by Dodge (2) are easily visible with a light microscope (Fig. 5). Only one pore is formed on each cell, and they appear to be formed unilaterally on the microsporophore, except above and below branches (Fig. 3).

Studies with an electron microscope. A vegetative hypha and one from a young microconidiophore may be observed in Fig. 6 and 7. The most striking difference between these is in the endoplasmic reticulum, which is much better developed in cells of the microconidiophore. The endoplasmic reticulum in these cells is of the rough type, and frequently is organized in parallel arrays as in Fig. 7. In fact, it will be seen that extensive development of the endoplasmic reticulum characterizes many subsequent stages in the development of microconidia as well. Furthermore, our observations suggest that the cells of the microconidiophore are uninucleate, in contrast to those of vegetative hyphae which are multinucleate.

Formation of a protuberance (Fig. 9) is the first visible sign that a cell of the microconidiophore will form a microconidium. The outer layers of the hyphal wall at this site seem to degenerate, and, as the incipient microconidium is forced out, flaps of densely staining tissue (Fig. 12) which comprise the remnants of these layers are thrust aside. Simultaneously, a collar is formed at the base of the developing microconidium by the deposition of more material (Fig. 10 and 11) which appears to be fibrous and continuous with the wall of the young spore (Fig. 10–13). At this stage, a nucleus enters the microconidium, and "membrane bodies" appear (Fig. 11) along with less well-defined clusters of membranes. The significance of these membranes is uncertain, for they may be artifacts of the preparative technique. A few small mitochondria enter the spore along with many ribosomes and endoplasmic reticulum.

The base of the young microconidium is separated from the microconidiophore by centripetal extension of the collar (Fig. 14). Thus, the entire microconidial wall seems to be formed from collar materials. The membranes, visible in the emerging spore (Fig. 11, 12, 14), disappear when the wall is completed (Fig. 14 and 15). After the wall of the microconidium is fully formed, the connection between the collar and this wall is eroded (Fig. 15 and 16), facilitating release of the spore.

A mature microconidium and macroconidium may be compared in Fig. 16 and 17. Much of the volume of the microconidium is occupied by its single nucleus. "Lipid inclusions" (based on observational data), endoplasmic reticulum, ribosomes, and a few small mitochondria make up the rest of the contents. On the other hand, a much greater proportion of the multinucleate macroconidium is occupied by mitochondria which usually are large. Moreover, macroconidia have vacuoles and less extensive endoplasmic reticulum.

DISCUSSION

The differences between microconidia and macroconidia are summarized in Table 1. Another difference is that it takes longer for microconidia to germinate (3), the reason for which may lie in the small amount of cytoplasm, in which few and small mitochondria are found. However, studies of the germination process will have to be performed to decide this point.

That the formation of microconidia takes 8 to 10 days at 25 C, as compared with the 2 to 4 days for macroconidia (3), is explained by the greater complexity of the former process. Thus, microconidiophores must be formed after which the cells of these specialized hyphae undergo the changes described herein.

TABLE 1. *Summary of differences between microconidia and macroconidia of Neurospora crassa*

Character	Microconidia	Macroconidia
Size	2.5 × 3.5 μ (1, 2, 3)[a]	5–9 μ
Shape	Ovoid to pear-shaped	Spherical or barrel-shaped to irregular
Endoplasmic reticulum	Extensive	Sparse to moderate
Mitochondria	Few and small (approx 1 μ long)	Many and large (>2 μ)
Nuclear number	Uniformly uninucleate (1; Baylis and De Busk, *personal communication*)	Multinucleate; average number varies in different populations (Pittenger, *personal communication*)

[a] Numbers refer to Literature Cited.

FIG. 10. *Early stage in formation of microconidium of N. crassa (pe, fl). Arrow indicates collar where heavy deposition of wall material has occurred.* × *32,000.*

FIG. 11. *Stage in formation of microconidium of N. crassa (pe, fl). Nucleus appears to be entering spore. Note membrane body (mb).* × *23,000.*

FIG. 12. *Stage in formation of microconidium of N. crassa (pe, fl). Note large amount of membranes at base of spore and continuity of collar with spore wall. Note densely-staining material from degenerating wall (arrow).* × *26,000.*

FIG. 13. *Stage in formation of microconidium of N. crassa (pe, fl). Nucleus has entered spore and spore wall is becoming well-defined.* × *26,000.*

