JOURNAL OF BACTERIOLOGY
Vol. 88, No. 5, p. 1497-1502 November, 1964

FINE STRUCTURE OF BASIDIOSPORES OF *SCHIZOPHYLLUM COMMUNE*

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Received for publication 6 July 1964

ABSTRACT

VOELZ, HERBERT (University of West Virginia, Morgantown), AND DONALD J. NIEDERPRUEM. Fine structure of basidiospores of *Schizophyllum commune*. J. Bacteriol. **88**:1497-1502. 1964.—The fine structure of basidiospores of the wood-rotting mushroom *Schizophyllum commune* was elucidated. Ungerminated spores were characterized by a fibrous cell wall, a cytoplasmic membrane, electron-dense granules, nuclei surrounded by nuclear envelopes with pores, and poorly defined mitochondria. Young germlings were still binucleate, whereas mitochondria appeared highly organized. Vacuolization appeared with germination, and elaborate pore structures were associated with septa.

Binucleate spores of the wood-rotting basidiomycete *Schizophyllum commune* germinate in a simple nutrient medium to form septate, homokaryotic hyphae. Although histochemical studies shed some light on the nuclear apparatus of the basidiospore (Ehrlich and McDonough, 1949) and subsequent morphological changes which occur during germination (Bakerspigel, 1959), no specific information is available concerning the fine structure of these reproductive units. Recent physiological studies provided data which support the idea that basidiospores of *S. commune* are endowed with certain enzymes of both carbohydrate utilization and terminal respiration (Niederpruem, 1964). The present report offers electron microscopic observations of basidiospore germination in this mushroom.

MATERIALS AND METHODS

S. commune Fr. was cultured and mated on a minimal medium which contained (per liter of distilled water): D-glucose, 20 g; asparagine (Difco), 2 g; thiamine hydrochloride, 100 μg; KH_2PO_4, 0.46 g; K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; and Noble's agar, 20 g. Basidiospores were collected solely from the cross, 699 $A^{41}B^{41} \times$ 845 $A^{51}B^{51}$. Basidiospore germination was studied in liquid minimal medium at 30 C by incubating culture flasks on a Gyrotory shaker (New Brunswick Scientific Co., New Brunswick, N.J.).

Basidiospores were fixed for electron microscopy by placing aqueous $KMnO_4$ (2%, w/v) in petri dish covers below the sporulating fruits. In this procedure, the freshly shed basidiospores immediately dropped into the fixative. Spores were washed free from fixative by centrifugation, with distilled water as the suspending medium. The concentrated spore pellet was dehydrated in alcohol and imbedded in Epon-Araldite (Voelz and Dworkin, 1962). Germlings were washed twice in 0.066 M phosphate buffer (pH 7.2), and were fixed, dehydrated, and embedded as above. Thin sections of basidiospores and germlings were observed under an electron microscope (model EMV-3E; Radio Corporation of America, Camden, N.J.) at 50 kv.

RESULTS AND DISCUSSION

Fine structure of ungerminated basidiospores. A short protuberance at the proximal end of the basidiospores represents the former conjunction (hilum) with the sterigma of the fruitbody (Fig. 1). The basidiospore cell wall consisted of one fibrous layer approximately 730 A wide. The cytoplasmic membrane measured 60 to 80 A, and appeared to be a typical unit membrane composed of two electron-dense lines bordering a transparent layer. Two nuclei were present in each spore. The nuclei distinctly exhibited a dense and a transparent area. Similar findings in yeast by Hashimoto et al. (1960) and Yotsuyanagi (1960) were interpreted as nucleolar material for the dense area and chromosomal matter for the transparent zone. The nuclear envelope consisted of a pair of unit membranes with rather wide pores (Fig. 2).