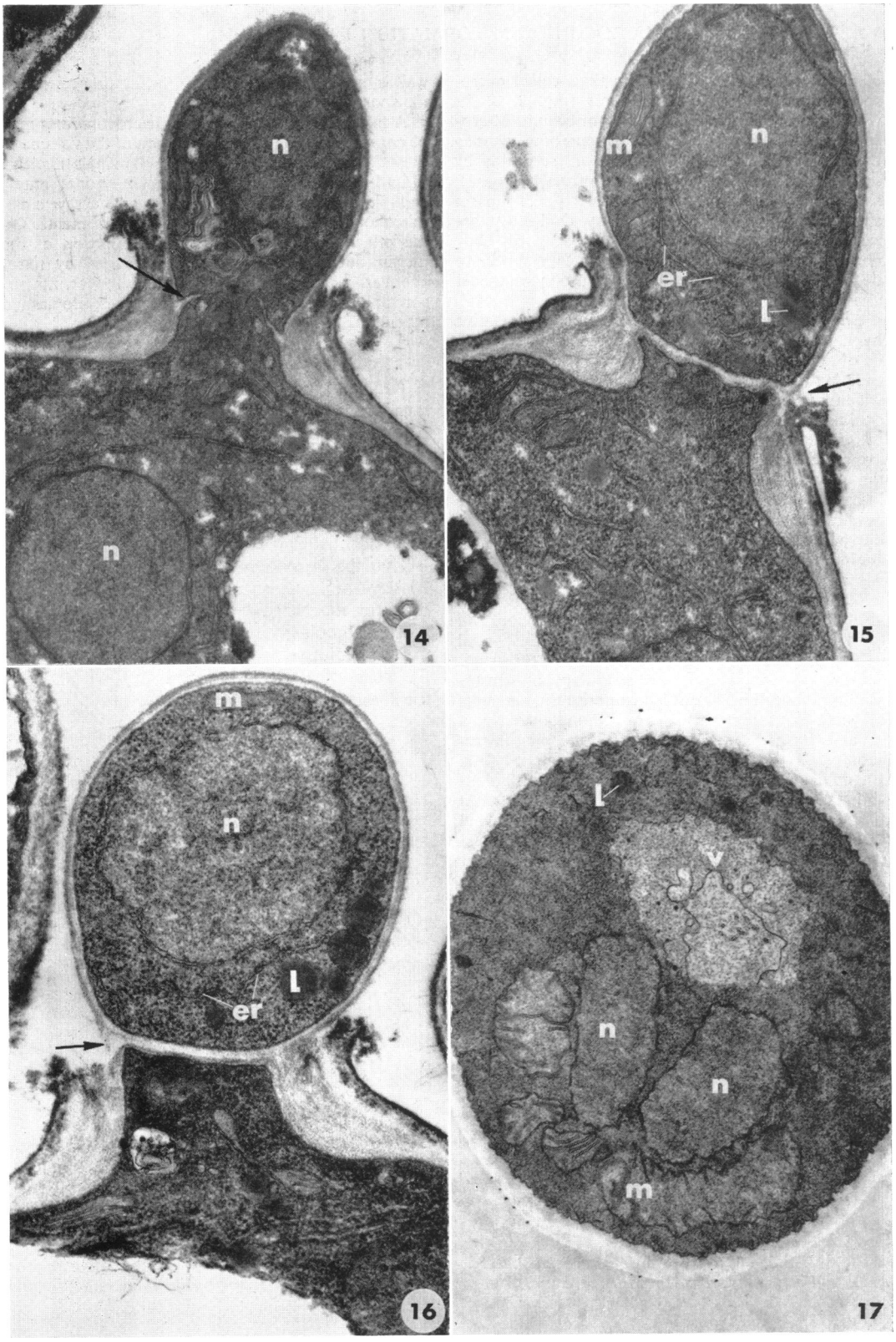

FIG. 14–17

The fact that the cells of the microconidiophore are uninucleate requires that the nuclei of vegetative hyphae be segregated and partitioned when these cells are formed. It is possible that the frequent occurrence of obstructions in the septa of microconidiophore cells (Fig. 3) provides a means through which partitioning can be accomplished, but the mechanism by which nuclei are segregated is unknown. We have observed dividing nuclei in cells of the microconidiophore, but whether division is initiated by microconidium formation, or the reverse, if they are associated at all, is not known.

Although these results show a very great difference in the size of the mitochondria of macroconidia and microconidia, they must be interpreted with caution. First, the macroconidia studied were from *inos* and the microconidia from *pe, fl*. It is conceivable that a strain difference could account for these results, though both Weiss and Turian (10) and Richmond, Somers, and Millington (5), working with macroconidia from strains Lindegren + and Em5297a respectively, also found many large mitochondria in such spores. Second, Luck (4) showed that the size and numbers of mitochondria in *Neurospora* can vary widely depending upon its nutrient status. Further experiments are indicated before the differences in mitochondria can be considered certain.

A number of unresolved questions are raised by this work. For example, how does the microconidiophore arise from vegetative hyphae? Inasmuch as each of the cells of the microconidiophore seems to be uninucleate, how do these arise from multinucleate hyphae? Given uninucleate cells of the microconidiophore, what is the pattern of segregation of nuclear types in relation to the position of these cells along the microconidiophore? And, how many microconidia arise from each cell of the microconidiophore? Dodge (1932) claimed that many do, and our observations support his. Studies are now in progress to answer these questions, which will extend our knowledge of this interesting type of spore and, perhaps, will enhance their usefulness in genetic studies.

ACKNOWLEDGMENT

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FIG. 14. *Stage in formation of microconidium of N. crassa (pe, fl). Note formation of wall which cuts off microconidium at base (arrow) and the prominent groups of membranes.* × *26,000.*

FIG. 15. *Maturing microconidium of N. crassa (pe, fl). Inclusion bodies are present (I) at this stage and subsequently. The connection between the collar and spore wall appears to be breaking down (arrow).* × *25,000.*

FIG. 16. *Mature microconidium of N. crassa (pe, fl). Several inclusion bodies (I) are visible and the connection between the collar and the spore wall is disintegrating (arrow).* × *36,000.*

FIG. 17. *Macroconidium of N. crassa (inos). Vacuole (v) is present as well as an inclusion body. Note nucleus or nuclei (n). Compare size and shape of mitochondria (m) in macroconidium and in microconidium in Fig. 15.* × *22,000.*