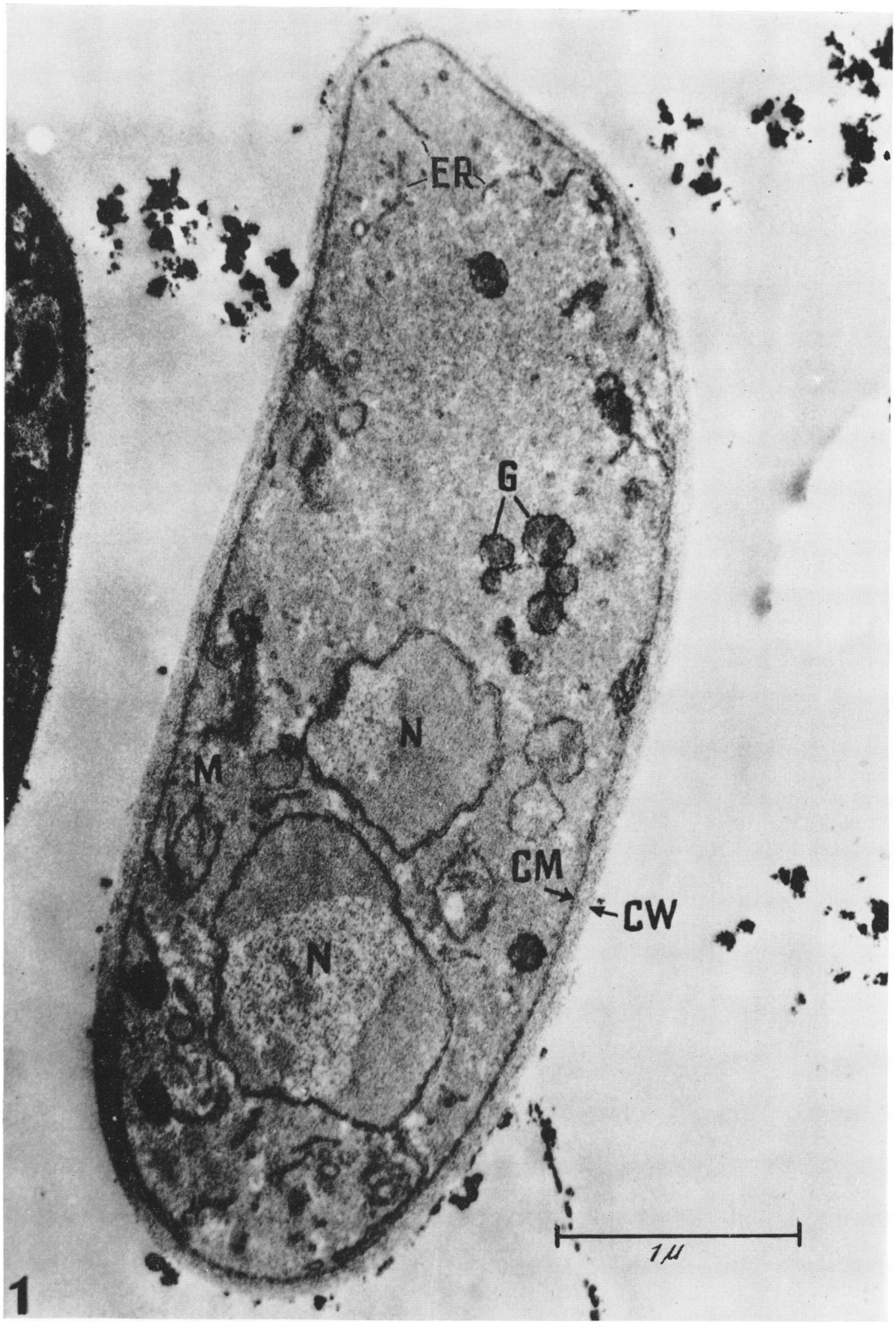

FIG. 1. *Longitudinal section of an ungerminated basidiospore of Schizophyllum commune. N, nuclei; M, mitochondrion; G, granules; CW, cell wall; CM, cytoplasmic membrane; and ER, endoplasmic reticulum.*

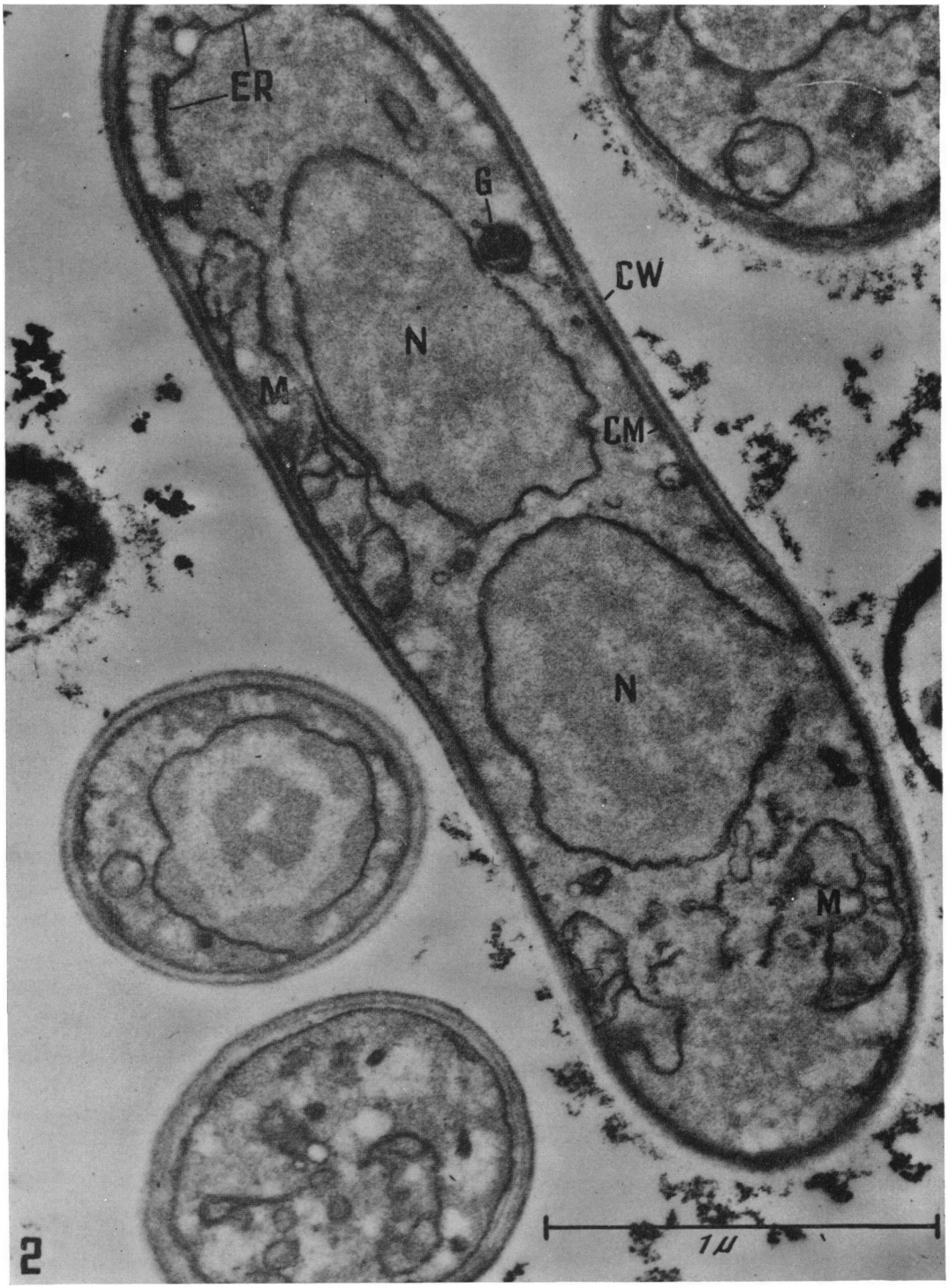

FIG. 2. *Longitudinal section of a 5-hr germling.*

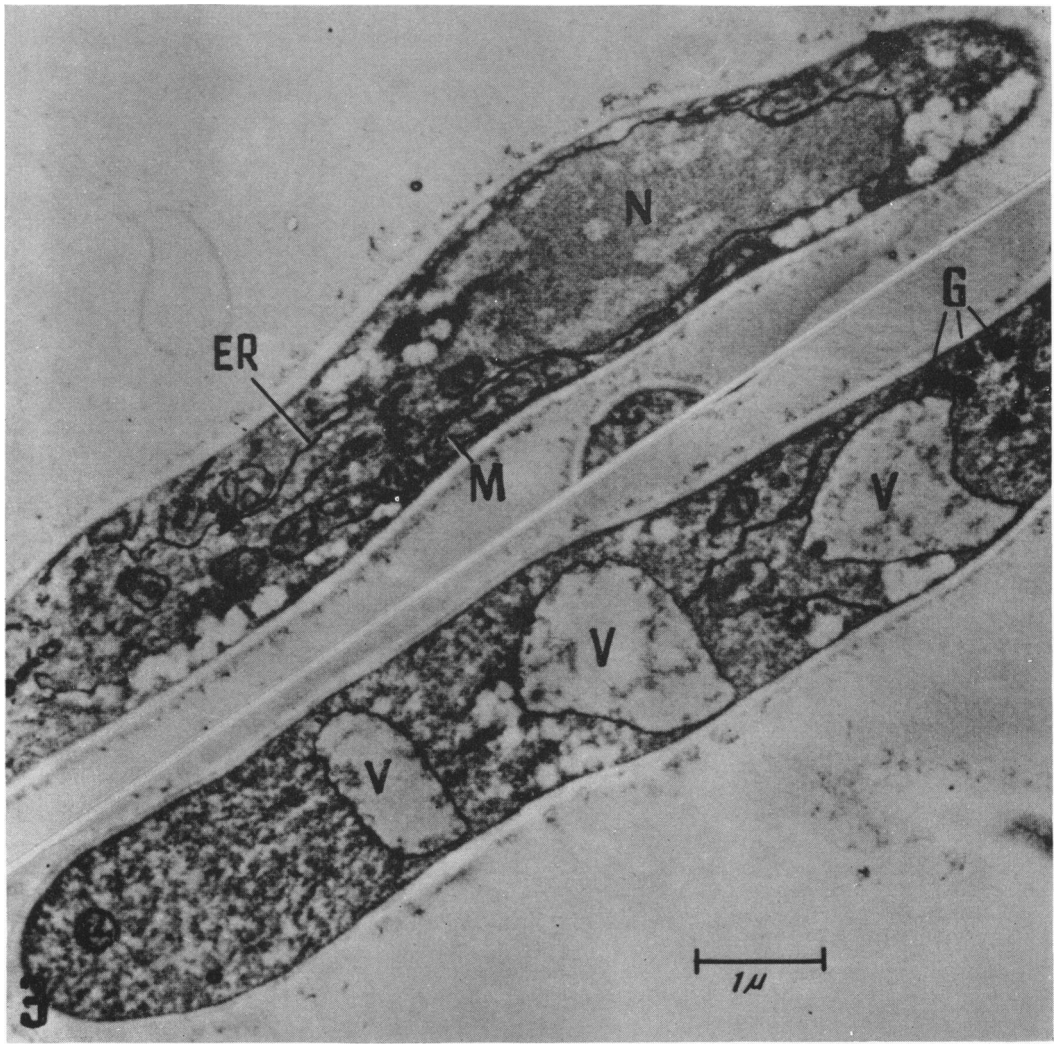

FIG. 3. *Longitudinal section of a 12-hr germling, cut in two portions. V, vacuoles.*

Electron-dense granules present in the cytoplasm of the basidiospore (Fig. 1) were round to oval with a diameter of approximately 1,700 A. Also noted was a pronounced dense "membrane" (70 A thick) surrounding the granule. Portions of the granular material evaporated under the electron beam, leaving the granule membrane apparently unaltered. Whether these residual structures are only interfaces occurring as dense precipitants or true membranes has not been determined. Certain histochemical procedures (Toluidine Blue or azure A) have similarly shown granular material in fresh basidiospores, whereas a marked metachromasy was evident with Bismarck Brown (Niederpruem, 1964).

The mitochondria of fresh basidiospores were poorly defined, and appeared as loosely assorted membranes or vesicles. The transparent areas within the cytoplasm may represent carbohydrate rather than lipids reported in yeast (Conti, Thyagarajan, and Naylor, 1963). Additional support for this view is the finding that fresh spores of *S. commune* stained positively with the periodic acid-Schiff procedure, and exhibited a

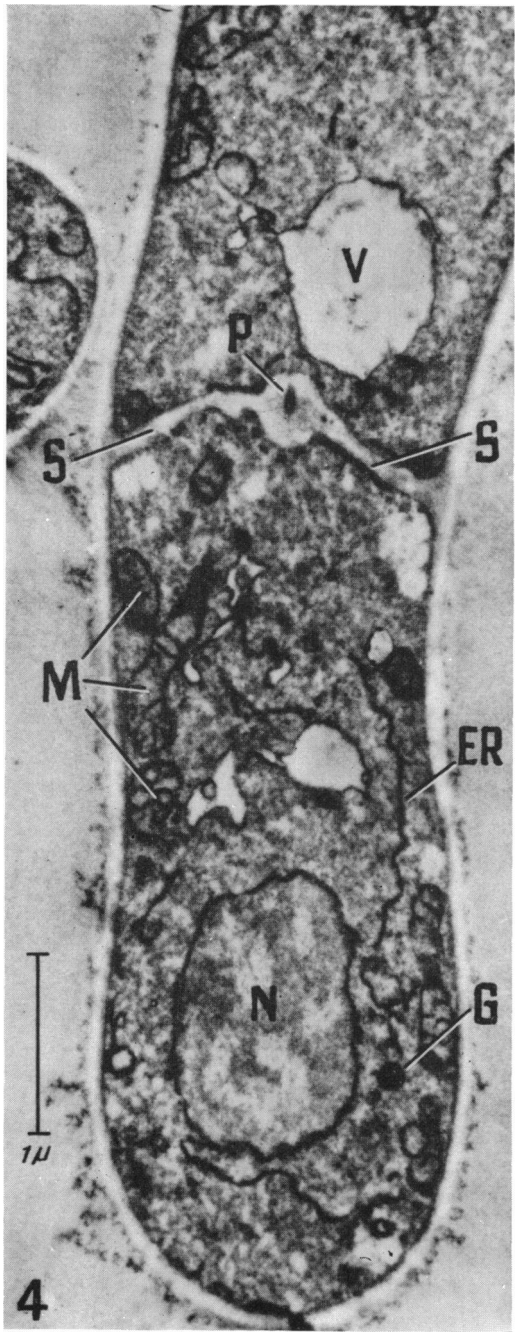

FIG. 4. *Longitudinal section of a hypha 16 hr after basidiospore germination. S, septum; P, pore apparatus.*

basal respiration characterized by a respiratory quotient (RQ = CO_2/O_2) of near unity (Niederpruem, 1964).

Fine structure of germlings. During germination, a marked alteration in the form of the basidiospore occurred; in addition, the cell wall of the germling was exceedingly difficult to stain by the fixative employed. The binucleate condition (Fig. 2) was still evident here, as were electron-dense granules. Moreover, the mitochondria appeared to be more organized at this stage of development. The apparent structural changes associated with the mitochondria may bear a relation to the finding that basal respiration increases several-fold during basidiospore germination in *S. commune* (Ratts et al., 1964). In addition, germlings showed definite signs of vacuolization (Fig. 3). In this connection, histochemical studies indicated that germlings stain positively with Sudan black B, suggesting the presence of a lipid reserve (Egbert and Niederpruem, *unpublished data*).

After 18 hr of germination, the cells reached a stage at which septa were formed. The elaborate pore apparatus (Fig. 4) resembled that described in other basidiomycetes (Girbardt, 1961; Moore and McAlear, 1962).

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research grants FR-05071 and E-4603.

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